Official Title:	A Multi-center, Single-Dose and Repeat-Dose Over Eight Weeks, Sequential Cohort Study to Evaluate Safety and Tolerability as well as Pharmacokinetics of Two Different Doses of Alpha1-Proteinase Inhibitor Subcutaneous (Human) 15% Administered Subcutaneously in Subjects with Alpha1-Antitrypsin Deficiency
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STATISTICAL ANALYSIS PLAN (SAP)

GC2008

Sponsor Name: Grifols Therapeutics Inc.
79 TW Alexander Drive
Research Triangle Park, NC 27709

Study Title: A Multi-center, Single-Dose and Repeat-Dose Over Eight Weeks, Sequential Cohort Study to Evaluate Safety and Tolerability as well as Pharmacokinetics of Two Different Doses of Alpha1-Proteinase Inhibitor Subcutaneous (Human) 15% Administered Subcutaneously in Subjects with Alpha1-Antitrypsin Deficiency

Study Number GC2008

Investigational Product: $Alpha_1$-Proteinase Inhibitor Subcutaneous (Human) 15%

Development Phase: I/II

SAP Version: 1.0

SAP Effective Date: See left margin

Protocol Version: GC2008 Amendment 6/Version 7.0 Dated 19Sep2024

Author: [redacted]

DOCUMENT REVISION HISTORY

SAP Version	Approval Date	Description of Change
1.0	See left margin	N/A – first version

ABBREVIATIONS

$\%AUC_{ex}$	Percentage of AUC Extrapolated from time 0 to infinity
AATD	$Alpha_1$-Antitrypsin Deficiency
ADR	Adverse Drug Reaction
AE	Adverse Event
ALP	Alkaline Phosphatase
$Alpha_1$-PI	$Alpha_1$-Proteinase Inhibitor
Alpha-1 15%	$Alpha_1$-Proteinase Inhibitor Subcutaneous (Human) 15%
ALT	Alanine Aminotransferase
AR	Adverse Reaction
ARC	Absolute Reticulocyte Count
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
$AUC_{0\text{-}7\ days}$	Area Under the Concentration-Time Curve From 0 to 7 Days
$AUC_{0\text{-}\tau}$	Area Under the Concentration-Time Curve at Steady State Over the Dosing Interval (from time 0 to τ)
$AUC_{0\text{-}\infty}$	AUC Extrapolated to Infinity
BLQ	Below the Limit of Quantification
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
C_{last}	Last Measurable Concentration
C_{max}	Maximum Concentration
C_{trough}	Steady state Mean Trough Concentrations
CL	Total Clearance
CL/F	Apparent Clearance
COPD	Chronic Obstructive Pulmonary Disease
CSR	Clinical Study Report
CV	Coefficient of Variation
DBL	Database Lock
DBP	Diastolic Blood Pressure
dL	Deciliter
eCRF	Electronic Case Report Form
FEV_1	Forced Expiratory Volume in 1 Second
FVC	Forced Vital Capacity
GM	Geometric Mean
GMCV	Geometric Mean Coefficient of Variation
HR	Heart Rate
ICH	International Conference on Harmonisation
IP	Investigational Product/Study Drug
IV	Intravenous
ISR	Infusion Site Reaction

iSRC	Internal Safety Review Committee
kg	Kilogram
LLOQ	Lower Limit of Quantification
LSM	Least-Squares Mean
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean Corpuscular Hemoglobin
MCHC	MCH Concentration
mg	Milligram
mL	Milliliter
MPV	Mean Platelet Volume
PFT	Pulmonary Function Test
pH	Potential of Hydrogen; Acidity/Alkalinity Measure
PI	Proteinase Inhibitor
PK	Pharmacokinetic(s)
PT	Preferred Term
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
RR	Respiration Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SC	Subcutaneous
SC SD	SC Administration of Alpha-1 15% in the Single-Dose Phase
SC RD	SC Administration of Alpha-1 15% in the Repeat-Dose Phase
SD	Standard Deviation
SOC	System Organ Class
T	Temperature
TEAE	Treatment Emergent Adverse Event
t_{last}	Time to C_{last}
$t_{1/2}$	Terminal Elimination Half-Life
t_{max}	Time to Reach C_{max}
WBC	White Blood Cell
WHO-DD	World Health Organization Drug Dictionary
V_d	Volume of Distribution
V_d/F	Apparent Volume of Distribution

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1 Introduction

The purpose of this statistical analysis plan (SAP) is to provide detailed information to aid in the implementation of the statistical analysis of the study data. It briefly summarizes the protocol, describes the analysis sets that will be analyzed, and describes the analyses to be performed. The details of the specific statistical methods that will be used are provided. Post database lock (DBL), it may become apparent that the planned analyses should be modified, e.g., modeling assumptions are untenable. Any modification needed to the original SAP will be identified, and a new version of the SAP will be created and approved prior to conducting any analyses. This will also be described in the clinical study report (CSR).

Table, figure, and listing specifications are in separate documents. This SAP is meant to supplement the study protocol. If differences occur between analyses described in the SAP and the current protocol, those found in this SAP will assume primacy.

This SAP is written with due consideration of the recommendations outlined in the most recent International Conference on Harmonisation (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline, entitled Guidance for Industry: Structure and Content of Clinical Study Reports and will be finalized prior to DBL.

This SAP is based on Protocol GC2008 Version 7.0, dated 19Sept2024.

2 Protocol Objectives

2.1 Primary Objectives

To evaluate the safety and tolerability of 72 mg/kg and 180mg/kg Alpha1-Proteinase Inhibitor Subcutaneous (Human) 15% (Alpha-1 15%), administered as a single-dose subcutaneous (SC) infusion and subsequently as weekly SC infusions over 8 weeks in subjects with Alpha1-Antitrypsin Deficiency (AATD).

2.2 Pharmacokinetic Objectives

The pharmacokinetic (PK) objective is to evaluate the PK of Alpha1-Proteinase Inhibitor ($Alpha_1$-PI), measured using both antigenic content and functional activity assays, following a single-dose SC infusion and subsequently 8 weekly SC infusions of 72 mg/kg or 180 mg/kg Alpha-1 15% in subjects with AATD.

2.3 Exploratory Objective

The exploratory objective of this study is to assess subject satisfaction with SC treatment following 8 weeks of SC doses of 72 mg/kg or 180 mg/kg Alpha-1 15% in subjects with AATD.

An additional exploratory PK objective is to evaluate the PK of $Alpha_1$-PI, measured using both antigenic content and functional activity assays, following 8 weekly intravenous (IV) infusions of 60 mg/kg or 120 mg/kg Liquid $Alpha_1$-PI in subjects with AATD.

3 Study Endpoints

3.1 Primary (Safety) Endpoints

- Adverse events (AEs):
  1. AEs
  2. Suspected Adverse Drug Reactions (suspected ADRs, or potentially related AEs)
  3. Adverse Reactions (ARs)
  4. Infusion Site Reactions (ISRs) (to be reported as AEs)
  5. Serious Adverse Events (SAEs)
  6. AEs and SAEs leading to discontinuation
- Chronic Obstructive Pulmonary Disease (COPD) exacerbations
- Vital signs
  1. Heart Rate (HR)
  2. Blood Pressure (BP)
  3. Respiratory Rate (RR)
  4. Temperature (T)
- Pulmonary Function Test (PFT):
  1. Forced Expiratory Volume in 1 Second (FEV_1), absolute and predicted
  2. Forced Vital Capacity (FVC)
- Clinical laboratory parameters
  1. Chemistry
  2. Hematology
  3. Urinalysis
- Immunogenicity assessments (antibodies to $Alpha_1$-PI and neutralizing antibodies as required)

3.2 Pharmacokinetic Endpoints

PK variables include the following single-dose PK parameters of $Alpha_1$-PI, following the single dose of Alpha-1 15% at Single-Dose Week 1 Visit, measured using both antigenic content and functional activity assays:

- Area Under the Curve (AUC) from time 0 (pre-dose) to time of last quantifiable concentration (AUC_{0-t})
- AUC from time 0 (pre-dose) extrapolated to infinite time ($AUC_{0-\infty}$)
- Percentage of $AUC_{0-\infty}$ obtained by extrapolation ($\%AUC_{ex}$)
- AUC from time 0 to 7 days ($AUC_{0-7\ days}$)
- AUC_{0-t}/Dose
- $AUC_{0-\infty}$/Dose
- $AUC_{0-7\ days}$/Dose
- Maximum concentration (C_{max})
- C_{max}/Dose
- Time to reach C_{max} (t_{max})
- Apparent clearance (CL/F)
- Last measurable concentration (C_{last})

- Time to C_{last} (t_{last})
- Terminal elimination half-life ($t_{1/2}$)
- Apparent volume of distribution (V_d/F)
- Baseline-corrected AUC_{0-t}, $AUC_{0-\infty}$, $\%AUC_{ex}$, $AUC_{0-7\ days}$, C_{max}, CL/F, $t_{1/2}$, and V_d/F, where baseline is the endogenous $Alpha_1$-PI concentration measured at Single-Dose Week 5 (4 weeks after the single SC infusion at Single-Dose Week 1)

In addition, PK variables include the following steady-state PK parameters of $Alpha_1$-PI, following the last repeat dose at Repeat-Dose Week 8, measured using both antigenic content and functional activity assays:

- Steady-state AUC from time 0 (pre-dose) to time of last quantifiable concentration (AUC_{0-t})
- Steady-state AUC over the weekly dosing interval (from 0 to 7 days) ($AUC_{0-7\ days}$)
- AUC_{0-t}/Dose
- $AUC_{0-7\ days}$/Dose
- Steady-state mean trough level (C_{trough}), calculated as the average value of the trough measurements obtained at Repeat-Dose Weeks 6, 7, 8, and 9.
- C_{max}
- C_{max}/Dose
- t_{max}
- CL/F
- $t_{1/2}$
- V_d/F
- C_{last}
- t_{last}

- $R_{AUC\ (0-7\ days)}$: Accumulation ratio for $AUC_{0-7\ days}$, calculated as $AUC_{0-7\ days}$ following repeat-dose divided by $AUC_{0-7\ days}$ following single-dose
- $AUC_{0-7\ days}$ / $AUC_{0-\infty}$: Time-invariance ratio calculated as $AUC_{0-7\ days}$ following repeat-dose divided by $AUC_{0-\infty}$ following single-dose
- Baseline-corrected AUC_{0-t}, $AUC_{0-7\ days}$, mean trough (C_{trough}), C_{max}, CL/F, $t_{1/2}$, V_d/F, accumulation ratio, and time-invariance ratio, where baseline is the endogenous $Alpha_1$-PI concentration measured at Single-Dose Week 5 (4 weeks after the single SC infusion at Single-Dose Week 1)

NOTE: If the dosing interval (τ) for the repeat-dose SC regimen is different from weekly (i.e., 7 days), the calculation of mean trough above will be adjusted to use appropriate trough samples only, and $AUC_{0-\tau}$ will be calculated in place of $AUC_{0-7\ days}$.

3.3 Exploratory Variables

Subject satisfaction with SC IP via a written questionnaire following the Repeat-Dose Phase.

Additional exploratory PK endpoints include the following steady-state PK parameters of Alpha$_1$-PI, following the 8th weekly IV infusion of Liquid Alpha$_1$-PI at IV Week 8, measured using both antigenic content and functional activity assays:

- Steady-state AUC over the weekly dosing interval (from 0 to 7 days) ($AUC_{0\text{-}7}$ days)
- $AUC_{0\text{-}7\ days}$/Dose
- $AUC_{0\text{-}t}$
- $AUC_{0\text{-}t}$/Dose
- Steady-state mean trough level (C_{trough}), calculated as the average value of the trough measurements obtained at IV Weeks 8 and 9.
- C_{max}
- C_{max}/Dose
- t_{max}
- Total clearance (CL)
- $t_{1/2}$
- V_d
- Baseline-corrected $AUC_{0\text{-}7}$ days, mean trough (C_{trough}), C_{max}, CL, $t_{1/2}$, V_d, where baseline is the endogenous Alpha$_1$-PI concentration measured at Single-Dose Week 5 (4 weeks after the single SC infusion at Single-Dose Week 1)

4 Study Design

4.1 Design Overview

This is a multi-center, single-dose followed by a repeat-dose over 8 weeks, sequential cohort study evaluating safety, tolerability, and PK of 2 different doses of Alpha-1 15% administered subcutaneously in Alpha$_1$-PI augmentation treatment-experienced subjects with AATD.

Two cohorts will be evaluated, as follows. Subjects cannot be enrolled into both cohorts.

- Cohort 1 subjects will receive 72 mg/kg dose of Alpha-1 15%, and 60 mg/kg dose of Liquid Alpha$_1$-PI
- Cohort 2 subjects will receive 180 mg/kg dose of Alpha-1 15%, and 120 mg/kg dose of Liquid Alpha$_1$-PI.

Each cohort will include approximately 8 subjects.

Each cohort will consist of:

- Screening Phase, up to 30 days
- Single-Dose Phase (Treatment Period 1), 7 days
- Washout / Serial PK Phase, 21 days
- Single-Dose Data Evaluation Phase, up to 18 months of weekly IV infusions of Liquid Alpha$_1$-PI
- Repeat-Dose Phase (Treatment Period 2), 56 days of weekly SC infusions of Alpha-1 15%
- Off-treatment Follow-up Phase, 7 days

Subjects will receive their first SC dose of investigational product (IP) (Alpha-1 15%) at Single-Dose Week 1 (Baseline). Subjects will receive their last SC dose of IP at Repeat-Dose Week 8. The entire duration is approximately 668 days.

Safety and tolerability will be assessed by an internal Safety Review Committee (iSRC) in a staggered approach, as follows:

- For the first 4 subjects of Cohort 1, dosing in the Single-Dose Phase will be staggered subject by subject for the first 4 subjects with a 7-day waiting period in between each subject for iSRC assessment.
- The iSRC will determine if it is appropriate and safe for the last 4 planned subjects of Cohort 1 to enroll without staggering.
- After completion of the Single-Dose Phase and Washout/Serial PK phase, the subject will enter into the Single-Dose Data Evaluation Phase where Liquid Alpha$_1$-PI (weekly IV Infusions) will be administered, and PK samples will be collected.
- Once all the 8 subjects have completed the Single-Dose Phase, safety data will be evaluated by the iSRC to determine if subjects may proceed to the Repeat-Dose Phase (Treatment Period 2).
- The PK data from the Single-Dose Phase will be evaluated by the study team to determine the best dose for the Repeat-Dose Phase*.
- Following the completion of the Repeat-Dose Phase for all subjects in Cohort 1, the iSRC will evaluate safety data to determine if it is safe to proceed to Cohort 2 (180 mg/kg).
- The safety reviews conducted for Cohort 2 follow a similar pattern as those described above for Cohort 1, unless Cohort 1 is specifically mentioned*.

* 72 mg/kg of Alpha-1 15% for Cohort 1 subjects and 180 mg/kg of Alpha-1 15% for Cohort 2 subjects were determined to be the best doses.

Refer to the following diagram and protocol section 4.2 for additional details.

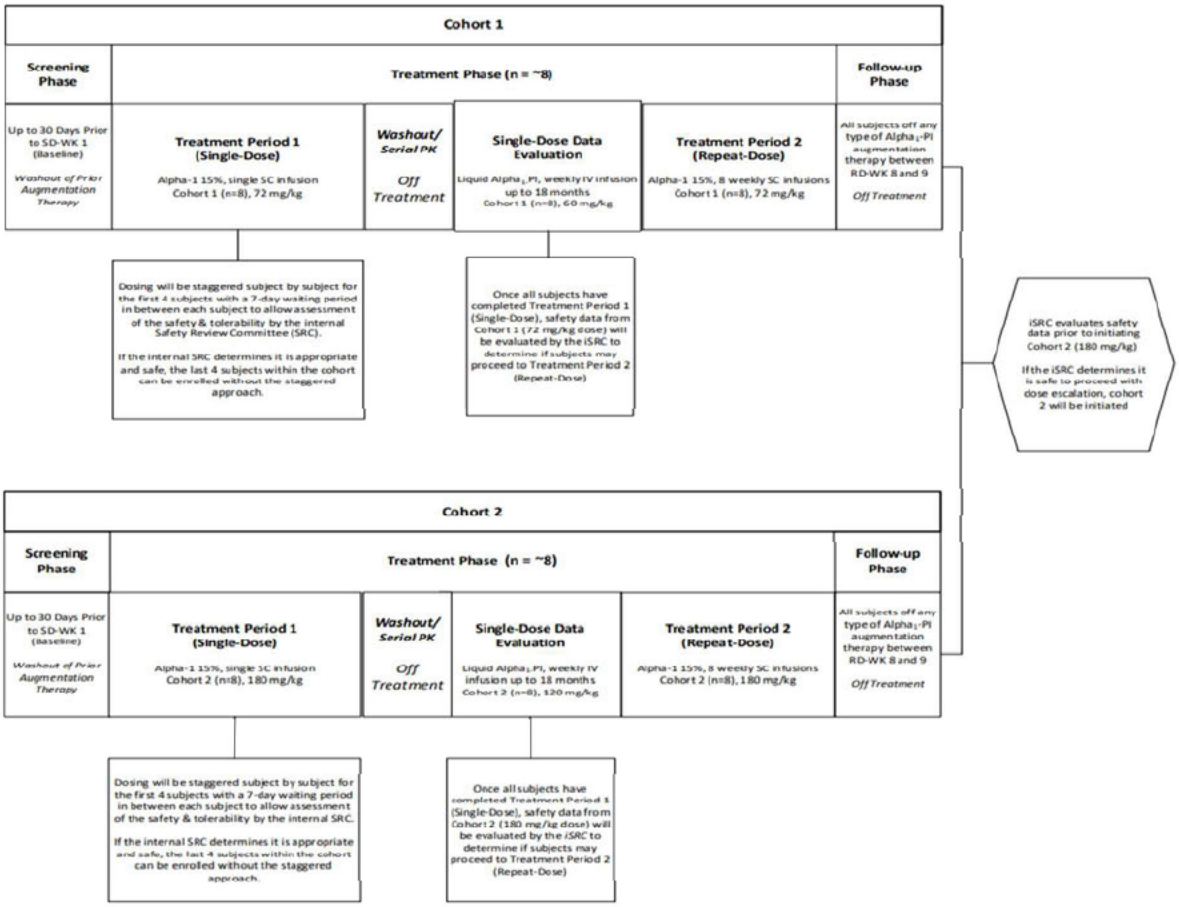


Figure 1 Overall Cohort Schema

5 General Statistical Considerations

Statistical analyses and data presentations will be generated using SAS version 9.4 or higher.

Unless otherwise noted, for continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation (SD), median, 25th percentile (Q1), 75th percentile (Q3), minimum, and maximum. In general, the mean, median, Q1, and Q3 will be presented to one decimal place greater than the original data, SD will be 2 decimal places greater than the original data, and minimum and maximum will have the same number of decimal places as the original data. For categorical/qualitative data, descriptive statistics will include counts and percentages per category. Percentages will not be presented for counts of 0, while percentages of 100 will be reported as a whole number (i.e. "100%"). All other percentages will be presented to 1 decimal place.

Data will be listed and summarized by cohort. Within cohort, data will be summarized for all subjects, and where applicable will be further summarized for the Single-Dose (SC Alpha-1 15%) Phase including the Washout Phase; the Single-Dose Data Evaluation Phase (IV Liquid

Alpha$_1$-PI); and the Repeat-Dose (SC Alpha-1 15%) Phase including the follow-up phase, as follows:

- For disposition summary, subject disposition will be presented for subjects who participate in the phase.
- Demographics and baseline characteristics will be summarized by cohort.
- By-visit and unscheduled assessments will be allocated to phase based on the assessment date.
- Adverse events and ISRs will be allocated to phase based on the AE/ISR start date.
- Concomitant medications will be allocated to phase based on the medication start and end date; a medication may be summarized in more than one phase.

5.1 Data Handling

5.1.1 Missing Data Imputation

Unless otherwise noted, if an observation is missing at a specific scheduled visit/time point, the value at that visit will not be imputed and will be set to missing.

5.1.2 Baseline Definition

Baseline is defined as the last available, valid, non-missing measurement taken prior to first IP administration date and time (including unscheduled assessments).

For PK, baseline is defined as the endogenous Alpha$_1$-PI concentration measured at Single-Dose Week 5 (4 weeks after the single SC infusion at Single-Dose Week 1/IV Week 1 visit prior to the IV#1 infusion).

5.1.3 Study days

Single-Dose Day 1 refers to the date of the first infusion in the Single-Dose phase Week 1.
Repeat-Dose Day 1 refers to the date of the first infusion in the Repeat-Dose phase Week 1.

All efficacy and safety assessments at all visits will be assigned a study day relative to Single Dose Study Day 1 (for assessment in the Single-Dose and Single-Dose Data Evaluation phase and Repeat-Dose Day 1 (for assessments in the Repeat-Dose phase), where relevant.

Study Day – Assessments in the Single-Dose and Single-Dose Data Evaluation phase:

- Study Day for an event/assessment visit on or after the Single-Dose Day 1 (≥ date of Single-Dose Day 1) is defined as: Event date/Assessment Visit date - (date of Single-Dose Day 1) + 1.

- Study Day for an event/assessment visit prior to the respective Single-Dose Day 1 (< date of Single-Dose Day 1): Event/Assessment Visit date - (date of Single-Dose Day 1).

Study Day – Assessments Repeat-Dose phase:

- Study Day for an event/assessment visit on or after the Repeat-Dose Day 1 (≥ date of Repeat-Dose Day 1) is defined as: Event date/Assessment Visit date - (date of Repeat-Dose Day 1) + 1

There is no 'Day 0'.

5.1.4 Visit Windows

As subjects do not always adhere to the timing of visits as scheduled in the study protocol, the following rules will be applied to map each subject's assessment visits to analysis visits (determined by analysis visit windows), based on the timing of their assessment visits, relative to each of their Single Dose Day 1 and their Repeat-Dose Day 1.

Both scheduled and unscheduled assessment visits, for each relevant assessment, will be mapped in this way for each subject. Details are provided in Section 13.2

For cases where multiple values are recorded for an assessment within a single analysis visit window (within a phase), the following rules will be applied for each parameter:

- If 2 or more assessments are mapped to the same visit window, then the non-missing assessment closest to the scheduled visit target day will be used in the analysis.
- If 2 assessments are equidistant from the visit target day, the later non-missing observation will be used in the analysis.

5.1.5 PK Samples Below the Limit of Quantification (BLQ) for Calculation of Concentration Summary Statistics

For PK samples with concentration values that are below the lower limit of quantification (LLOQ), the listing will designate that the sample is below the limit of quantification (BLQ); for summarization, the concentration will be imputed using the LLOQ value.

5.1.6 PK Samples Below the Limit of Quantification (BLQ) for Estimation of PK Parameters

PK samples with concentration values that are BLQ will be treated as missing. A concentration that is BLQ will be assigned a value of zero if it occurs in a profile before the first measurable concentration.

5.2 Determination of Sample Size

No formal power/sample size calculations were performed. The sample size for each cohort is primarily based on feasibility. A sample size of 8 Alpha-1 15% subjects per cohort is deemed sufficient to address the safety and PK objectives of this early phase, dose escalation study.

5.3 Analysis Populations

All analyses and summaries will be performed according to actual treatment received.

Safety Population
The safety population will include all subjects who receive any dose of IP (Alpha-1 15% or Liquid Alpha$_1$-PI) and will be used for all analyses and summaries other than those for PK.

PK Population
The PK population will include all subjects who receive IP (Alpha-1 15% or Liquid Alpha$_1$-PI) and have sufficient and valid Alpha$_1$-PI concentration data to allow calculation of at least 1 of the PK parameters of interest. PK analyses will be based on this population.

If any subjects are found to be noncompliant with respect to dosing, have incomplete data, or experience an event that is likely to affect PK, the Sponsor, the Pharmacokineticist and the Statistician will determine their inclusion into the population on a case-by-case basis. Subjects excluded from the PK analyses and their reasons for exclusion will be denoted and detailed.

6 Disposition

Subject disposition will include the number of subjects screened, screen failures, subjects rescreened, number of subjects treated, number and percentage of subjects in each analysis population, and number and percentage of subjects completing the study by cohort, study phase (e.g., Single-Dose Data Evaluation Phase etc.), and overall. The denominator for percentages will be the number of subjects treated.

The number and percentage of subjects discontinuing/withdrawing early from the study will be summarized for primary reasons of discontinuation/withdrawal by cohort, study phase (e.g., Single-Dose Data Evaluation Phase), and overall. The number and percentage of subjects discontinuing/withdrawing from the study due to COVID-19 restrictions will be summarized.

Also, the number and percentage of screening failures will be summarized with primary reasons of ineligibility.

The denominator for percentages will be the number of subjects treated except for percentage of screening failures where the denominator would be number of subjects screened but not treated.

Listings of subjects will be provided for the following

- Disposition status for all subjects.
- Subjects who prematurely discontinued the study.
- Subjects excluded from analysis populations as defined in Section 5.3

7 Protocol Deviations

Protocol deviations will be identified during the study and evaluated and documented before the DBL. The type/category of protocol deviations and severity (i.e., minor, major, or critical) will be summarized and listed.

Details of the protocol deviations, along with all details on how and where they are recorded are contained in the Protocol Deviations Management Plan (PDMP) v4.0 25 May 2025.

8 Demographics and Medical History

8.1 Demographics

Demographic and baseline characteristics including age (at the time of informed consent), sex, race, ethnicity, age categories (≥18 - ≤65, >, 65 - ≤80), height (cm), weight (kg), BMI (kg/m^2) and baseline Alpha1-PI level (mg/dL) will be summarized by treatment cohort and overall.

BMI will be calculated as weight at baseline (kg) / (height at baseline(m))^2.

All demographic data above, in addition to year of birth and fertility status, will be also reported in a subject listing.

In addition, the distribution of subjects by site and investigator will be summarized by treatment cohort for each analysis population.

8.2 Medical History

Medical history verbatim terms used in the eCRF reported by the investigators will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized/listed. A version of MedDRA within one year of DBL will be employed. The summary will present the number and percentage of subjects with at least one medical history event by treatment cohort, system organ class (SOC), and preferred term (PT).

8.3 Alpha$_1$-Antitrypsin Deficiency and Prior Alpha$_1$-Antitrypsin Augmentation History

A summary of AATD will be presented including congenital allelic combination and total Alpha$_1$-PI serum level documenting deficiency. This information along with date of total Alpha$_1$-PI serum level documenting deficiency and date of congenital allelic combination testing will be presented in a listing. Study day of first diagnosis will also be listed (calculated as date of diagnosis - date of start of IP).

Prior Alpha1-Antitrypsin augmentation therapy within 12 months of screening will be summarized and listed. Medications will be grouped based on medical review of the Trade name (medication/drug name)of to allow categorization by commercial product received.

9 Concomitant Medication and Treatment

9.1 Prior and Concomitant Medication (Excluding Prior Alpha1-Antitrypsin Augmentation Therapy)

All medications as documented by the investigator will be coded using Anatomical Therapeutic Chemical (ATC) classification codes via the World Health Organization Drug Classification Dictionary (WHO-DD). A version of WHO-DD within one year of DBL will be employed. All medications will be summarized and sorted alphabetically by medication class (i.e., ATC level 2) and medication sub-class (i.e., ATC level 4). If the ATC level 2 or 4 term is missing, the higher ATC level term will be used in the medication summary table and data listing.

Prior medications and concomitant medications will be summarized separately either by cohort (prior medications) or by cohort and study phase (concomitant medications).
Prior medications are defined as any medication ended prior to the start date/time of IP. Concomitant medications are defined as any medication started on or after the start date /time of IP in the respective study phase or any medication taken prior to the start date/time of IP in a study phase and continued after the start date/time of IP in the respective study phase.

The following conservative imputation rules will be used for missing or partial medication start/data time and end date/time information in order to determine whether a medication is prior or concomitant (i.e., the unknown portions of a medication start date/time will be assumed to be as early as possible and end date/time will be assumed to be as late as possible):

Note: year is required for the start and end time of a medication on the electronic Case Report Form (eCRF), except the end year for ongoing medication

Start Date/Time:

- If the month is missing, impute “January”
- If the day is missing, impute ‘01’
- If the hours are missing, impute “00”
- If the minutes are missing, impute “01”

End Date/time:

- If the entire end year, date and time values are missing (i.e., ongoing medication), then no imputation is performed, and the medication will be assigned to the “concomitant” category
- If the month is missing, impute “December”
- If the day is missing, impute the last day of the month (i.e., “28/29/30/31” depending on the year and month)

- If the hours are missing, impute "23"
- If the minutes are missing, impute "59"

The start/end dates/times reported on the eCRFs will be presented in the listings; imputed dates will only be used to determine prior or concomitant medication status.

At each level of summarization, a subject will be counted once per medication class or subclass.

Prior and concomitant medications will also be listed by cohort.

9.2 Extent of IP Exposure

All exposure details collected on the eCRF, and all derived exposure measures used to determine compliance, will be listed.

The following information will be summarized by cohort, separately for Alpha-1 15% SC infusions (overall, and for the Single-Dose Phase distinct from Repeat-Dose Phase as appropriate) and Liquid Alpha$_1$-PI IV infusions (Single-Dose Data Evaluation Phase), as detailed in Table 1

Table 1: Exposure Summaries

Measures of Exposure	**Alpha-1 15% SC infusions**	**Liquid Alpha$_1$-PI IV infusions**
Subject-Level Measures of Exposure (n will represent number of subjects)		
Duration of exposure (weeks)	x	x
Number of infusions received	x	x
Total volume infused (mL)	x	x
Number of infusions temporarily interrupted and permanently stopped	x	x
By-subject maximum volume infused per site per infusion (mL/site)	x	
By-subject maximum infusion rate per site per infusion (mL/hr/site)	x	
Distribution of SC infusion sites (location)	x	
Infusion-Level Measures of Exposure (n will represent number of infusions across all subjects)		
Duration of infusion (hours) per infusion	x	x
Volume infused per infusion (mL)	x	x
Dose per infusion (mg/kg)	x	x
Number of SC infusion sites per infusion	x	
Volume infused per site per infusion (mL/site)	x	
Infusion rate per site per infusion (mL/hr/site)	x	

For SC infusion at a given visit, 2 infusion pumps may be used; this will be counted as one infusion for number of infusions received and for per infusion measures. The infusion rate for

a single infusion is the sum of the infusion rates for each pump used. For each pump, the eCRF captures both an initial infusion rate and a final infusion rate; both will be listed, and for summary measures of exposure the final infusion rate will be used.
The dose per infusion (mg/kg) will be calculated as:

Vial Concentration (mg/ml) = potency per vial (mg)[1] / volume per vial (mL)[2]
Total mg per dose = vial concentration (mg/mL) * volume infused (mL)
Actual dose (mg/kg) = total mg per dose/weight (kg)[3]

[1]Note: potency per vial (mg) is lot-specific and is captured in Veeva Randomization and Trial Supply Management (RTSM) System for each vial administered.
[2]Note: volume per vial of Alpha-1 15% = 6.7mL, volume per vial of Liquid $Alpha_1$-PI = 20mL.
[3]Note: weight (kg) is the most recent weight collected prior to the infusion.

9.2.1 Duration of Exposure

The duration of exposure will include not only the total time between the first and last infusion of IP, but also include an additional 7 days to take into account total exposure time to the IP since each infusion is administered weekly.

Duration of exposure will be calculated in days for each treatment and phase as detailed in Table 2.

Table 2: Duration of IP Exposure per Study Phase

Treatment	Phase	Duration of Exposure (days)
SC infusion of Alpha-1 15%	Single-Dose Phase	7 days for all subjects; the Washout Phase is excluded. (This will be noted in footnote for relevant exposure summaries.)
IV infusion of Liquid $Alpha_1$-PI	Single-Dose Data Evaluation Phase	(Last Liquid $Alpha_1$-PI infusion date – first Liquid $Alpha_1$-PI infusion date at Single-Dose Week 5/IV Week 1) + 7
SC infusion of Alpha-1 15%	Repeat-Dose Phase	(Last Alpha-1 15% infusion date – first Alpha-1 15% infusion date during Repeat-Dose Phase) + 7

Duration of exposure in weeks will be calculated as:

Duration of exposure in days / 7

9.2.2 Duration of Infusion

Duration of infusion in minutes will be calculated for each infusion as:

Stop time of infusion – Start time of infusion

The duration of infusion and the dose per infusion (section 9.2) at the serial PK visits will be summarized by cohort and phase.

9.2.3 Per Infusion Measures for SC Administration of Alpha-1 15%

Volume infused per SC site per infusion will be calculated for each infusion as:
total volume infused (mL) / total number of infusion sites

Total infusion rate (mL/hr) will be calculated for each infusion as:
Sum of the final SC infusion rates for each pump used

Infusion rate per site will be calculated for each infusion as:
Total infusion rate (mL/hr) / total number of infusion sites SC

9.2.4 Treatment Compliance

Treatment compliance and infusion compliance (calculated separately for Alpha-1 15% single-dose SC infusions, Alpha-1 15% repeat-dose SC infusions, and Liquid $Alpha_1$-PI IV infusions) will be listed and summarized. In addition, the number and percentage of subjects with compliance <80%, 80% to ≤120%, and >120% will be presented.

Infusion compliance will be calculated as (number of actual infusions / number of infusions expected or planned) * 100.

- Alpha-1 15% SC infusions – Single-Dose Phase: All subjects who receive their single-dose SC infusion will have 100% compliance.
- Liquid $Alpha_1$-PI IV infusions: For subjects that complete the study, the number of infusions expected may vary, and may be up to 78.
  - For subjects that complete the IV Single-Dose Data Evaluation Phase and continue to the SC Repeat-Dose Phase, the number of infusions expected will be calculated as the integer value of [(start date of SC Repeat-Dose Phase – start date of IV Single-Dose Data Evaluation Phase) / 7 + 1].
  - For subjects who discontinue during the IV Single-Dose Data Evaluation Phase and do not start Alpha-1 15% SC repeat dosing, the number of planned doses will be calculated as the integer value of [(study discontinuation/withdrawal date or start date of ISR (or AE or COPD exacerbation) as noted in ISR (or AE or COPD exacerbation) eCRF page that lead to IP withdrawal date (whichever is earlier) – start date of Single-Dose Data Evaluation Phase) / 7]. The integer value is obtained using the SAS 'CEIL' function.

- Alpha-1 15% SC infusions – Repeat-Dose Phase: For subjects that complete the study, the number of infusions expected is 8. For subjects who drop out from the study early, the number of infusions expected or planned will depend on the number of weeks the subjects are in the Alpha-1 15% Repeat-Dose phase.

Treatment compliance will be calculated as (total volume infused / total volume prepared) * 100. Missing visits will be excluded in the calculation.

Overall compliance will be calculated as (treatment compliance * infusion compliance)/100.

10 PK Analysis

The analyses of PK parameters will be based on the PK population.

Serial PK Blood Sample Timepoints

Alpha$_1$-PI concentrations in serum will be measured by validated antigenic content assay. Alpha$_1$-PI concentrations in plasma will be measured by validated functional activity assay and will be provided by the lab in serum equivalents calculated as plasma values divided by 0.9. See Table 3 for PK Blood sample timepoints.

Table 3: PK Blood Sample Timepoints

Treatment	Treatment Phase/Serial PK Sampling Week	Collection Time
Alpha-1 15% SC	Single-Dose Phase / Week 1; Repeat-Dose Phase / Week 8	Within 25 minutes prior to infusion Within 5 minutes after SC infusion completion 1 hour (±15 minutes) 4 hours (±30 minutes) 8 hours (±2 hours) 1 day (24±4 hours) 2 days (48±4 hours) 3 days (72±4 hours) 4 days (96±4 hours) 5 days (120±4 hours) 6 days (144±4 hours) 7 days (+1 day) [1,2] 14 days (+1 day) [1] 21 days (+1 day) [1] 28 days (+1 day)[3]
Liquid Alpha$_1$-PI IV	Single-Dose Data Evaluation Phase / IV Week 8	Within 25 minutes prior to infusion Within 5 minutes following completion (after NaCl flush) 15 minutes (±5 minutes) 30 minutes (±5 minutes) 1 hour (±10 minutes) 2 hours (±15 minutes) 4 hours (±30 minutes) 8 hours (±2 hours) 1 day (24 ±4 hours) 2 days (48 ±4 hours) 5 days (120 ±4 hours) 7 days (+1 day)[4]

[1] The PK serial sample taken at 7, 14, and 21 days following completion of the Single-Dose Week 1 infusion will be drawn at the Single-Dose Weeks 2, 3, and 4, respectively.
[2] The PK serial sample taken at 7 days following completion of the Repeat-Dose Week 8 infusion will be drawn at the Repeat-Dose Week 9 Visit (Final Visit).
[3] A blood sample will be collected for endogenous Alpha$_1$-PI concentration 28 days following completion of the Single-Dose Week 1 infusion (Note: sample is considered the last serial PK sample following single-dose. In addition, it will be used to calculate the baseline-corrected PK parameters of Alpha$_1$-PI for both single-dose and repeat-dose SC administrations as well as for repeat-dose IV administration). This sample will be taken at the Single-Dose Week 5/Intravenous Week 1 visit prior to the IV#1 infusion.
[4] Sample will be drawn at the IV Week 9 Visit prior to the IV#9 IP infusion

10.1 Calculation of PK Parameters

PK parameters for Alpha1-PI measured using both antigenic content and functional activity assays will be estimated through non-compartmental (NCA) methods using Phoenix® WinNonlin® software, version 8.3 or later (Certara USA, Inc. [Princeton, NJ]). PK analysis will be performed using actual sampling times relative to dosing times recorded in the raw data. If an actual collection time or a dosing time is missing, the nominal time may be used.

10.1.1 PK Parameters for SC Administration of Alpha-1 15%

For the Single-Dose Phase, absolute/uncorrected and baseline-corrected PK parameters (as data permits) will be calculated for Alpha1-PI following the single dose Alpha1-15% Week 1 (Cohort 1 & Cohort 2) based on Alpha1-PI concentrations as measured by antigenic content as well as functional activity assays. The single-dose PK parameters are listed in Table 4.

For the Repeat-Dose Phase, steady-state uncorrected/absolute and baseline-corrected PK parameters of Alpha1-PI from weekly SC administration of Alpha-1 15% in subjects with AATD will be evaluated. The PK profiles of Alpha1-PI following the last repeat dose at Repeat-Dose Week 8 will be characterized by PK parameters listed in Table 4.

In addition, for the Repeat-Dose Phase the trough concentration (C_{trough}) will be calculated as the average value of the pre-dose Alpha1-PI concentrations measured at Repeat-Dose Weeks 6, 7, 8, and 9 (Final/Early Withdrawal Visit).

10.1.2 PK Parameters for IV Administration of Liquid Alpha1-PI

For the Single-Dose Data Evaluation Phase, the steady-state absolute/uncorrected and baseline corrected PK parameters of Alpha1-PI from weekly IV administration of Liquid Alpha1-PI in subjects with AATD will be evaluated. The PK profiles of Alpha1-PI following the 8th weekly IV infusion at IV Week 8 will be characterized by PK parameters.
In addition, mean trough concentrations (C_{trough}) will be calculated as the average value of the trough Alpha1-PI concentrations measured at IV Weeks 8 and 9.

10.1.3 List of PK Parameters for Each Treatment and Phase

Table 4 describes the PK parameters to be calculated for SC Administration of Alpha-1 15% in the Single-Dose Phase and the Repeat-Dose Phase , as well as for IV Administration of Liquid Alpha1-PI in the Single-Dose Data Evaluation Phase. The PK parameters will be calculated in Phoenix WinNonlin® using the linear up log down methodology. All parameters will be measured using both antigenic content and functional activity assays. All parameters except t_{max} will be calculated as both absolute/uncorrected and baseline-corrected measures. Additional PK parameters may be estimated if deemed necessary.

Table 4: PK Parameters in Each Study Phase

PK Parameter	Definition	SC SD	SC RD	IV RD
AUC_{0-t}	Area under the plasma concentration-time curve (AUC) from time 0 to time of the last measurable concentration	X	X	X
AUC_{0-t}/Dose	AUC_{0-t} divided by the dose administered (Dose normalized AUC)	X	X	X
$AUC_{0-\infty}$	AUC from time 0 to infinity	X		
$AUC_{0-\infty}$ /Dose	$AUC_{0-\infty}$ divided by the dose administered	X		
$AUC_{0-7\ days}$	AUC from time 0 to 7 days after dosing	X	X	X
$AUC_{0-7\ days}$ /Dose	$AUC_{0-7\ days}$ divided by the dose administered	X	X	X
C_{max}	Observed maximum $Alpha_1$-PI concentration following infusion obtained directly from the experimental data without interpolation, expressed in concentration units (e.g., mg/dL).	X	X	X
C_{max} /Dose	C_{max} divided by the dose administered	X	X	X
t_{max}	Observed time to reach maximum $Alpha_1$-PI concentration obtained directly from the experimental data without interpolation, expressed in time units (hour). If there is more than one maximum observed concentration, the t_{max} is the time to the first observed maximum concentration. t_{max} will not be reported following baseline-correction.	X	X	X
$t_{1/2}$	Half-life associated with the terminal slope of the semi-logarithmic drug concentration-time curve	X	X	X
CL/F	Apparent clearance of $Alpha_1$-PI after SC administration.	X	X	
V_d/F	Apparent volume of distribution	X	X	
CL	Total plasma clearance			X
V_d	Volume of distribution			X
C_{last}	Last measurable observed concentration	X	X	X
t_{last}	Time of last measurable observed concentration	X	X	X
Accumulation Ratio	$R_{AUC\ (0-7\ days)}$: $AUC_{0-7\ days}$ following repeat-dose divided by $AUC_{0-7\ days}$ following single-dose		X	
Time-invariance ratio	$AUC_{0-7\ days}$ / $AUC_{0-\infty}$: $AUC_{0-7\ days}$ following repeat-dose divided by $AUC_{0-\infty}$ following single-dose		X	

SC SD = SC Administration of Alpha-1 15% in the Single-Dose Phase; SC RD= SC Administration of Alpha-1 15% in the Repeat-Dose Phase; IV-RD= IV Administration of Liquid Alpha1-PI in the Single-Dose Data Evaluation Phase.
NOTE: if the dosing interval (τ) for the repeat-dose SC regimen is different from weekly (i.e., 7 days), AUC0-τ will be calculated in place of AUC_{0-7} days.

Table 5 describes additional PK parameters that will be calculated for diagnostic purposes and will be listed, but not summarized. All parameters will be measured using both antigenic content and functional activity assays. All parameters will be calculated as absolute/uncorrected and baseline-corrected measures.

Table 5: Additional PK Parameters in Each Study Phase

Parameter	Definition	SC SD	SC RD	IV RD
λz	Apparent first-order rate constant associated with the terminal portion of the curve	X	X	X
λz lower	Lower limit of time (hours) included in the calculation of λz	X	X	X
λz N	Number of data points used in the calculation of λz	X	X	X
λz upper	Upper limit of time (hours) included in the calculation of λz	X	X	X
R_2adj	Regression coefficient for the calculation of λz adjusted for λz N	X	X	X
%AUC_{ex}	Percentage of $AUC_{0-\infty}$ obtained by extrapolation from the last quantifiable time point to infinity	X		

SC SD = SC Administration of Alpha-1 15% in the Single-Dose Phase; SC RD= SC Administration of Alpha-1 15% in the Repeat-Dose Phase; IV-RD= IV Administration of Liquid Alpha1-PI in the Single-Dose Data Evaluation Phase.

Assessments of λz will include the following key considerations:

- The λz must be positive and must be calculated from at least 3 data points in the terminal phase of the concentration-time profile (in which the first time point must be greater than the t_{max}).
- The C_{max} and points prior to the C_{max} will not be used in the calculation of λz.
- Terminal data points will be selected automatically based on the default algorithm within the Phoenix WinNonlin® software.
- Adjusted coefficient of determination (R_2adj) ≥ 0.800 will be used as acceptance criteria for λz and λz-dependent parameters (e.g., CL/F [single dose], V_d/F, $t_{1/2}$, and $AUC_{0-\infty}$).
- λz and other λz-related parameters (e.g., CL/F [single dose], V_d/F, $t_{1/2}$, $AUC_{0-\infty}$) will be listed but excluded from summary statistics for concentration-time profiles that do not exhibit an elimination phase in the concentration-time profile or if the fit of the terminal slope data is poor (i.e., if adjusted R_2adj < 0.800 for the log-linear regression analysis of λz).
- Single dose $AUC_{0-\infty}$, $t_{1/2}$, V_d/F, and CL/F will be listed but may be excluded from summary statistics for concentration profiles where > 20% of the $AUC_{0-\infty}$ is extrapolated.

10.2 Summary of PK Concentrations and PK Parameters

$Alpha_1$-PI concentrations (absolute/uncorrected and baseline-corrected) at each timepoint and PK parameters will be listed and summarized using descriptive statistics including n, mean, SD, % Coefficient of Variation (CV), % Geometric Mean Coefficient of Variation (GMCV), median, minimum, and maximum. The geometric mean (GM) and 90% confidence interval (CI) for the GM will also be calculated for all PK parameters (except t_{max}).

A data listing will include the Alpha$_1$-PI concentrations (both antigenic content and functional activity assays) at each time point and the baseline-corrected concentrations, where the correction is performed by subtracting the baseline concentrations of Single-Dose Week 5/IV Week 1 measurements (after 4-week washout following the end of dosing at Single-Dose Week 1) from the uncorrected concentrations at each time point.
All Alpha$_1$-PI concentrations (absolute/uncorrected and baseline-corrected) for both antigenic content and functional activity versus time curves will be plotted on both the linear and semi-logarithmic scales. Alpha$_1$-PI concentration versus time curves for individual subjects will be presented with the actual elapsed time from the start of the Single-Dose Week 1, IV Week 8, or Repeat-Dose Week 8 infusions plotted on the x-axis. Individual concentration versus actual time plots will also be presented for all subjects on the same figure (spaghetti plot), separately for each study phase. For all subjects combined, mean Alpha$_1$-PI concentration versus nominal time curves (including absolute/uncorrected and baseline-corrected overlays) will be presented by study phase in one figure with the nominal time plotted on the x-axis. Similarly, mean and individual trough concentration versus time plots (including absolute/uncorrected and baseline-corrected and IV versus SC overlays) will also be generated.

Box and scatter plots of the PK parameters for SC single dose (C_{max}, C_{max}/Dose, $AUC_{0\text{-}7days}$, $AUC_{(0\text{-}7days)}$/Dose, $AUC_{0\text{-}\infty}$, and $AUC_{0\text{-}\infty}$/Dose) and SC and IV repeat-dose (C_{max}, C_{max}/Dose, $AUC_{0\text{-}7days}$ and $AUC_{0\text{-}7days}$/Dose) will be presented.

The percentage of subjects with a C_{trough} Alpha1-PI Concentrations above 11 µM and 20 µM will also be presented. Alpha1-PI concentrations (antigenic content and functional activity) are reported in the unit of mg/mL. The following conversion factors will be used to calculate the above thresholds:

- Antigenic content :1 mg/mL alpha$_1$-PI is equivalent to 22.6 µM, calculated based on the molecular weight of alpha$_1$-PI without glycosylation (approximately 44.3 KDa). Therefore, 11 µM=0.49 mg/mL.
- Functional Activity: 1 mg/mL alpha$_1$-PI is equivalent to 19.2 µM, calculated based on the molecular weight of alpha$_1$-PI with glycosylation (approximately 52 KDa). Therefore, 11 µM=0.57 mg/mL.

10.3 Statistical Analysis of PK Parameters

10.3.1 Dose Proportionality

Dose proportionality will be separately assessed for single-dose and repeat-dose SC Administrations (for both absolute/corrected and uncorrected measures) by comparing dose-normalized SC PK parameters between the 72 mg/kg and 180 mg/kg dose levels.

Assessment of dose proportionality will be explored based on natural log transformed C_{max} and AUC parameters and for dose normalized C_{max} and AUC parameters for Alpha$_1$-PI

concentrations as measured by antigenic content and functional activity assays. A t-test will be used to compare the doses of SC administered (72 mg/kg vs.180 mg/kg) using the natural log-transformed values of the PK parameters. The t-test will include dose as a fixed effect and the alpha level will be 0.1 (alpha=0.05 for each side).

The following sample SAS pseudocode (using dose normalized C_{max} as an example) will be used for the analyses:

```
    proc glm;
        class DOSE;
           model LOG(DOSE NORMALIZED Cmax )= DOSE ;
           lsmeans DOSE/ diff=control("72mg/kg") CL ALPHA=0.1;
run;
```

where DOSE is the dose level of SC administrated (72 mg/kg or 180 mg/kg), the 72 mg/kg dose will be considered as the Reference dose and the 180 mg/kg dose will be treated as the Test dose. The analysis will be repeated for all AUC parameters.

The t-test will provide the least-squares means (LSM) of the natural log transformed PK parameters, the differences between the adjusted means and the standard error associated with these differences. The Dose effect will be estimated by exponentiation of the difference in least squares means between treatments (Test-Reference) and the corresponding 90% CI for the geometric LSM ratio between treatments (Test/Reference) for each PK parameter analyzed.

The analysis described above is exploratory in nature and no statistical inferences will be made based on the results.

10.3.2 Linear Regression Model: Relationship Between PK Parameter and Infusion Volume and Duration for repeat-dose SC

The impact of the volume and duration of infusion on the PK endpoints, clearance (CL/F) and dose normalized $AUC_{0\text{-}7days}$, following SC administration will be evaluated by using a linear regression model fitting natural log-transformed SC PK parameters as the response variable and volume and duration of infusion as the explanatory variables.

The following sample SAS pseudocode (using CL/F as an example) can be used for the analyses:

```
proc reg;
      model LOG(CL/F) = DUCR VOL DUCR*VOL;
run;
```

where LOG(CL/F) is the natural log-transformed CL/F, DUCR is duration of infusion, VOL is volume of infusion and DUCR*VOL is the interaction between duration and volume. Plots of clearance (Y-Axis) and Infusion Duration (X-Axis) and clearance (Y-Axis) and Infusion Volume (X-Axis) will be presented separately. If the duration by volume interaction is significant (<0.05), a plot of clearance (Y-Axis) and Infusion Duration (X-Axis) grouped by volume (<30mL, 30mL-40mL, 41mL-50mL, >50mL) will be displayed. The coefficients,

standard errors and p-value for infusion duration, infusion volume and the duration by volume interaction from the model will also be presented in a table. The analysis will be repeated for dose normalized $AUC_{0\text{-}7days}$.

10.3.3 Mixed-Effect Model: Relationship Between Steady-State PK Parameters and Mode of Administration

To compare the steady-state PK between the IV administration (in the Single-Dose Data Evaluation Phase) and SC administration (in the Repeat-Dose Phase), natural log-transformed dose normalized $AUC_{0\text{-}7days}$, dose normalized C_{max} and steady-state mean trough values (C_{trough}) parameters will be analyzed by analysis of variance with a mixed-effect model. The model will include route of administration (repeat-dose Alpha-1 15% SC or repeat-dose Liquid $Alpha_1$-PI IV) as a fixed effect, and subject as a random effect. From this model, the geometric least-squares mean ratio between SC and IV administration, and the corresponding 90% CI will be calculated. This analysis can be implemented by the following sample SAS pseudocode:

```
proc mixed;
      class TRT SUBJID;
      model LOG(AUC0-7days ) = TRT;
      random SUBJID;
      lsmeans TRT / pdiff cl alpha = 0.1;
      estimate 'Alpha-1 15% vs Liquid Alpha1-PI' TRT -1 1 /
               cl alpha = 0.1;
run;
```

where $LOG(AUC_{0\text{-}7days})$ is the natural log-transformed $AUC_{0\text{-}7days}$, TRT is route of administration, and SUBJID is subject number. The analysis will be repeated for dose normalized C_{max} and steady-state mean trough values (C_{trough}).

The administration route effect will be estimated by exponentiation of the difference in least squares means between treatments (SC-IV) and the corresponding 90% CI for the geometric LSM ratio between treatments (SC/IV) for each PK parameter analyzed.

10.4 Exploratory PK Parameters

Exploratory PK endpoints include the following absolute/uncorrected and baseline corrected steady-state PK parameters of $Alpha_1$-PI, following the 8th weekly IV infusion of Liquid $Alpha_1$-PI at IV Week 8: Steady-state AUC of $Alpha_1$-PI over the weekly dosing interval from 0 to 7 days $AUC_{0\text{-}7days}$ ($AUC_{0\text{-}\tau}$), steady-state mean trough $Alpha_1$-PI level (C_{trough}), C_{max}, $AUC_{0\text{-}t}$/Dose, $AUC_{0\text{-}7\ days}$/Dose ($AUC_{0\text{-}\tau}$ /Dose), C_{max}/Dose, CL, $t_{1/2}$, V_d and t_{max}, (will not be reported following baseline-correction), based on $Alpha_1$-PI concentrations as measured by antigenic content as well as functional activity assays. Descriptive statistics as mentioned in SAP Section 10.2 will be used to summarize these parameters by Cohort.

11 Primary (Safety) Analysis

Safety analyses will be based on the Safety population.

Safety analyses for all subjects will be addressed by listing and tabulation of AEs (including suspected ADRs and ARs), clinical laboratory tests, vital signs, and physical assessments. Data will be summarized using descriptive analyses, and treatment comparisons will be based on review of descriptive statistics.

Safety data will be analyzed separately for treatment-72 mg/kg and treatment-180 mg/kg subjects and Liquid 60mg/kg and 120mg/kg treatments. Safety data for each cohort will be analyzed separately for single-dose SC administration Alpha-1 15% administration and for IV administration of Liquid Alpha$_1$-PI during the Single-Dose Data Evaluation Phase, and safety data for SC administration will be analyzed separately for repeat-dose administration of Alpha-1 15% within each cohort.

11.1 Adverse Events, Infusion Site Reactions, and COPD Exacerbations

Common Definitions

In this subsection, the term "event" refers to AEs, ISRs, and COPD exacerbations. All reported events will be coded and summarized by SOC and PT according to MedDRA. The number of subjects and percentage of subjects will be computed by SOC and PT to show the incidence of events by treatment.

When the causal relationship of an event is classified by the investigator as definitely or possibly related, the event will be defined as a suspected ADR. A suspected ADR with a causal relationship of "definitely related" will be defined as an AR.

For summary purposes, events will be classified as treatment emergent or non-treatment-emergent depending on the comparison of event onset date/time with the start date/time of IP. A treatment emergent event will be defined as an event with an onset date/time on or after the start of IP up to on or before 28 days after the last dose of IP. Events will be considered treatment emergent if the start date is missing, or the start date is non-missing and is the same as first dose date, but the time is partial/missing. Note: the eCRF does not permit entry of partial dates, only partial times are permitted.

A non-treatment emergent event will be defined as an event which with an onset date/time prior to the start of IP or more than 28 days after the last dose of IP. Non-treatment emergent events and treatment emergent events will be summarized separately.
Treatment-emergent events will be further attributed to a treatment by comparing the event onset date/time to the start date/time of the first infusion in each study phase. Treatment-emergent events will be attributed to treatment phase as detailed in Table 6:

Table 6: Treatment Emergent Definitions by Treatment Phase

Treatment Phase	Definition
Single-Dose Phase and Washout	Onset date/time on or after the start date/time of the first SC Alpha-1 15% infusion and prior to the start date/time of the first Liquid Alpha$_1$-PI infusion, or up to 28 days after the last dose of IP for subjects who prematurely discontinue.

Single-Dose Data Evaluation Phase (IV Liquid Alpha1-PI infusions)	Onset date/time on or after the start date/time of the first IV Liquid $Alpha_1$-PI infusion and prior to the start date/time of the first SC Alpha-1 15% infusion during the Repeat-Dose Phase, or up to 28 days after the last dose of IP for subjects who prematurely discontinue.
Repeat-Dose Phase	Onset date/time on or after the start date/time of the first SC Alpha-1 15% infusion during the Repeat-Dose Phase and up to on or before 28 days after the last dose of IP.

Temporally associated events are defined as those events occurring during or within 72 hours following the end of the IP infusion. If an event occurs more than 72 hours after the end of the IP infusion, it will be considered as treatment-emergent (as per the above definitions) but will not be considered as temporally associated.

<u>All Adverse Events – AEs and ISRs</u>

An overall summary of AEs (including ISRs), by treatment phase as defined in Table 6, will present the number of AEs and the number and percentage of subjects with AEs, in the following categories:

- Treatment Emergent Adverse Events (TEAEs)
- Suspected ADR
- AR
- TEAE Leading to Death
- Serious TEAE
- Serious Suspected ADR
- Serious AR
- Non-Serious TEAE
- TEAE Leading to IP Interruption
- TEAE Leading to IP Withdrawal
- TEAE Leading to Study Withdrawal
- Serious TEAE Leading to Study Withdrawal
- ISRs
- Temporally Associated TEAEs

AEs with onset prior to treatment and with an onset great than 28 days after end of treatment will also be presented.

These overall summaries will be repeated for AEs excluding COPD exacerbations.

In addition, TEAEs (including ISRs), by treatment phase as defined in Table 6, will be summarized in the following ways:

- SOC / PT – summary of number of TEAEs and number/percentage of subjects with at least one TEAE by treatment, SOC, and PT
    - A select subset of TEAE summaries will also include number of events by SOC and PT

- PT – summary of number of TEAEs and number/percentage of subjects with at least one TEAE by treatment and PT
- SOC/PT/Maximum Severity – summary of number of TEAEs and number/percentage of subjects with at least one TEAE by treatment, SOC, PT, and severity
- SOC/PT/Maximum Causality – summary of number of TEAEs and number/percentage of subjects with at least one TEAE by treatment, SOC, PT, and causality
- PT Rates – summary of number of infusions, number of AEs, rate of AEs per infusion, and rate of AEs per exposure week by PT. Rate per infusion is defined as total number of events divided by total number of infusions received. Rate per exposure week is defined as total number of events divided by total duration of exposure in weeks.

At each level of summarization, a subject will only be counted once per SOC or PT, using the most severe AE or the AE with the strongest causal relationship to the treatment as applicable.

In addition, non-treatment emergent AEs will be flagged in the listing of all AEs.

Table 7 identifies the subsets AEs that will be presented for each type of summaries.

Table 7: AE Summaries

	TEAE Summary					
Adverse Event Subset	**SOC / PT**	**PT**	**SOC / PT / Maximum Severity**	**SOC / PT / Maximum Causality**	**PT Rates**	**Listing**
AE with Onset Prior to Treatment	x					x*
AE with Onset Greater than 28 Days after End of Treatment	x					x*
TEAEs	x	x	x	x	x	x (All AEs)
Suspected ADR	x	x	x		x	
AR	x	x	x		x	
Serious TEAEs	x	x		x		x
Non-serious TEAEs	x					
TEAEs Leading to Study Withdrawal	x					x
Serious TEAEs Leading to Study Withdrawal	x					
TEAEs Leading to Death	x					x
Infusion Site Reactions	x					x
COPD exacerbations			x			x
Temporally Associated AEs	x					

* Non- TEAEs will be flagged in the listing of all AEs

For AEs that occur during IP infusion, the infusion rate in effect at the time of onset of the AE, the time when the AE is first reported and the time when the AE changes materially in intensity and/or resolves will be listed.

11.1.1 Infusion Site Reactions

Local ISRs will be summarized separately by cohort and infusion week. The summaries will be presented by PT and infusion site and include the number and percentage of subjects with any event and the total number of events.

The rate of local ISRs will also be summarized separately by cohort and treatment phase, and will be presented by PT and infusion site.

The percentage of subjects with local ISRs and the rate per infusion will also be plotted vs. infusion week number.

All local ISRs will be presented in a data listing separately from other AEs.

11.1.2 COPD Exacerbations

All COPD exacerbations will be summarized separately by cohort and treatment phase (see Table 6) at the time of the start of a COPD exacerbation. Summaries will include the number and percentage of subjects with any event and the total number of events by severity. COPD exacerbations will be presented in a data listing separately from other AEs

11.2 Clinical Laboratory Assessments

The following tests will be conducted at the assigned visits according to the protocol:

- Hematology (See Section 13.1)
-
- Clinical chemistry (See Section 13.1)
- Urinalysis (See Section 13.1)
- Pregnancy testing will be performed for child-bearing potential females only and will be analyzed by a local laboratory, local urine testing at Screening and Baseline visit.
- $Alpha_1$-PI Immunogenicity
- Urine cotinine
- Blood for biomarkers
- $Alpha_1$-PI Concentration (refer to section 10)
- Genotyping/phenotyping
- Virus safety retain samples

11.2.1 Chemistry / Hematology / Urinalysis / Pregnancy

The hematology, clinical chemistry, and urinalysis parameters will be summarized at each visit with number of subjects, mean, SD, median, minimum, and maximum values.

Change from baseline (screening) to all subsequent visits, change from the from Single Dose Week 5/IV Week 1 to Repeat-Dose Phase week 1, and change from Repeat-Dose week 1 to Repeat-Dose week 9 will also be presented.

For hematology and clinical chemistry, shift from baseline (screening) tables (cross tabulations of baseline results as normal, abnormal low, or abnormal high and post-baseline results as normal, abnormal low, or abnormal high) will be summarized at each visit by cohort for each parameter with normal ranges.
Similarly defined shift tables will be also presented for, shift from Single Dose Week 5/IV Week 1 to Repeat-Dose Phase Week 1 and Shift from Repeat-Dose Week 1 to Repeat-Dose Week 9.

All laboratory data will be presented in data listings. Pregnancy test results will be listed but not summarized.

11.2.2 Immunogenicity

Blood samples for immunogenicity testing will be drawn prior to IP administration at the Single-Dose Weeks 1 and 5/IV Week 1, Repeat-Dose Weeks 1, 4, and 9 (Final/Early Withdrawal Visit). The samples collected at Repeat-Dose Week 4 will be tested for immunogenicity only if deemed appropriate (e.g., unexpected PK profile).

All immunogenicity results will be listed, and new positive immunogenicity results will be tabulated.

11.3 Vital Signs

Vital sign data [BP (systolic blood pressure (SBP) and diastolic blood pressure (DBP), HR, T, RR, weight] will be summarized by cohort and visit. Summaries will be presented for the original value and change from baseline (Single-Dose week 1) to each post-baseline visit, change from Single Dose Week 5/IV Week1 to Repeat-Dose Week 1 and change from Repeat-Dose Week 1 to Repeat-Dose Week 9. Height will be summarized with baseline characteristics.

All vital sign data will be listed.

11.4 Pulmonary Function Tests

Pulmonary function tests (FEV_1, FVC, and FEV_1 % predicted) will be listed for each subject and summarized by cohort and visit, separately for pre- and post- bronchodilator (BD) assessments. The difference between pre- and post-BD assessments of FEV_1 and FVC at each visit will also be presented (calculated as post-BD assessment - pre-BD assessment).
Summaries will be presented for the original value and change from baseline (screening) to each post-baseline visit, change from Single Dose Week 5/IV Week 1 to Repeat-Dose Week

1, and change from Repeat-Dose Week 1 to Repeat-Dose Week 9. FEV_1 and FVC units will be reported in liters and FEV_1 predicted as a percentage.

11.5 Physical Examinations

Abnormal physical examination findings at screening will be summarized using counts and percentages of subjects, by body system. Entries for 'Other' body systems will be grouped together; a subject with 2 or more 'Other' entries will be counted only once. Counts and percentages of subjects with new or worsening findings at Repeat Dose Week 9 will be presented by cohort.

All physical assessment data will be listed.

12 Other Exploratory Analysis

12.1 Satisfaction with Subcutaneous Treatment

Subjects' responses to 6 questions collected on the 'Subject satisfaction with treatment' questionnaire following the Repeat-Dose Phase will be summarized using counts and percentages of subjects giving each response. Satisfaction response data will also be listed.

13 Supporting Documentation

13.1 Appendix 1 Clinical Laboratory Parameters

Category	Laboratory Parameter	Summary	Shift Table
Chemistry	ALT	x	x
	Albumin	x	x
	ALP	x	x
	AST	x	x
	Bicarbonate	x	x
	Urea Nitrogen	x	x
	Calcium	x	x
	Creatinine	x	x
	Glucose	x	x
	Potassium	x	x
	Protein	x	x
	Sodium	x	x
	Bilirubin	x	x
Hematology	Basophils	x	x
	Basophils/Leukocytes	x	x
	Eosinophils	x	x
	Eosinophils/Leukocytes	x	x
	Hematocrit	x	x
	Hemoglobin	x	x
	Lymphocytes	x	x
	Ery. Mean Corpuscular Hemoglobin	x	x
	Ery. Mean Corpuscular HGB Concentration	x	x
	Ery. Mean Corpuscular Volume	x	x
	Monocytes	x	x
	Mean Platelet Volume (MPV)	x	x
	Neutrophils	x	x
	Nucleated Erythrocytes/Leukocytes	x	x
	Platelets	x	x
	Erythrocyte Cell Morphology	x	x
	Erythrocytes Distribution Width	x	x
	Erythrocytes	x	x
	Reticulocytes	x	x
	Immature Granulocytes/Leukocytes	x	x
	Lymphocytes/Leukocytes	x	x
	Monocytes/Leukocytes	x	x
	Neutrophils/Leukocytes	x	x
	Leukocytes	x	x

Category	Laboratory Parameter	Summary	Shift Table
Urinalysis - Numeric	Urine pH	x	
Urinalysis - Categorical	Amorphous Crystals	x	
	Calcium Carbonate Crystals	x	
	Calcium Oxalate Crystals	x	
	Calcium Phosphate Crystals	x	
	Cysteine Crystals	x	
	Granular Casts	x	
	Hyaline Casts	x	
	Leucine Crystals	x	
	RBC Casts	x	
	Renal Epithelial Cells	x	
	Squamous Epithelial Cells	x	
	Transitional Epithelial Cells	x	
	Triple Phosphate Crystals	x	
	Tyrosine Crystals	x	
	Uric Acid Crystals	x	
	Bacteria	x	
	Glucose	x	
	Protein	x	
	Erythrocytes	x	
	Leukocyte	x	
	Waxy Casts	x	
	WBC Casts	x	
	Occult Blood	x	
	Urinary Mucus	x	
	Yeast Cells	x	
Miscellaneous	Urinary Cotinine	x	
Referral	Alpha-1-Antitrypsin Proteotype S/Z Reflex to Alpha-1-Antitrypsin Phenotype		
	Alpha-1-Antitrypsin Proteotype S/Z, Alpha-1-Antitrypsin		
	Alpha-1-Antitrypsin Proteotype S/Z, S mutation		
	Alpha-1-Antitrypsin Proteotype S/Z, Z mutation		
	SERPINA1 Gene, Full Gene Analysis, Interpretation		
	SERPINA1 Gene, Full Gene Analysis, Result		
	SERPINA1 Gene, Full Gene Analysis, Result REFLEX Amino Acid Change		

Category	Laboratory Parameter	Summary	Shift Table
	SERPINA1 Gene, Full Gene Analysis, Result REFLEX Classification		
	SERPINA1 Gene, Full Gene Analysis, Result REFLEX DNA Change		
	SERPINA1 Gene, Full Gene Analysis, Result Summary		
	SERPINA1 Gene, Full Gene Analysis, Reviewer		

13.2 Appendix 2 Analysis Visit Windows

Table 8: Scheduled Visits and Analysis Visit Windows Weight

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Baseline[$]	Week 1	Baseline	1*	<=1
SDDE	Week 5	IV-WK 1	29*	[2 to (Repeat-Dose Day 1 -1)]
RD	RD Week 1	RD-WK1	1[#]	[>= Repeat-Dose Day 1]

$ see Section 5.1.2
* Relative to Single-Dose Day 1.
[#] Relative to Repeat-Dose Day 1
RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

Table 9: Scheduled Visits and Analysis Visit Windows for Urine Pregnancy Tests

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Baseline[$]	Week 1	Baseline	1*	<=1*
SDDE	Week 5	IV-WK 1	29*	[2* to Repeat-Dose Day 1 - 1)]
RD	RD Week 1	RD-WK1	1[#]	[Repeat-Dose Day 1 to 29[#]]
	RD Week 9	RD-WK9	57[#]	[>=30[#]]

$ see Section 5.1.2
* Relative to Single-Dose Day 1.
[#] Relative to Repeat-Dose Day 1
RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

Table 10: Scheduled Visits and Analysis Visit Windows for PFTs (FEV_1 and FVC)

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Screening		Baseline	Up to -1*	<=1*
SDDE	Week 5	IV-WK 1	29*	[2* to Repeat-Dose Day 1 - 1)]

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
RD	RD Week 1	RD-WK1	1#	[Repeat-Dose Day 1 to 11#]
	RD Week 4	RD-WK4	22#	[12# to 39#]
	RD Week 9	RD-WK9	57#	[>=40#]

* Relative to Single-Dose Day 1.
# Relative to Repeat-Dose Day 1
FEV_1=Force forced expiratory volume in 1 second; FVC= Forced Vital Capacity; PFT=Pulmonary Function Test.
RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

Table 11: Scheduled Visits and Analysis Visit Windows for Hematology, Chemistry, and Urinalysis

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Screening		Baseline	Up to -1*	<=1*
SDDE	Week 5	IV-WK 1	29*	[2* to Repeat-Dose Day 1 - 1)]
RD	RD Week 1	RD-WK1	1#	[Repeat-Dose Day 1 to 29#]
	RD Week 9	RD-WK9	57#	[>=30#]

* Relative to Single-Dose Day 1.
# Relative to Repeat-Dose Day 1
RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

Table 12: Scheduled Visits and Analysis Visit Windows for Vital Signs

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Baseline$	Week 1	Baseline	1*	<=1*
SDDE	Week 5	IV-WK 1	29*	[2* to 32*]
	Week 6	IV-WK 2	36*	[33 to 39]
	…			..
	Week X	IV-WK X-4	7*X – 6*	[[7*X - 9 to 7*X – 3] *
	Last Week in SDDE phase			See note
RD	RD Week 1	RD-WK 1	1#	[Repeat-Dose Day 1 to 4#]
	RD Week 2	RD-WK 2	8#	[5# to 11#]
	RD Week 3	RD-WK 3	15#	[12# to 18#]
	RD Week 4	RD-WK 4	22#	[19# to 25#]
	RD Week 5	RD-WK 5	29#	[26# to 32#]
	RD Week 6	RD-WK 6	36#	[33# to 39#]
	RD Week 7	RD-WK 7	43#	[40# to 46#]
	RD Week 8	RD-WK 8	50#	[47# to 53#]
	RD Week 9	RD-WK 9	57#	[>=54#]

Note: the analysis visit window for the last visit for a patient in the SDDE phase will be the lower bound presented, up to (Repeat-Dose Day 1 - 1).
$ see Section 5.1.2
* Relative to Single-Dose Day 1.
# Relative to Repeat-Dose Day 1

RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

Table 13: Scheduled Visits and Analysis Visit Windows for Alpha-1 MP Immunogenicity

Analysis Period	Scheduled Time Point	Analysis Visit (label on output)	Target Time Point (Day)	Analysis Visit Window (Day)
Baseline[$]	Week 1	Baseline	1[*]	<=1[*]
SDDE	Week 5	IV-WK 1	29[*]	[2[*] to Repeat-Dose Day 1 - 1)]
RD	RD Week 1	RD-WK 1	1[#]	[Repeat-Dose Day 1 to 11[#]]
	RD Week 4	RD-WK 4	22[#]	[12[#] to 39[#]]
	RD Week 9	RD-WK 9	57[#]	[>=40[#]]

$ see Section 5.1.2
* Relative to Single-Dose Day 1.
# Relative to Repeat-Dose Day 1
RD = Repeat-Dose; SDDE = Single Dose Data Evaluation.

13.3 Appendix 3 Changes from the Protocol

Not Applicable

14 References

(Quanjer PH 2012) Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.

(Implementing GLI-2012) Philip H. Quanjer, Sanja Stanojevic, Tim J. Cole, Janet Stocks. Implementing GLI-2012 regression equations. Global Lung Function Initiative.