PROTOCOL AVRO-RD-01-201

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

AMENDMENT 5

Sponsor:

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This protocol contains confidential information and is provided for exclusive use of Investigators. This information may only be disclosed to those persons involved in this study who have a need to know with the obligation not to further disseminate this information. This information may not be disclosed to other individuals unless such disclosure is required by country, federal, or state law or regulations subject to the foregoing. These restrictions on disclosure will apply equally to all future oral or written information supplied by AVROBIO that is designated as "privileged" or "confidential."

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: 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 NUMBER: AVRO-RD-01-201 AMENDMENT 5

[redacted]

[redacted]

AVROBIO, Inc.

8 April 2020

Date

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for AVR-RD-01. I have read the AVRO-RD-01-201 study protocol and agree to conduct the study in accordance with this protocol, all applicable government regulations, the principles of the International Council for Harmonisation (ICH) E6 Guidelines for Good Clinical Practices (GCP), and the principles of the World Medical Association Declaration of Helsinki. I also agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

_____

Site Number

1. SYNOPSIS

Protocol Number: AVRO-RD-01-201

Name of Sponsor/Company: AVROBIO, Inc.

Name of Investigational Product: AVR-RD-01

Name of Active Ingredient: The active substance in AVR-RD-01 is autologous CD34+-enriched cell fraction transduced with lentiviral vector (LV)/alpha galactosidase A (AGA) containing a ribonucleic acid (RNA) transcript that, after reverse transcription, results in codon-optimized complementary deoxyribonucleic acid (cDNA) that, upon its integration into the human genome, encodes for functional human AGA.

Title of Study: 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

Study Center(s): This is a multinational study

Length of Study: Approximately 3 years

Planned Duration of Treatment: The duration of each subject's participation in this study will be approximately 64 weeks (or 1 year, 12 weeks), comprised of an approximately 8-week Screening Period, 1- to 3-day Baseline Period, 6- to 8-week Pre-transplant Period, 1-day Transplant Period (during which time one AVR-RD-01 infusion will be administered), and 48-week Post-transplant Follow-up Period.

After study completion, consenting subjects will continue periodic safety and efficacy assessments for approximately 14 years (for a total of 15 years post-transplant follow-up) in long-term follow-up study AVRO-RD-01-LTF01.

Objectives:

Primary Objectives:

The primary objectives of this study are to:

- Evaluate the effect of AVR-RD-01 on substrate (ie, 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)

Secondary Objectives:

The secondary objectives of this study are to:

- Evaluate AGA enzyme activity in plasma and peripheral blood leukocytes
- Evaluate the effect of AVR-RD-01 on biomarkers for Fabry disease (ie, Gb3 and its deacylated form, globotriaosylsphingosine [lyso-Gb3]) in plasma and urine

- Evaluate the effect of AVR-RD-01 on substrate (ie, 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

Exploratory Objectives:

The exploratory objectives of this study are to:

- Evaluate the effect of AVR-RD-01 on the following:
  - 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 disease 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 (ie, 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)
- 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)
- Assess the impact of the conditioning regimen on reproductive potential

Study Design and Methodology:

This is a multinational, open-label study to assess the efficacy and safety of AVR-RD-01 in approximately 8 to 12 adult (defined as either ≥18 years of age; or ≥16 and <18 years of age and postpubertal, 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-transplant, Transplant, and Post-transplant 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-transplant baseline. Once baseline assessments are complete, the subject will enter the Pre-transplant Period (approximately 6 to 8 weeks) during which time mobilization, apheresis, AVR-RD-01 preparation and testing for release, conditioning regimen administration to achieve myeloablation, and conditioning washout period will take place. Following completion of the Pre-transplant Period, the subject will enter the Transplant Period (1 day) during which time AVR-RD-01 infusion will take place. After AVR-RD-01 infusion, the subject will enter the Post-transplant 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-transplant. Post-transplant 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-transplant, Transplant, and Post-transplant 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 transplant 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 (SAEs) related to AVR-RD-01 transplantation, 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 (SAEs related to AVR-RD-01 transplantation), 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 (ie, 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-transplant follow-up) in long-term follow-up study AVRO-RD-01-LTF01.

Number of Subjects (planned): Approximately 8 to 12 adult male subjects with a confirmed diagnosis of classic Fabry disease.

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion Criteria:

Subjects must meet all of the following inclusion criteria for participation in this study:

1. Subject is an adult male ≤50 years of age at the time of Screening. For this study, adult is defined as:
   - ≥18 years of age (mandated for subjects enrolled in the United States); or
   - ≥16 and <18 years of age and postpubertal, defined as having adult male testicular volume (where permitted by region)
2. Subject has a confirmed diagnosis of classic Fabry disease based on deficient AGA enzyme activity in peripheral blood leukocytes (defined as <1% of normal) at the time of Screening.
3. Subject with female spouse/partner of childbearing potential (defined as all women physiologically capable of becoming pregnant; this includes any female who has experienced menarche and who has not undergone successful surgical sterilization [ie, hysterectomy, bilateral tubal ligation, or bilateral ovariectomy] or is not postmenopausal [defined as amenorrhea >12 consecutive months and increased serum follicle-stimulating hormone level (ie, >30 IU/L) on at least 2 occasions (in the absence of hormone replacement therapy, dietary phytoestrogens) or estradiol concentration <10 pg/mL]) must be willing to remain sexually abstinent or use double-barrier method while sexually active from conditioning therapy administration until 48 weeks post transplantation (ie, the Week 48 study visit). Barrier contraception is required even with documented medical assessment of surgical success of a vasectomy. For female spouse/partner, acceptable methods of barrier contraception include diaphragm, cervical cap, or contraceptive sponge.
4. Subject must be willing to refrain from donating sperm any time after receiving conditioning therapy. For subjects planning on (or for whom there is a possibility of) fathering children in the future, sperm banking prior to administration of the conditioning regimen will be recommended.
5. Subject must be willing to refrain from donating blood, organs, tissues, or cells for transplantation at any time after AVR-RD-01 transplant.
6. Subject and/or parent (or legal guardian) must be willing and able to provide written informed consent (and assent, if applicable) for the study in accordance with applicable regulations and guidelines and to comply with all study visits and procedures, including the use of any data collection device(s) that may be used to directly record subject data.

Exclusion Criteria:

Subjects will be excluded from participation in this study if they meet any of the following exclusion criteria:

1. Subject has a *GLA* gene mutation associated with late-onset cardiac variant Fabry disease, including:
   - N215S (c.644A>G [p.Asn215Ser])
   - M296V (c.886A>G [p.Met296Val])

- R301Q (c.902G>A [p.Arg301Gln])
- Q279E (c.835C>G [p.Gln279Glu])
- A20P (c.58G>C [p.Ala20Pro])

2. Subject has received any ERT and/or chaperone therapy for Fabry disease within 3 years of Screening. Subjects who received ERT and/or chaperone therapy more than 3 years from Screening are eligible for study participation as long as all other study inclusion/exclusion criteria are met.
3. Subject has histopathologic findings on skin biopsy at Screening that indicate no or trace Gb3 accumulation (ie, score of 0 as defined in Thurberg, 2011).
4. Subject has tested positive for anti-AGA antibodies at the time of Screening.
5. Subject has eGFR <60 mL/min/1.73 m² (ie, chronic kidney disease [CKD] stage ≥3) at Screening.
6. Subject has a prior history of significant proteinuria (≥0.5 grams/24 hours) or equivalent (urine protein/creatinine ratio ≥50) and has not been on a stable dose of an appropriate anti-proteinuric medication for at least 3 months. Subjects with significant proteinuria (≥0.5 grams/24 hours) or equivalent (urine protein/creatinine ratio ≥50) found at Screening may be rescreened for study participation after they have been on a stable dose of an appropriate anti-proteinuric medication for at least 3 months.
7. Subject has evidence of any clinically relevant renal disease, in the opinion of the Investigator, not attributed to Fabry disease (eg, nephrotic syndrome, repeated episodes of pyelonephritis, obstructive uropathy, red blood cell [RBC] casts on urinalysis, kidney size discrepancy >1.5 centimeters, evidence of other renal disease on kidney biopsy performed at Screening).
8. Subject has a prior history of myocardial infarction (MI).
9. Subject has a history of coronary artery disease (CAD) with angina requiring percutaneous transluminal coronary angioplasty (with or without stent placement) and/or coronary artery bypass graft (CABG).
10. Subject has a history of moderate to severe valvular heart disease requiring valve replacement.
11. Subject has a history of heart failure, moderate to severe diastolic dysfunction, and/or left ventricular ejection fraction (LVEF) ≤45% on echocardiogram (ECHO) performed at rest at Screening.
12. Subject has a history of clinically significant cardiac arrhythmia (eg, heart block [second or third degree], atrial fibrillation requiring therapy [history of intermittent atrial fibrillation not requiring treatment is allowed], ventricular fibrillation, ventricular tachycardia, supraventricular tachycardia, or cardiac arrest).
13. Subject has uncontrolled hypertension (defined as systolic blood pressure ≥150 mmHg or diastolic blood pressure ≥90 mmHg) at Screening. Subjects with hypertension found at screening may be rescreened for study participation after they have been on a stable dose of an appropriate antihypertensive medication for at least 3 months.
14. Subject is on (or requires treatment with) any anticoagulant (warfarin, dabigatran, or other oral anticoagulant; or heparin) from 30 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 48 study visit. Use of antiplatelet agents are permitted, however should be withheld in the presence of bleeding or platelet counts <50 x 10^9/L.
15. Subject has a prior history of stroke and/or transient ischemic attack (TIA).

16. Subject has aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN) at Screening.
17. Subject has forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and/or diffusing capacity of the lung for carbon dioxide (DLCO) ≤50% predicted value (corrected for hemoglobin) on pulmonary function testing performed at Screening.
18. Subject has diabetes mellitus (type 1 or type 2).
19. Subject has an active chronic infection during the Screening, Baseline, or Pre-transplant Period of the study.
20. Subject has an active uncontrolled acute bacterial, viral, fungal, parasitic, or prion-associated infection during the Screening, Baseline, or Pre-transplant Period of the study.
21. Subject has a history of (or current) tuberculosis.
22. Subject tests positive for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV, type 1 or 2), human T-cell lymphotropic virus (HTLV)-1, HTLV-2, and/or syphilis on Venereal Disease Research Laboratory (VDRL) test, chemiluminescent microplate immunoassay (CMIA), or enzyme immunosorbent assay (EIA) at Screening.
23. Subject has a prior history of (or current) malignancy; the one exception is a prior history of resected basal cell carcinoma.
24. Subject has any other medical condition that predisposes him to (or conveys increased risk of) malignancy.
25. Subject has undergone, or is scheduled to undergo, bone marrow transplant and/or solid organ transplant.
26. Subject has hematological compromise evidenced by white blood cell count (WBC) <3.0 x 10^9/L, platelet count <100 x 10^9/L, hemoglobin <100 g/L, and/or uncorrected bleeding disorder from signing informed consent at Screening (ie, study enrollment) through the Transplant Period of the study (ie, the day of AVR-RD-01 infusion).
27. Subject has clinically significant immunodeficiency disease or condition, in the opinion of the Investigator, at Screening.
28. Subject is on (or requires treatment with) cytotoxic or immunosuppressive agents from 60 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 48 study visit; the one exception is treatment with cytotoxic or immunosuppressive agents required per protocol for stem cell transplant.
29. Subject has contraindication to iohexol.
30. Subject has any condition that makes it impossible to perform MRI studies (including allergies to anesthetics).
31. Subject has medical conditions(s) and/or is receiving medication(s) that would contraindicate ability to undergo mobilization (including contraindication to granulocyte-colony stimulating factor [G-CSF] and/or plerixafor), apheresis, or receive myeloablative therapy for conditioning.
32. Subject has any medical, psychological, or other condition that, in the opinion of the Investigator:
    - Might interfere with the subject's participation in the study (including consenting to procedures); and/or
    - Poses any additional risk to the subject; and/or
    - Might confound the results of any study-required assessments.

33. Subject has previously received treatment with AVR-RD-01 or any other gene therapy.
34. Subject is participating in (or plans to participate in) any other investigational drug trial, or plans to be exposed to any other investigational agent, device and/or procedure, from 30 days prior to signing informed consent at Screening (ie, study enrollment) through study completion.

Investigational Product, Dosage, and Mode of Administration: Between 3 and 20 x 10^6 autologous CD34+ cell-enriched population that contains cells transduced with LV/AGA containing a RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human AGA (ie, AVR-RD-01)/kg body weight will be administered once by intravenous (IV) infusion.

Reference Therapy, Dosage, and Mode of Administration: Not applicable

Endpoints:

Efficacy:

Primary

- Change from baseline in the average number of Gb3 inclusions (ie, myelinosomes) per kidney peritubular capillary (PTC) per subject at Week 48

Secondary

Secondary efficacy endpoints include the following:

- Change from baseline in AGA enzyme activity level in plasma and peripheral blood leukocytes at Weeks 24 and 48
- Change from baseline in biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma and urine at Weeks 24 and 48
- Change from baseline in substrate (ie, 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

Exploratory

Exploratory efficacy endpoints include the following:

- Change from baseline in 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 (ie, 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
- Changes from baseline in peripheral blood cell subpopulations (ie, T cells [CD3+], natural killer [NK] cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (ie, 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 (ie, T cells [CD3+], NK cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (ie, 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)
- 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)

Safety:

Primary

Primary safety endpoints include the following:

- Incidence and severity of AEs and 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

- Change from baseline in sperm count, sperm motility, and sperm morphology

Statistical Methods:

Data collected will be presented in summary tabulations. All data, as well as any outcomes derived from the data, will be presented in detailed data listings. Graphical displays will also be provided, where appropriate. All analyses will be performed using validated statistical software. Continuous variables will be summarized using descriptive statistics, with the number of non-missing observations, mean, standard deviation, median, minimum, and maximum values provided. Categorical variables will be summarized by counts and by percentage of subjects.

Subject disposition and demographic and baseline data will be summarized.

The primary efficacy endpoint, change in the average number of Gb3 inclusions (ie, myelinosomes) per kidney PTC per subject at Week 48, will be summarized using descriptive statistics. All secondary and exploratory efficacy endpoints will also be summarized.

Safety summaries will be provided for AEs, SAEs, and AEs leading to study discontinuation. Adverse events will be coded by Medical Dictionary for Regulatory Activities (MedDRA) primary System Organ Class and Preferred Term. Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary. Changes from baseline in vital signs, clinical laboratory assessments (including serum chemistry, hematology, urinalysis, and immunogenicity testing for the presence of anti-AGA antibodies), and abnormal ECG findings will be summarized. Results of testing for the presence of RCL and vector ISA will be provided in a listing.

Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 48 assessments (or prematurely discontinue the study) and the clinical database has been locked. Prior to finalizing and locking the database, all decisions concerning the inclusion or exclusion of data from the analysis for each subject will be determined by appropriate medical and statistical personnel. Any and all exclusions will be documented in subject listings.

Full analytical details for the study, including definition of analysis populations and procedures for handling missing data, will be prospectively outlined in a statistical analysis plan for the study.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADL	Activities of daily living
AE	Adverse event(s)
AGA	Alpha-galactosidase A
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical (classification)
ATS	American Thoracic Society
AUC	Area-under-the-curve
BID	Twice a day
BLISS	Barisoni Lipid Inclusion Scoring System
BMT	Bone marrow transplant
BPI-SF	Brief Pain Inventory-Short Form
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CBER	Center for Biologics Evaluation and Research
c/dg	Copy per diploid genome
cDNA	Complementary deoxyribonucleic acid
cGMP	Current Good Manufacturing Practice
CIOMS	Council for International Organizations of Medical Sciences
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology
CMIA	Chemiluminescent microplate immunoassay
CS	Clinically significant
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
ddPCR	Droplet digital polymerase chain reaction
DIBSS-D	Diary for Irritable Bowel Syndrome Symptoms-Diarrhea
DLCO	Diffusing capacity of the lung for carbon dioxide
DMC	Data Monitoring Committee
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	Electronic case report form

Abbreviation	Definition
eGFR	Estimated glomerular filtration rate
EIA	Enzyme immunosorbent assay
EIU	Exposure in-utero
EMA	European Medicines Agency
ERS	European Respiratory Society
ERT	Enzyme replacement therapy
EU	European Union
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
Gb3	Globotriaosylceramide
GCP	Good Clinical Practices
G-CSF	Granulocyte-colony stimulating factor
GI	Gastrointestinal
GLP	Good Laboratory Practices
HBV	Hepatitis B virus
HCT	Hematopoietic cell transplant
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HREC	Human research ethics board
HSC	Hematopoietic stem cell(s)
HSCT	Hematopoietic stem cell transplantation
HTLV	Human T-cell lymphotropic virus
IBS	Irritable bowel syndrome
ICF	Informed consent form
ICH	International Council for Harmonisation
IRB	Institutional review board
ISA	Integration site analysis
IV	Intravenous
LSD	Lysosomal storage disorder
LV	Lentiviral vector
LVEF	Left ventricular ejection fraction
LVMI	Left ventricular mass index
Lyso-Gb3	Globotriaosylsphingosine

Abbreviation	Definition
MCS	Mental Component Summary
t-MDS	Therapy-related myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
mGFR	Measured glomerular filtration rate
MI	Myocardial infarction
MLD	Metachromatic leukodystrophy
MRI	Magnetic resonance imaging
MS/MS	Tandem mass spectrometry
NAT	Nucleic acid-amplification testing
NCS	Not clinically significant
NHANES	National Health and Nutrition Examination Survey
NK	Natural killer
NOD	Nonobese diabetic
NSF	Nonobese diabetic (NOD)/severe combined immunodeficiency (SCID)/Fabry
PCS	Physical Component Summary
PI	Principal Investigator
PK	Pharmacokinetics
PTC	Peritubular capillary
q6h	Every 6 hours
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell
RCL	Replication competent lentivirus
REB	Research ethics board
RNA	Ribonucleic acid
SAE	Serious adverse event(s)
SAP	Statistical analysis plan
SC	Subcutaneously
SCD	Sickle cell disease
SCID	Severe combined immunodeficiency
scRNAseq	Single-cell RNA sequencing
SD	Standard deviation
SF-36	Short Form 36
SmPC	Summary of Product Characteristics

Abbreviation	Definition
SUSAR	Suspected unexpected serious adverse reaction(s)
TDM	Therapeutic drug monitoring
TDT	Transfusion-dependent β-thalassemia
TEAE	Treatment-emergent adverse event
TIA	Transient ischemic attack
UHN	University Health Network
ULN	Upper limit of the normal range
VCN	Vector copy number
VDRL	Venereal Disease Research Laboratory
WBC	White blood cell
WHO	World Health Organization

4. INTRODUCTION

Fabry disease is a rare lysosomal storage disorder (LSD), associated with significant morbidity and early mortality, that is caused by deficiency of alpha-galactosidase A (AGA) enzyme activity due to mutations in the *GLA* gene (MIM 301500). This deficiency in enzyme activity leads to accumulation of glycosphingolipids (eg, 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 (Germain, 2010). Variant late-onset Fabry disease phenotypes also exist, with disease limited primarily to cardiac or renal involvement (Mehta, 2002; Germain, 2010).

Although therapeutic options are currently available for treatment of Fabry disease, including two enzyme replacement therapies (ERT) and one chaperone therapy, there are limitations associated with each of these therapies for which consideration of alternative treatment strategies is warranted.

Despite availability of the ERTs Fabrazyme® (agalsidase beta) and Replagal® (agalsidase alfa), some patients have proven either refractory to or have achieved suboptimal response to ERT (Germain, 2010; Biffi, 2016). Due to its very short half-life in plasma (a few hours), ERT provides only periodic therapeutic levels of AGA to patients over a very short duration; given that current dosing is via intravenous (IV) infusion every two weeks, patients are left with sub-therapeutic levels of enzyme for a significant period of time between scheduled infusions. Hence, at best, ERT can only slow disease progression. Enzyme replacement therapies also exhibit safety concerns, particularly early in the treatment course; in the pivotal clinical studies of both Fabrazyme and Replagal, over 50% of patients treated experienced mild to moderate infusion reactions (Eng, 2001; Schiffmann, 2001). With both ERTs, some patients have also demonstrated immunogenic response to treatment (IgG seroconversion). Importantly, a few patients treated with Fabrazyme have also tested positive for plasma IgE antibodies and/or have had a positive skin test in conjunction with urticaria or skin rash (Germain, 2010), posing a challenge for continued treatment.

Galafold™ (migalastat), an oral small molecule pharmacological chaperone therapy, is also approved for treatment of Fabry disease. Galafold (migalastat) works by binding and stabilizing specific mutant forms of the AGA enzyme in the endoplasmic reticulum, thereby preventing misfolding and subsequent premature degradation, and facilitating trafficking to lysosomes for functional enzyme expression and resulting substrate reduction. Although an alternative treatment strategy for Fabry disease, its utility is limited to the Fabry patient population (approximately 50%) who have specific *GLA* gene mutations (amenable mutations) associated with mutant forms of the AGA enzyme that have been confirmed to be amenable to binding and stabilization (Benjamin, 2017). Furthermore, data on the long-term safety and efficacy of Galafold (migalastat) treatment are currently limited.

Considering the limitations of the currently approved treatments for Fabry disease, AVROBIO is developing AVRO-RD-01, an *ex vivo*, lentiviral vector (LV)-mediated gene-modified autologous

cell therapy. This therapy involves the autologous transplantation of CD34+ cells augmented with a therapeutic version of the *GLA* gene inserted into their genome via a LV. Mobilized autologous CD34+ hematopoietic stem cells (HSCs) obtained from the patient's peripheral blood are genetically modified *ex vivo* with a LV containing a ribonucleic acid (RNA) transcript that, after reverse transcription, results in codon-optimized complementary deoxyribonucleic acid (cDNA) that, upon its integration into the human genome, encodes for functional human AGA. After the patient has undergone a conditioning regimen to achieve myeloablation, the genetically-modified cells are returned to the patient by IV infusion. This infusion of genetically-modified cells is intended to permanently engraft into the bone marrow, asymmetrically divide, and differentiate, effecting 'metabolic cooperativity' (also known as 'cross correction'), wherein functional hydrolase AGA is secreted from augmented cells, circulated, and taken up through mannose-6-phosphate receptors and trafficked to the lysosomes of bystander cells. Once inside the lysosome, the functional enzyme reduces glycosphingolipid storage, allowing the reconstitution of lysosomal function, cell survival, and avoidance of end-organ damage associated with early morbidity and mortality in affected patients. Unlike ERTs, AVR-RD-01 is intended for single administration, having the potential to provide sustained therapeutic levels of functional AGA over the patient's lifetime, thus potentially eliminating the need for bi-weekly IV infusions, resulting in a beneficial effect on patient quality of life and healthcare burden associated with Fabry disease management.

AVROBIO obtained exclusive licensing rights to the *ex vivo*, LV-mediated gene-modified autologous cell therapy program for Fabry disease from University Health Network (UHN). University Health Network has completed a number of nonclinical studies to support this program. Results of completed nonclinical studies in mice suggest that HSCs transduced with a LV containing a RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the genome, encodes for functional human AGA, can produce sustained levels of functional human AGA for up to 6 months, thus demonstrating proof-of-concept. No overt toxicity nor tumorigenicity attributed to test article were observed in concert with stable and persistent expression of AGA enzyme activity for up to 6 months (the last time point evaluated) in male Fabry mice and up to 12 weeks (the median lifespan in sublethally irradiated highly-immunodeficient mice) in non-obese diabetic (NOD)/severe combined immunodeficiency (SCID)/Fabry (ie, NSF) mice. In both animal models, concomitant reductions in enzyme substrate (ie, Gb3) were also observed. In two independent (non-Good Laboratory Practice [GLP]) toxicology studies, neither test article-related toxicity nor adverse changes were observed.

Currently, there is one other ongoing clinical study (Study OZM-074), UHN-sponsored in Canada, to support the *ex vivo*, LV-mediated gene-modified autologous cell therapy program for Fabry disease. This study is a multi-center, non-randomized, open-label prospective pilot study that enrolled a total of five adult male subjects with classic Fabry disease on ERT. The primary objective of the study is to evaluate the overall safety and tolerability of autologous stem cell transplantation with mobilized CD34+ cells transduced with a LV containing a RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human AGA, after conditioning. The secondary objective of the study is to obtain evidence of the efficacy of the transplantation procedure in subjects with Fabry disease. Preliminary results from this study indicate presence of vector and a trend in increased AGA enzyme activity post-AVR-RD-01 transplant in enrolled subjects,

providing early evidence that the LV is effective in delivering and integrating the *GLA* transgene into the human genome, enabling expression of endogenous functional AGA. From a safety perspective, preliminary data indicate AVR-RD-01 was generally well-tolerated and confirm the feasibility of the pre-treatment plan involving CD34+ cell mobilization, apheresis, and conditioning for the Fabry patient population under study.

Further details on the completed nonclinical studies and the ongoing clinical studies supporting the AVR-RD-01 program, as well as information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) of AVR-RD-01, can be found in the current edition of the Investigator's Brochure for AVR-RD-01.

5. STUDY OBJECTIVES AND ENDPOINTS

Study objectives and associated endpoints are presented in Sections 5.1 and 5.2, respectively; the frequency and timing of study measurements are provided in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3). Information regarding sample and data collection are presented in Section 9.

5.1. Study Objectives

5.1.1. Primary Objectives

The primary objectives of this study are to:

- Evaluate the effect of AVR-RD-01 on substrate (ie, Gb3) in kidney biopsies
- Evaluate the safety and tolerability of AVR-RD-01, including, but not limited to:
  - Evaluation of 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)

5.1.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate AGA enzyme activity in plasma and peripheral blood leukocytes
- Evaluate the effect of AVR-RD-01 on biomarkers for Fabry disease (ie. Gb3 and its deacylated form, lyso-Gb3) in plasma and urine
- Evaluate the effect of AVR-RD-01 on substrate (ie, 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

5.1.3. 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 disease 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 (ie, 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)
- 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)
- Assess the impact of the conditioning regimen on reproductive potential

5.2. Endpoints

5.2.1. Efficacy Endpoints

5.2.1.1. Primary Efficacy Endpoint

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

5.2.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- Change from baseline in AGA enzyme activity level in plasma and peripheral blood leukocytes at Weeks 24 and 48
- Change from baseline in biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma and urine at Weeks 24 and 48
- Change from baseline in substrate (ie, 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

5.2.1.3. Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include the following:

- Change from baseline in 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 (ie, 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)
- Changes from baseline in peripheral blood cell subpopulations (ie, T cells [CD3+], natural killer [NK] cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (ie, 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 (ie, T cells [CD3+], NK cells [CD56+], B cells [CD19+], granulocytes [CD15+], and monocytes [CD14+]) and bone marrow CD34+ subtypes (ie, 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)

5.2.2. Safety Endpoints

5.2.2.1. Primary Safety Endpoints

Primary safety endpoints include the following:

- Incidence and severity of AEs and SAEs
- Change from baseline in clinical laboratory values relevant to safety
- Incidence of 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)

5.2.2.2. Exploratory Safety Endpoint

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

6. INVESTIGATIONAL PLAN

6.1. Summary of Study Design

This is a multinational, open-label study to assess the efficacy and safety of AVR-RD-01 in approximately 8 to 12 adult (defined as either ≥18 years of age; or ≥16 and <18 years of age and postpubertal, 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 ERT and/or chaperone therapy for Fabry disease within 3 years of Screening.

Five study periods (Screening, Baseline, Pre-transplant, Transplant, and Post-transplant 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-transplant baseline. Once baseline assessments are complete, the subject will enter the Pre-transplant Period (approximately 6 to 8 weeks) during which time mobilization, apheresis, AVR-RD-01 preparation and testing for release, conditioning regimen administration to achieve myeloablation, and conditioning washout period will take place (see Section 8.1.1). Following completion of the Pre-transplant Period, the subject will enter the Transplant Period (1 day) during which time AVR-RD-01 infusion will take place (see Section 8.1.2). After AVR-RD-01 infusion, the subject will enter the Post-transplant Follow-up Period (approximately 48 weeks) (see Section 8.1.3), during which time periodic safety and efficacy assessments will be performed to assess measures of engraftment, clinical response, and safety post-transplant. Post-transplant 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.

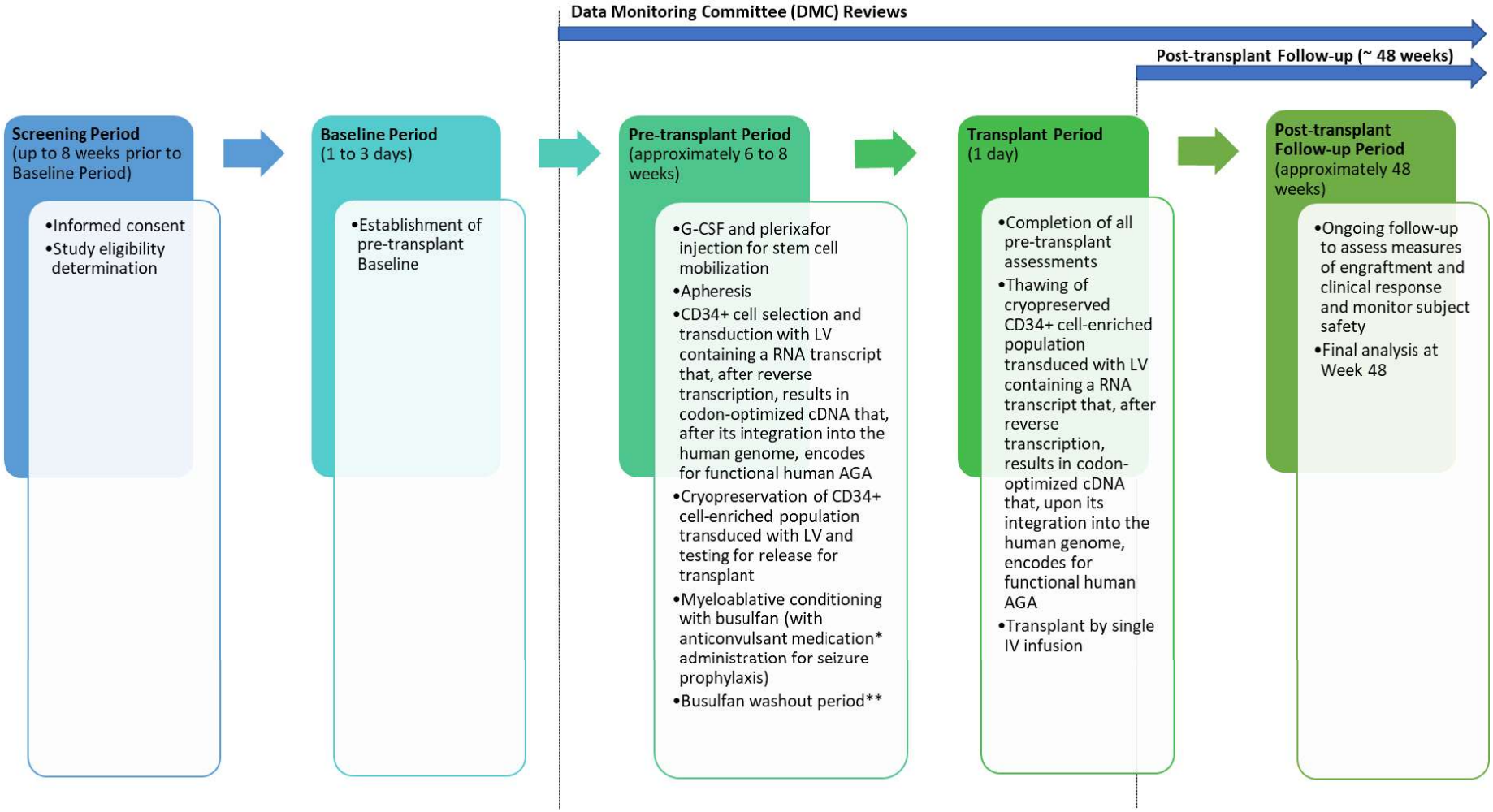
An independent Data Monitoring Committee (DMC) will be established for the study to review safety information during the Pre-transplant, Transplant, and Post-transplant Follow-up Periods to assess for signals that may preclude continued study enrollment and/or necessitate changes to the protocol. A safety review meeting will take place to review the first subject's safety data after the subject has received the transplant 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 (SAEs related to AVR-RD-01 transplantation), 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 (SAEs related to AVR-RD-01 transplantation), 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 (ie, 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). Full analytical details for the study will be outlined in a statistical analysis plan (SAP).

An overview of the overall study design can be found in Figure 1 (with a detailed overview of the Pre-transplant and Transplant Periods provided in Figure 2).

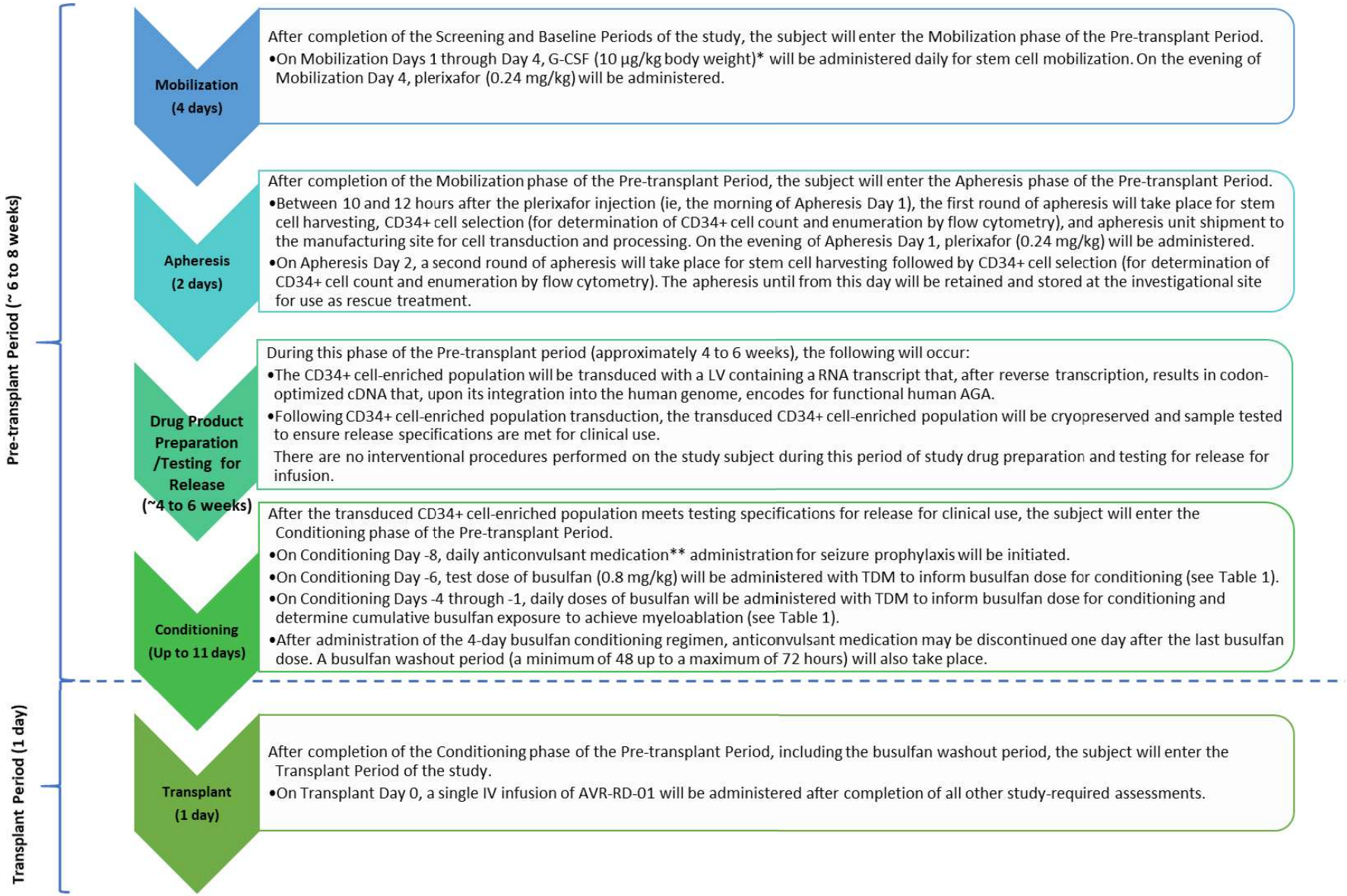
Figure 1: Overall Study Design for AVRO-RD-01-201



*Levetiracetam will be administered. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used.
**The busulfan washout period prior to AVR-RD-01 infusion must be a minimum of 48 up to a maximum of 72 hours.
The independent DMC will review safety information during the Pre-transplant, Transplant, and Post-transplant Follow-up Periods of the study to assess for signals that may preclude continued enrollment and/or necessitate changes to the protocol. Safety review meetings will take place as described above to review safety data. Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 48 assessments (or prematurely discontinue the study).

Abbreviations: AGA = alpha-galactosidase A; cDNA = complementary deoxyribonucleic acid; G-CSF = granulocyte-colony stimulating factor; IV = intravenous; LV = lentiviral vector; RNA = ribonucleic acid

Figure 2: Overview of the Pre-transplant and Transplant Periods of Study AVRO-RD-01-201



*For investigational sites with only prefilled syringes, a G-CSF dose from 10 µg/kg body weight up to 12 µg/kg body weight, administered in either a single or divided twice a day (BID) dose, may be administered. The G-CSF dose may be administered subcutaneously (SC) or IV.
**Levetiracetam will be administered. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used.
Abbreviations: AGA = alpha-galactosidase A; cDNA = complementary deoxyribonucleic acid; G-CSF = granulocyte-colony stimulating factor; IV = intravenous(ly); LV = lentiviral vector; RNA = ribonucleic acid; TDM = therapeutic drug monitoring

An overview of required assessments for the study (including the timing of each) can be found in Section 14.1 (Schedule of Assessments from Screening through Investigational Product Preparation and Testing for Release in the Pre-Transplant Period), Section 14.2 (Schedule of Assessments from the Pre-Transplant Conditioning Period through Transplant [Day 0]), and Section 14.3 (Schedule of Assessments for the Post-Transplant Follow-up Period [Study Week 1 (Day 1) through Study Week 48 (Day 336)]).

After study completion, consenting subjects will continue periodic safety and efficacy assessments for approximately 14 years (for a total of 15 years post-transplant follow-up) in long-term follow-up study AVRO-RD-01-LTF01.

6.2. Discussion of Design and Control

6.2.1. Overall Study Design and Study Population

The open-label design without concurrent control was deemed appropriate for the early phase, proof-of-concept nature of the study. Only male subjects with classic Fabry disease who are treatment-naïve (ie, received no ERT and/or chaperone therapy for Fabry disease within 3 years of Screening) are being included in this study, to eliminate the variability in heterozygous subjects and to provide the best opportunity to evaluate AVR-RD-01 without the potential confounding effects associated with prior Fabry disease-specific therapies.

Only subjects at least 18 years of age (the mandated lower age limit for inclusion in the United States) or ≥16 and <18 years of age and postpubertal (where permitted by region) are being included in the study to provide the best opportunity to evaluate AVR-RD-01. Subjects ≥16 and <18 years of age with full pubertal development are considered appropriate for inclusion, where permitted by region; this approach is a physiologic/developmental approach, rather than a more arbitrary age cutoff approach. This approach is validated, in part, by ICH E11(R1), Addendum, Clinical Investigation of Medicinal Products in the Pediatric Population, 2017, which states that chronological age alone may not serve as an adequate categorical determinant to define developmental subgroups, and that physiological development and maturity of organs are factors to be considered. In some regions, the age of 16 years constitutes a regulatory definition for the end of pediatrics (ICH E11, Clinical Investigation of Medicinal Products in the Pediatric Population, 2000). With regard to Fabry disease, the average age at diagnosis for patients with the classic form of the disease is in the late teens to early 20s (Eng, 2007; Waldek, 2009); thus, inclusion of subjects ≥16 years is an important consideration in identifying subjects that are ERT and/or chaperone therapy-naïve for recruitment into the study.

For the first three subjects who receive the AVR-RD-01 transplant, each subject's safety data will be reviewed by the DMC after the subject receives the transplant and completes the Week 4 follow-up visit. The DMC will review the Week 4 safety data and mobilization of additional subjects will be staggered based on the schedule described in Section 6.1. Assessment of safety data through Week 4 post-transplant for the first three subjects allows for appropriate evaluation of short-term safety related to the mobilization, conditioning, and infusion of AVR-RD-01 prior to continuing mobilization for additional subjects. This time interval also allows for early evaluation of engraftment of transduced cells and enzyme production via those cells.

6.2.2. Stem Cell Mobilization and Conditioning Regimens

6.2.2.1. Stem Cell Mobilization

Both granulocyte-colony stimulating factor (G-CSF) and plerixafor are being used in the study for stem cell mobilization. Superiority of G-CSF and plerixafor over G-CSF alone in stem cell mobilization of patients has been demonstrated (Yannaki, 2013; Baiamonte, 2015; Goker, 2015); in combination with G-CSF, plerixafor has been shown to increase peripheral blood CD34+ cell counts, including increased frequency of more primitive CD34+ cell subtypes and clonogenic capacity.

6.2.2.2. Conditioning

A four-day conditioning regimen of IV busulfan using a prior weight-based test dose of 0.8 mg/kg on Day -6 of Conditioning will be used in this study. The myeloablative cumulative target for busulfan exposure in the AVRO-RD-01-201 study will be a cumulative area-under-the curve (AUC) of $90_{(day\ 0\text{-}4)}$ +/- 0% mg•hr/L. Busulfan will be administered once-daily, with the initial doses on Days -4 and -3 of Conditioning based on the pharmacokinetics (PK) from the busulfan test dose. The United States FDA, European Medicines Agency (EMA), and Health Canada recommend an initial IV busulfan weight-based dose of 0.8 mg/kg IV every 6 hours (q6h), or 3.2 mg/kg IV daily for conditioning, with consideration for modified dosing strategies for patients with less than ideal body-weight (Busulfan Prescribing Information, Busulfan Product Monograph, Busulfan Summary of Product Characteristics [SmPC]). However, Bartelink et al. (Bartelink, 2012) investigated the covariates related to the PK of busulfan and determined that body-weight was the most predictive covariate that explained variability in busulfan exposure between patients. The relationship between body-weight and clearance was characterized by an allometric equation with a scaling component (Bartelink, 2012). The scaling range for adolescents and adults was from 2.7 to 4.1 mg/kg/day, and allowed for individualized initial dosing based on body-weight. However, further studies evaluating the PK of busulfan by Davis et al. (Davis, 2018) and Weil et al. (Weil, 2017) determined that administration of a busulfan test dose prior to the 4 day myeloablative conditioning regimen allowed for the use of a personalized PK-guided dosing strategy, based on the PK from the test dose and the patients individual body-weight, which resulted in significant improvement in target AUC attainment and less interpatient variability compared to using the weight-based dosing regimen alone as described by Bartelink et al. (Bartelink, 2012). The personalized PK- and weight-based guided dosing strategy as suggested by Weil et al. (Weil, 2017) and Davis et al. (Davis, 2018), in addition to therapeutic drug monitoring (TDM) of the PK of the test dose and during conditioning, has resulted in more accurate cumulative AUC outcomes versus previous weight-based and TDM-guided dosing without test dose.

This PK- and weight-based guided, individualized dosing regimen of busulfan defines a once-daily regimen for myeloablative conditioning at an $AUC_{(day0\text{-}4)}$ of 90 mg•h/L, which equates to 21.6 µmol•min/L total, or 5400 µmol•min/L. Because busulfan PK display large interpatient and intrapatient variability, TDM of busulfan is routinely performed as standard clinical care in patients undergoing hematopoietic stem cell transplantation (HSCT) (Long-Boyle, 2015).

For this study, busulfan TDM will be performed on the test dose on Conditioning Day -6 to inform the dose on Conditioning Days -4 and -3. Busulfan TDM will also be performed on Conditioning Days -4 and -3 to inform the doses on Days -2 and -1, respectively. Collective information from TDM on all days during Conditioning (Days -4 to Day -1) will be used to calculate the cumulative AUC in Study AVRO-RD-01-201, with plasma samples taken at periodic intervals post-busulfan administration as described in Section 8.1.1.3 (and the Schedule of Assessments in Section 14.2). All laboratories performing TDM will use validated methods to quantify busulfan in plasma, according to GLP.

The busulfan dose-versus-concentration relationship over time will be evaluated to obtain an individualized dosing strategy that balances efficacy and toxicity. Optimizing the target for cumulative busulfan exposure has a significant impact on outcomes (Bartelink, 2016). In transfusion-dependent β-thalassemia (TDT) patients who underwent autologous HSCT for LV-mediated gene therapy, a four-day myeloablative conditioning regimen, with an average busulfan daily dose range of 4,670 to 5,212 µmol•min/L per day, was used (Thompson, 2018). In 400 children and young adults who underwent allogeneic HSCT for non-oncology indications, a busulfan AUC of 78 to 101 mg•hr/L was considered optimal for targeting a balance between acute toxicity and patient outcomes, including graft failure (Bartelink, 2016).

In the AVRO-RD-01-201 study, a centralized, web-based program will be used to determine the daily busulfan dose required by each subject to achieve the busulfan target cumulative AUC. Using this centralized, web-based program, each site will enter each subject's busulfan level for each time point collected. Based on the data entered, the investigative site will be provided with each subject's next day's busulfan dose recommendation as determined by the centralized model-based Bayesian forecasting method. Reports in the literature indicate that cumulative AUCs are sometimes calculated with methods that vary by institution, and use of a PK modeling software program can provide a consistent, standardized approach in dosing strategies between sites (Bartelink, 2016).

Busulfan does cross the blood-brain barrier and can cause neurotoxicity, including seizures, when administered at higher doses. Therefore, anticonvulsant medication is routinely prescribed for patients undergoing myeloablative therapy with busulfan in preparation for HSCT. Consistent with this practice, anticonvulsant prophylaxis will be administered concurrent with busulfan administration for conditioning in this study. Levetiracetam will be administered in this study as described in Section 8.1.1.3. The dosing regimen selected for levetiracetam has been shown in the literature to be effective at preventing seizures in adults receiving conditioning with busulfan in preparation for HSCT, and to have an acceptable safety profile when administered in combination with busulfan (Akiyama, 2018). For subjects with contraindication to levetiracetam, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used before and after busulfan treatment (Busulfan Prescribing Information).

The most frequent, serious consequence of treatment with busulfan at the approved dose and schedule is profound myelosuppression, occurring in all patients. Hematologic changes have been observed in subjects receiving myeloablative busulfan conditioning regimens used in other autologous LV-mediated gene therapy studies for TDT, sickle cell disease (SCD), and metachromatic leukodystrophy (MLD) (Thompson, 2018; Biffi, 2013; Tisdale, 2018; see also the Investigator's Brochure for AVR-RD-01).

In addition, busulfan may be a human carcinogen as secondary malignancy has been reported in patients treated with busulfan. Recently a case of therapy-related myelodysplastic syndrome (t-MDS) was reported in an individual who underwent busulfan conditioning before LV-mediated gene therapy for SCD (Kanter, 2018). While secondary malignancies have been reported among individuals exposed to busulfan during treatment for hematologic cancers, less is known about the carcinogenic risk among individuals exposed to busulfan conditioning in the management of non-malignant disorders. To address this concern, AVROBIO conducted a literature review of busulfan conditioning in conjunction with bone marrow transplant (BMT) or hematopoietic cell transplant (HCT) for non-malignant conditions (literature references available upon request). Similarly, AVROBIO reviewed the literature for busulfan conditioning before LV-mediated gene therapy for non-malignant conditions (literature references available upon request). With the exception of the single case of t-MDS noted above, no other cases of t-MDS, leukemia, cancer, or pre-cancerous lesions were reported in over 700 exposures to busulfan conditioning for non-malignant conditions. Based on this literature review, the risk of t-MDS or leukemia due to busulfan conditioning before LV-mediated gene therapy for non-malignant conditions appears to be low. Nevertheless, bone marrow aspirate and peripheral blood samples will be collected at baseline and stored for subsequent evaluation should hematologic cancer be observed.

The potential impact, if any, of the conditioning regimen used in this study is not fully understood and may result in infertility. To off-set the potential risk of infertility in the study, males will be offered sperm cryopreservation. The impact of the conditioning regimen on the subjects' reproductive potential will be assessed during the study by evaluating the change from baseline in sperm count, morphology, and motility. Reproductive outcomes, if any, will be monitored and recorded during this study.

6.2.3. Efficacy and Safety Measures

In the AVRO-RD-01-201 study, measures of engraftment of gene-augmented HSCs will be evaluated by determining average VCN in peripheral blood and bone marrow stem and progenitor cells using qPCR and/or ddPCR. To ensure subject safety, a CD34+ stem-cell back-up of nontransduced cells will be collected and stored at the investigational site for use as rescue treatment in the unlikely event that there is a transplant failure or prolonged bone marrow aplasia after AVR-RD-01 transplant (see Section 8.1.1.1).

Efficacy measures chosen for the planned AVRO-RD-01-201 study have been commonly used to measure treatment effects in trials of ERT and chaperone therapy for Fabry disease (Eng 2001; Schiffmann 2001; Ries 2006; Germain 2007; Germain 2012; Ramaswami 2007; van Breemen 2011; Hughes 2017). The following were considered in the choice of efficacy and safety endpoints, and the timing of related assessments:

- The natural history of Fabry disease, and the clinical progression of signs/symptoms known to impact morbidity and mortality in patients with the disease.
- Regulatory guidelines for follow-up of subjects receiving gene therapy products (ie, United States Department of Health and Human Services, FDA, Center for Biologics Evaluation and Research [CBER], Guidance for Industry, Long Term Follow-Up After Administration of Human Gene Therapy Products, January 2020; United States Department of Health and Human Services, FDA, CBER, Guidance for Industry,

Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up, January 2020; EMA, Guideline on Follow-up of Patients Administered with Gene Therapy Medicinal Products, October 2009).

7. STUDY POPULATION

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.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

7.1. Inclusion Criteria

Subjects must meet all of the following inclusion criteria for participation in this study:

1. Subject is an adult male ≤50 years of age at the time of Screening. For this study, adult is defined as:
   - ≥18 years of age (mandated for subjects enrolled in the United States); or
   - ≥16 and <18 years of age and postpubertal, defined as having adult male testicular volume (where permitted by region)
2. Subject has a confirmed diagnosis of classic Fabry disease based on deficient AGA enzyme activity in peripheral blood leukocytes (defined as <1% of normal) at the time of Screening.
3. Subject with female spouse/partner of childbearing potential (defined as all women physiologically capable of becoming pregnant; this includes any female who has experienced menarche and who has not undergone successful surgical sterilization [ie, hysterectomy, bilateral tubal ligation, or bilateral ovariectomy] or is not postmenopausal [defined as amenorrhea >12 consecutive months and increased serum follicle-stimulating hormone level (ie, >30 IU/L) on at least 2 occasions (in the absence of hormone replacement therapy, dietary phytoestrogens) or estradiol concentration <10 pg/mL]) must be willing to remain sexually abstinent or use double-barrier method while sexually active from conditioning therapy administration until 48 weeks post transplantation (ie, the Week 48 study visit). Barrier contraception is required even with documented medical assessment of surgical success of a vasectomy. For female spouse/partner, acceptable methods of barrier contraception include diaphragm, cervical cap, or contraceptive sponge.
4. Subject must be willing to refrain from donating sperm any time after receiving conditioning therapy. For subjects planning on (or for whom there is a possibility of) fathering children in the future, sperm banking prior to administration of the conditioning regimen will be recommended.
5. Subject must be willing to refrain from donating blood, organs, tissues, or cells for transplantation at any time after AVR-RD-01 transplant.
6. Subject and/or parent (or legal guardian) must be willing and able to provide written informed consent (and assent, if applicable) for the study in accordance with applicable regulations and guidelines and to comply with all study visits and procedures, including the use of any data collection device(s) that may be used to directly record subject data.

7.2. Exclusion Criteria

Subjects will be excluded from participation in this study if they meet any of the following exclusion criteria:

1. Subject has a *GLA* gene mutation associated with late-onset cardiac variant Fabry disease, including:
   - N215S (c.644A>G [p.Asn215Ser])
   - M296V (c.886A>G [p.Met296Val])
   - R301Q (c.902G>A [p.Arg301Gln])
   - Q279E (c.835C>G [p.Gln279Glu])
   - A20P (c.58G>C [p.Ala20Pro])
2. Subject has received any ERT and/or chaperone therapy for Fabry disease within 3 years of Screening. Subjects who received ERT and/or chaperone therapy more than 3 years from Screening are eligible for study participation as long as all other study inclusion/exclusion criteria are met.
3. Subject has histopathologic findings on skin biopsy at Screening that indicate no or trace Gb3 accumulation (ie, score of 0 as defined in Thurberg, 2011).
4. Subject has tested positive for anti-AGA antibodies at the time of Screening.
5. Subject has eGFR <60 mL/min/1.73 m² (ie, chronic kidney disease [CKD] stage ≥3) at Screening.
6. Subject has a prior history of significant proteinuria (≥0.5 grams/24 hours) or equivalent (urine protein/creatinine ratio ≥50) and has not been on a stable dose of an appropriate anti-proteinuric medication for at least 3 months. Subjects with significant proteinuria (≥0.5 grams/24 hours) or equivalent (urine protein/creatinine ratio ≥50) found at Screening may be rescreened for study participation after they have been on a stable dose of an appropriate anti-proteinuric medication for at least 3 months.
7. Subject has evidence of any clinically relevant renal disease, in the opinion of the Investigator, not attributed to Fabry disease (eg, nephrotic syndrome, repeated episodes of pyelonephritis, obstructive uropathy, red blood cell [RBC] casts on urinalysis, kidney size discrepancy >1.5 centimeters, evidence of other renal disease on kidney biopsy performed at Screening).
8. Subject has a prior history of myocardial infarction (MI).
9. Subject has a history of coronary artery disease (CAD) with angina requiring percutaneous transluminal coronary angioplasty (with or without stent placement) and/or coronary artery bypass graft (CABG).
10. Subject has a history of moderate to severe valvular heart disease requiring valve replacement.

11. Subject has a history of heart failure, moderate to severe diastolic dysfunction, and/or left ventricular ejection fraction (LVEF) ≤45% on echocardiogram (ECHO) performed at rest at Screening.
12. Subject has a history of clinically significant cardiac arrhythmia (eg, heart block [second or third degree], atrial fibrillation requiring therapy [history of intermittent atrial fibrillation not requiring treatment is allowed], ventricular fibrillation, ventricular tachycardia, supraventricular tachycardia, or cardiac arrest).
13. Subject has uncontrolled hypertension (defined as systolic blood pressure ≥150 mmHg or diastolic blood pressure ≥90 mmHg) at Screening. Subjects with hypertension found at screening may be rescreened for study participation after they have been on a stable dose of an appropriate antihypertensive medication for at least 3 months.
14. Subject is on (or requires treatment with) any anticoagulant (warfarin, dabigatran, or other oral anticoagulant; or heparin) from 30 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 48 study visit. Use of antiplatelet agents are permitted, however should be withheld in the presence of bleeding or platelet counts $<50 \times 10^9$/L.
15. Subject has a prior history of stroke and/or transient ischemic attack (TIA).
16. Subject has aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN) at Screening.
17. Subject has forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and/or diffusing capacity of the lung for carbon dioxide (DLCO) ≤50% predicted value (corrected for hemoglobin) on pulmonary function testing performed at Screening.
18. Subject has diabetes mellitus (type 1 or type 2).
19. Subject has an active chronic infection during the Screening, Baseline, or Pre-transplant Period of the study.
20. Subject has an active uncontrolled acute bacterial, viral, fungal, parasitic, or prion-associated infection during the Screening, Baseline, or Pre-transplant Period of the study.
21. Subject has a history of (or current) tuberculosis.
22. Subject tests positive for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV, type 1 or 2), human T-cell lymphotropic virus (HTLV)-1, HTLV-2, and/or syphilis on Venereal Disease Research Laboratory (VDRL) test, chemiluminescent microplate immunoassay (CMIA), or enzyme immunosorbent assay (EIA) at Screening.
23. Subject has a prior history of (or current) malignancy; the one exception is a prior history of resected basal cell carcinoma.
24. Subject has any other medical condition that predisposes him to (or conveys increased risk of) malignancy.
25. Subject has undergone, or is scheduled to undergo, bone marrow transplant and/or solid organ transplant.

26. Subject has hematological compromise evidenced by white blood cell count (WBC) <3.0 x 10^9/L, platelet count <100 x 10^9/L, hemoglobin <100 g/L, and/or uncorrected bleeding disorder from signing informed consent at Screening (ie, study enrollment) through the Transplant Period of the study (ie, the day of AVR-RD-01 infusion).
27. Subject has clinically significant immunodeficiency disease or condition, in the opinion of the Investigator, at Screening.
28. Subject is on (or requires treatment with) cytotoxic or immunosuppressive agents from 60 days prior to signing informed consent at Screening (ie, study enrollment) through the Week 48 study visit; the one exception is treatment with cytotoxic or immunosuppressive agents required per protocol for stem cell transplant.
29. Subject has contraindication to iohexol.
30. Subject has any condition that makes it impossible to perform MRI studies (including allergies to anesthetics).
31. Subject has medical conditions(s) and/or is receiving medication(s) that would contraindicate ability to undergo mobilization (including contraindication to G-CSF and/or plerixafor), apheresis, or receive myeloablative therapy for conditioning.
32. Subject has any medical, psychological, or other condition that, in the opinion of the Investigator:
    - Might interfere with the subject's participation in the study (including consenting to procedures); and/or
    - Poses any additional risk to the subject; and/or
    - Might confound the results of any study-required assessments.
33. Subject has previously received treatment with AVR-RD-01 or any other gene therapy.
34. Subject is participating in (or plans to participate in) any other investigational drug trial, or plans to be exposed to any other investigational agent, device and/or procedure, from 30 days prior to signing informed consent at Screening (ie, study enrollment) through study completion.

7.3. Rationale for Inclusion or Exclusion of Certain Study Candidates

AVR-RD-01 is an *ex vivo* LV-mediated gene-modified autologous cell therapy intended for the durable provision of functional AGA for patients with Fabry disease. It was determined that for this early phase proof-of-concept study, the most robust assessment of safety and efficacy would be generated in adult male subjects with classic Fabry disease without any recent ERT and/or chaperone therapy for Fabry disease (ie, a treatment-naïve Fabry disease patient population), to eliminate the variability in heterozygous subjects and to provide the best opportunity to evaluate AVR-RD-01 without the potential confounding effects associated with prior Fabry disease-specific therapies. Thus:

- Inclusion criteria [1] and [2] specify the study population as adult male subjects with "classic" Fabry disease.

- Exclusion criterion [1] explicitly states subjects with certain mutations that have been associated with late-onset cardiac variant Fabry disease are excluded from the study population.
- Exclusion criterion [2] eliminates subjects that have received any ERT and/or chaperone therapy for Fabry disease within 3 years of Screening.
  - Both ERT and chaperone therapy have been shown to have an effect on increasing functional AGA enzyme (either through direct replacement or modification of endogenous enzyme), with the therapeutic goal of reducing the substrates (glycosphingolipids) that accumulate in Fabry disease, including Gb3 and its deacylated form, lyso-Gb3. Considering this, inclusion of subjects with any recent exposure to ERT and/or chaperone therapy for Fabry disease may confound efficacy results for Study AVRO-RD-01-201. Based on the progression of renal Fabry disease described in natural history studies, as well as the data evaluating the impact of previous ERT shortage (Smid, 2011; Weidemann, 2014), one would expect reaccumulation of substrate to pre-treatment levels within 3 years in patients who discontinue ERT or chaperone therapy.
- Exclusion criterion [3] eliminates subjects that have histopathologic findings on skin biopsy at Screening that indicate no or trace Gb3 accumulation (ie, score of 0 as defined in Thurberg, 2011).
  - Histopathologic findings on skin biopsy at Screening that are essentially normal suggest that these subjects may have insufficient substrate accumulation to permit evaluation of substrate-related efficacy endpoints (including the primary efficacy endpoint) for the study.
- Exclusion criterion [4] eliminates subjects that test positive for anti-AGA antibodies at the time of Screening
  - Inclusion of subjects with recent ERT and/or chaperone therapy for Fabry disease may confound safety results, considering Study AVRO-RD-01-201 is assessing development of immunogenic response to AVR-RD-01 through anti-AGA antibody assay.

Other exclusion criteria are related to the subjects' fitness for the conditioning regimen and transplantation.

7.4. Discontinuations

7.4.1. Discontinuation of Subjects

The criteria for enrollment must be followed explicitly. If it is discovered that a subject who did not meet enrollment criteria was inadvertently enrolled prior to AVR-RD-01 infusion, the subject will be discontinued from the study. If the subject received any study-related treatments or experienced an AE, they will be followed for 30 days or until the AE resolves, whichever is longer (see Section 9.3).

Subjects who discontinue from the study prior to infusion of AVR-RD-01 will be replaced.

In addition, subjects may be discontinued from the study in the following circumstances:

- The subject or the subject's legal representative (ie, parents or legal guardian) requests to be withdrawn from the study
- Sponsor Decision
  - The Sponsor or its designee discontinues the study or discontinues the subject's participation in the study for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and Good Clinical Practice (GCP)
  - The Sponsor or its designee discontinues the clinical study at a particular investigative site
- Adverse Event
  - If the Investigator decides that the subject should be withdrawn because of an SAE or a clinically significant laboratory value, study-related procedures are to be discontinued and appropriate safety measures taken. The Sponsor or its designee is to be alerted immediately.

7.4.2. Discontinuation of Study Sites/Site Terminated by Sponsor

Study site participation may be discontinued if the Sponsor or its designee, the Investigator, or the Research Ethics Board/Human Research Ethics Committee/Institutional Review Board (REB/HREC/IRB) of the study site judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

7.4.3. Discontinuation of the Study/Study Terminated by Sponsor

The study will be discontinued if the Sponsor or its designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

7.4.4. Study Stopping Rules

If either of the following occur, transplantation procedures in additional subjects will be temporarily suspended, and a safety review meeting will take place with the DMC and the Sponsor:

- Death of a subject that is attributed as probably or definitely related to AVR-RD-01
- Any individual subject shows no evidence of engraftment at the Week 4 or Week 24 assessments (based on average VCN or AGA enzyme activity in plasma)

Transplantation procedures may resume if appropriate changes to study conduct are identified and implemented to reduce any risks to subjects going forward.

8. TREATMENT

8.1. Treatments Administered

The Sponsor identifier for the investigational product is AVR-RD-01. The active substance in AVR-RD-01 is autologous CD34+-enriched cell fraction transduced with LV/AGA containing a RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human AGA. The finished investigational product is presented as a cryopreserved cell solution which is thawed immediately prior to use. The investigational 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, subjects will receive the following as part of the autologous transplant procedure in this study:

- Four to eight G-CSF and two plerixafor injections for HSC mobilization prior to AVR-RD-01 infusion
  - Additional G-CSF injections may also be administered beginning Day 5 after AVR-RD-01 transplant until defined absolute neutrophil count (ANC) is achieved
- Five IV infusions of busulfan, one test dose to inform the conditioning dose and four for myeloablative conditioning, prior to AVR-RD-01 transplant
- Anticonvulsant medication administration, concurrent with busulfan administration, for seizure prophylaxis
  - Levetiracetam will be administered. If levetiracetam is contraindicated, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used.

Further details on treatment administration (including dosing regimens) are described in Sections 8.1.1 through 8.1.3. Additional details can also be found in the Study Treatment Manual.

8.1.1. Pre-transplant Period (Approximately 6 to 8 Weeks)

8.1.1.1. Mobilization and Apheresis

Mobilization of the subject's HSC will take place at the participating investigational site.

On Mobilization Days 1 through 4, subjects meeting eligibility criteria for study participation will receive a daily G-CSF dose of 10 µg/kg body weight administered IV or subcutaneously (SC) (for sites with only prefilled syringes, a G-CSF daily dose from 10 µg/kg body weight up to 12 µg/kg body weight, administered in either a single or divided twice a day [BID] dose, may be administered). On the evening of Mobilization Day 4, subjects will receive an injection of plerixafor (0.24 mg/kg).

Between 10 and 12 hours after the plerixafor injection (ie, the morning of Apheresis Day 1), mononuclear cells enriched with CD34+ stem cells will be harvested from the subject's peripheral blood by apheresis, using standard institutional procedures at the participating investigational study site. The apheresis unit obtained on Apheresis Day 1 will be shipped to the

manufacturing site for cell transduction and processing (see Section 8.1.1.2). On the evening of Apheresis Day 1, subjects will receive a second injection of plerixafor (0.24 mg/kg).

On the morning of Apheresis Day 2, subjects will undergo an additional harvest of mononuclear cells enriched with CD34+ stem cells from the subject's peripheral blood by apheresis, using standard institutional procedures at the participating investigational study site. CD34+ cells harvested from this apheresis (nontransduced cells) will be retained and stored at the investigational site for use as rescue treatment in the unlikely event that there is a transplant failure or prolonged bone marrow aplasia after AVR-RD-01 transplant.

After each apheresis (ie, on Apheresis Days 1 and 2), total cell count (per µL) and CD34+ cell enumeration by flow cytometry will be performed at the participating investigational study site to ensure an adequate number of cells have been procured for CD34+ cell selection. The target number of cells for the Apheresis Day 1 collection is 6.5 x 10^{10} total cells and 6.5 x10^{8} CD34+ cells (shipped to the manufacturing site for transduction) and the target number of cells for the Apheresis Day 2 collection is at least 3 x 10^{6} CD34+ cells/kg body weight (retained at the site).

Details on timing for mobilization and apheresis are outlined in the Schedule of Assessments in Section 14.1, and details on the processes may be found in the Study Treatment Manual.

8.1.1.2. Investigational Product Preparation/Testing for Release

Following harvest on Apheresis Day 1, the apheresis unit from that day will be transported under temperature-controlled conditions (2 to 8ºC) to a manufacturing site for further processing. The manufacturing of AVR-RD-01 investigational product is compliant with applicable current Good Manufacturing Practices (cGMP).

AVR-RD-01 investigational product will be tested for identity, potency, purity, and safety prior to release for clinical use. Transduction efficiency and the number of transduced cells for each product are reported on certificate of analysis. AVR-RD-01 Drug Product lot must meet drug product release specification prior to use in the clinic.

Once the release testing is complete, AVR-RD-01 investigational product will be dispositioned for clinical use and sent to the investigational site in a dry shipper with Certificate of Analysis(es), documenting that all release testing specifications for clinical use have been met, and that the investigational product can be used in humans.

8.1.1.3. Conditioning

After receipt of the investigational product dispositioned for clinical use at the investigational site, the subject will enter the Conditioning phase. During this phase of the study, subjects will receive a 4-day myeloablative conditioning regimen of IV busulfan, informed by a weight-based test dose of 0.8 mg/kg administered on Conditioning Day -6, prior to the 4-day myeloablative conditioning regimen administered on Conditioning Days -4 to -1. The test dose will serve to establish the predicted PK for each individual subject, as part of the TDM.

For Conditioning Days -4, -3, -2, and -1, a centralized, web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC. The myeloablative cumulative target AUC for busulfan is $90_{(\text{day } 0\text{-}4)}$ +/- 0% mg•hr/L. Therapeutic drug monitoring will be performed to target the cumulative busulfan AUC for the

4 days of conditioning. Once-daily dosing of IV busulfan with TDM will be administered as follows (see also Table 1):

- On Conditioning Days -4 and -3, a PK- and weight-based guided IV busulfan dose, based on TDM PK of the test dose on Day -6 and the respective calculated AUC generated from the web-based dose simulation program, will be administered (Table 1).
- For Conditioning Day -2, the web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose and Day -4 (Table 1).
- For Conditioning Day -1, the web-based dose simulation program will be used to determine the busulfan dose required for each subject to achieve the busulfan target AUC based on TDM PK of the test dose, Day -4, and Day -3 (Table 1).
- If AUC of the previous dose is outside of +/- 0% of the AUC_{target} determined by the web-based dose simulation program, the busulfan dose will be adjusted accordingly.

Table 1: PK- and Weight-based Guided Busulfan Conditioning Regimen

Dosing Day	Busulfan Dose (mg/kg/day)	TDM PK Schedule for Busulfan Dose Determination
Day -6	Test dose of 0.8 mg/kg	PK sampling at 1, 3, 5 and 7 hours post dose; TDM PK results will be used to inform the dose on Day -4 and Day -3
Day -4	Dose based on TDM PK of test dose	PK sampling at 1, 3 and 5 hours post dose; TDM PK results will be used to inform the dose on Day -2 together with TDM PK results from the test dose
Day -3	Dose based on TDM PK of test dose	PK sampling at 1, 3 and 5 hours post dose; TDM PK results will be used to inform the dose on Day -1 together with TDM PK results from the test dose and Day -4
Day -2	Dose based on TDM PK of test dose and Day -4	PK sampling at 1, 3 and 5 hours post dose; TDM PK results will be used to inform the cumulative AUC
Day -1	Dose based on TDM PK of test dose, Day -4, and Day -3	PK sampling at 1, 3 and 5 hours post dose; TDM PK results will be used to inform the cumulative AUC

Abbreviations: AUC = area-under-the curve; PK = pharmacokinetics; TDM = therapeutic drug monitoring

Anticonvulsant medication will also be administered for seizure prophylaxis concurrent with busulfan administration. Levetiracetam will be administered at a dosage of 1000 mg per day, beginning on Conditioning Day -8 (ie, two days prior to administration of the busulfan test dose), and continuing until one day after administration of the 4-day busulfan conditioning regimen. For subjects with a contraindication to levetiracetam, alternative anticonvulsant medications (eg,

benzodiazepines or valproic acid) may be used before and after busulfan treatment (Busulfan Prescribing Information).

After the 4-day conditioning regimen is administered, a busulfan washout period of a minimum of 48 up to a maximum of 72 hours, is required prior to AVR-RD-01 administration.

Details on the timing of all Conditioning procedures, including the busulfan washout period, are outlined in the Schedule of Assessments in Section 14.2, with additional information included in the Study Treatment Manual.

8.1.2. Transplant Period (1 Day)

After completion of the busulfan washout period (ie, a minimum of 48 up to a maximum of 72 hours after the last busulfan infusion), AVR-RD-01 will be administered once by IV infusion. Prior to the IV infusion, subject identifiers on the AVR-RD-01 investigational product must be checked against the intended recipient, and investigational product must be thawed at 37ºC.

Further details on the transplant process can be found in the Study Treatment Manual.

8.1.3. Post-transplant Follow-up Period (Approximately 48 Weeks)

After AVR-RD-01 infusion, the subject will enter the Post-transplant Follow-up Period, during which time periodic safety and efficacy assessments will be performed to monitor safety post-transplant, and to assess measures of engraftment and clinical response.

In particular, safety laboratory assessments will be performed daily for the first 7 days after AVR-RD-01 transplant. If, on Day 5 after transplant, a subject's ANC is $\leq 1.5 \times 10^9$/L, G-CSF injections of 5 µg/kg body weight (for sites with only prefilled syringes, a G-CSF dose from 4 µg/kg body weight up to 6 µg/kg body weight, administered in either a single or divided BID dose, may be administered) will be administered every morning, and a blood sample for ANC collected, until the subject achieves an ANC $>1.5 \times 10^9$/L.

8.2. Materials and Supplies

AVR-RD-01

The finished investigational product is presented as a cryopreserved product which is thawed at 37ºC immediately prior to use. The thawed investigational product is intended for one-time IV infusion of between 3 and 20 x 10^6/kg body weight autologous CD34+-enriched HSCs that have been genetically modified.

Upon receipt at the investigational site, cryopreserved investigational product must be maintained cryopreserved in vapor-phase liquid nitrogen until ready to thaw for clinical use. Thawed investigational product maintained at controlled room temperature in cryopreservation media is stable for up to 3 hours.

Traceability from the initiation of autologous HSC procurement through the completion of AVR-RD-01 infusion must be ensured through labeling and documentation control at each step. Investigational sites are required to maintain labeling control and documentation to support these steps.

Any unused AVR-RD-01 investigational product should be disposed of in accordance with local biosafety requirements.

Further details can be found in the Study Treatment Manual.

Other Study Treatments

For storage requirements, handling, and stability of other study treatments (ie, pre-transplant G-CSF and plerixafor injections, busulfan infusions, and levetiracetam [or other anticonvulsant medications that may be administered]), see the manufacturers' packaging.

8.3. Dosing Considerations

8.3.1. Rationale for Selection of Doses in the Study

The goal of the transplant is to provide as many CD34+ transduced cells as possible. Due to variables in individual apheresis results, the actual number of infused transduced cells will vary per subject. The AVR-RD-01 dose range to be administered is between 3 and 20×10^6/kg body weight autologous CD34+-enriched cells that are genetically modified *ex vivo* with a LV containing a RNA transcript that, after reverse transcription, results in codon-optimized cDNA that, upon its integration into the human genome, encodes for functional human AGA. This is based on well-established and accepted safe practices commonly used in autologous HSCT and has been shown to safely achieve hematopoietic reconstitution along with long-term engraftment. There is general consensus that a minimum dose of 3×10^6 cells/kg of mobilized CD34+ cells is required for favorable engraftment. Therefore, to be consistent with best practice, the lowest dose of AVR-RD-01 selected for the study is 3×10^6 cells/kg. The upper dose limit of 20×10^6 CD34+ cells/kg is supported by preclinical safety studies conducted to date, which demonstrated tolerability at twice the maximum planned dose.

8.3.2. Other Important Treatment Considerations

AVR-RD-01 is not tested for transmissible infectious agents. Healthcare professionals handling AVR-RD-01 should therefore take appropriate precautions to avoid potential transmission of infectious diseases.

A summary of precautions required and key toxicities associated with G-CSF, plerixafor, busulfan, and levetiracetam (or other anticonvulsant medication used) can be found in the Investigator's Brochure for AVR-RD-01. Investigators should refer to the most current local prescribing information for full details regarding precautions required and a complete list of toxicities associated with G-CSF, plerixafor, busulfan, and levetiracetam (or other anticonvulsant medication used).

Precautions should be taken during apheresis to avoid conditions such as hypotension, electrolyte abnormalities, citrate toxicity, thrombocytopenia, and coagulation abnormalities.

Stem cell infusion can cause tightness in chest, hypotension, coughing, chest pain, decreased urine output, weakness, hypersensitivity reactions, electrolyte disturbances, and rarely, engraftment syndrome (fever, rash, non-cardiogenic pulmonary edema). Subjects should be cautioned on these and other potential side effects of stem cell infusion.

Complete details regarding study treatments are contained in the Study Treatment Manual.

8.4. Blinding

This is an open-label study.

8.5. Concomitant Medications and Non-Pharmacologic Therapies and Procedures

Treatment with ERT and chaperone therapy for Fabry disease is excluded from 3 years prior to signing informed consent (ie, study enrollment at Screening) through the Week 48 study visit. Treatment with cytotoxic or immunosuppressive agents is excluded from 60 days prior to signing informed consent (ie, study enrollment) through the Week 48 study visit; the one exception is treatment with cytotoxic or immunosuppressive agents required per protocol for stem cell transplant. Vaccinations post-transplant may be administered in accordance with standard of care. Treatment with anticoagulants (warfarin, dabigatran, or other oral anticoagulant; or heparin) is excluded from 30 days prior to signing informed consent (ie, study enrollment) through the Week 48 study visit due to bleeding risks and contraindications associated with study procedures, in particular, kidney biopsy. Use of antiplatelet agents are permitted, however should be withheld in the presence of bleeding or platelet counts $<50 \times 10^9$/L.

Certain medications must be discontinued temporarily prior to obtaining skin and renal biopsy samples. Aspirin, other platelet inhibitors, and anticoagulants must be discontinued at least 10 days prior to the biopsies, and non-steroidal anti-inflammatory drugs must be discontinued at least 3 days prior to the procedure. Full details for collecting biopsy samples are contained in the Laboratory Manual.

Subjects are prohibited from undergoing bone marrow transplant and/or solid organ transplant through the Week 48 study visit.

9. STUDY ASSESSMENTS

An overview of required assessments for the study (including the timing of each) can be found in Section 14.1 (Schedule of Assessments from Screening through Investigational Product Preparation and Testing for Release in the Pre-Transplant Period), Section 14.2 (Schedule of Assessments from the Pre-Transplant Conditioning Period through Transplant [Day 0]), and Section 14.3 (Schedule of Assessments for the Post-Transplant Follow-up Period [Study Week 1 (Day 1) through Study Week 48 (Day 336)]).

9.1. Efficacy Measures

9.1.1. Primary Efficacy Measure: Globotriaosylceramide (Gb3) Inclusions in Peritubular Capillaries (PTC) on Kidney Biopsy

Kidney biopsies will be collected at the times shown in the Schedule of Assessments (Sections 14.1 and 14.3).

Electron microscopic images of kidney biopsy samples will be taken and read centrally by two independent renal pathologists. The renal pathologists will score the average number of Gb3 inclusions (ie, myelinosomes) per kidney PTC per subject using a quantification method similar to the Barisoni Lipid Inclusion Scoring System (BLISS) previously described for light microscopy (Barisoni 2012).

Details on collection, processing, and shipment of kidney biopsy samples will be included in the Laboratory Manual.

9.1.2. Secondary Efficacy Measures

The following secondary efficacy measures will be collected at the times shown in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3):

- AGA enzyme activity in plasma and peripheral blood leukocytes: AGA activity will be measured in plasma and peripheral blood leukocytes using standard methods in an accredited central laboratory.
- Biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma and urine: Gb3 and lyso-Gb3 will be measured in plasma and urine using tandem mass spectrometry (MS/MS) analyses as described in the Laboratory Manual. Urine samples must be obtained from a second or third morning void.
- Biomarkers for Fabry disease (ie, Gb3) in skin biopsy samples: Gb3 will be measured in skin from biopsy samples using MS/MS analyses. Details for collecting biopsy samples can be found in the Laboratory Manual.
- Clinical laboratory measures of renal function:
  - mGFR: The mGFR will be determined using plasma clearance of iohexol (Delanaye, 2016a; Delanaye, 2016b).
  - eGFR: The eGFR will be determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for adults (Levey, 2009), using serum creatinine collected with serum chemistry, normalized for age.

  - Urine protein/albumin: Urine protein and albumin will be determined using samples from an overnight void.

- Cardiac structure assessed by LVMI on cardiac MRI.
- GI symptoms assessed by the DIBSS-D: The DIBSS-D is an event-driven, daily diary developed to measure changes in the severity of symptoms experienced by patients with diarrhea-predominant irritable bowel syndrome (IBS). This scale includes measures of bowel function (stool frequency, consistency, urgency) and abdominal symptoms (abdominal pain, discomfort, bloating, and cramping). Stool consistency and abdominal pain will be evaluated as secondary endpoints.
- Pain assessed by the BPI-SF questionnaire: The BPI-SF is a 9-item self-administered questionnaire used to evaluate the severity of a subject's pain and the impact of this pain on the subject's daily functioning on an 11-point scale.
- Functional status assessed by the SF-36: The SF-36 is a 36-item, patient-reported measure yielding eight subscale scores (Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning, and Mental Health) and two component summary scores (Physical Functioning and Mental Functioning). The secondary efficacy measure will be the component scores (ie, Physical Functioning and Mental Functioning).
- Engraftment: Engraftment of gene-augmented HSCs will be assessed by determining average VCN in peripheral blood leukocytes and bone marrow stem and progenitor cells using qPCR and/or ddPCR analysis. Only peripheral blood leukocytes with an average VCN ≥0.01 as determined by the central laboratory, suggesting ≥1% of the sampled blood cells are vector positive, with an average of 1 copy per diploid genome (c/dg), will undergo ISA at the times specified in the Schedule of Assessments.

9.1.3. Exploratory Efficacy Measures

The following exploratory efficacy measures will be collected at the times shown in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3):

- Microscopic findings on kidney biopsy: Kidney biopsy samples will be evaluated for microscopic findings (eg, total number of globally sclerotic glomeruli, morphological finding in glomeruli, degree of tubular atrophy and interstitial fibrosis [scale of 0 to 3], and vascular intimal fibrosis [scale of 0 to 3]).
- AGA enzyme activity in skin and kidney from biopsy samples: AGA activity will be measured in skin and kidney using standard methods in an accredited central laboratory. Details for collecting biopsy samples can be found in the Laboratory Manual.
- Podocytes in urine: The number of podocytes per mL of urine, and per gram of urine creatinine, will be determined using immunofluorescence techniques on samples obtained from a second morning void.
- GI symptoms assessed by the DIBSS-D: Stool frequency, recurrent bowel movements, urgency, and the abdominal symptom subscale score (abdominal pain, discomfort, bloating, and cramping) will be evaluated.

- Functional status assessed by the SF-36: The exploratory efficacy measure will be the eight subscale scores (Vitality, Physical Functioning, Bodily Pain, General Health Perceptions, Physical Role Functioning, Emotional Role Functioning, Social Role Functioning, and Mental Health).
- To assess reconstitution dynamics, immunophenotyping of the fluorescence-activated cell-sorted peripheral blood subpopulation, as well as scRNAseq, will be performed, with the following subpopulations analyzed: T cells (CD3+), NK cells (CD56+), B cells (CD19+), granulocytes (CD15+), and monocytes (CD14+). Immunophenotyping and scRNAseq of the fluorescence-activated bone marrow CD34+ subpopulation will also be performed, with the following populations analyzed: 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. No whole genome sequencing will be performed. Details on peripheral blood and bone marrow sample collection, processing, and shipment will be included in the Laboratory Manual for the study.
- To assess engraftment dynamics, average VCN will be determined, and ISA performed, on fluorescence-activated cell-sorted peripheral blood subpopulations and bone marrow CD34+ subpopulations, with the following analyzed for peripheral blood: T cells (CD3+), NK cells (CD56+), B cells (CD19+), granulocytes (CD15+), and monocytes (CD14+). For bone marrow, the following will be analyzed: 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. Details on peripheral blood and bone marrow sample collection, processing, and shipment will be included in the Laboratory Manual for the study.
- Subject-reported (and caregiver- or other subject observer-reported) experience with AVR-RD-01 treatment and Fabry disease burden and quality of life assessed by qualitative interviews: With separate written informed consent, subjects will complete a series of qualitative interviews intended to collect direct feedback about their experience in the study, particularly with respect to AVR-RD-01 treatment and perception of changes in Fabry disease burden (eg, pain [including general and chronic pain, and pain crises]; gastrointestinal symptoms; fatigue; hearing loss and inner ear-related symptoms; cognitive, neurological and mood-related disease manifestations) and quality of life. With separate consent, caregivers or other individuals (observers) familiar enough with the daily life of participating subjects will also be asked to complete the same series of interviews. Casimir (Plymouth, MA, USA), an organization specializing in the capture of patient- and caregiver-reported data, will schedule and conduct the interviews. Interviews

will be conducted using a secure, web-based conferencing system, and only Casimir staff trained in qualitative research methods will conduct the interviews. Interviews will be conducted with a semi-structured interview guide, recorded and transcribed for analysis. Baseline interviews will assess disease-related burden and quality of life experience over the month preceding the interview, while Follow-up interviews will assess disease-related burden and quality of life experience since the preceding interview, as well as perceptions of change in their disease burden and quality of life. In addition, Follow-up interviews also assess subject experience with pre-treatment conditioning. Each interview takes approximately 60 minutes to complete.

- Exploratory biomarkers for Fabry disease in plasma and urine: With separate subject consent (Optional Genetic Biomarker Research ICF), samples will be collected in plasma and urine for assessment of biomarkers for Fabry disease that may become available during or after the study. Samples obtained will be labeled for 'Biorepository'. Subjects may withdraw consent for use of biorepository samples at any time, as described in the Optional Genetic Biomarker Research ICF. Biorepository samples will be used only for this specific analysis, and will be destroyed 5 years after the Clinical Study Report (CSR) is finalized.

9.2. Safety Assessments

9.2.1. Physical Examinations

A physical examination will be performed at the times specified in Sections 14.1, 14.2, and 14.3. Each examination will include the following assessments: general appearance; skin; head, ears, eyes, nose, and throat; neck; lymph nodes; chest; heart; abdomen; neurologic examination; and musculoskeletal system.

If clinically significant changes from screening are noted, the changes will be documented as AEs in the AE electronic case report form (eCRF). Conditions present at the time informed consent is signed will be documented in the Medical History eCRF. Clinical significance is defined as any variation in physical findings that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the subject until the parameter returns to baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.2.2. Vital Signs

The following vital signs will be recorded at the times specified in Sections 14.1, 14.2, and 14.3: heart rate (beats/minute), systolic and diastolic blood pressure (mmHg), respiratory rate (breaths/minute), and temperature (°C or °F). Vital signs will be obtained after the subject has been supine or seated. On Days 6 and 7 of the Apheresis phase of the Pre-transplant Period, vital signs must be taken prior to apheresis. On Day 0 of the Transplant Period (ie, the day of AVR-RD-01 infusion), vital signs must be taken pre-infusion (window -10 minutes) and post-infusion (window +10 minutes). Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice for transplantation.

If clinically significant vital sign changes as compared to screening are noted, the changes will be documented as AEs in the AE eCRF. Conditions present at the time informed consent is

signed will be documented in the Medical History eCRF. Clinical significance is defined as any variation in vital signs that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the subject until the parameter returns to baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.2.3. Serum Chemistry, Hematology, and Urinalysis

Serum chemistry, hematology, and urinalysis will be performed at the times specified in Sections 14.1, 14.2, and 14.3. In particular, safety laboratory assessments will be performed daily for the first 7 days post-transplant (see Section 8.1.3).

Serum chemistry includes AST, ALT, gamma-glutamyl transferase, amylase, alkaline phosphatase, lipase dehydrogenase, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Electrolytes include sodium, potassium, chloride, and bicarbonate. Hematology includes hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, ANC, and flow cytometric analysis of peripheral blood lymphocyte subsets (CD3, CD4, CD8, CD19, and CD16/56). Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated).

Laboratory assessments for safety will be tested at local laboratories.

It is anticipated that some laboratory values may be outside of the normal value range due to the underlying disease or procedures associated with AVR-RD-01 transplant. As in routine practice, the Investigators should use their medical judgment when assessing clinical significance. Clinical significance is defined as any variation in laboratory measurements which has medical relevance and which results in a change in medical care. If clinically significant laboratory changes from the time informed consent is signed are noted, the changes will be documented as AEs in the AE eCRF. Any Grade 3 or higher platelet count, grade 3 or higher WBC count, and grade 2 or higher neutrophil count (grade determined by Common Terminology Criteria for Adverse Events [CTCAE] v4.03; see Section 9.3.1.2) must also be documented as an AE in the AE eCRF, regardless of whether considered clinically significant by Investigator report (see Section 9.3.1). For all clinically significant out-of-range values (and Grade 3 or higher platelet count, grade 3 or higher WBC count, and grade 2 or higher neutrophil count) documented as AEs, the Investigator will assess the relationship to study treatment (see Section 9.3.1.3). The Investigator will also continue to monitor the subject with additional laboratory assessments until (1) values have reached normal range and/or baseline, or (2) in the judgment of the Investigator, the values are not related to the administration of study drug or other protocol-specific procedures.

9.2.4. Electrocardiograms

For each subject, 12-lead digital ECGs will be collected according to the Schedule of Assessments (Sections 14.1, 14.2, and 14.3) as single ECGs for over-read. Subjects must be supine for approximately 5 to 10 minutes before ECG collection and remain supine but awake during ECG collection.

ECGs may be obtained at additional times, when deemed clinically necessary. Collection of more ECGs than expected at a given time point is allowed when needed to ensure high quality records.

ECGs will be interpreted by a qualified physician (the Investigator or qualified designee) at the site as soon after the time of ECG collection as possible, and ideally while the subject is still present, to determine whether the subject meets entry criteria and for immediate subject management, should any clinically relevant findings be identified.

9.2.5. Pulmonary Function

Spirometry will be performed according to the Schedule of Assessments (Sections 14.1, 14.2, and 14.3) per American Thoracic Society (ATS)/European Respiratory Society (ERS) Standards, using the National Health and Nutrition Examination Survey (NHANES) III reference standard. FEV1, FVC, and FEV1/FVC ratio will be collected; diffusing capacity of the lung for carbon dioxide (DLCO) must also be collected if FEV1 is not available. Actual values and percent predicted values will be recorded.

9.2.6. Chest X-ray

Standard chest x-rays will be performed according to the Schedule of Assessments (Sections 14.1, 14.2, and 14.3).

9.2.7. Immunogenicity

Blood samples for immunogenicity testing will be collected at the times specified in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3) to assess for the presence of anti-AGA antibodies. Immunogenicity will be assessed using a qualified assay designed to detect anti-AGA antibodies.

9.2.8. Testing for Replication Competent Lentivirus

Peripheral blood samples will be collected for RCL testing at the times specified in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3) to determine infection of non-target cells. Testing will be performed by qPCR; possible future serology testing may also be performed.

Blood samples for qPCR will be tested centrally. Blood samples for possible future serology testing will be centrally stored. Details on blood sample collection, processing, and shipment will be included in the Laboratory Manual.

9.2.9. Reproductive Potential

Sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology will be collected at the times specified in the Schedule of Assessments (Sections 14.1, 14.2, and 14.3). Samples should be collected after at least 2 to 3 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation. For subjects continuing in the AVRO-RD-01-LTF01 study with reduced fertility at Week 48, sperm samples must also continue to be collected in that study until the subject demonstrates recovery.

9.2.10. Samples to be Archived for Possible Future Testing

Peripheral blood and bone marrow aspirate samples will be collected at Baseline, as specified in the Schedule of Assessments (Section 14.1), and archived for possible future testing should a subject develop hematologic cancer.

Details on sample collection, processing, and shipment will be included in the Laboratory Manual.

9.3. Safety Monitoring

Investigators are responsible for monitoring the safety of subjects who have entered this study and for alerting the Sponsor or its designee to any event that seems unusual, even if the event may be considered an unanticipated benefit to the subject.

The Investigator is responsible for the appropriate medical care of subjects during the study.

The Investigator remains responsible for following, through an appropriate health care option, AEs that are serious, considered related to the study treatment or the study, and that caused the subject to discontinue before completing the study. The subject should be followed for 30 days or until the event is resolved, whichever is longer. Frequency of follow-up evaluation is left to the discretion of the Investigator.

The Medical Monitor will monitor safety data throughout the course of the study.

The Sponsor or designee will review SAEs within time frames mandated by company procedures. The Medical Monitor will, as appropriate, periodically review:

- Trends in safety data
- Laboratory analytes
- Adverse events, including monitoring of infections and malignancies

If a subject experiences elevated ALT or AST >3X ULN or elevated total bilirubin >2X ULN, clinical and laboratory monitoring should be initiated by the Investigator. Details for hepatic monitoring depend upon the severity and persistence of observed laboratory test abnormalities. To ensure subject safety and comply with regulatory guidance, the Investigator is to consult with the Sponsor's designated Medical Monitor regarding collection of specific recommended clinical information and follow-up laboratory tests.

9.3.1. Adverse Events

An AE is any untoward medical occurrence in a clinical study subject who has been administered a medicinal product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product.

For the purposes of this study, 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 v4.03; see Section 9.3.1.2), regardless of whether considered clinically significant by Investigator report.

The Sponsor has standards for reporting AEs that are to be followed, regardless of applicable regulatory requirements that may be less stringent.

Cases of pregnancy that occur during paternal exposures to study treatment are to be reported (see Section 9.3.2). Data on fetal outcome are collected for regulatory reporting and drug safety evaluation.

Study site personnel will record the occurrence and nature of each subject's preexisting condition(s), including clinically significant signs and symptoms of the disease under treatment in the study. After the ICF is signed, site personnel will record any change in the condition(s) and the occurrence and nature of any AEs. Exceptions to this requirement are outlined in Section 9.3.1.1.

9.3.1.1. Exemptions to Adverse Event Recording/Reporting

For the purposes of this study, the following will not be considered AEs:

- Situations where an untoward medical occurrence did not occur (eg, social and/or convenience admission to a hospital, inpatient busulfan conditioning and management), and anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen over the course of the study.
- Lack of drug effect (because one of the objectives of this study is to establish the effect of AVR-RD-01 transplant).
- Individual Fabry disease-related signs and symptoms assessed during the periodic visits for the study, which include physical examinations and laboratory studies. The physical examination content is outlined in Section 9.2.1. Key Fabry disease signs and symptoms will also be evaluated through the efficacy endpoints outlined in Section 9.1, which include patient-reported outcome measures for key Fabry disease symptoms such as pain, gastrointestinal abnormalities, and mental and physical functioning.

9.3.1.2. Severity Assessment

Adverse event severity will be graded using the CTCAE v4.03. The CTCAE is a descriptive terminology utilized for AE reporting. A grading (severity) scale is provided for each AE term (each CTCAE v4.03 term is a Medical Dictionary for Regulatory Activities [MedDRA] Lowest Level Term).

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5, with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).[a]

Grade 3 Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.[b]

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

[a] Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[b] Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Change in severity of an AE should be documented based on specific guidelines in the eCRF completion guidelines.

Severity and seriousness must be differentiated: severity describes the intensity of an AE, while the term seriousness refers to an AE that has met the criteria for an SAE (see Section 9.3.1.5).

9.3.1.3. Causality Assessment

Investigators will be instructed to report to the Sponsor or its designee their assessment of the potential relatedness of each AE (both serious and non-serious) to protocol procedures and/or study treatment. Causality assessments will be provided on the eCRF (and any additional forms, as appropriate). Causality relationship will be classified according to the definitions in Table 2.

Table 2: Causality Definitions

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 participant'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
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

If a subject's transplant is canceled or discontinued as a result of a protocol procedure- or pre-infusion-related AE prior to the transplant, study site personnel must clearly report to the Sponsor or its designee via the eCRF the circumstances and data leading to any such cancelation or discontinuation of treatment.

Additional details can be found in the eCRF completion guidelines.

9.3.1.4. Outcome Assessment

Investigators will be instructed to report to the Sponsor or its designee their assessment of the outcome of each AE (both serious and non-serious). Definitions for possible AE outcomes include:

- Recovered/Resolved: the event has improved or the subject recuperated
- Recovered/Resolved with Sequelae: the subject recuperated but retained pathological conditions directly resulting from the disease or injury
- Recovering/Resolving: the event is improving

- Not Recovered/Not Resolved: the event has not improved, or the subject has not recuperated
- Fatal: termination of life as a result of an AE. There should be only one AE marked with this outcome
- Unknown: not known, not observed, not recorded, or refused

Additional details can be found in the eCRF completion guidelines.

9.3.1.5. Serious Adverse Events

Serious adverse event collection begins after the subject has signed informed consent. Planned surgeries and/or hospitalizations should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study.

Study site personnel must alert the Sponsor or its designee of any SAE within 24 hours of Investigator awareness of the event via a Sponsor-approved method. This 24-hour notification requirement refers to the initial SAE information.

An SAE is any AE from this study that results in 1 or more of the following outcomes:

- Results in death
- Requires or prolongs hospitalization
- Is life threatening (that is, immediate risk of dying)
- Persistent or significant disability/incapacity
- Congenital anomaly or birth defect
- Other medically important serious event

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug events when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Serious adverse events occurring up to and including the subject's last study visit will be collected, regardless of the Investigator's opinion of causation.

If an Investigator becomes aware of SAEs occurring to a subject after the subject's participation in the study has ended (including any protocol-required post-treatment follow-up), the Investigator should report the SAEs to the Sponsor, regardless of the Investigator's opinion of causation. For subjects consenting and meeting eligibility for participation in the long-term follow-up study to AVRO-RD-01-201 (ie, Study AVRO-RD-01-LTF01), SAEs occurring after AVRO-RD-01-201 study completion should be reported in the AVRO-RD-01-LTF01 study.

Information on SAEs expected in the study population independent of study treatment, and that will be assessed by the Sponsor in aggregate periodically during the course of the study, may be found in the Reference Safety Information section of the Investigator's Brochure.

9.3.1.6. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the Investigator's Brochure and that are identified as related to study treatment. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. The Sponsor has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

9.3.1.7. Reporting Adverse Events

All non-serious AEs must be recorded in the eCRF upon awareness.

All SAEs must be reported to the Sponsor or designee, via the SAE contact in the Safety Management Plan, within 24 hours of the Investigator or their staff becoming aware of them. The Investigator must complete, sign, and date the SAE pages; verify the accuracy of the information recorded on the SAE pages with the corresponding source documents; and send a copy via electronic submission to the safety database or email. Facsimile transmission may be used in the event of electronic submission or email failure.

When further information becomes available, the SAE Form should be updated with the new information and reported immediately via the same methods as the initial information.

Additional follow-up information, if required or available, should be reported to the Sponsor or designee within 24 hours of the Investigator or their staff becoming aware of this additional information via the same manner as above.

These timelines for reporting information to the Sponsor must be followed for all initial SAE reports and for all follow-up versions of the initial reports.

SAE Forms should be kept within the appropriate section of the study files. SAEs should be reported to your local REB/HREC/IRB per institutional guidelines (see Section 9.3.1.9).

All SAEs (related and unrelated) will be recorded from the signing of informed consent until the Week 48 (or Early Termination) Visit.

For all SAEs the Investigator must provide the following:

- Appropriate and requested follow-up information in the time frame detailed above
- Causality of the SAE(s)
- Outcome of the SAE(s)
- Redacted medical records and laboratory/diagnostic information

9.3.1.8. Sponsor Reporting Requirements

The Sponsor or its legal representative is responsible for notifying the relevant regulatory authorities of SAEs meeting the reporting criteria. This protocol will use the current Investigator's Brochure as the Reference Safety Document. The expectedness and reporting criteria of an SAE will be determined by the Sponsor from the Reference Safety Document.

9.3.1.9. Investigator Reporting Requirements

The Investigator must fulfill all local regulatory obligations required for study Investigators. It is the Principal Investigator's (PI) responsibility to notify the REB/HREC/IRB of all SAEs that occur at his or her site. Investigators will also be notified of all SUSAR events that occur during the clinical study. Each site is responsible for notifying its REB/HREC/IRB of these additional SAEs according to local requirements.

9.3.2. Exposure During Pregnancy

Pregnancy data will be collected during this study for all subjects. Should it occur, exposure during pregnancy (also referred to as exposure in-utero [EIU]) for this study would result from transmission of any study treatments via semen following paternal exposure.

If a subject's partner becomes pregnant while exposed to study treatment, the Investigator must submit a pregnancy form to the Sponsor or designee via the same method as SAE reporting (see Section 9.3.1.7). When the outcome of the pregnancy becomes known (ie, spontaneous miscarriage, elective termination, normal birth, or congenital abnormality), the pregnancy form must be completed and returned to the Sponsor or designee. If additional follow-up is required, the Investigator will be requested to provide the information.

For any partner pregnancies reported during the study, the pregnancy must be followed until the outcome of the pregnancy is known, even if the subject discontinues from the study.

Pregnancy in itself is not regarded as an AE unless there is a suspicion that study treatment may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs, and many may meet criteria for an SAE (such as ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly). Elective abortions without complications should not be reported as AEs

9.4. Appropriateness of Measurements

Efficacy and safety measures chosen for this study have been commonly used to measure treatment effects in trials of ERT for Fabry disease (van Breemen, 2011; Germain, 2007; Eng, 2001; Schiffmann, 2001; Ries, 2006; Ramaswami, 2007). The following were considered in choosing efficacy and safety endpoints, and the timing of related assessments:

- The natural history of Fabry disease, and the clinical progression of sign/symptoms known to impact morbidity and mortality in patients with the disease.
- Regulatory guidelines for follow-up of subjects receiving gene therapy products (ie, United States Department of Health and Human Services, FDA, CBER, Guidance for Industry, Long Term Follow-Up After Administration of Human Gene Therapy Products, January 2020; United States Department of Health and Human Services, FDA, CBER, Guidance for Industry, Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up, January 2020; EMA, Guideline on Follow-up of Patients Administered with Gene Therapy Medicinal Products, October 2009).

10. DATA QUALITY ASSURANCE

To ensure accurate, complete, and reliable data, the Sponsor or its representatives will do the following:

- Provide instructional material to the study sites, as appropriate
- Provide start-up training to instruct the Investigators and study coordinators. This training will give instruction on the protocol, the completion of the eCRFs, and study procedures.
- Make periodic visits to the study site
- Be available for consultation and stay in contact with the study site personnel by email, telephone, and/or fax
- Review and evaluate eCRF data and use standard computer edits to detect errors in data collection
- Conduct a quality review of the database

In addition, the Sponsor or its representatives will periodically check a sample of the subject data recorded against source documents at the study site. The study may be audited by the Sponsor or its representatives, and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of participants in the study, and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the study. If requested, the Investigator will provide the Sponsor, applicable regulatory agencies, and applicable REBs/HRECs/IRBs with direct access to original source documents.

10.1. Data Collection and Storage

All clinical raw data will be recorded promptly, accurately, and legibly, either directly into a data capture system as e-source data, or indelibly on paper (eg, ECG readings). A detailed list of the type (electronic or paper) and location for all source data will be included in the Trial Master File. When recorded electronically, CRFs will be electronically generated. All raw data will be preserved in order to maintain data integrity. The Investigator or designee will assume the responsibility of ensuring the completeness, accuracy, and timeliness of the clinical data recording.

At each scheduled monitoring visit, the Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of data capture.

The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE and concomitant medication reporting, raw data collection forms) designed to record all observations and other pertinent data for each subject.

The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the REB/HREC/IRB to have direct access to all documents pertaining to the study.

11. STATISTICAL METHODS AND PLANNED ANALYSES

11.1. General Considerations

Prior to the analysis of the study data, a detailed SAP will be written describing all analyses to be performed. The SAP will contain any modifications to the analysis plan described below. Any changes to the statistical plans will be described and justified in the CSR.

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, safety and tolerability endpoints will be summarized descriptively at each protocol-scheduled time point and as change from baseline. Continuous data will be summarized using descriptive summary statistics (number of non-missing observations, mean, standard deviation [SD], minimum, and maximum). Categorical data will be summarized as frequency counts and percentages at each protocol-scheduled time point.

For all safety endpoints, baseline will be defined as the last available, non-missing observation prior to first study treatment administration, unless specifically mentioned otherwise. For all efficacy endpoints, baseline will be defined as the last available, non-missing observation prior to AVR-RD-01 administration. In general, Unknown, Not Done, Not Applicable, and other classifications of missing data will not be considered when calculating baseline observations. However, valid categorical observations will be considered for baseline calculations. In addition, non-missing results from unscheduled assessments prior to first study drug administration may also be considered in the calculation of baseline observations.

Only data from nominal protocol-scheduled visits will be included in summary tables. Data from unscheduled visits will not be included in the summary tables but will be included in figures and listings.

No formal hypothesis tests or statistical models are planned.

11.2. Determination of Sample Size

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.

11.3. Analysis Sets

Subject inclusion into each population defined below will be determined after database lock and prior to commencing the analysis.

All Subjects Population

The All Subjects population will consist of all enrolled subjects. The All Subjects population will be used for the summaries of all disposition and demographic and baseline data. In addition, all listings will also be produced using the All Subjects population.

Safety Population

The Safety population will consist of all enrolled subjects who receive any amount of study treatment. The Safety population will be used for the summaries of all safety data.

Efficacy Population

The Efficacy population will consist of all enrolled subjects who received AVR-RD-01 transplant, have no major protocol deviations, and have efficacy data both at baseline and at least one post-baseline assessment. The Efficacy population will be used for the summaries of all efficacy endpoints.

11.4. Demographics and Baseline Characteristics

Demographic (age, race, ethnicity, height, weight, and body mass index) and baseline data, including disease history and characteristics, will be summarized. Medical and surgical history will be coded using the most current MedDRA and summarized by System Organ Class and Preferred Term. All other data recorded at the screening assessment will be listed only.

11.5. Subject Disposition

The total number of subjects enrolled, treated, and included in each analysis population will be summarized. The number of subjects discontinuing the study early, along with the reason for early study discontinuation, will also be summarized.

11.6. Concomitant Medications and Non-Pharmacologic Therapies and Procedures

Medications used in this study will be coded using the most current World Health Organization Drug Dictionary Enhanced (WHO).

The start dates of medications will be used to assign medications into the following categories:

- Prior medication: any medication that started before initial dosing of study treatment, regardless of when it ended
- Concomitant medication: medication continued or newly received at or after initial dosing of study treatment

Medications will be classified as a prior medication and/or a concomitant medication. If a medication has a missing or partial missing start/end date or time, and it cannot be determined whether it was taken before initial treatment or concomitantly, it will be considered as prior and concomitant. Prior and concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) classification and drug preferred term; the summary tables will show the number and percentage of subjects taking each medication by ATC and preferred term. For subjects who take the same medication (in terms of the ATC and preferred term) more than once, the subject will be counted only once for that medication.

Prior medication and concomitant medication will be listed and summarized separately.

11.7. Treatment Compliance

All study treatment administration data will be listed. As all study treatment will be administered at the investigational site, treatment compliance will not be determined for this study.

11.8. Efficacy Analyses

All efficacy analyses will be conducted on the Efficacy Population. All raw and derived efficacy data will be listed.

11.8.1. Primary Efficacy Analysis

As previously reported (see Section 9.1.1), 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 (ie, myelinosomes) per kidney PTC per subject using a quantification method similar to the BLISS previously described for light microscopy (Barisoni, 2012).

The average number of Gb3 inclusions (ie, myelinosomes) per kidney PTC per subject at Baseline and Week 48 will be summarized using descriptive statistics. Observed values and changes from baseline will be summarized at each protocol-scheduled time point.

Additional details on statistical analysis will be included in the SAP for the study.

11.8.2. Secondary Efficacy Analyses

Observed values and changes from baseline will be summarized at each protocol-scheduled time point. For questionnaire data, the appropriate scores will be calculated, and data will be summarized overall and for each domain, where relevant.

11.8.3. Exploratory Efficacy Analyses

For quantitative exploratory efficacy data collected, observed values and changes from baseline will be summarized at each protocol-scheduled time point.

Descriptive summaries of average VCN and ISA results at each timepoint in each population analyzed will be provided by-subject for qualitative exploratory efficacy and safety assessment.

For qualitative interviews conducted, thematic analysis will be performed to assess perception of changes over time in Fabry disease burden and quality of life, including impact of pre-treatment conditioning on participating subjects, to augment other clinical data collected in the study.

11.9. Safety Analyses

All safety analyses will be conducted on the Safety Population.

11.9.1. Physical Examination and Vital Signs

All individual vital signs results will be presented in data listings. Observed vital signs parameter values and changes from baseline will be summarized at each protocol-scheduled time point.

Physical examination data will be listed only.

11.9.2. Serum Chemistry, Hematology, and Urinalysis

For serum chemistry, hematology (including peripheral blood lymphocyte subsets [CD3, CD4, CD8, CD19, and CD16/56]), and urinalysis, all individual clinical laboratory results will be

presented in data listings. Values outside the laboratory reference range will be flagged (low-L, high-H).

Observed values and changes from baseline in clinical laboratory data will be summarized at each protocol-scheduled time point. Frequency tabulations of the number of normal and abnormal (low and high) records, as well as the number of clinically significant (CS) and not clinically significant (NCS) records, will also be summarized at each protocol-scheduled time point. Urinalysis results will be summarized at each protocol-scheduled time point, using frequency tabulations, and any available microscopic urinalysis results will be listed.

In addition, shift tables presenting the number of subjects with abnormal values compared to baseline will be produced.

11.9.3. Adverse Events

Adverse events will be coded using MedDRA. Adverse events will be grouped by System Organ Class and Preferred Term and summarized. The summary tables will present the frequency and percentage of total subjects, by System Organ Class and Preferred Term.

For the summaries of AEs, subjects who experience the same AE (in terms of the MedDRA Preferred Term) more than once will be counted only once for that event in the number of subjects; however, all occurrences of the same event will be counted in the number of events.

Adverse event summaries will be restricted to treatment-emergent AEs (TEAEs) only. Treatment emergent events are defined as AEs that commence on or after the time of first study treatment administration. Adverse events without an onset date or time will be defined as treatment emergent except where an incomplete date (eg, month and year) clearly indicates that the event started prior to the start of first study treatment administration, or the AE stop date indicates that the event started and/or stopped prior to the start of first study treatment administration. Adverse events will be summarized by study period (ie, Pre-transplant [Mobilization, Apheresis, or Conditioning], Transplant, or Post-transplant Follow-up).

Incidence of AEs, as well as the duration, severity, relationship to study treatment, outcome, and actions taken, will be listed for each subject. In addition, listings of AEs leading to discontinuation of the study, SAEs, and deaths will be provided if applicable.

The following AE summaries will be provided:

- Overall summary of TEAEs:
  - Any TEAE
  - Any severe/≥CTCAE Grade 3 TEAE
  - Any TEAE considered as related to any study treatment (assessed by the Investigator as being possibly related, probably related, or definitely related)
  - Any TEAE leading to discontinuation from the study
  - Any serious TEAE
  - Any life-threatening TEAE
  - Any TEAE leading to death

- TEAEs overall and by System Organ Class and Preferred Term
- TEAEs related to any study treatment, overall and by System Organ Class and Preferred Term (a TEAE related to study treatment is defined as any TEAE assessed by the Investigator as being possibly related, probably related, or definitely related to any study treatment)
- TEAEs by severity/CTCAE Grade, overall and by System Organ Class and Preferred Term
- TEAEs leading to study discontinuation, overall and by System Organ Class and Preferred Term
- Serious TEAEs, overall and by System Organ Class and Preferred Term

In the summary tables, AEs will be presented by decreasing frequency across all subjects.

11.9.4. Reproductive Potential

Reproductive potential will be evaluated based on sperm count, morphology, and motility.

All individual results collected will be presented in data listings. Values outside the laboratory reference range will be flagged (low-L, high-H), where applicable.

Observed values and changes from baseline in reproductive endpoints will be summarized at each protocol-scheduled time point. Frequency tabulations of the number of normal and abnormal (low and high) records, as well as the number of CS and NCS records, will also be summarized at each protocol-scheduled time point.

11.9.5. Other Safety Assessments

All individual ECG parameter results and the clinical assessment of the ECG will be presented in data listings. Observed ECG parameter values and changes from baseline will be summarized at each protocol-scheduled time point. Clinical assessment of the ECG (Normal, Abnormal NCS, Abnormal CS) will be summarized using frequency tabulations.

Anti-AGA antibody data will also be summarized.

All other safety data, including results of vector ISA and testing for presence of RCL, will be listed only.

11.10. Subgroup Analyses

No subgroups are defined for this study.

11.11. Other Statistical Issues

11.11.1. Significance Levels

Due to the small sample size, no formal statistical testing will be conducted.

11.11.2. Missing or Invalid Data

For subjects who are withdrawn from the study prior to study completion, all data compiled up to the point of discontinuation will be used for analysis. All withdrawals will be included in all

analyses up to the time of withdrawal. There will be no imputation for missing data, unless otherwise stated.

11.12. Interim Analyses

In this open-label study, after AVR-RD-01 transplant, 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. Final statistical analysis for the study will be performed after all enrolled subjects complete the Week 48 assessments (or prematurely discontinue the study). Full analytical details for the study will be outlined in the SAP.

12. INFORMED CONSENT, ETHICAL REVIEW, AND REGULATORY CONSIDERATIONS

12.1. Informed Consent

The Investigator is responsible for ensuring that the subject understands the potential risks and benefits of participating in the study (including participating in the optional qualitative interviews and genetic biomarker research outlined in Section 9.1.3), including answering any questions the subject may have throughout the study, and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the study and optional qualitative interviews and genetic biomarker research.

Three ICFs, one for the study, one for the optional qualitative interviews, and one for the optional genetic biomarker research, will be used to explain the potential risks and benefits of study and optional qualitative interview and genetic biomarker research participation to the subject in simple terms before the subject is entered into the study, and to document that the subject is satisfied with his or her understanding of the risks and benefits of participating in the study and optional qualitative interviews and genetic biomarker research, and desires to participate.

The Investigator is responsible for ensuring that informed consent is given by each subject or legal representative. This includes obtaining the appropriate signatures and dates on the ICF(s) prior to the performance of any protocol procedures and prior to the administration of investigational product.

A legal representative must give informed consent for a minor to participate in this study. In addition to informed consent given by the legal representative, the minor may be required to give documented assent.

For any caregivers or other qualified observers of subjects who opt to participate in qualitative interviews being conducted for the study, written informed consent will also be obtained for participation.

As used in this protocol, the term "informed consent" includes all consent and assent given by subjects or their legal representatives, as well any caregivers or other qualified observers of subjects who opt to participate in the qualitative interviews for the study.

12.2. Data Monitoring Committee

An independent DMC has been established for the study. The DMC will consist of clinical trial experts in stem cell transplantation, gene therapy, and Fabry disease independent of the study. The DMC will monitor the safety aspects of the trial, including review of safety data (including all SAEs) after subjects complete the Week 4 follow-up visit in accordance with the schedule outlined in Section 6.1. The DMC will also convene for a safety review if stopping rules for the study are met (see Section 7.4.4). Additional meetings may be scheduled as necessary.

The specific responsibilities of the DMC are described in the DMC Charter, which is maintained as a separate document.

12.3. Ethical Review

The Sponsor or its representatives must approve all ICFs before they are used at investigative sites(s). All ICFs must be compliant with the International Council for Harmonisation (ICH) guideline on GCP.

Documentation of REB/HREC/IRB approval of the protocol and the ICF must be provided to the Sponsor before the study may begin at the investigative site(s). The REBs/HRECs/IRBs will review the protocol as required.

The study site's REBs/HRECs/IRBs should also be provided with the following:

- The current Investigator's Brochure for AVR-RD-01
- ICF
- Relevant curricula vitae

12.4. Regulatory Considerations

This study will be conducted in accordance with:

1. Consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
2. The ICH GCP Guideline (E6)
3. Applicable laws and regulations

The Investigator or designee will promptly submit the protocol to applicable REBs/HRECs/IRBs.

Some of the obligations of the Sponsor will be assigned to a Third Party Organization.

An identification code assigned to each subject will be used in lieu of the subject's name to protect the subject's identity when reporting AEs and/or other study-related data.

12.4.1. Investigator Information

One PI will oversee the trial at each investigative site. The PI undertakes to perform the study in accordance with this clinical trial protocol, ICH GCP Guidelines, and the applicable national regulations and local REB/HREC/IRB requirements.

The PI may appoint other individuals as he/she deems appropriate to assist in the conduct of the study. All appointed designees will be listed in the site delegation log. The appointed designates will be supervised by and under the responsibility of the PI.

12.4.2. Protocol Signatures

The Sponsor's responsible medical officer will approve the protocol, confirming that, to the best of her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, each PI will sign the protocol signature page and send a copy of the signed page to a Sponsor representative.

12.4.3. Final Report Signature

The CSR coordinating Investigator will sign the final CSR for this study, indicating agreement that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

The Sponsor's responsible medical officer and statistician will approve the final CSR for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

12.5. Publication Policy

The full terms for publication are included in the clinical trial agreement between the Sponsor and the Investigator.

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14. APPENDICES

14.1. Study AVRO-RD-01-201: Schedule of Assessments (Screening through Investigational Product Preparation and Testing for Release in the Pre-Transplant Period)

Study Assessment	Study Period									
			Pre-transplant							
			Mobilization				Apheresis		IP Preparation and Testing for Release for Infusion[c]	Early Termination[d]
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---	---
Assessment Window	- 8W[a]	---	---	---	---	---	---	---	---	---
Informed consent/assent	X									
Demographics	X									
General- and Fabry-specific medical and surgical history[e]	X									
Mutation analysis[f,h]	X									
Transmittable disease testing (including HIV)[g]	X									
ECHO	X									
PTT and INR	X		X							
Efficacy Assessments										
Blood sample collection for AGA activity in plasma[h]	X	X								X
Blood sample collection for AGA activity in peripheral blood leukocytes[h]	X	X								X
Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma[h]		X								X
Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine[h,i]		X								X

Study Assessment	Study Period									
			Pre-transplant							
	Screening	Baseline[b]	Mobilization				Apheresis		IP Preparation and Testing for Release for Infusion[c]	Early Termination[d]
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---	---
Assessment Window	**- 8W[a]**	---	---	---	---	---	---	---	---	---
Blood sample collection for exploratory biomarkers for Fabry disease[h,j]		X								
Urine sample collection for exploratory biomarkers for Fabry disease[h,j]		X								
Renal biopsy[k]	X									
Skin biopsy[k]	X									
GFR (iohexol-measured)		X								
GFR (estimated using serum creatinine, normalized for age)	X									X
Overnight urine collection for protein/albumin excretion	X	X								X
Urine collection for creatinine and podocyte assessment[h,l]		X								X
Cardiac MRI[m]		X								
DIBSS-D[n]	X⇒⇒	X								
BPI-SF questionnaire[o]		X								
SF-36[o]		X								
Qualitative interview[p]		X								
Peripheral blood sample collection for VCN, ISA, immunophenotyping, and sample archiving[h,q]		X[q]								X

Study Assessment	Study Period									
			Pre-transplant							
	Screening	Baseline[b]	Mobilization				Apheresis		IP Preparation and Testing for Release for Infusion[c]	Early Termination[d]
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---	---
Assessment Window	**- 8W[a]**	---	---	---	---	---	---	---	---	---
Bone marrow aspirate collection for VCN, ISA, immunophenotyping, and sample archiving[h,r]		X[r]								
Safety Assessments										
Physical examination	X	X								X
Weight		X								X
Height		X								
Vital signs[s]	X	X	X	X	X	X	X	X		X
Chest X-ray	X	X								
PFT by spirometry[t]	X	X								
12-lead ECG	X	X								
CBC with differential[u]	X	X	X	X	X	X	X	X		X
Blood sample collection for T and B cell counts[v]	X	X								X
Serum chemistry[w]	X	X								X
Electrolytes, BUN, and serum creatinine[x]	X	X	X							X
Urinalysis[y]	X	X								X
Blood sample collection for immunogenicity testing[h,z]	X									X
Peripheral blood sample collection for RCL[h,aa]		X								X
Measures of reproductive potential[bb]		X								X

Study Assessment	Study Period									
			Pre-transplant							
	Screening	Baseline[b]	Mobilization				Apheresis		IP Preparation and Testing for Release for Infusion[c]	Early Termination[d]
Study Day [D]	---	B ---	M D1	M D2	M D3	M D4	A D1	A D2	---	---
Assessment Window	- 8W[a]	---	---	---	---	---	---	---	---	---
AVR-RD-01 Drug Product Preparation Procedures for Transplant										
G-CSF injection[cc]			X	X	X	X				
Plerixafor administration[dd]						X	X			
Apheresis for stem cell harvesting[ee]							X	X		
CD34+ cell count/µL							X	X		
CD34+ cell enumeration by flow cytometry							X	X		
Continuous Monitoring Procedures										
Prior and concomitant medications and therapies[ff]	Continuous monitoring									X
Adverse events[gg]	Continuous monitoring									X

Abbreviations: A = apheresis Ab= antibody; Ag = antigen; AGA = alpha-galactosidase A; ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANC = absolute neutrophil count; anti-HBc = antibodies (IgG and IgM) to hepatitis B core antigen; AST = aspartate aminotransferase; B = Baseline; BPI-SF = Brief Pain Inventory Short Form; BUN = blood urea nitrogen; CBC = complete blood cell count; c/dg = copy per diploid genome; CMIA = chemiluminescent microplate immunoassay; D = day; DIBSS-D = Diary for Irritable Bowel Syndrome Symptoms - Diarrhea; DLCO = diffusing capacity of the lung for carbon dioxide; ECG = electrocardiogram; ECHO = echocardiogram; EIA = enzyme immunosorbent assay; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; Gb3 = globotriaosylceramide; G-CSF = granulocyte colony-stimulating factor; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; HSV = herpes simplex virus; HTLV = human T-cell lymphotropic virus; INR = international normalized ratio; IP = investigational product; ISA= integration site analysis; IV = intravenous(ly); LDH = lactate dehydrogenase; lyso-Gb3 = globotriaosylsphingosine (deacylated form of Gb3); M = Mobilization; MRI = magnetic resonance imaging; PCR = polymerase chain reaction; PFT = pulmonary function testing; PTT = partial thromboplastin time; qPCR = quantitative polymerase chain reaction; RBC = red blood cell count; RCL = replication competent lentivirus; SAE = serious adverse event; SC = subcutaneously; SF-36 = 36-Item Short Form Health Survey; VCN = vector copy number; VDRL = Venereal Disease Research Laboratory; VSV = vesicular stomatitis virus; W = week; WBC = white blood cell count

Footnotes:

[a] Unless otherwise specified, Screening assessments must be performed within 8 weeks of the Baseline visit.

[b] The Baseline visit may be conducted over the course of up to 3 days.

[c] Between the Apheresis and Conditioning phases of the Pre-transplant Period, there is an approximately 4-6 week period during which time AVR-RD-01 is tested for release for infusion.

[d] Assessments required for those subjects who prematurely discontinue the study after initiation of the Pre-transplant Period. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made.

[e] Medical history will include retrospective collection of Fabry-disease specific medical and surgical history, including historical results of any AGA enzyme activity testing.

[f] Documented historical results of mutation analysis from a certified laboratory will be recorded if available. Mutation analysis must also be performed at Screening, regardless of the availability of historical results.

[g] Transmittable disease testing includes serological analysis for HIV Ag/Ab, HIV nucleic acid-amplification testing (NAT), HBsAg, anti-HBc, HBV NAT, anti-HCV antibody, HCV NAT, syphilis test (VDRL, CMIA, or EIA test), cytomegalovirus (CMV) IgG Ab, CMV IgM Ab, HTLV-1 Ab, HTLV-2 Ab, HSV-1 IgG Ab, and, if applicable, VSV IgG Ab. If applicable, testing for West Nile virus by PCR must also be performed. Testing is performed for study eligibility determination and/or AVR-RD-01 manufacturing purposes. Transmittable disease testing will be performed locally. Subjects who test positive for HBV, HCV, HIV (type 1 or 2), HTLV-1, HTLV-2, and/or syphilis on VDRL, CMIA, or EIA test will be discontinued from the study.

[h] Blood or urine sample(s) will be tested centrally. Details on collection, processing, and shipment will be included in the Laboratory Manual.

[i] Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine must be the second or third morning void.

[j] For consenting subjects, samples will be collected in plasma and urine for assessment of biomarkers for Fabry disease that may become available during or after the study. Samples obtained will be labeled for 'Biorepository'. Biorepository samples will be used only for these specific analyses, as applicable, and will be destroyed 5 years after the Clinical Study Report (CSR) is finalized.

[k] Skin and renal biopsies will ideally be the last assessment performed at Screening, and will be analyzed centrally for substrate (ie, Gb3) and AGA activity. Renal biopsy will also be analyzed centrally to exclude other renal disease. Details on collection, processing, and shipment will be included in the Laboratory Manual.

[l] Urine for podocytes and creatinine should be second morning void.

[m] Cardiac MRI will be analyzed centrally.

[n] Baseline DIBSS-D diary data will be collected for two consecutive weeks during the Screening period.

[o] BPI-SF questionnaire and SF-36 should be completed prior to any other required assessments at the study visit.

[p] Interviews will be scheduled and conducted by Casimir for both subjects and caregivers, or other qualified observers, of subjects who opt to participate, provide written informed consent is obtained.

[q] Integration site analysis will only be performed on peripheral blood leukocyte samples with an average VCN $\geq$0.01 as determined by the central laboratory, suggesting $\geq$1% of the sampled blood cells are vector positive, with an average of 1 c/dg. At Baseline only, a peripheral blood sample will be archived for possible future testing should a subject develop hematologic cancer.

[r] At Baseline only, a bone marrow sample will be archived for possible future testing should a subject develop hematologic cancer.

[s] Vital signs include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be obtained with the subject supine or seated. On Days 1 and 2 of the Apheresis phase of the Pre-transplant Period, vital signs must be taken prior to apheresis. Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice.

[t] Pulmonary function tests include FEV1, FVC, and FEV1/FVC ratio; DLCO must also be collected if FEV1 is not available. Both results and percent predicted results should be recorded. A $\leq$50% predicted value (corrected for hemoglobin) for one or more of the following at Screening is an exclusion criterion for the study: FEV1, FVC, and DLCO. PFT at Baseline is only required if there is a change in physical examination or new onset pulmonary or other medical condition relevant to testing since the Screening PFT.

[u] CBC with differential includes the following: hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, and ANC count. Samples will be tested locally.

[v] Assessment will also include routine flow cytometric analysis of peripheral blood lymphocyte subsets. Panel will include: CD3, CD4, CD8, CD19, and CD16/56. Samples will be tested locally.

[w] Serum chemistry includes AST, ALT, GGT, amylase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Samples will be tested locally.

[x] Electrolytes include sodium, potassium, chloride, and bicarbonate. Samples will be tested locally.

[y] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Samples will be tested locally.

[z] Blood sample collection for immunogenicity testing must be collected for all subjects at Screening, regardless of whether or not they have received previous treatment with enzyme replacement therapy.

[aa] Samples will be collected for RCL testing to determine infection of non-target cells. Testing will be performed by qPCR; possible future serology testing may also be performed. Blood samples for possible future serology testing will be centrally stored.

[bb] Sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 2 to 3 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation.

[cc] On Mobilization Days 1 through 4 of the Pre-transplant Period, a daily G-CSF dose of 10 µg/kg body weight will be administered (for sites with only prefilled syringes, a G-CSF daily dose from 10 µg/kg body weight up to 12 µg/kg body weight, administered in either a single or divided twice a day (BID) dose, may be administered). The G-CSF dose may be administered IV or SC. The 'G-CSF Diary' should be completed by subjects self-administering.

[dd] On the evening of Mobilization Day 4 and on the evening of Apheresis Day 1, plerixafor (0.24 mg/kg) will be administered SC.

[ee] On the morning of Apheresis Day 1 (ie, between 10 and 12 hours after plerixafor administration on Mobilization Day 4), cells will be harvested from the subject's peripheral blood; this apheresis unit will be shipped to the manufacturing site for cell transduction and processing. On the morning of Apheresis Day 2 (ie between 10 and 12 hours after plerixafor administration on Apheresis Day 1), an additional harvest of cells will occur; cells harvested from this apheresis (nontransduced cells) will be retained and stored at the investigational site for use as rescue treatment (if needed). Standard institutional procedures at the participating investigational study site will be used for both apheresis procedures.

[ff] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.

[gg] All AEs (including SAEs) will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.

14.2. Study AVRO-RD-01-201: Schedule of Assessments (Pre-Transplant Conditioning Period through Transplant [Day 0])

Study Assessment	Study Period										
	Pre-transplant									IP Transplant[b]	Early Termination[c]
	Conditioning (including washout period)[a]										
Study Day [D]	C D-8	C D-7	C D-6	C D-5	C D-4	C D-3	C D-2	C D-1	Conditioning Washout Period	T D0	---
Study Week [W]	---	---	---	---	---	---	---	---	---	W1	---
Assessment Window	---	---	---	---	---	---	---	---	Minimum of 48 up to Maximum of 72 hours[a]	---	---
Efficacy Assessments											
Blood sample collection for AGA activity in plasma[d]		X								X	X
Blood sample collection for AGA activity in peripheral blood leukocytes[d]		X								X	X
Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma[d]		X								X	X
Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine[d,e]		X								X	X
GFR (estimated using serum creatinine, normalized for age)											X
Overnight urine collection for protein/albumin excretion											X
Urine collection for creatinine and podocyte assessment[d,f]											X
Peripheral blood sample collection for VCN, ISA, and immunophenotyping[d,g]										X	X
Safety Assessments											
Physical examination		X								X	X

Study Assessment	Study Period										
	Pre-transplant									IP Transplant[b]	Early Termination[c]
	Conditioning (including washout period)[a]										
Study Day [D]	C D-8	C D-7	C D-6	C D-5	C D-4	C D-3	C D-2	C D-1	Conditioning Washout Period	T D0	---
Study Week [W]	---	---	---	---	---	---	---	---	---	W1	---
Assessment Window	---	---	---	---	---	---	---	---	Minimum of 48 up to Maximum of 72 hours[a]	---	---
Weight			X							X	X
Vital signs[h]		X			X	X	X	X		X	X
Chest X-ray		X									
PFT by spirometry[i]		X									
12-lead ECG		X									
CBC with differential[j]		X				X	X	X	X[j]	X	X
Blood sample collection for T and B cell counts[k]		X									X
Serum chemistry[l]		X				X	X	X	X[l]	X	X
Electrolytes, BUN, and serum creatinine[m]		X				X	X	X	X[m]	X	X
Urinalysis[n]											X
PTT and INR		X									
Blood sample collection for immunogenicity testing[d]											X
Peripheral blood sample collection for RCL[d,o]											X
Measures of reproductive potential[p]											X
Conditioning, Conditioning Washout Period, and AVR-RD-01 Transplant											
IV busulfan administration for conditioning[q]			X (TD)		X	X	X	X			
Serial blood sample collection for TDM (for busulfan dose determination and calculation of cumulative AUC from Day -4 to Day -1)[r]			X		X	X	X	X			

Study Assessment	Study Period										
	Pre-transplant									IP Transplant[b]	Early Termination[c]
	Conditioning (including washout period)[a]										
Study Day [D]	**C D-8**	**C D-7**	**C D-6**	**C D-5**	**C D-4**	**C D-3**	**C D-2**	**C D-1**	**Conditioning Washout Period**	**T D0**	**---**
Study Week [W]	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**W1**	**---**
Assessment Window	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**Minimum of 48 up to Maximum of 72 hours[a]**	**---**	**---**
Levetiracetam administration for prophylaxis[s]	X	X	X	X	X	X	X	X	X[s]		
AVR-RD-01 infusion[t]										X	
Continuous Monitoring Procedures											
Prior and concomitant medications and therapies[u]	Continuous monitoring										X
Adverse events[v]	Continuous monitoring										X

Abbreviations: AGA = alpha-galactosidase A; ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; AUC = area-under-the-curve; BUN = blood urea nitrogen; C = Conditioning; CBC = complete blood cell count; c/dg = copy per diploid genome; D = day; DLCO = diffusing capacity of the lung for carbon dioxide; ECG = electrocardiogram; ECHO = echocardiogram; eCRF = electronic case report form; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; Gb3 = globotriaosylceramide; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; INR = international normalized ratio; ISA = integration site analysis; IV = intravenous(ly); LDH = lactate dehydrogenase; lyso-Gb3 = globotriaosylsphingosine (deacylated form of Gb3); PFT = pulmonary function testing; PTT = partial thromboplastin time; qPCR = quantitative polymerase chain reaction; RBC = red blood cell count; RCL = replication competent lentivirus; SAE = serious adverse event; T = Transplant; TD = test dose; TDM = therapeutic drug monitoring; VCN = vector copy number; W = week; WBC = white blood cell count

Footnotes:

[a] The Pre-transplant Conditioning period includes both conditioning procedures and a washout period from conditioning. The washout period must be a minimum of 48 up to a maximum of 72 hours prior to AVR-RD-01 infusion.

[b] During the Transplant Period (Day 0), all blood and urine samples for clinical laboratory testing must be collected (and other required assessments performed) prior to AVR-RD-01 infusion.

[c] Assessments required for those subjects who prematurely discontinue the study after initiation of the Pre-transplant Period. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made. For subjects who undergo transplantation, every effort should be made to continue to follow the subject for safety monitoring (including any indication(s) of loss of gene therapy effect).

[d] Blood or urine sample(s) will be tested centrally. Details on collection, processing, and shipment will be included in the Laboratory Manual.

[e] Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine must be the second or third morning void.

[f] Urine for podocytes and creatinine should be second morning void.

[g] Integration site analysis will only be performed on peripheral blood leukocyte samples with an average VCN ≥0.01 as determined by the central laboratory, suggesting ≥1% of the sampled blood cells are vector positive, with an average of 1 c/dg.

[h] Vital signs include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be obtained with the subject supine or seated. On Day 0 of the Transplant Period (ie, the day of AVR-RD-01 infusion), vital signs must be taken pre-infusion (window -10 minutes) and post-infusion (window +10 minutes). Additional vital signs may be taken if clinically indicated and/or mandated per local institution practice for transplantation.

[i] Pulmonary function tests include FEV1, FVC, and FEV1/FVC ratio; DLCO must also be collected if FEV1 is not available. Both results and percent predicted results should be recorded.

[j] CBC with differential includes the following: hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, and ANC count. During the Conditioning washout period, testing should be performed daily. Samples will be tested locally.

[k] Assessment will also include routine flow cytometric analysis of peripheral blood lymphocyte subsets. Panel will include: CD3, CD4, CD8, CD19, and CD16/56. Samples will be tested locally.

[l] Serum chemistry includes AST, ALT, GGT, amylase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. During the Conditioning washout period, testing should be performed daily. Samples will be tested locally.

[m] Electrolytes include sodium, potassium, chloride, and bicarbonate. During the Conditioning washout period, testing should be performed daily. Samples will be tested locally.

[n] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Samples will be tested locally.

[o] Samples will be collected for RCL testing to determine infection of non-target cells. Testing will be performed by qPCR; possible future serology testing may also be performed. Blood samples for possible future serology testing will be centrally stored.

[p] Sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 2 to 3 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation.

[q] A conditioning regimen of IV busulfan, including a test dose of 0.8 mg/kg on Day -6, will be administered. The Day -4 and Day -3 busulfan dose will be PK- and weight-guided based on the test dose and the respective calculated AUC using a web-based dose simulation program. The busulfan doses for Days -2 and -1 of Conditioning will be based on an AUC calculation generated from a web-based dose simulation program that will use measurements of busulfan in serial blood samples drawn over several hours (see footnote 'r') on Conditioning Days -6 (test dose), -4 (for the Day -2 dose), and -3 (for the Day -1 dose). If the $AUC_{previous\ dose}$ is outside of +/- 0% of the AUC_{target} determined by the program, the busulfan dose will be adjusted accordingly. See Section 8.1.1.3 (and the Study Treatment Manual) for further details on busulfan dose determination.

[r] Blood samples will be collected 1, 3, 5, and 7 hours post-busulfan test dose administration for busulfan TDM for dose determination (see footnote 'q'). Blood samples will be collected 1, 3, and 5 hours post-busulfan dose administration on Days -4, -3, -2, and -1 for busulfan TDM for dose determination (ie, Days -2 and -1) and actual cumulative AUC calculations (ie, Days -4, -3, -2 and -1) (see also footnote 'q'). Laboratories performing TDM must use validated methods to quantify busulfan in plasma according to Good Laboratory Practice.

[s] Levetiracetam will be administered at a dosage of 1000 mg per day beginning two days prior to administration of the busulfan test dose (ie, on Conditioning Day -8), and continuing until one day after administration of the 4-day busulfan conditioning regimen (ie, until one day into the Conditioning washout period). For subjects with contraindication to levetiracetam, an alternative anticonvulsant medication (eg, benzodiazepines or valproic acid) may be used. Details on medication administered must be captured in the eCRF.

[t] A minimum of 48 up to a maximum of 72 hour busulfan washout period is required before administration of the AVR-RD-01 infusion.

[u] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.

[v] All AEs (including SAEs) will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.

14.3. Study AVRO-RD-01-201: Schedule of Assessments for the Post-Transplant Follow-up Period (Study Week 1 [Day 1] through Study Week 48 [Day 336])

Study Assessment	Study Period															ET[c]
	Post-transplant Follow-up[b]															
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D28**	**D56**	**D84**	**D168**	**D252**	**D336**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W4**	**W8**	**W12**	**W24**	**W36**	**W48[d]**	---
Assessment Window (Days)	---	---	---	---	---	---	---	**± 1D**	**± 1D**	**± 3D**	**± 3D**	**± 3D**	**± 7D**	**± 7D**	**±7D**	---
Efficacy Assessments																
Blood sample collection for AGA activity in plasma[e]				X			X		X	X	X	X	X	X	X	X
Blood sample collection for AGA activity in peripheral blood leukocytes[e]				X			X		X	X	X	X	X	X	X	X
Blood sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in plasma[e]				X			X		X	X	X	X	X	X	X	X
Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine[e,f]				X			X		X	X	X	X	X	X	X	X
Blood sample collection for exploratory biomarkers for Fabry disease[e,g]													X		X	
Urine sample collection for exploratory biomarkers for Fabry disease[e,g]													X		X	
Renal biopsy[h]															X	
Skin biopsy[h]													X		X	
GFR (iohexol-measured)															X	
GFR (estimated using serum creatinine, normalized for age)										X	X	X	X	X	X	X

Study Assessment	Study Period															
	Post-transplant Follow-up[b]															ET[c]
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D28**	**D56**	**D84**	**D168**	**D252**	**D336**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W4**	**W8**	**W12**	**W24**	**W36**	**W48[d]**	---
Assessment Window (Days)	---	---	---	---	---	---	---	**± 1D**	**± 1D**	**± 3D**	**± 3D**	**± 3D**	**± 7D**	**± 7D**	**±7D**	---
Overnight urine collection for protein/albumin excretion													X		X	X
Urine collection for creatinine and podocyte assessment[e,i]													X		X	X
Cardiac MRI[j]															X	
DIBSS-D[k]										X		X	X	X	X	
BPI-SF questionnaire[l]													X		X	
SF-36[l]															X	
Qualitative interview[m]												X	X		X	
Peripheral blood sample collection for VCN, ISA, and immunophenotyping[e,n]									X	X	X	X	X	X	X	X
Bone marrow aspirate collection for VCN, ISA, and immunophenotyping[e]													X		X	
Safety Assessments																
Physical examination	X[o]	X	X	X	X	X	X			X		X	X		X	X
Weight	X									X			X		X	X
Vital signs[p]	X[o]	X	X	X	X	X	X			X		X	X		X	X
Chest X-ray													X		X	
PFT by spirometry[q]													X		X	
12-lead ECG													X		X	
CBC with differential[r]	X[o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
G-CSF injection[s]					Daily administration until ANC > 1.5 x 10^9/L achieved											
Blood sample collection for T and B cell counts[t]									X	X	X	X	X	X	X	X

Study Assessment	Study Period															
	Post-transplant Follow-up[b]															ET[c]
Study Day [D][a]	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D10**	**D14**	**D28**	**D56**	**D84**	**D168**	**D252**	**D336**	---
Study Week [W][a]	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W1**	**W2**	**W2**	**W4**	**W8**	**W12**	**W24**	**W36**	**W48[d]**	---
Assessment Window (Days)	---	---	---	---	---	---	---	**± 1D**	**± 1D**	**± 3D**	**± 3D**	**± 3D**	**± 7D**	**± 7D**	**±7D**	---
Serum chemistry[u]							X		X	X	X		X		X	X
Electrolytes, BUN, and serum creatinine[v]	X[o]	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Urinalysis[w]										X	X		X		X	X
Blood sample collection for immunogenicity testing[e]										X			X		X	X
Peripheral blood sample collection for RCL[e,x]												X	X		X	X
Measures of reproductive potential[y]															X	X
Continuous Monitoring Procedures																
Prior and concomitant medications and therapies[z]	Continuous monitoring															X
Adverse events (AEs)[aa]	Continuous monitoring															X

Abbreviations: AE = adverse event; AGA = alpha-galactosidase A; ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; BPI-SF = Brief Pain Inventory Short Form; BUN = blood urea nitrogen; CBC = complete blood cell count; c/dg = copy per diploid genome; D = day; DIBSS-D = Diary for Irritable Bowel Syndrome Symptoms-Diarrhea; ECG = electrocardiogram; ET = early termination; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; Gb3 = globotriaosylceramide; G-CSF = granulocyte colony-stimulating factor; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; GI = gastrointestinal; ISA = integration site analysis; LDH = lactate dehydrogenase; lyso-Gb3 = globotriaosylsphingosine (deacylated form of Gb3); MRI = magnetic resonance imaging; PFT = pulmonary function testing; qPCR = quantitative polymerase chain reaction; RBC = red blood cell count; RCL = replication competent lentivirus; SAE = serious adverse event; SF-36 = 36-Item Short Form Health Survey; VCN = vector copy number; W = week; WBC = white blood cell count

Footnotes:

[a] In the Post-transplant Follow-up Period, study days (and weeks) are relative to Day 0 in the Transplant Period (ie, the day of AVR-RD-01 infusion).

[b] During the Post-transplant Follow-up Period, additional assessments may be performed at any time for clinical management or safety concerns at the discretion of the Investigator.

[c] Assessments required for those subjects who prematurely discontinue the study. Assessments should be completed as soon as feasible after the decision to discontinue the study has been made. Every effort should be made to continue to follow the subject for safety monitoring (including any indication(s) of loss of gene therapy effect).

[d] The week 48 (Day 336) visit may be conducted over the course of up to 3 days.

[e] Blood or urine sample(s) will be tested centrally. Details on collection, processing, and shipment will be included in the Laboratory Manual.
[f] Sample collection for biomarkers for Fabry disease (ie, Gb3 and its deacylated form, lyso-Gb3) in urine must be second or third morning void. Samples will be tested centrally.
[g] For consenting subjects, samples will be collected in plasma and urine for assessment of biomarkers for Fabry disease that may become available during or after the study. Samples obtained will be labeled for 'Biorepository'. Biorepository samples will be used only for these specific analyses, as applicable, and will be destroyed 5 years after the Clinical Study Report (CSR) is finalized.
[h] Skin and renal biopsies will be analyzed centrally for substrate (ie, Gb3) and AGA activity. Details on collection, processing, and shipment will be included in the Laboratory Manual.
[i] Urine for podocytes and creatinine should be second morning void.
[j] Cardiac MRI will be analyzed centrally.
[k] DIBSS-D diary data will be collected over the 2 weeks following the site visit, except at the Week 48 visit when the DIBSS-D diary data will be collected over the 2 weeks prior to the site visit.
[l] BPI-SF questionnaire and SF-36 should be completed prior to any other required assessments at the study visit.
[m] Interviews will be scheduled and conducted by Casimir for both subjects and caregivers, or other qualified observers, of subjects who opt to participate, provide written informed consent is obtained. For subjects who enrolled and received AVR-RD-01 treatment in the study prior to approval of Amendment 4, the first interview will take place upon Amendment 4 approval, unless the subject is within one month of the next regularly scheduled interview.
[n] Integration site analysis will only be performed on peripheral blood leukocyte samples with an average VCN $\geq$0.01 as determined by the central laboratory, suggesting $\geq$1% of the sampled blood cells are vector positive, with an average of 1 c/dg.
[o] At a minimum, physical examinations, vital signs collection, and blood sample collection for CBC with differential and electrolytes must occur daily through a minimum of 7 days post-transplantation (ie, until Day 7 of the Post-transplant Follow-up Period). If clinically indicated and/or mandated per local institution practice post-transplantation, additional physical examinations, vital signs collection, and blood sample collection for CBC with differential and electrolytes should also take place.
[p] Vital signs include heart rate, blood pressure, respiratory rate, and temperature. Vital signs will be obtained with the subject supine or seated.
[q] Pulmonary function tests include FEV1, FVC, and FEV1/FVC ratio (both results and percent predicted results should be collected).
[r] CBC with differential includes the following: hematocrit, hemoglobin, platelet count, WBC count (total and differential), RBC count, and ANC count. If ANC is $\leq 1.5 \times 10^9$/L on Day 5 of the Post-transplant Follow-up Period, blood sample for ANC should continue to be collected (and G-CSF injection administered [see footnote s]) until the subject achieves an ANC $>1.5 \times 10^9$/L. Samples will be tested locally.
[s] G-CSF injection of 5 µg/kg body weight (for sites with only prefilled syringes, a G-CSF dose from 4 µg/kg body weight up to 6 µg/kg body weight, administered in either a single or divided twice a day (BID) dose, may be administered) will be administered every morning from Day 5 post-transplant until the subject achieves an ANC $>1.5 \times 10^9$/L. The 'G-CSF Diary' should be completed by subjects self-administering.
[t] Assessment will also include routine flow cytometric analysis of peripheral blood lymphocyte subsets. Panel will include: CD3, CD4, CD8, CD19, and CD16/56. Samples will be tested locally.
[u] Serum chemistry includes AST, ALT, GGT, amylase, ALP, LDH, bilirubin, calcium, albumin, phosphate, magnesium, uric acid, and glucose. Samples will be tested locally.
[v] Electrolytes include sodium, potassium, chloride, and bicarbonate. Samples will be tested locally.
[w] Urinalysis includes blood, glucose, protein, specific gravity, and microscopic examination (if clinically indicated). Samples will be tested locally.
[x] Samples will be collected for RCL testing to determine infection of non-target cells. Testing will be performed by qPCR; possible future serology testing may also be performed. Blood samples for possible future serology testing will be centrally stored.
[y] Sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology will be collected. Samples should be collected after at least 2 to 3 days of abstinence. Samples will be tested locally. For samples that yield abnormal results, an additional sample must be collected and tested to confirm the initial results. If test results indicate reduced fertility (eg, oligospermia or azoospermia, decreased sperm motility, and/or abnormal sperm morphology), investigational sites should follow institutional guidelines for further evaluation. For subjects continuing in the AVRO-RD-01-LTF01 study with reduced fertility at Week 48, sperm samples for volume, sperm count, sperm concentration, total motility, progressive motility, and morphology must also continue to be collected in that study until the subject demonstrates recovery.
[z] Prior and concomitant medications and therapies will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.

[aa] All AEs (including SAEs) will be recorded from the time of signing of informed consent until completion of the Week 48 assessments for the study.