Official Title: A Phase 2a, Single-Blind, Placebo-Controlled, Parallel-Group Study to Assess Safety, Tolerability, and Pharmacokinetics of Brensocatib Tablets in Adults With Cystic Fibrosis

NCT Number: NCT05090904

Document Dates: SAP Version 1.0: 21-October-2022

STATISTICAL ANALYSIS PLAN

A Phase 2a, Single-Blind, Placebo-Controlled, Parallel-Group Study to Assess Safety, Tolerability, and Pharmacokinetics of Brensocatib Tablets in Adults with Cystic Fibrosis

Study Number: INS1007-211

IND Number: 133790

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Version Number	Amendment	Date
FINAL 1.0		21 Oct 2022

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1. LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AESI	adverse event of special interest
ATC	Anatomical Therapeutic Chemical
AUC_{0-24}	area under the concentration-time curve from time 0 to 24 hours
AUC_{last}	area under the concentration-time curve from time 0 to the last timepoint with measurable concentration
AUC_{∞}	area under the concentration-time curve from time 0 to infinity
BDRM	Blind Data Review Meeting
BLQ	below limit of quantification
CatG	cathepsin G
C_{av}	average concentration over the dosing interval
CDISC	Clinical Data Interchange Standards Consortium
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CI	confidence interval
CL/F	total clearance following oral administration
C_{max}	maximum observed plasma concentration in a dosing interval
C_{min}	minimum observed plasma concentration in a dosing interval
C_{trough}	concentration at the end of a dosing interval taken directly before next administration
COVID-19	corona virus disease 2019
CSP	clinical study protocol
CSR	clinical study report
DBL	database lock
DLT	dose limiting toxicity
eCRF	electronic case report form
ECG	electrocardiogram
EoS	end of study
EoT	end of treatment
$FEF_{(25-75\%)}$	forced expiratory flow between 25% and 75% of forced vital capacity

Abbreviation	Term
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
gCV%	geometric coefficient of variation percentage
GM	geometric mean
ICE	intercurrent event
ICH	International Council for Harmonisation
ITT	Intent-to-Treat
IP	investigational product
IWRS	interactive web response system
λ_z	elimination rate constant
LL	lower limit
LLOQ	lower limit of quantification
LS	least squares
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NC	not calculable
NCA	non-compartmental analysis
NCFBE	non-cystic fibrosis bronchiectasis
NE	neutrophil elastase
NSP	neutrophil serine protease
PD	pharmacodynamic
PK	pharmacokinetics
$ppFEV_1$	percent predicted forced expiratory volume in 1 second
PR3	proteinase 3
PT	Preferred Term (MedDRA)
QD	once daily
QoL	quality of life
$R_{ac,Cmax}$	accumulation ratio based on C_{max}
$R_{ac,AUC}$	accumulation ratio calculated from AUC_{0-24} at steady state divided by AUC_{0-24} after single dose
SD	standard deviation
SAE	serious adverse event

Abbreviation	Term
SAP	statistical analysis plan
SOC	System Organ Class (MedDRA)
SRC	Safety Review Committee
$t_{½}$	elimination half-life
TEAE	treatment-emergent adverse event
TEAESI	treatment-emergent adverse event of special interest
TLF	table, listing, and figure
t_{max}	time to maximum observed plasma concentration
t_{last}	time of the last measurable (positive) concentration
UL(N)	upper limit (of normal)
Vd/F	volume of distribution at terminal phase following oral administration
WHO	World Health Organization

2. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to describe the planned analyses to be included in the Clinical Study Report (CSR) for study INS1007-211.

The SAP complies with the International Council for Harmonisation (ICH) E9 'Statistical Principles for Clinical Trials' and E9(R1) 'Statistical Principles for Clinical Trials: Addendum on Estimands and Sensitivity Analysis in Clinical Trials', and is based upon the following study documents:

- Clinical Study Protocol (CSP) Amendment 2 dated 26 Jul 2022
- electronic Case Report Form (eCRF) dated 17 Aug 2022

All decisions regarding the final analysis of the study results, as defined in this SAP, will have been made before database lock (DBL) of the study data.

Please refer to the CSP for details on the conduct of the study, operational aspects of clinical assessments, and the time schedule of assessments.

The SAP is intended to be aligned with the CSP. The SAP typically contains more details than the CSP or other types of analyses. When differences exist in descriptions or explanations provided in the CSP and this SAP, the SAP prevails; the differences will be detailed in the CSR. Minor changes or additional exploratory analyses will be listed in Section 13 and described in the CSR.

Details pertaining to the scope of analyses for Safety Review Committee (SRC) review are provided in the SRC Charter and the corresponding mock table, listing, and figure (TLF) shells document.

3. STUDY OBJECTIVES AND ESTIMANDS

3.1. Primary Objectives and Estimands: Pharmacokinetics and Safety

Pharmacokinetic Objective(s)	
To evaluate the pharmacokinetics (PK) of brensocatib in participants with cystic fibrosis (CF) following once daily (QD) oral administration of study drug	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP who received brensocatib
Treatment Regimen	1 tablet QD of brensocatib (actual treatment)
Endpoints	C_{max}, t_{max}, AUC_{0-24}, and $t_{½}$ on Day 1 and Day 28
Population Level Summary	Mean brensocatib dose levels for C_{max}, AUC_{0-24}, and $t_{½}$, median for t_{max}
Intercurrent Event (ICE)	• Early discontinuation from brensocatib for any reason • Not on stable cystic fibrosis transmembrane conductance regulator (CFTR) modulators for participants on modulators
Strategy	For the first ICE, the While-On-Treatment strategy (including up to Day 35 follow-up period) will be applied. For the second ICE, the Treatment Policy strategy will used.

C_{max} = maximum plasma concentration, t_{max} = time to maximum plasma concentration, AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours post-dose, $t_{½}$ = elimination half-life.

Safety Objective(s)	
To evaluate the safety of brensocatib compared to placebo in participants with CF	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP
Treatment Regimen	1 tablet QD of brensocatib or placebo (actual treatment)
Endpoints	Participant reported treatment-emergent adverse event (TEAE) and type of TEAE
Population Level Summary	Number and percentage of participants with each type of TEAEs and number of TEAEs
ICE	• Early discontinuation from randomized investigational product (IP) for any reason
Strategy	The While-On-Treatment strategy (including the 28-days follow-up) will be applied.

3.2. Secondary Objective and Estimand: Pharmacokinetics

Pharmacokinetic Objective(s)	
To evaluate the dose-dependency of brensocatib exposure	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP who received brensocatib
Treatment Regimen	1 tablet QD of brensocatib (actual treatment)
Endpoints	C_{max}, AUC_{0-24}, and AUC_{last} on Day 1 and Day 28
Population Level Summary	Slope from a statistical model (if data allow estimation based on a statistical model) or ratio of geometric means (GMs)
ICE	• Early discontinuation from brensocatib for any reason • Not on stable CFTR modulators for participants on-modulators
Strategy	For the first ICE, the While-On-Treatment strategy (including up to Day 35 follow-up period) will be applied. For the second ICE, the Treatment Policy strategy will be used.

C_{max} = maximum plasma concentration, AUC_{0-24} = area under the concentration-time curve from time 0 to 24 hours post-dose, AUC_{last} = area under the concentration-time curve from time 0 to the last timepoint with measurable concentration.

3.3. Exploratory Objectives and Estimands

Pharmacodynamic Objective(s)	
To evaluate the effect of brensocatib compared with placebo on the concentration of Neutrophil Elastase (NE), Cathepsin G (CatG), and Proteinase 3 (PR3) in sputum over the 28-day treatment period	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP
Treatment Regimen	1 tablet QD of brensocatib or placebo (actual treatment)
Endpoints	Percentage inhibition from baseline to Day 14 and Day 28 for concentration of NE, CatG, and PR3 in sputum
Population Level Summary	Treatment means for observed values and for percent inhibition from baseline
ICE	• Early discontinuation from randomized IP for any reason, • Use of antibiotics during an acute event that could affect neutrophil serine protease (NSP) levels
Strategy	The While-On-Treatment strategy (including up to Day 35 follow-up period) will be applied for both ICEs.

Pharmacodynamic Objective(s)	
To evaluate the effect of brensocatib compared with placebo on the concentration of NE, CatG, and PR3 in blood over the 28-day treatment period	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP

Treatment Regimen	1 tablet QD of brensocatib or placebo (actual treatment)
Endpoints	Percentage inhibition from baseline to Day 14 and Day 28 for concentration of NE, CatG, and PR3 in blood
Population Level Summary	Treatment means for observed values and for percent inhibition from baseline
ICE	• Early discontinuation from randomized IP for any reason, • Use of antibiotics during an acute event that could affect NSP levels
Strategy	The While-On-Treatment strategy (including up to Day 35 follow-up period) will be applied for both ICEs.

Efficacy Objective(s)	
To assess the effect of brensocatib compared with placebo on lung function, as measured by change in pulmonary function tests including $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$ at Day 28	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP
Treatment Regimen	1 tablet QD of brensocatib or placebo (randomized treatment)
Endpoints	Change from Baseline to Day 28 in prebronchodilator $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$
Population Level Summary	Treatment means or difference in means if data allow estimation based on a statistical model
ICE	• Early discontinuation from randomized IP for any reason
Strategy	The While-On-Treatment strategy will be applied.

FEV_1 = forced expiratory volume in 1 second, $ppFEV_1$ = percent predicted forced expiratory volume in 1 second, FVC = forced vital capacity, $FEF_{(25-75\%)}$ = forced expiratory flow between 25% and 75% of forced vital capacity.

Efficacy Objective(s)	
To evaluate the effect of brensocatib compared with placebo on quality of life (QoL) as measured by cystic fibrosis questionnaire (revised version) (CFQ-R)[1] through the 4-week treatment period	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP
Treatment Regimen	1 tablet QD of brensocatib or placebo (randomized treatment)
Endpoints	Change from Baseline to Day 14 and Day 28 for the CFQ-R Respiratory Domain score
Population Level Summary	Treatment means or difference in means if data allow estimation based on a statistical model
ICE	• Early discontinuation from the randomized IP for any reason
Strategy	The While-On-Treatment strategy (including up to Day 35 follow-up period) will be applied.

CFQ-R = Cystic Fibrosis Questionnaire-Revised.

Microbiology Objective(s)	
To evaluate the effect of brensocatib on sputum microbiology	
Population	Participants with CF defined by the inclusion and exclusion criteria of the CSP
Treatment Regimen	1 tablet QD of brensocatib or placebo (randomized treatment)
Endpoints	Change from Baseline to Day 28 in number of colony-forming units for microbial species
Population Level Summary	Treatment means and change from baseline
ICE	• Early discontinuation from randomized IP for any reason
Strategy	The While-On-Treatment strategy will be applied.

4. STUDY DESIGN

4.1. Summary of Study Design

This is a Phase 2a, single-blind, randomized, placebo-controlled, parallel-group, multiple-dose study to assess the PK, PD, and safety of brensocatib 10, 25, and 40 mg QD in participants with CF. Following review of PK and safety data by a SRC, and provided that the SRC deems it appropriate to administer the next dose, a cohort of participants will receive brensocatib 65 mg QD on the same schedule as the previous cohorts. A tablet formulation of brensocatib 65 mg is not yet available; therefore, participants assigned to the 65 mg dose cohort will receive 1 tablet of brensocatib 25 mg and 1 tablet of brensocatib 40 mg.

Overall, the study will enroll approximately 25 to 34 participants. The first three brensocatib arms (10 mg, 25 mg, and 40 mg QD) will enroll approximately 7 participants each, and the placebo arm will enroll approximately 4 participants. For the active treatment arms, there will be 2 strata: 5 participants who have previously received and will continue to receive CFTR modulators as concomitant medication (CFTR modulator stratum), and approximately 2 participants who have either never previously received CFTR modulators or have not been administered CFTR modulators within 30 days prior to Screening (Visit 1) and will not receive CFTR modulators during the study (non-modulator stratum). For the placebo arm, approximately 3 participants will be randomized into the CFTR modulator stratum and 1 participant in the non-modulator stratum.

Each dose group will enroll participants in a 5:1 ratio (active : placebo) for the CFTR modulator stratum. For the non-modulator stratum, approximately 6 participants will be randomized to receive active treatment (10 mg, 25 mg, or 40 mg brensocatib) and approximately 1 participant to placebo.

If there are no safety issues observed in the first 3 dose cohorts, a 65 mg dose cohort will be similarly randomized: 7 participants (5 participants CFTR modulator and 2 participants non-modulator stratum) in active treatment and 2 participants in placebo (1 participant each in the CFTR modulator and non-modulator strata), increasing the sample size from approximately 25 to 34 participants.

The overview below illustrates the prospective number of participants based on approximately 25 participants in the first 3 cohorts (10 mg, 25 mg, and 40 mg) and approximately 9 participants in the fourth cohort (65 mg).

Table 1: Participant Randomization Scheme by Dose Cohort and CFTR Modulator Status

CFTR Modulator Status	Active (Brensocatib)				Placebo	Total Number of Participants**
	10 mg	25 mg	40 mg	65 mg*		
CFTR Modulator	5	5	5	5	4	24
Non-Modulator	2	2	2	2	2***	10
Total Number of Participants**	7	7	7	7	6	34

CFTR = cystic fibrosis transmembrane conductance regulator.
* Participants will only be randomized to the 65 mg dose cohort if there are no safety issues observed and the PK data demonstrates a need in the first 3 dose cohorts (10 mg, 25 mg, and 40 mg).
** Approximate number of participants.
*** Overall, approximately 1 participant will be randomized to receive placebo in the non-modulator stratum from the first 3 dose cohorts (10 mg, 25 mg, and 40 mg). Pending no safety issues approximately 1 participant will be randomized in the 65 mg dose cohort to receive placebo in the non-modulator stratum.

Including the Screening (up to 28 days), Treatment (28 days), and Follow-Up Periods (28 days), the study participation duration is expected to be 84 days or less. At the end of study (EoS), all ongoing AEs will be assessed by the investigators whether they are resolved and stable or not clinically significant. Any study-related safety issues or serious events that extend past Study Day 56 (EoS) will be followed to resolution.

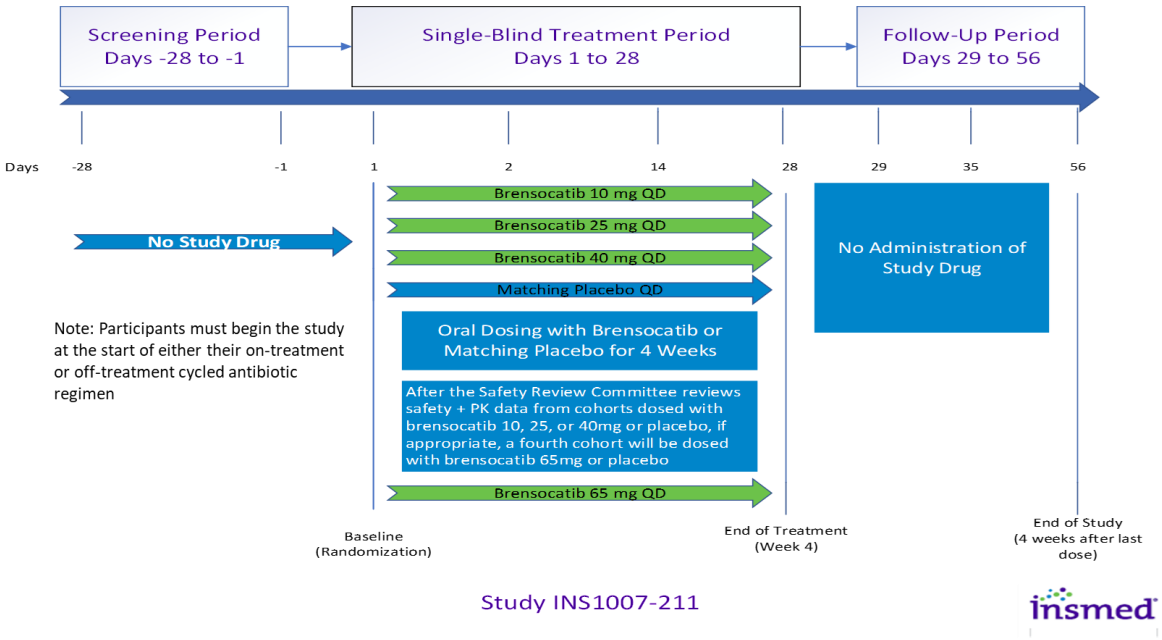
IP will be administered to the participant by the study center staff on Day 1 (Baseline), and at all in-clinic visits after Day 1 (Days 2, 14, and 28). On days when there is no in-clinic visit, participants will self-administer IP at home.

A SRC will review safety and PK data of completed cohorts exposed to brensocatib 10, 25, and 40 mg QD in an unblinded manner to determine whether brensocatib has an acceptable safety and PK profile and can be escalated to the 65 mg dose as planned (Section 5.1).

The purpose, function, conduct and structure of the SRC are further described in the SRC Charter.

The procedures and assessments conducted at each study visit in the study are provided in Section 1.3 of the CSP. Please refer to Figure 1 for a schematic diagram of the study design.

Figure 1: Study Schema



Brensocatib dose and placebo cohorts stratified to include participants who have previously received and will continue to receive CFTR modulators as concomitant medication (CFTR modulator stratum), and participants who have either never previously received CFTR modulators or have not been administered CFTR modulators within 30 days prior to Screening (Visit 1) and will not receive CFTR modulators during the study (non-modulator stratum).

4.2. Sample Size Determination

Overall, the study will initially enroll approximately 25 participants. The 10 mg, 25 mg, and 40mg arm will enroll approximately 7 participants each, and the placebo arm will enroll approximately 4 participants. For the active treatment arms, there will be 2 strata: approximately 5 participants who have previously received and will continue to receive CFTR modulators as concomitant medication and 2 participants who have either never previously received CFTR modulators or have not been administered CFTR modulators within 30 days prior to Screening (Visit 1) and will not receive CFTR modulators during the study (non-modulator stratum). For the placebo arm, approximately 3 participants will be randomized into the CFTR modulator stratum and 1 participant into the non-modulator stratum.

If there are no safety issues observed with the first 3 doses, an additional cohort will be similarly randomized: 7 participants (5 participants CFTR modulator and 2 participants non-modulator stratum) on active treatment with 65 mg and 2 participants on placebo (1 participant each in the CFTR modulator and non-modulator strata), increasing the sample size from approximately 25 to 34 participants.

A formal sample size calculation has not been performed. The number of participants has been chosen based on feasibility, as is typical and standard for such multiple ascending dose study designs and is considered sufficient to meet the study objectives.

Brensocatib AUC_{0-24} and C_{max} will be summarized by dose group and by dose group within each stratum (CFTR modulator and non-modulator) using the GM. The precision, as measured by the width of confidence intervals (CIs), for these GMs will depend on the observed variability and sample size. The following tables present descriptions of 95% CIs for a range of possible values of the geometric coefficient of variation percentage (gCV%) of the GM. Table 2 presents participants treated with brensocatib per dose group (N=10 and N=7), and Table 3 presents participants treated with brensocatib per stratum (N=5) (CFTR modulator) within dose group.

Table 2: 95% CIs for Values of Inter-Participant gCV% of the AUC_{0-24} and C_{max} GM in Each Actively Treated Cohort

N	gCV%	GM 95% CI LL	GM 95% CI UL
10	30	GM÷1.234	GM×1.234
	40	GM÷1.317	GM×1.317
	50	GM÷1.402	GM×1.402
7	30	GM÷1.312	GM×1.312
	40	GM÷1.428	GM×1.428
	50	GM÷1.548	GM×1.548

Note: Assumes N=number of participants treated with brensocatib per dose group.
gCV% = geometric coefficient of variation percentage, CI = confidence interval, GM = geometric mean, LL = lower limit, UL = upper limit.

Table 3: 95% CIs for Values of Inter-Participant gCV% of the AUC_{0-24} and C_{max} GM for Each Stratum Within Each Actively Treated Cohort

gCV%	GM 95% CI LL	GM 95% CI UL
30	GM÷1.440	GM×1.440
40	GM÷1.613	GM×1.613
50	GM÷1.798	GM×1.798

Note: Assumes N=5 (number of participants treated with brensocatib per stratum in each dose group).

The range of values for gCV% is based on the results of Day 29 AUC_{last} and C_{max} in a study of patients with non-cystic fibrosis bronchiectasis (NCFBE) (INS1007-201) and on the results of Day 28 AUC_{0-24} and C_{max} in a multiple-dose study of healthy volunteers (D6190C00001).

For the primary evaluation of safety, N=10 or 7 participants per brensocatib dose group provides 80% or 68% probability, respectively, to detect an adverse event (AE) that occurs with an incidence rate of 15%, and 90% or 80% probability, respectively, to detect an AE that occurs with an incidence rate of 21%.

4.3. Randomization

After meeting all inclusion criteria and none of the exclusion criteria, participants in each stratum (CFTR modulator and non-modulator) will be randomized by an interactive web

response system (IWRS) in a 1:1:1 ratio to 1 of the first 3 dose cohorts (brensocatib 10, 25, or 40 mg); each cohort, will enroll participants in a 5:1 ratio (active : placebo) for the CFTR modulator stratum. For the non-modulator stratum, approximately 6 participants will be randomized to active treatment in the first 3 cohorts and 1 participant will be assigned placebo. If there are no safety issues in these cohorts, a 65 mg dose cohort will be similarly randomized: 7 participants (5 participants CFTR modulator and 2 participants non-modulator stratum) in active treatment and 2 participants in placebo (1 participant each in the CFTR modulator and non-modulator strata), raising the total sample size from approximately 25 to 34 participants.

This is a single-blind study: participants are blinded to the IP to which they are randomly assigned, as are the study center staff and the Investigator. The site will contact the IWRS prior to the start of IP administration for each participant. The site will record the assigned IP label number on the applicable eCRF, if the participant receives randomized treatment. Unblinding is only to occur in the case of participant emergencies and at the conclusion of the study.

Selected individuals not involved in the conduct of the study may have access to unblinded data as needed for safety review or other data review. These individuals comprise the SRC members and the Independent Clinical Pharmacologist (refer to CSP section 10.1.8).

5. PLANNED ANALYSES

5.1. Interim Analysis / Safety Review Committee Monitoring

No formal interim analysis is planned.

Safety monitoring of the study will be conducted by an SRC. There will be a first initiation meeting without review of data. A second meeting will take place after the first 3 cohorts (brensocatib 10, 25, and 40 mg QD) have completed the study (Day 56). The SRC will review available data (PK, PD, and safety data [vital signs, clinical laboratory test values, electrocardiograms (ECG), and AEs]) in an unblinded manner from all cohorts to determine if brensocatib has shown an acceptable safety and PK profile and whether the brensocatib dose should be escalated to 65 mg QD as planned. If significant safety issues arise in between scheduled meetings, ad hoc meetings will be scheduled to review those data.

The decision on whether the maximum tolerated dose (MTD) has been reached will be based on the dose limiting toxicity (DLT). The MTD is defined in this study as the highest single dose tested without incurring DLT. However, it is recognized that the MTD may differ for patients on CFTR modulators versus those not on CFTR modulators. The signs and symptoms to consider for DLT include any events that in the opinion of the SRC are relevant to brensocatib exposure, including but not limited to hyperkeratosis, severe infection, and periodontal disease. The PK profile will also be considered and compared with those of other populations (healthy volunteers and patients with NCFBE) to assess whether similar levels of exposure have been achieved with the evaluated doses.

5.2. Final Analyses

If the 65 mg QD cohort will not be conducted, the final DBL will occur after the participants in the first three cohorts have completed the study. If the 65 mg cohort will be conducted, the final DBL will occur after the participants in all four cohorts (10 mg, 25 mg, 40 mg, and 65 mg QD) have completed the study.

6. ANALYSIS SETS

6.1. Screened Analysis Set

The Screened Analysis Set comprises all participants who provide written informed consent.

Technical Note: Participants with non-missing date of informed consent will be included.

6.2. Randomized Analysis Set

The Randomized Analysis Set comprises all randomized participants. Participants will be analyzed using the treatment to which they were randomized, regardless of the treatment received.

Technical Note: Participants with a randomization number will be included.

6.3. Intent-to-Treat Analysis Set

The Intent-to-Treat (ITT) Analysis Set comprises all randomized participants who receive at least 1 dose of the IP with at least 1 pre-dose and 1 post-dose efficacy measure. Participants will be analyzed using the treatment to which they were randomized. However, per the While-On-Treatment strategy, data after participants receive a treatment that is inconsistent with their assignment will be excluded.

Technical Note: Randomized participants who received at least 1 dose of IP and have at least 1 pre-dose and 1 post-dose assessment of the same pulmonary function test or CFQ-R will be included.

6.4. Safety Analysis Set

The Safety Analysis Set comprises all randomized participants who receive at least 1 dose of IP. Participants will be analyzed using the actual treatment they received.

Technical Note: Randomized participants who received at least 1 dose of IP will be included.

6.5. Pharmacokinetic Analysis Set

The PK Analysis Set comprises all randomized participants who receive at least 1 dose of brensocatib and have sufficient data to calculate at least 1 PK parameter.

Technical Note: Randomized participants who received at least 1 dose of brensocatib (actual treatment) and for whom at least 1 PK parameter was derived will be included. Protocol deviations will be assessed on a case-by-case basis for any potential exclusion from the analysis. Participants in this analysis set will be used for all PK summaries and statistical analyses.

6.6. Pharmacodynamic Analysis Set

The PD Analysis Set comprises all randomized participants who receive at least 1 dose of IP with at least 1 pre-dose and 1 post-dose PD measure. Participants will be analyzed using the actual treatment they received.

Technical Note: Randomized participants who received at least 1 dose of IP and who have at least 1 pre-dose and 1 post-dose PD assessment of the same PD marker will be included. Participants in this analysis set will be used for all PD summaries and statistical analyses. Separate PD analysis sets for PD in blood and PD in sputum will be defined.

7. GENERAL STATISTICAL CONSIDERATIONS

7.1. General

Tables and figures will be provided by treatment group/dose level (10 mg, 25 mg, 40 mg, and 65 mg [if conducted] QD brensocatib, pooled placebo except for PK output). If sample sizes permit, summaries will be further stratified by CFTR modulator and non-modulator. Treatment arm/dose levels will be presented in this stratified analysis if the number of observations is ≥ 2. If the assignment to a stratum differs between the randomization file and the eCRF, the randomization file will prevail. In the listings, CFTR modulator use will be indicated by + for participants on CFTR modulator and by - for the non-modulator stratum following the Subject Identifier. For tables in the demographic and safety sections, also a Total column will be presented.

Descriptive statistics for continuous variables will be calculated at scheduled visits (nominal timepoints for PK) and will include the number of participants in the treatment group/dose level of the respective analysis set (N in column header of the table), the number of non-missing- observations (n), the number of observations below limit of quantification (BLQ) for PK and PD tests, arithmetic mean, standard deviation (SD), median, minimum, and maximum. For brensocatib plasma concentrations, the GM with 95% CI and gCV%, for PK parameters (except for t_{max} and t_{last}), and PD tests, the GM and gCV% will be reported in addition. The GM will be derived by calculating the arithmetic mean of the natural log-transformed observations and then back-transforming the mean by exponentiation. The gCV% will be calculated as: 100*sqrt (exp [variance of the log-transformed values] - 1). GM and gCV% will only be calculated for quantifiable values above 0. For t_{max} and t_{last}, only n, median, minimum, and maximum will be presented. For PK parameters, no descriptive statistics will be calculated when fewer than three individual PK parameter values are available. Summaries of continuous variables other than plasma concentrations that have some values recorded using approximate values (e.g., < or >) will use the numeric part of the value in calculations. Rules for plasma concentrations BLQ or missing are given in Section 11.

Categorical variables will be summarized by counts and by percentage of participants in the corresponding categories. The number of participants in the treatment group/dose level of the respective analysis set (N in column header of the table) and the number and percentage of non-missing observations in each category will be provided. A category including missing assessments may be added if appropriate. Footnotes will specify the base for the percentages.

Data from unscheduled visits will not be used in summaries, except for the derivation of baseline.

Listings corresponding to all summaries will be provided. Listings will also show data from unscheduled visits. Listings will be presented by treatment group/dose level, Subject Identifier, and date or visit. Listings will present the data as collected in the eCRF along with variables derived from the eCRF data.

The baseline value for safety data will be the most recent non-missing value prior to the first dose of IP.

The baseline value for efficacy data will be the most recent non-missing value prior to randomization. Note that for PFT, the most recent best effort prior to randomization will be selected.

Change from baseline at visit (i) = observed value at visit (i) – baseline value.
Percent change from baseline at visit (i) =
100* [(observed value at visit (i) – baseline value) / baseline value].

Percent inhibition from baseline at visit (i) = 100 * [1 – fold change from baseline at visit (i)], where fold change from baseline at visit (i) = observed value at visit (i) / baseline value.

7.2. Analysis Methods for Estimands

This is a Phase 2a study. No formal hypothesis will be tested and there will not be a confirmatory treatment comparison.

The following ICEs have been identified for this study (see Section 3):

1. Early discontinuation from the randomized IP for any reason,
2. Not on stable CFTR modulators for participants on modulators,
3. Use of antibiotics during an acute event that could affect NSP levels.

The table below gives an overview which analysis set(s) and strategy to deal with related ICEs will be used for the Estimands.

Estimand	Analysis Set	Strategy for ICEs
Primary, Secondary, and Exploratory PK	PK Analysis Set	For the first ICE, While-on-Treatment strategy: data collected prior to the occurrence of the ICE, i.e., to discontinuation of IP, will be included in the analysis. For the second and third ICE, the Treatment Policy strategy will be used, i.e., all data will be included in the analysis.
Safety	Safety Analysis Set	For the first ICE, While-on-Treatment strategy: data collected prior to the occurrence of the ICE, i.e., to discontinuation of IP, will be included in the analysis. For the second and third ICE, the Treatment Policy strategy will be used, i.e., all data will be included in the analysis.
Exploratory PD	PD Analysis Set	For the first and the third ICE, While-on-Treatment strategy: data collected prior to the occurrence of the ICE, i.e., to discontinuation of IP or to the use of antibiotics with impact on NSP levels, will be included in the analysis.

		For the second ICE, the Treatment Policy strategy will be used, i.e., all data will be included in the analysis.
Exploratory Efficacy, Microbiology	ITT Analysis Set	For the first ICE, While-on-Treatment strategy: data collected prior to the occurrence of the ICE, i.e., to discontinuation of IP, will be included in the analysis. For the second and third ICE, the Treatment Policy strategy will be used, i.e., all data will be included in the analysis.

The While-on-Treatment strategy will be applied for the first ICE: data collected after discontinuation of IP will be excluded from the derivation of PK parameters and from statistical analysis for PK, safety, PD, and efficacy analysis.

The Treatment Policy strategy will be applied for the second ICE: data will be included in derivation of PK parameters and in the PK analysis irrespective of changes in CFTR modulator use.

For the third ICE which is applicable for PD, Insmed clinical team will review by participant listings of concomitant medication to judge whether antibiotics have been administered which may invalidate the use of the PD concentrations for the analysis. If this is the case, PD data after the start of such concomitant medication will be excluded from the statistical analysis.

Before DBL, the participants' data will be discussed during a Blind Data Review Meeting (BDRM). It will be decided whether ICEs occurred and up to which timepoint data for the While-On-Treatment strategy will be included in any analysis. The protocol deviation tracker will also be reviewed and discussed during the BDRM. The decisions will be documented in a Data Review Report (meeting minutes). Unblinding after DBL can only occur when the Data Review Report has been agreed upon. This will be documented by email approval. After unblinding, it will be checked if participants received treatment other than the randomized. Furthermore, it will be confirmed that the reasons to exclude participants or data records from an analysis set or analysis, respectively, are still in the same way valid as before unblinding. Any deviations between the pre-DBL and post-DBL data with an impact on decisions made during the BDRM will be documented in the final Data Review Report. If decisions made during the BDRM must be revised this will also be documented in the final Data Review Report and a rationale will be provided for the revision. The Data Review Report will only be finalized and signed after DBL.

7.3. Multiple Comparisons and Multiplicity

There is no formal hypothesis-testing in this study, and no inferential conclusions will be drawn from the study results. Therefore, corrections for multiple testing are not applicable.

8. STUDY POPULATION SUMMARIES

For the general layout of the tables see Section 7.1.

The study population summaries will be reported for the PK Analysis Set unless otherwise stated and will be repeated for the Safety Analysis Set. If both analysis sets comprise the same participants, only the table for the PK Analysis Set will be produced.

8.1. Subject Disposition

For participants screened, the number of participants who are screened and the number of participants who failed screening, the reasons for screen failure and whether it is related to corona virus disease 2019 (COVID-19) will be summarized.

Subject disposition will be summarized to present the number and percentage of participants (overall and within randomization strata CFTR modulator / non-modulator)

- randomized,
- randomized and treated,
- who completed/discontinued from IP and the study with reason for discontinuation.

Percentages will be based on the Screened Analysis Set. Percentages of reasons for discontinuation of the study/IP will be based on the participants who discontinued the study/IP.

Number and percentage of participants included in each of the analysis sets described in Section 6 will also be presented (overall and within randomization strata CFTR modulator / non-modulator).

Reasons for exclusion from analysis sets will be summarized using number and percentage of participants. The percentages will be based on the Screened or Randomized Analysis Sets.

8.2. Protocol Deviations

Deviations from the CSP will be monitored throughout the conduct of the study. Rules and definitions are included within the study document Study Deviation Rules.XLSX.

Significant protocol deviations will be summarized by number and percentage of participants. All protocol deviations will be listed. A separate listing for protocol deviations due to COVID-19 will be provided.

8.3. Demographic and Baseline Characteristics

The following demographic and baseline characteristic variables will be summarized:

- age and age category (< 30, ≥ 30 years),
- sex,
- ethnicity and race,
- height, weight, and body mass index at Screening,
- reported diagnosis of bronchiectasis,
- type of CFTR modulator (categories),
- type of antibiotics for stable CF treatment (inhaled, oral, nasal),
- use of pancreas enzymes,

- Pre-bronchodilator FEV_1 and $ppFEV_1$ at Baseline,
- NE, CatG, and PR3 range categories in sputum at Baseline.

The information about the bronchiectasis diagnosis will be obtained from the medical history eCRF, the type of antibiotics for stable CF treatment, and the use of pancreas enzymes from the concomitant medication eCRF reviewed and selected by Insmed.

8.4. Medical History

All medical history entries will be coded using Medical Dictionary for Regulatory Activities (MedDRA). Number and percentage of participants with medical history findings will be summarized by System Organ Class (SOC) and Preferred Term (PT).

Participants will be counted once in each SOC and in each PT.

8.5. Prior and Concomitant Medications and Procedures

Prior and concomitant medications will be coded using the World Health Organization (WHO) Drug dictionary.

The reported medication will be classified as 'Prior only', 'Prior and concomitant' or 'Concomitant only'. These 3 categories are mutually exclusive:

- 'Prior only': if the participant has not taken any IP; or if the medication end date is before IP start date. If the medication end date is partially missing, the medication will only be assigned to 'Prior only' if the partial date gives clear evidence that the medication stopped before IP start.
- 'Concomitant only': if the medication start date is on or after IP start date. If the medication start date is partially missing, the medication will only be assigned to 'Concomitant only' if the partial date gives clear evidence that the medication started on or after IP start date.
- 'Prior and concomitant': if the medication start date is before IP start date and the medication end date is on or after IP start date. If the medication start date or end date are partially or completely missing, the medication will be assigned to 'Prior and concomitant' unless there is clear evidence that the medication stopped before IP start date (that is, the medication should be assigned to 'Prior only') or that the medication started after IP start date (that is, the medication should be assigned to 'Concomitant only').

Concomitant medications (i.e., medication classified as 'Concomitant only' or 'Prior and concomitant') will be summarized showing the number and percentage of participants taking concomitant medications by Anatomical Therapeutic Chemical (ATC) classification level 4 and PT within ATC4. If the ATC level 4 coding is not available for a PT, the next available lower-level ATC code will be used.

Non-pharmacological procedures will be listed but not summarized.

8.6. COVID-19 Impact

Potential COVID-19 impact is captured in the eCRF and will be summarized by participant.

Subject disposition summary and listing will include (see Section 8.1):

- Screen failure related to COVID-19
- Early termination of study related to COVID-19

AE overview table and listings will include (see Section 10.2):

- AEs that fall into the category of COVID-19 related

Summary table and listing of dose interruption will include (see Section 10.1):

- Dose interruption related to COVID-19

Summary and listing of protocol deviations will include (see Section 8.2):

- Protocol deviations due to COVID-19

Early termination of study related to COVID-19 will be determined using the AE eCRF form: if the question "was the AE COVID-19 related?" is answered with "yes" and this AE caused early termination of the study, this is considered early termination of the study related to COVID-19.

9. EFFICACY

For the general layout of the tables see Section 7.1.

The primary and secondary endpoints are related to PK and Safety (AEs) (see Section 11 and Section 10.2) and will be analyzed using the PK and Safety Analysis Sets, respectively. The efficacy in this study is exploratory and will be analyzed using the ITT Analysis Set.

For the exploratory endpoints related to PD, refer to Section 12. Analysis will be based on the PD Analysis Set.

The strategies for ICEs as outlined in detail for the analysis of the endpoints related to spirometry and CFQ-R in Section 3.3 and Section 7.2 will be applied.

9.1. Spirometry

Spirometry tests include the following: prebronchodilator FEV_1, prebronchodilator $ppFEV_1$, FVC, and $FEF_{(25-75\%)}$.

Observed values and change from baseline at Day 28 will be summarized descriptively by scheduled visit. If applicable (more than 5 participants who prematurely discontinued the study) a separate analysis using the end of treatment (EoT) assessment, i.e., Day 28 or Early Discontinuation, will be done.

An analysis of covariance will be performed for change from baseline at Day 28 including the baseline value in the model as fixed covariate and the treatment group/dose level as fixed factor. The analysis will be stratified by CFTR modulator / non-modulator if sample sizes permit (see Section 7.1). Least Squares (LS) means for each treatment group and dose levels with 95% CIs and LS mean differences between each Brensocatib dose level and placebo with 95% CIs will be provided.

9.2. CFQ-R

The CFQ-R (Appendix A) is a disease-specific QoL measure with 9 QoL domains (Physical Functioning, Role Limitations, Vitality, Emotional State, Social Limitations, Body Image, Eating Disturbances, Treatment Burden, Health Perception) that assess the impact of CF on overall health, daily life, and perceived well-being. There are 3 symptom scales: weight, respiratory, and digestion. Scores range from 0 to 100, with higher scores indicating better health.

Observed values and change from baseline for the 9 QoL domains and the 3 symptom scales will be summarized descriptively by scheduled visit.

A repeated measurement model will be used for the analysis of change from baseline in CFQ-R respiratory symptom scale. The model will include the baseline value as a fixed covariate and the treatment group/dose level, visit, and treatment group/dose level-by-visit interaction as fixed factors. Given the small sample size in each dose level, estimation of a complex variance-covariance structure with many parameters will be untenable. Therefore, the variance-covariance structure will be compound symmetric with the robust sandwich variance estimator.

This analysis will be performed using the MIXED procedure in SAS. If sample sizes permit, the analysis may be stratified by CFTR modulator / non-modulator. LS means for each treatment group and dose levels with 95% CIs and LS mean differences between each Brensocatib dose level and placebo with 95% CIs will be provided. The analysis will only be provided if data allow for such an analysis.

9.3. Microbiology

Microbiology results will be presented by descriptive statistics for the ITT Analysis Set.

Number and percentage of participants with any microbial detection in quantitative measures will be summarized by scheduled visit. Descriptive statistics will be provided for change from baseline in the number of colony-forming units.

For microbials in qualitative measures, a shift summary for the categories will be provided from Baseline to Day 28/Last Visit.

10. SAFETY

For the general layout of the tables see Section 7.1.

Analyses will be based on the Safety Analysis Set, unless otherwise stated.

Unless otherwise stated all summaries will have corresponding listings.

10.1. Study Drug Exposure and Compliance

10.1.1. Treatment and Study Duration

Duration of study, actual and planned treatment are Safety components and will be analyzed for the Safety and PK Analysis Sets.

Study duration will be calculated as number of days between randomization and EoS visit:

study duration (days) = date of EoS visit – date of randomization + 1

Actual treatment duration will be calculated as

actual treatment duration (days) = date of last dose – date of first dose + 1

This definition does not account for protocol-allowed dose interruptions for safety reasons.

IP exposure duration will be calculated as:

exposure duration (days) =actual treatment duration – number of missed doses if reason was AE.

Descriptive statistics will be provided for duration of

- study,
- treatment and
- exposure.

Number and percentage of participants will be presented for categories of treatment duration

- ≤ 7 days,
- >7 to ≤ 14 days,
- >14 to ≤ 21 days,
- >21 to ≤ 28 days,
- >28 days.

The last category is inserted to confirm compliance with the planned treatment duration.

10.1.2. Dose Interruptions

Dose interruptions will be recorded in the eCRF as number of missed doses, reason, and start and end date of dose interruption. The number and percentage of participants with missed IP doses by reason and descriptive statistics for the number of missed doses by reason will be presented. Summary tables will be given for the PK and the Safety Analysis Sets.

10.1.3. Compliance

Compliance summary tables will be provided for the PK Analysis Set. The listings will be provided for the Safety Analysis Set. The actual treatment will be compared with the planned treatment.

Participants will be required to bring all their used and unused IP supplies to in-clinic study visits during the treatment period or, if the visit is being conducted virtually, have all used and unused IP supplies available to be accounted for.

The number of expected doses will be calculated based on the planned treatment duration (since the therapy is prescribed QD) and the number of missed doses due to AE (see Section 10.1.1). At days of interruption of IP due to safety reasons (reason for interruption = 'Adverse Event') no doses are expected. Drug is dispensed at the Day 1 and Day 14 visit and returned drug is counted at the Day 14 and Day 28 visit, respectively. To assess compliance, the planned treatment duration will be calculated for these periods based on the visit dates as:

Day 1 to Day 13:
Planned treatment period (days) = Day 14 visit date – Day 1 visit date.

Day 14 to Day 28:
Planned treatment period (days) = Day 28 visit date – Day 14 visit date + 1.

Day 1 to Day 28:
Planned treatment period (days) = Day 28 visit date – Day 1 visit date + 1.

The planned treatment duration from Day 1 to Day 28 may vary between 24 and 32 days due to a time window of ± 2 days allowed at the Day 14 and Day 28 visit. If a participant has a planned treatment duration of less than 24 or more than 32 days, the planned treatment duration will be set to 24 or 32 days, respectively, for the calculation of compliance.

Expected doses = planned treatment period - number of missed doses if reason was AE.

Compliance (%) = 100 × (number of randomized doses / number of expected doses).

If the actual treatment differs from the randomized treatment, tablets in the actual treatment will not be counted for the number of randomized doses.

Compliance to IP will be categorized (<80%, 80-120%, >120%) and summarized by number and percentage of participants in the categories and descriptive statistics for the continuous compliance value.

10.2. Adverse Events

The second primary endpoint is the incidence of TEAEs. The strategies as outlined in Section 3.1 and Section 7.2 will be applied for ICEs.

The AE verbatim descriptions (Investigator terms from the eCRF) will be coded by standardized medical terminology using MedDRA. AEs will be coded to primary SOC and PT using the most recent MedDRA version.

A TEAE is defined as any AE that occurs on or after the first dose of IP and within 28 days after the last dose of IP. AEs that occur between the time the participant had signed the Informed Consent Form for the study and before the participant receives the first dose of IP on Study Day 1 will be summarized as medical history and not as an AE unless the event meets the definition of a serious adverse event (SAE).

Where AE start dates are missing or partially missing, AEs will be assumed to be treatment-emergent, unless the partial start date or the AE end date gives clear evidence that the AE started before the first administration of IP.

Table 4: TEAE Assignment in Case of Missing AE Start Date Elements

Missing elements of AE start	Rule (IP start = first dose of IP)	Classification
Regardless of any missing information for AE start: AE end date < IP start date		non-TEAE
Otherwise (i.e., if AE end date ≥ IP start date)		
- day/month/year	all AEs	TEAE
- day and month	AE start year ≥ IP start year	TEAE
	AE start year < IP start year	non-TEAE
- day	AE start month/year ≥ IP start month/year	TEAE
	AE start month/year < IP start month/year	non-TEAE

Duration of AEs will be calculated in days as:

AE end date - AE start date + 1.

If the start or the end date of the AE is not complete, the duration cannot be calculated and will be missing.

Summaries that are displayed by SOC and PT or by PT and will be ordered by descending order of total number of participants of SOC and PT within each SOC or by descending order of total number of participants of PT. SOCs and PTs with the same number of participants will further be ordered by number of events. In the case of the same number of events, SOCs and PTs will be ordered alphabetically.

Summary tables will present number and percentage of participants with TEAEs and number of events.

The following tables will be presented:

- Overall summary of TEAEs, specifically participants with at least one:
  - TEAE,
  - TEAE related to IP,
  - serious TEAE,
  - serious TEAE related to IP,
  - TEAE resulting in death,
  - TEAE of special interest (TEAESI),
  - TEAE leading to withdrawal from the study/IP, and
  - TEAE related to COVID-19.
- Participant incidence of TEAEs and number of TEAEs by MedDRA SOC and PT (for ordering see specification above):
  - TEAEs,

- TEAEs related to IP,
- TEAEs by severity,
- TEAEs leading to study/IP withdrawal,
- TEAEs related to COVID-19,
- serious TEAEs, and
- serious TEAEs related to IP.

- Participant incidence of TEAEs and number of TEAEs by MedDRA PT (for ordering see specification above).

All AEs will be listed.

10.2.1. Adverse Events of Special Interest

AESIs include the following:

- Periodontitis/gingivitis
- Hyperkeratosis
- Serious infections
- Pneumonia

AESIs will be identified by the entries in the corresponding eCRF pages.

Hyperkeratosis TEAESIs will be summarized by location and signs. Dental (periodontitis / gingivitis) will be summarized by PT and by the outcome of the Investigator evaluation. Serious infections will be summarized by body system and PT. Pneumonia will be summarized by symptoms / laboratory findings and PT.

10.2.2. Deaths

A listing of all deaths (from the eCRF "Death details") including AEs resulting in death will be provided.

10.3. Clinical Laboratory Evaluations

For the general layout of the tables see Section 7.1.

Continuous laboratory tests (hematology, clinical chemistry, and coagulation) will be summarized using descriptive statistics at scheduled visits (Screening, baseline, and EoT [Day 28 or Early Discontinuation]) for observed values and changes from baseline.

Shift tables (i.e., low-normal-high at baseline versus low-normal-high at EoT in a 3-by-3 contingency table) will be provided to assess changes in laboratory values from baseline to last visit with respect to the reference range. Reference ranges established by the central laboratory will be used to determine the categories. Values below the lower limit (LL) of the reference range will be classified as "low". Values above the upper limit (UL) of the reference range will be classified as "high". Values within the reference range will be classified as "normal".

Clinical laboratory results will be provided in participant listings.

Participants experiencing alanine aminotransferase or aspartate aminotransferase ≥ 3 × upper limit of normal (ULN) and total bilirubin > 2 × ULN according to Hy's Law will be listed.

10.4. Vital Signs

Systolic and diastolic blood pressure, pulse, respiratory rate, oxygen saturation, temperature, height, and weight will be summarized by scheduled visits.

Descriptive summaries of observed values and absolute and percentage changes from baseline will be based on available data.

10.5. Electrocardiogram

ECG will be summarized by scheduled visits. ECG will be collected in triplicates. The mean of the triplicates will be summarized, and all measurements will be listed.

Descriptive summaries for the mean of the triplicate collections of the 12-lead ECG tracings for observed values and changes from baseline will be based on available data. Number and percentage of participants with ECG findings will be summarized by scheduled visit and timepoint for investigator and vendor interpretation.

Number and percentage of participants experiencing extraordinary ECG values will be summarized by visit:

- QTcF > 450 ms, QTcF > 480 ms,
- change from baseline in QTcF > 30 ms, change from baseline in QTcF > 60 ms.

10.6. Other Safety Assessments

10.6.1. Physical Examination

Any abnormalities noticed by the investigator during the Physical Examination at Screening will be recorded in the medical history, or as an AE if occurring or worsening after signing the informed consent form.

Dates of Physical Examination will not be listed.

10.6.2. Pregnancy

Pregnancy results will be listed for all participants with outcome.

11. PHARMACOKINETICS

Individual PK parameters of brensocatib will be determined by non-compartmental analysis (NCA) using Phoenix® WinNonlin® (WNL) version 8.2 or later (Certara L.P, Princeton, New Jersey). PK outputs will be prepared using SAS Version 9.4 or higher (SAS Institute, Inc., Cary, North Carolina). The primary PK endpoints are brensocatib C_{max}, t_{max}, and AUC_{0-24} after single dose administration on Day 1; and C_{max}, t_{max}, AUC_{0-24}, and $t_{½}$ after multiple dose administration on Day 28. Brensocatib plasma concentrations and PK parameters, including the ones that are not included in the primary endpoint, will be summarized as described in Section 7.1. Statistical summary tables and mean plasma concentration by time figures will be presented based on the PK Analysis Set. Brensocatib plasma concentration by time figures for individual participants and listings will be based on the Safety Analysis Set. PK parameters will be listed for the PK Analysis Set.

Strategies for ICEs as outlined in Section 3.1, 3.2, and 7.2 will be applied.

If calculable, the following PK parameters listed in Table 5: will be determined for brensocatib in plasma following single oral dose administration (Day 1).

Table 5: Plasma PK Parameters After Single Dose Administration or First Dose Administration of Multiple Dose Study

Parameter	WNL Name	CDISC Name	Definition
C_{max}	Cmax	CMAX	Maximum observed plasma concentration
t_{max}	Tmax	TMAX	Time to C_{max}
t_{last}	Tlast	TLST	Time of the last measurable (positive) concentration
AUC_{0-24}	AUC0_24	AUC24	AUC from time T1=0 to T2=24 hours
AUC_{last}	AUClast	AUCLST	AUC from time T1=0 to T2=the last timepoint with measurable concentration

CDISC = Clinical Data Interchange Standards Consortium.

If calculable, the PK parameters listed in Table 6 will be calculated for brensocatib in plasma following multiple oral dose administration after last dose administration (Day 28).

Reasons for PK parameters not calculable will be listed.

Table 6: Plasma PK Parameters after Multiple Dose Administration after Last Dose

Parameter	WNL Name	CDISC Name	Definition
C_{trough}	Ctrough	CTROUGH	Concentration at the end of a dosing interval [taken directly before next administration]; Obtained on Days 2, 14, 28 and 29

Parameter	WNL Name	CDISC Name	Definition
C_{max}	Cmax	CMAX	Maximum observed plasma concentration in a dosing interval
C_{min}	Cmin	CMIN	Minimum observed plasma concentration in a dosing interval
t_{max}	Tmax	TMAX	Time to C_{max}
t_{last}	Tlast	TLST	Time of the last measurable (positive) concentration
$t_{½}$	HL_Lambda_z	LAMZHL	Elimination half-life
λ_z	Lambda_z	LAMZ	Elimination rate constant
AUC_{0-24}	AUC0_24	AUC24	AUC from time T1=0 to T2=24 hours
AUC_{last}	AUClast	AUCLST	AUC from time T1= 0 to T2=the last timepoint with measurable concentration
AUC_{∞}	AUC∞	AUCINF	AUC from time T1=0 to T2=infinity
CL/F	CLss_F	CLFTAU	Total clearance following oral administration
V_d/F	Vz_F	VZFTAU	Volume of distribution at terminal phase following oral administration
C_{av}	Cavg	CAVG	Average concentration over the dosing interval
$R_{ac,AUC}$	RacAUC*	ARAUC	Accumulation ratio calculated from AUC_{0-24} at steady state divided by AUC_{0-24} after single dosing
$R_{ac,Cmax}$	RacCmax*	ARCMAX	Accumulation ratio based on C_{max} calculated from C_{max} at steady state divided by C_{max} after single dosing

* Obtained through additional calculations in WNL outside of NCA model.

11.1. Pharmacokinetic Analysis, Concentration, and Parameter Tables, Listings, and Figures, and Statistical Analysis of Pharmacokinetic Parameters for Final Analysis

Any anomalous PK plasma concentration values observed at pre-dose on Day 1 will be identified in the CSR and reported in listings. All pre-dose values (BLQ and any anomalous values) will be set to zero.

PK plasma concentrations that are collected outside the acceptable collection windows (Section 1.3.1, CSP) will be flagged in the listing with a footnote that these concentrations are excluded from the concentration summary.

11.1.1. Pharmacokinetic Concentrations

Concentration Listings:

Concentration listings will include nominal PK sampling time, actual sampling times relative to dose administration, deviation from nominal time, percent deviation from nominal time, and concentrations. Plasma concentrations below the lower limit of quantification (LLOQ) will be presented as BLQ in the listings and the LLOQ value presented as a footnote. Missing PK samples will be reported as no sample or not reportable as appropriate and considered excluded from PK analysis.

Concentration Summary Tables:

Source data as reported from the laboratory will be used for calculation of concentration summary statistics. Concentrations for brensocatib will be summarized by use of CF modulator, brensocatib dose level, day, and nominal timepoint. See Section 7.1 for details of the statistics to be included.

For summary tables, all BLQs will be considered zero, and the number of BLQs and non-BLQs at each scheduled timepoint will be reported. Summary statistics will not be calculated if the number of non-BLQ concentrations at a scheduled timepoint is <3 and will be reported as not calculable (NC).

The rules followed for calculation and presentation of concentration data with regards to the number of decimal places/significant digits for the listings of participant level concentrations and summary tables of concentration are as follows:

Concentration Listings and Tables	Rounding
Individual Concentrations	*n* s.d. as supplied by bioanalytical laboratory
Minimum and Maximum	*n* s.d. capped at 4
Mean, SD, Median, GM	*n+1* s.d. capped at 4
CV%, gCV%	*1* d.p.
N, n	whole number

s.d. = significant digits, d.p. = decimal place.

Concentration Figures:

For arithmetic mean linear/linear graphs, all BLQ values will be substituted with zero for calculation of arithmetic mean and for log/linear graphs the mean of natural log transformed values will be displayed (this should not include zero).

For individual linear/linear and log/linear graphs all BLQ values will be substituted as follows:

- BLQ values on Day 1 pre-dose will be set to 0. When using log/linear scale, these values will be considered missing.
- BLQ values post-dose will be set to missing.

To visualize participant-level concentrations and the comparison between groups for each treatment, the descriptive PK graphs listed below will be generated. LLOQ will be given in a footnote.

- Individual participant profiles for plasma concentration data over time on linear/linear and log/linear scale (log scale for concentration data, time on linear scale), stratified by PK Day (Day 1 and Day 28).
- Mean (+ SD) profiles for plasma concentrations over time on linear/linear and log/ linear scale (SD will only be displayed on linear/linear scale) by dose level overlaid in the same figure, for each PK Day [Day 1 and Day 28].
- Mean (+ SD) profiles for plasma trough concentrations (pre-dose values) over time from Day 2 to Day 29.

Figures will be generated using unique line style and marker for each plot in the graph. For all PK concentration-time plots, linear scale will be used for the x-axis (i.e., do not use an ordinal scale). Actual sampling time will be used for individual plots while scheduled sampling time will be used for mean plots.

11.1.2. Pharmacokinetic Parameters

PK parameters will be calculated by NCA methods from the concentration-time data as specified in beginning of Section 11 following these guidelines:

- Actual time from dose will be used in the calculation of all derived PK parameters, except when parameters are calculated for safety/dose escalation meetings when nominal times may be used.
- There will be no imputation of missing data.
- Handling of BLQ samples for derivation of plasma PK parameters after single and multiple dose administration:
    - BLQs for Day 1 at the beginning of a participant profile (i.e., pre-dose and leading BLQs) will be set to zero.
    - BLQs between evaluable concentrations (embedded BLQs), will be set to missing before the calculation of the PK parameters.
    - Terminal BLQs (trailing BLQs at the end of participant profile) will be set to missing.

Participants with partial data will be evaluated on a case-by-case basis to determine if sufficient data are available for reliable estimation of PK parameters. PK parameters will be estimated according to the guidelines presented in Table 7: .

Table 7: PK Parameter and Estimation

Parameter	Guideline for Derivation
C_{max}, C_{min}, t_{max}, C_{trough}, t_{last},	Obtained directly from the observed concentration-time data

Parameter	Guideline for Derivation
AUC_{last}	The AUC from zero time (pre-dose) to the time of last quantifiable concentration will be calculated by a combination of linear and logarithmic trapezoidal methods. Unless specifically requested and justified, the linear up/log down trapezoidal method will be employed for all AUC calculations. The AUC_{last} is the sum of areas up to the time of the last quantifiable sample: $$AUC_{last}=\int_0^t C_{last} * dt$$
AUC_{0-24}	The AUC from zero time to the specific time x is the sum of areas up to the specific time x sample, where x=actual time at 24h: $$AUC_{0-x} = \int_0^x Cx * dx$$ If the PK sample at 24h is collected out of time window, AUC_{0-24} and its related parameters will be flagged in the listing but included in the analysis.
$AUC\infty$	The area from zero time extrapolated to infinite time (AUC_∞) will be calculated as follows: $AUC_\infty=AUC_{0-last}+C_{last}/\lambda z$ where C_{last} is the last observed quantifiable concentration.
λ_z and $t_{½}$	1. The terminal phase first-order rate-constant (λ_z) will be estimated. 2. A minimum number of three data points in the terminal phase will be used in calculating λ_z with the adjusted correlation coefficient (R^2 adjusted) ≥ 0.8. Otherwise, λ_z will be considered not calculable, and all the λ_z dependent parameters (i.e., $t_{½}$, CL/F, and Vd/F) will be flagged in listings and excluded accordingly from summary tables and statistical analysis of PK parameters. The reason for exclusion will be listed/footnoted in parameter listings. *3.* Unless otherwise determined by PK Scientist's best knowledge and judgment, the interval used to determine λ_z should be equal or greater than 1.5-fold the estimated $t_{½}$, and if less than 1.5-fold, λ_z will be flagged in listings and might be excluded (based on sponsor specific requirements) from summary tables and statistical analysis of PK parameters. All the derived parameters (i.e., $t_{½}$, CL/F, and Vd/F) may also be flagged in listings and excluded from statistical analysis of PK parameters. The reason for exclusion will be listed/footnoted in parameter listings. 4. The $t_{½}$ will be calculated as follows: $t_{½} = \ln 2/\lambda_z = 0.693/\lambda_z$.
CL/F	Following extravascular (e.g., oral) administration, apparent systemic clearance of parent drug following steady state administration will be calculated from: $$CL/F = \frac{Dose}{AUC_{0-24}}$$ The administered brensocatib dose will be used for the calculation.
V_d/F	Volume of distribution at terminal phase following either intravascular or extravascular dosing after steady state administration may be calculated from: $$V_d/F = \frac{Dose}{\lambda_z \times AUC_{0-24}} = (CL/F)/\lambda_z$$

Parameter	Guideline for Derivation
C_{av}	Average concentration at steady state (Day 28) will be calculated as $C_{av} = AUC_{0-24}/24$
R_{ac}	Accumulation ratio, calculated from comparison of single dose and steady state data: R_{acAUC} = *Steady state* AUC_{0-24}/ *Single dose* AUC_{0-24} R_{acCmax} = *Steady state* C_{max}/ *Single dose* C_{max}

PK Parameters Listings:

PK parameters will be listed by participant. PK parameters that will be flagged and/or excluded from summary tables and statistical analyses of PK parameters will be flagged and footnoted with the reason for flagging/exclusion.

The Phoenix NCA core output will be provided as a Word document.

PK Parameter Summary Tables:

Biostatistics group will consider the derived PK parameters as source data and will use this data without rounding for calculation of PK parameters summary statistics tables.

PK parameters will be summarized as specified in Section 7.1.

The rules followed for presentation of PK parameters data with regards to the number of decimal places/significant digits for the listings of participant level PK parameters and summary tables of PK parameters are as follows:

Table 8 Presentation of PK Parameters in Tables and Listings

PK Parameter Tables and Listings	Rounding
Derived Individual Parameters	3 s.d.
Directly Derived Individual Parameters (C_{max}, C_{trough}, C_{min})	*n* s.d. as supplied by the analytical laboratory but not more than 3 s.d.
Minimum and Maximum	3 s.d.
Mean, SD, Median, GM	3 s.d.
CV%, gCV%	1 d.p.
Comparative Estimates (e.g., Ratios)	3 d.p.
CI and Percentages	2 d.p.
p-Values	4 d.p.
N/n	whole number
Exceptions for PK Tables	
t_{max} Individuals, and Minimum, Maximum	2 d.p.
t_{max} Median Only	2 d.p.

s.d. = significant digits, d.p. = decimal place

PK Parameter Figures:

Steady state will be assessed based on determination of individual half-lives. Dose-proportionality plots are described in the following section.

11.1.3. Assessment of Dose Proportionality

Analysis of dose dependency for brensocatib $AUC_{0\text{-}24}$, AUC_{last}, and C_{max} after single-dose administration and at steady state (Day 28) will be performed using a power law model[2]. The logarithm of the PK parameter will be linearly related to the logarithm of the dose using a linear regression analysis in the following way:

$$\ln(PK) = ß_0 + ß_1 \cdot \ln(dose),$$

where ln(PK) represents the natural log transformed $AUC_{0\text{-}24}$, AUC_{last}, and C_{max} parameter and ln(dose) represents the natural log transformed dose as fixed factor.

LS estimates of the slope of the regression and corresponding 90% CIs will be obtained.

Dose proportionality plots for C_{max}, $AUC_{0\text{-}24}$, and AUC_{last} at Day 1 (after single dose administration) and at Day 28 (after multiple dose administration): the natural log-transformed PK parameter versus natural log-transformed dose will be displayed with the fitted line from the power law model. The individual natural log-transformed data will be displayed around the fitted line. The CFTR modulator / non-modulator groups will be displayed on the same plot.

A categorical covariate for CFTR modulator stratum or separate analyses by stratum may be conducted if the C_{max}, $AUC_{0\text{-}24}$, and AUC_{last} values between strata are dissimilar, as assessed by visual inspection of the above dose proportionality plots for C_{max}, $AUC_{0\text{-}24}$, and AUC_{last} parameters. A clear separation of the individual data for CFTR modulator / non-modulator groups suggests dissimilarity in the PK profile between the two CFTR strata. Analyses will be repeated for brensocatib PK parameters after multiple-dose administration on Day 28.

In addition, pairwise comparisons of dose dependency among brensocatib dose levels may be performed based on dose-normalized C_{max}, $AUC_{0\text{-}24}$, and AUC_{last} separately for Day 1 and Day 28. An analysis of variance will be run with the natural-log transformed ratio of the PK parameter divided by the dose as dependent variable and the dose level as independent fixed effect. The LS means and LS mean difference will be back transformed to obtain the geometric means and ratio of geometric means along with corresponding 90% CI. If dose proportionality holds between the 2 dose levels being compared, the ratio of geometric means will be approximately 1.00.

If dose proportionality applies, data within stratum (CFTR modulator and non-modulator) will be pooled across dose levels. The log-transformed dose-adjusted C_{max}, $AUC_{0\text{-}24}$, and AUC_{last} for Day 1 and Day 28 will be used as dependent variable in an analysis of variance with the CFTR stratum as independent variable. The LS mean for the difference between strata with 90% CI will be estimated from the model. The estimate and lower and upper confidence limits will be back transformed by exponentiation. The back-transformed difference between the strata is the GM ratio of the strata.

11.1.4. Pharmacokinetic-Pharmacodynamic Analysis

Additional PK-PD evaluations will be specified in a separate PK/PD Analysis Plan:

- To evaluate the PK/PD relationship between AUC/dose and biomarker activity (blood and/or sputum),
- To evaluate the PK/PD relationship between systemic exposure and safety (e.g., AESIs of hyperkeratosis, periodontitis/gingivitis, and infections),
- To evaluate the PK/PD relationship between systemic exposure and clinical efficacy (i.e., $ppFEV_1$ and CFQ-R).

12. PHARMACODYNAMICS

For the general layout of the tables see Section 7.1.

PD Tests NE, CatG, and PR3 will be assessed in blood and in sputum. Baseline is defined in Section 7.1. The most recent non-missing value before the first dose of IP will be used as baseline which is either the assessment at Day 1 pre-dose or if this is not available the assessment at Screening. If both assessments are not available, the participant will not be included in the PD analysis set.

The strategies for the ICEs as outlined in Section 3.3 and Section 7.2 will be applied.

Descriptive statistics (including GM and gCV% for observed values and number of values BLQ) will be provided for observed values and %inhibition from baseline by scheduled visit (on natural scale). %Inhibition from baseline will be calculated as 100 * (1 - fold change from baseline), where fold change = post baseline value / baseline value. Observed values that are BLQ will be set to 0 in the calculation of descriptive statistics. If sample protein concentration data are available, the activity of NE, CatG and PR3 should be normalized by the protein concentration, to generate activity values of ng/ml/mg protein, and the normalized NSP data will be presented in the same way as described above.

The neutrophil count summary will be included in the safety (hematology) analyses.

The following figures will be provided for each PD test for concentrations in blood and in sputum:

- Mean (+/- SD) over time using scheduled sampling time on natural scale by dose level overlaid in the same figure.
- Mean %inhibition (+/- SD) values over time using scheduled sampling time by dose level overlaid in the same figure.
- Individual participant profiles for PD data over time at scheduled visits – the participant's dose levels will be distinguished by different colors (spaghetti plots).

13. CHANGES TO PROTOCOL-SPECIFIED ANALYSES

Currently, no changes from the protocol-specified analyses are planned.

A Screened Analysis Set was defined in Section 6.1 in addition to the analysis sets defined in the CSP.

14. REFERENCES

1. Henry B, Aussage P, Grosskopf C, Goehrs JM. Development of the Cystic Fibrosis Questionnaire (CFQ) for assessing quality of life in pediatric and adult patients. Qual Life Res. 2003; 12:63-76.
2. Smith BP, Vandenhende FR, DeSante KA, Farid NA, Welch PA, Callaghan JT, Forgue, ST. Confidence Interval Criteria for Assessment of Dose Proportionality. Pharmaceutical Research 2000; 17: 1278-1283.

APPENDIX A. CFQ-R

The items in the CFQ-R are expressed either "negatively" or "positively". To align the direction of expression, some of the items must be recoded before the scores for the domains are calculated. The items 6, 10, 13, 15, 17, 18, 23, 28, 30, 32, 34, and 35 will be re-coded by using the formula: recoded score = 5 - original score.

The QoL domains and symptom scores will be derived in the following way:

CFQ-R Domain/ Symptom Score	**Formula**[1] [1] CFQRx: answer to CFQ-R question from eCRF. CFQRx*: recoded question.
Physical Functioning	100*((mean of (CFQR1, CFQR2, CFQR3, CFQR4, CFQR5, CFQR13*, CFQR19, CFQR20) - 1)/3) if at least 50% of the questions are non-missing
Role Limitations	100*((mean of (CFQR35*, CFQR36, CFQR37, CFQR38) - 1)/3) if at least 50% of the questions are non-missing
Vitality	100*((mean of (CFQR6*, CFQR9, CFQR10*, CFQR11) - 1)/3) if at least 50% of the questions are non-missing
Emotional State	100*((mean of (CFQR7, CFQR8, CFQR12, CFQR31, CFQR33) - 1)/3) if at least 50% of the questions are non-missing
Social Limitations	100*((mean of (CFQR22, CFQR23*, CFQR27, CFQR28*, CFQR29, CFQR30*) - 1)/3) if at least 50% of the questions are non-missing
Body Image	100*((mean of (CFQR24, CFQR25, CFQR26) - 1)/3) if at least 2 questions are non-missing
Eating Disturbances	100*((mean of (CFQR14, CFQR21, CFQR50) - 1)/3) if at least 2 questions are non-missing
Treatment Burden	100*((mean of (CFQR15*, CFQR16, CFQR17*) - 1)/3) if at least 2 questions are non-missing
Health Perception	100*((mean of (CFQR18*, CFQR32*, CFQR34*) - 1)/3) if at least 2 questions are non-missing
Weight	100*(CFQR39-1)/3 if CFQR39 is non-missing
Respiratory	100*((mean of (CFQR40, CFQR41, CFQR42, CFQR44, CFQR45, CFQR46) - 1)/3) if at least 50% of the questions are non-missing
Digestion	100*((mean of (CFQR47, CFQR48, CFQR49) - 1)/3) if at least 2 questions are non-missing