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: Protocol Version 3.0: 26-July-2022

Signature Page for INS1007-211 Protocol Amendment No. 2

Approve	[redacted] Clinical Development 26-Jul-2022 17:27:17 GMT+0000

CLINICAL STUDY PROTOCOL

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

Protocol Number:	INS1007-211
Version Number:	3.0
Amendment Number:	2 (global)
Content Final Date:	26 JUL 2022
Compound:	Brensocatib (INS1007)
Brief Title:	A Safety, Tolerability, and Pharmacokinetic Study of Brensocatib in Adult Participants With Cystic Fibrosis
Study Phase:	2a
Sponsor Name:	Insmed Incorporated
Legal Registered Address:	700 US Highway 202/206 Bridgewater, NJ 08807-1704 USA
Manufacturer:	Insmed Incorporated
Regulatory Agency Identifier Number(s):	IND: 133790

Important Contact Information

Name	Title	Telephone (Office/Mobile)	e-Mail Address
[redacted]	[redacted]	[redacted] (mobile only)	[redacted]
[redacted]	[redacted]	[redacted] (mobile)	[redacted]

Protocol Amendment Summary of Changes

DOCUMENT HISTORY	
Document	**Date**
Version 3.0, Amendment 2 (global)	26 JUL 2022
Version 2.0, Amendment 1 (global)	01 JUL 2021
Version 1.0, Original Protocol	05 FEB 2021

Version 3.0, Global Amendment 2: 26 JUL 2022

This amendment is considered to be substantial.

Overall Rationale for the Amendment:

The primary driver for the changes in the protocol amendment is to align the study to the cystic fibrosis population at large with regards to the proportion of patients treated with cystic fibrosis transmembrane conductance regulator (CFTR) modulators, which is considered a cornerstone of their treatment (van der Meer et al., 2021), (CF Foundation Registry, 2020).

Grammatical and typographical errors were corrected throughout the document.

The summary of major changes in this amendment, compared to Amendment 1 of the protocol, is shown below:

Description of Change	Brief Rationale	Section Number
Senior Director, Clinical Operations updated	Personnel change	Important Contact Information
Amended trial design as follows: • CFTR non-modulator strata definition revised to include 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 them during the study • Revised the number of participants randomized to each strata to 5 participants (CFTR modulator stratum)	To align the study to the cystic fibrosis population at large with regards to the low proportion of patients (approximately only 14% of the eligible population) who are not prescribed a CFTR modulator	Section 1.1 Section 1.2 Section 4.1 Table 1 Section 5

Description of Change	Brief Rationale	Section Number
and 2 participants (non-modulator stratum) • Revised the number of participants randomized to the placebo cohort to 3 participants (CFTR modulator stratum) and 1 participant (non-modulator stratum) • Revised the overall number of study participants to approximately 25 to 34 participants		
Updated sputum sample collection for microbiology to include a sample collection at the Early Discontinuation visit	Omission from initial sampling collection schedule	Section 1.3
Table footnote **a** updated to state the Pharmacokinetic (PK) sampling window permitted for Visit 7 (from the PK Sampling Schedule in Section 1.3.1)	To clarify study procedure	Section 1.3
Footnote added for the pharmacodynamic (PD) sample collection description to confirm that the defined PK sampling timepoint windows do not apply to PD sample collections	To clarify study procedure	Section 1.3.1
Updated details that participants must begin the study at the start of either their on-treatment or off-treatment cycled antibiotic regimen (previously referred to their inhaled antibiotic regimen)	Cycled antibiotic regimen used to cover the option that participants may be on inhaled or antibiotic regimens.	Section 4.1.2 Section 6.8 Section 1.2
Updated exclusion criterion 17 to include participants who are expected to have periodontal disease-related procedures	To align with changes made to the monitoring of suspected periodontitis/gingivitis (adverse event of special interest)	Section 5.2

Description of Change	Brief Rationale	Section Number
within the study period (as well as those who are under active treatment for periodontal disease)		
Updated details of randomization for CFTR modulator and non-modulator strata	To align the study to the cystic fibrosis population at large with regards to the low proportion of patients (approximately only 14% of the eligible population) who are not prescribed a CFTR modulator	Section 6.3
Updated reporting details such that any clinical signs and symptoms associated with an overdose should be reported via the SAE form within 24 hours of awareness, even if no seriousness criteria for the events are met	To align with updated reporting procedures	Section 6.7
Revision to PD sample collection such that sputum sample collection will be attempted on all participants, but sputum sample collection will not be required for participants who cannot provide sputum	Although sputum collection is important to the exploratory PD analysis, in light of the potential challenges obtaining an adequate sample due to the concomitant administration of CFTR modulators, sputum sample collection will be attempted on all participants but not required for participants who cannot provide sputum	Section 8 Section 1.3
Updated the monitoring details to cover cases of suspected periodontitis/gingivitis reported during the study whereby participants should be referred to a dentist/periodontist for evaluation, diagnosis, and treatment	As an adverse event of special interest, cases of suspected periodontitis/gingivitis will be monitored and referred for evaluation	Section 8.2.7.2

Description of Change	Brief Rationale	Section Number
Clarification that the administration of a bronchodilator as part of a chronic and stable regimen is not prohibited	To clarify study procedure	Section 8.8.1
Revisions to sample size determination to account for the trial design changes, including the number of participants randomized	To align the study to the cystic fibrosis population at large with regards to the low proportion of patients (approximately only 14% of the eligible population) who are not prescribed a CFTR modulator	Section 9.2 Table 5
Updated details of exploratory endpoint analyses to include analysis by treatment group within strata (CFTR modulator and non-modulator, pooled data across all dose levels)	Proposed change due to the anticipated smaller number of participants in the CFTR non-modulator stratum	Section 9.4.1 Section 9.4.4
Clarification that no formal interim analysis is planned	To clarify study procedure	Section 9.5
Liver safety suggested actions and stopping rules updated from Hy's Law details to the Council for International Organizations of Medical Sciences (CIOMS) Drug-Induced Liver Injury (DILI) guidelines (CIOMS, 2020)	Updated in line with Sponsor's protocol template and agreed use of stopping rule criteria as defined in the CIOMS DILI guidelines.	Section 10.6
Summary of changes for Global Amendment 1 (dated 01 Jul 2021) moved to the Protocol Amendment History (Appendix 7)	Placement of previous protocol amendments	Section 10.7

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANC	absolute neutrophil account
ARDS	acute respiratory distress syndrome
AST	aspartate aminotransferase
ATS	American Thoracic Society
AUC	area under the concentration-time curve
$AUC_{0-\infty}$	AUC from time 0 to infinity
AUC_{0-24}	AUC from time 0 to 24 hours postdose
AUC_{last}	AUC from time 0 to the last timepoint with measurable concentration
BCRP	breast cancer resistance protein
BID	twice a day
CatG	cathepsin G
CF	cystic fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CFTR	cystic fibrosis transmembrane conductance regulator
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology
CL/F	apparent total clearance of drug from plasma after extravascular administration
C_{max}	maximum plasma concentration
C_{min}	minimum plasma concentration
C_{trough}	plasma concentration before the next dose
COVID-19	coronavirus disease 2019
CRF	case report form
CRO	contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
CV	percent coefficient of variation
CYP	cytochrome P450
DLT	dose limiting toxicity
DPP1	dipeptidyl peptidase 1
ECG	electrocardiogram

Abbreviation	Definition
EDC	electronic data capture
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EOS	End of Study
EOT	End of Treatment
ERS	European Respiratory Society
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
$FEF_{(25-75\%)}$	forced expiratory flow between 25% and 75% of forced vital capacity
FSH	follicle stimulating hormone
FVC	forced vital capacity
GM	geometric mean
HBcAb	hepatitis B core antibody
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
HRQOL	health-related quality of life
IC_{50}	concentration of a drug causing half-maximal inhibitory effect
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	investigational medicinal product
IRB	Institutional Review Board
IWRS	Interactive Web Response System
MATE	multidrug and toxin extrusion protein
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NCFBE	non-cystic fibrosis bronchiectasis
NCI	National Cancer Institute
NE	neutrophil elastase
NOAEL	no-observed-adverse-effect level
NSAID	nonsteroidal anti-inflammatory drug
NSP	neutrophil serine protease
OAT	organic anion transporters
OATP	organic anion-transporting polypeptide

Abbreviation	Definition
OCT	organic cation transporter
PD	pharmacodynamic(s)
PE	pulmonary exacerbation
PEFR	peak expiratory flow rate
PFT	pulmonary function test
Pgp	P-glycoprotein
PK	pharmacokinetic(s)
PLS	Papillon-Lefèvre syndrome
$ppFEV_1$	percent predicted forced expiratory volume in 1 second
PR3	proteinase 3
PT	preferred term
QD	once daily
QTc	corrected QT interval
QTcF	corrected QT interval by Fridericia's formula
$R_{ac(AUC)}$	accumulation ratio for AUC
$R_{ac(Cmax)}$	accumulation ratio for C_{max}
ROS	reactive oxygen species
SAE	serious adverse event
SAP	statistical analysis plan
SoA	schedule of assessments and procedures
SOC	System Organ Class
SpO_2	oxygen saturation
SRC	Safety Review Committee
SUSAR	suspected unexpected serious adverse reaction
$t_{½}$	elimination half-life
TB	tuberculosis
TEAE	treatment-emergent adverse event
t_{max}	time to maximum plasma concentration
ULN	upper limit of normal
US	United States
V_d/F	apparent volume of distribution
WOCBP	women of childbearing potential

1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol 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

Brief Title:

A Safety, Tolerability, and Pharmacokinetic Study of Brensocatib Tablets in Adults With Cystic Fibrosis

Rationale:

Study INS1007-211 is a randomized, single-blind, placebo-controlled study. Placebo control eliminates bias and ensures that any differences among treatment groups are due to the active drug. The use of multiple doses of study drug to assess the pharmacokinetics (PK) and pharmacodynamics (PD) of brensocatib in a specific patient population (in this case, participants with cystic fibrosis [CF]) is a standard practice to determine the dose and dosing regimen that achieves target drug exposures in the relevant population.

Safety parameters are standard and meet the International Council for Harmonisation requirements for safety reporting. In a single-blind study, participants are blinded to their assigned study drug and dose, as are the study center staff and the Investigator; select Sponsor personnel, including the Independent Clinical Pharmacologist and SRC members, are not blinded. Blinding is maintained by means of an Interactive Web Response System for assigning treatment.

Efficacy measures include spirometry, which is a validated method for assessing respiratory function, and the Cystic Fibrosis Questionnaire Revised (CFQ-R) Respiratory Domain, a validated instrument for assessing quality of life for patients with CF.

Objectives and Endpoints:

Objectives	Endpoints
Primary	
To evaluate the PK of brensocatib in participants with CF following once daily oral administration of study drug	C_{max}, t_{max}, AUC_{0-24}, and $t_{½}$ on Day 1 and Day 28
To evaluate the safety of brensocatib compared to placebo in participants with CF over the 4-week treatment period	Frequency of treatment-emergent adverse events[a]
Secondary	
To evaluate the dose-dependency of brensocatib exposure	C_{max}, AUC_{0-24}, and AUC_{last} on Day 1 and Day 28

Objectives	Endpoints
Exploratory	
To evaluate the effect of brensocatib compared with placebo on the concentration of NE, CatG, and PR3 in sputum over the 28 day treatment period	Change from Baseline to Day 14, Day 28, and over the 28 day treatment period for concentration of NE, CatG, and PR3 in sputum
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	Change from Baseline to Day 14, Day 28, and over the 28 day treatment period for concentration of NE, CatG, and PR3 in blood
To assess the effect of brensocatib compared with placebo on lung function, as measured by change in pulmonary function parameters, including $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$ at Day 28	Change from Baseline to Day 28 in $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$
To evaluate the effect of brensocatib compared with placebo on quality of life as measured by CFQ-R through Week 4	Change from Baseline to Day 14 and Day 28 for the CFQ-R Respiratory Domain score
To evaluate the PK/PD relationship between AUC/dose and biomarker activity (blood and/or sputum)	NE, CatG, and PR3 concentrations between Day 14 and Day 28 in blood and sputum vs. brensocatib exposure at steady state
To evaluate the PK/PD relationship between systemic exposure and safety (eg, AESIs of hyperkeratosis, periodontitis/gingivitis, and infections)	AESIs during the 28-day treatment period and their relationship to brensocatib exposure at steady state
To evaluate the PK/PD relationship between systemic exposure and clinical efficacy (ie, $ppFEV_1$ and CFQ-R)	$ppFEV_1$ and CFQ-R during the 28-day treatment period and their relationships with brensocatib exposure at steady state
To evaluate the effect of brensocatib on sputum microbiology	Change from Baseline to Day 28 in number of colony forming units for microbial species.

[a] Frequency of TEAEs includes clinically significant vital signs and laboratory abnormalities.
AESI = adverse events of special interest, AUC = area under the concentration-time curve, AUC_{last} = AUC from time 0 to the last timepoint with measurable concentration, AUC_{0-24} = AUC from time 0 to 24 h postdose, CatG = cathepsin G, CFQ-R = Cystic Fibrosis Questionnaire-Revised, C_{max} = maximum plasma concentration, $FEF_{(25-75\%)}$ = forced expiratory flow between 25% and 75% of forced vital capacity, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, NE = neutrophil elastase, PD = pharmacodynamic, PK = pharmacokinetic(s), $ppFEV_1$ = percent predicted forced expiratory volume in 1 second, PR3 = proteinase 3, $t_{½}$ = elimination half-life, t_{max} = time to maximum plasma concentration.

Overall 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 once daily (QD) in participants with CF. Following review of PK and safety data by a Safety Review Committee (SRC; Section 4.1.5), 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.

A figure of the study schema is presented in Section 1.2.

Overall, the study will enroll approximately 25 to 34 participants. The first three brensocatib dose cohorts (10 mg, 25 mg, and 40 mg QD) will enroll approximately 7 participants each, and the placebo cohort will enroll approximately 4 participants. For the active treatment dose cohorts, there will be 2 strata: approximately 5 participants who have previously received and will continue to receive cystic fibrosis transmembrane conductance regulator (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 cohort, approximately 3 participants will be randomized into the CFTR modulator stratum and 1 participant in the non-modulator stratum.

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 receive active treatment (10 mg, 25 mg, and 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.

Including the Screening (up to 28 days), Treatment (28 days), and Follow-Up Period (28 days), the study participation duration is expected to be 84 days or less. At the EOS (End of Study), all ongoing adverse events (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.

Study drug 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 study drug at home.

An 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.

The purpose, function, and conduct of the SRC are further described in Section 4.1.5.

The structure of the SRC is described in Section 10.1.8.

Brief Summary:

Study INS1007-211 is designed to evaluate the PK of brensocatib in participants with CF (primary objective), and consists of a 28-day Screening Period, a 28-day Treatment Period (visits at Days 1, 2, 14, and 28), and a 28-day Follow-Up Period (visits at Days 29, 35, and 56), for a total study duration of 84 days or less.

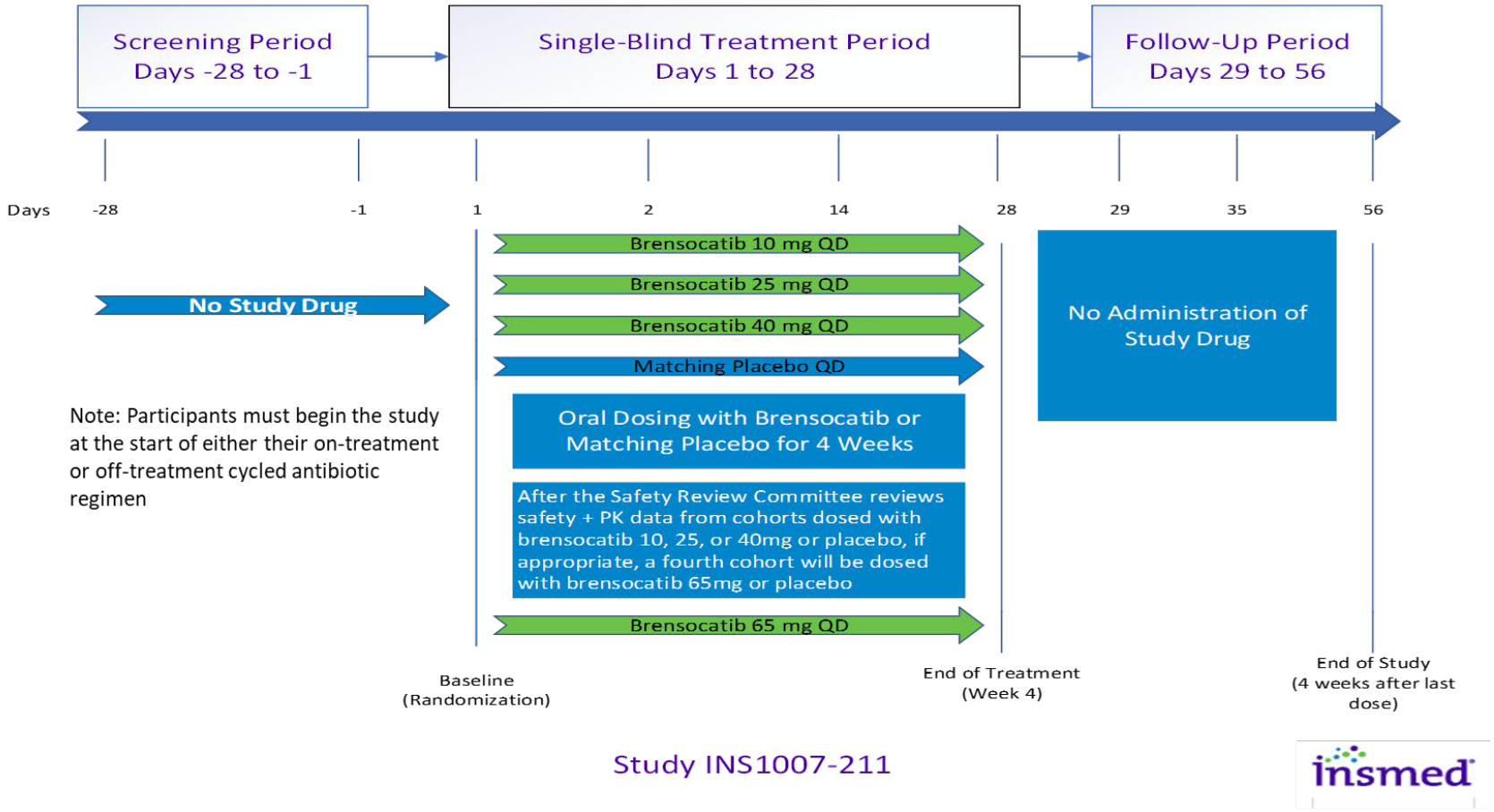
Eligible participants will be randomized to receive brensocatib 10, 25, or 40 mg or placebo, administered QD for 28 days. Approximately 25 participants will be randomized for the first 3 dose cohorts: 7 participants per active treatment dosing cohort (5 CFTR modulator: 2 non-modulator participants), and a total of 4 participants assigned to receive placebo (3 CFTR modulator: 1 non-modulator participant[s]). If there are no safety issues with the first 3 dose cohorts (brensocatib 10, 25, and 40 mg), a fourth cohort (65 mg) will be added, with the same active treatment allocation rules used in the first 3 cohorts (5 CFTR modulator: 2 non-modulator participants) and 2 participants randomized to receive placebo (1 CFTR modulator: 1 non-modulator participant), increasing the sample size from approximately 25 to 34 participants.

The primary PK endpoints are C_{max}, T_{max}, AUC_{0-24} and $t_{1/2}$ on Day 1 and Day 28. A secondary analysis of dose-dependency will be performed based on AUC_{last}, AUC_{0-24} and C_{max} for Day 1 and Day 28. Additional PK parameters, such as AUC_{last}, AUC_{inf}, CL/F, Vd/F, C_{min}, C_{trough}, $R_{ac(AUC)}$ and $R_{ac(Cmax)}$, will be determined when data permit. The definition of the PK parameters can be found in the abbreviation list and in PK sections.

The primary safety endpoint is the frequency of treatment-emergent adverse events.

The Schedule of Assessments and Procedures is presented in Section 1.3.

1.2. Study Schema



Brensocatib dose and placebo cohorts stratified to include participants who have previously received and will continue to receive cystic fibrosis transmembrane conductance regulator (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).

1.3. Schedule of Assessments and Procedures

Period	**Screening Period**	**Treatment Period**				**Follow-Up Period**			**E/D**
Days Windows	**-28 to -1**	**1**	**2**	**14 (±2 days)**	**28 (EOT) (±2 days)**	**29 (±2 days)**	**35 (±3 days)**	**56 (EOS) (±2 days)**	
Visit	**1**	**2**	**3**	**4**	**5**	**6**[a]	**7**[a]	**8**[b]	
Informed consent	X								
CFQ-R[c]		X		X	X		X		X
Inclusion and exclusion criteria	X	X							
Demographics	X								
Medical history[d]	X								
Substance usage assessment[e]	X	X							
Prior and concomitant medications	→								
COVID-19 test[f]	X	X							
Complete physical examination[g]	X	X	X	X	X	X	X		X
Height, weight, BMI[h]	X	X			X		X		X
Serology testing[i]	X								
Pregnancy test (WOCBP only)[j]	X	X		X	X		X		X
Serum FSH level[k]	X								
12-Lead ECG[l]	X	X		X	X				X
Vital signs[m]	X	X	X	X	X	X	X		X

Period	**Screening Period**	**Treatment Period**				**Follow-Up Period**			**E/D**
Days Windows	**-28 to -1**	**1**	**2**	**14 (±2 days)**	**28 (EOT) (±2 days)**	**29 (±2 days)**	**35 (±3 days)**	**56 (EOS) (±2 days)**	
Visit	**1**	**2**	**3**	**4**	**5**	**6**[a]	**7**[a]	**8**[b]	
Laboratory tests (hematology, coagulation, urinalysis, clinical chemistry)[n]	X	X			X				X
eGFR	X	X			X				X
Spirometry	X	X			X				X
AEs	→	→	→	→	→	→	→	→	X
SAEs	→	→	→	→	→	→	→	→	X
PK sample collection[o]		X	X	X	X	X	X		X
PD sample collection (blood and sputum) [o]	X	X	X	X	X	X	X		X
Sputum collection for microbiology[o]		X			X				X
Randomization		X							
Study drug dispense[p]		X		X					
Study drug accountability				X	X				X
Telephone safety follow-up[q]								X	

[a] Visit 6 and Visit 7: MUST be scheduled in relation to the actual day of the EOT Visit for PK timing purposes. Visit 6 MUST occur 24 hours after Visit 5, and Visit 7 MUST occur 7 days (±24 hours) after Visit 5 for PK sampling purposes (as described in the PK sampling schedule Section 1.3.1 shown below).
[b] Visit 8 (EOS Visit): Must occur 4 weeks after the last dose of study drug (4 weeks after Visit 5).
[c] CFQ-R should be completed as the first procedure of the visit per FDA guidelines.
[d] The diagnosis of bronchiectasis must be confirmed as part of medical history documentation; imaging performed to confirm diagnosis of CF-related lung disease should be reported.
[e] Substance use is collected on a form separate from the Medical History eCRF.

[f] COVID-19 rapid test should be performed at Screening and on Day 1. Test results should be negative prior to participant randomization.

[g] Physical examination: Includes examination of the head (external), eyes, ears, nose, throat, mouth (including gums and teeth), lungs, cardiovascular system, abdomen, musculoskeletal system, skin, lymph nodes, and central nervous system.

[h] Height, weight, BMI: BMI will be calculated at Screening.

[i] Serology testing: HBcAb, HBsAb, HBsAg, HCV, and HIV testing at Screening only.

[j] Pregnancy test: Serum pregnancy test at Screening, and urine pregnancy test at Day 1 (Baseline), Day 14, Day 28, Day 35, and at the E/D Visit, if appropriate.

[k] Serum FSH level: Female participants are considered postmenopausal if at Screening they have had 12 months of consecutive spontaneous amenorrhea, OR < 12 months of consecutive amenorrhea AND a serum FSH level > 40mIU/mL.

[l] See the table in Section 1.3.1 for detailed information on ECG, PK, and PD sample collection.

[m] Vital Signs: Include body temperature (°C), pulse rate (bpm), respiratory rate (breaths/min), blood pressure (systolic and diastolic [mm Hg]), and oxygen saturation (SpO_2). Vital signs will be collected at Screening; predose on Days 1, 2, 14, 28, 29, and 35; and at the E/D Visit, if appropriate.

[n] Clinical laboratory tests: Section 8.1.4 contains the description of the clinical laboratory parameters.

[o] Sputum for microbiology should be collected at Days 1, 28, and E/D Visit; PD sputum collection should be prioritized if participants are unable to produce a sufficient sputum sample. Sputum sample collection will be attempted on all participants per local practice, however, sputum sample collection will not be required for participants who cannot provide sputum.

[p] Dispense Study Drug: Participants will receive study drug supply on Days 1 and 14. The study drug supply on both days will be sufficient for once daily dosing for the entire Treatment Period duration of 28 days (±2 days). Participants will be administered the first dose of study drug on Day 1, at the study center by the study center staff. On days of in-clinic visits (except for the Screening Visit), the study center staff will administer the study drug to the participants. On Days 1 and 28 participants should fast for 8 hours prior to receiving study drug. On days when there is no in-clinic visit, the participant will self-administer the study drug with or without food. Instructions on study drug administration are provided in Table 2.

[q] Telephone Follow-Up Call: Participants will receive a telephone call from the study center on Day 56 to assess the participant's well-being.

Note: All participants are to fast 8 hours prior to attending Day 1 and Day 28 in-clinic visits.

AE = adverse event, BMI = body mass index, CF = cystic fibrosis, CFQ-R = Cystic Fibrosis Questionnaire-Revised, COVID-19 = coronavirus disease 2019, ECG = electrocardiogram, eCRF = electronic case report form, E/D = Early Discontinuation, eGFR = estimated glomerular filtration rate, EOS = End of Study, EOT = End of Treatment, FSH = follicle stimulating hormone, HBcAb = hepatitis B core antibody, HBsAb = hepatitis B surface antibody, HBsAg = hepatitis B surface antigen, HCV = hepatitis C virus, HIV = human immunodeficiency virus, PD = pharmacodynamic, PK = pharmacokinetic, SAE = serious adverse event, WOCBP = women of childbearing potential.

1.3.1. PK, PD, and ECG Sampling Schedule

Day	Timepoint (window)	PK[a]	PD[b] (sputum)	PD[b] (blood)	ECG[c]
Screening Period Days -28 to -1			X	X	X
Day 1	Pre-dose	X	X	X	X
	0.5 h (± 5 min)	X			
	1 h (± 5 min)	X			
	2 h (± 5 min)	X			X
	4 h (± 15 min)	X			
	6 h (± 15 min)	X			X
	8 h (± 15 min)	X			
Day 2	24 h (± 1 h)	X	X	X	
Day 14	Pre-dose	X	X	X	X
	2 h (± 5 min)	X			X
Day 28	Pre-dose	X	X	X	X
	0.5 h (± 5 min)	X			
	1 h (± 5 min)	X			
	2 h (± 5 min)	X			X
	4 h (± 15 min)	X			

Day	Timepoint (window)	PK[a]	PD[b] (sputum)	PD[b] (blood)	ECG[c]
	6 h (± 15 min)	X			X
	8 h (± 15 min)	X			
Day 29	24 h (± 1 h)	X	X	X	
Day 35	168 h (± 24 h)	X	X	X	
E/D		X	X	X	X

[a] PK Sample Collection: Day 1 (predose and at 0.5, 1, 2, 4, 6, 8, and 24 hours postdose [24-hour postdose collection is Day 2 predose collection]), Day 14 (predose and at 2 hour postdose), Day 28 (predose and at 0.5, 1, 2, 4, 6, 8, 24 (collected on Day 29), and 168 hours postdose (±24 hours; collected on Day 35). The collection windows will be up to 30 minutes prior to dosing for the predose sample, ±5 minutes for the 0.5, 1, and 2-hour samples, ±15 minutes for the 4-, 6-, and 8-hour samples, ± 1 hour for the 24-hour sample, and ± 24 hours for the 168-hour sample.

[b] PD Sample Collection: Includes collection of 1 sputum sample and 1 blood sample at Screening, and at predose on Days 1, 2, 14, 28, 29, and 35; in addition, at E/D Visit, if applicable (the defined pre- and post-dose PK sampling timepoint windows do not apply to PD sample collections). A 6 ml sample of blood should be collected at each timepoint.

[c] ECG: 12-lead ECG will be performed at Screening, on Day 1 (predose [0 hour], at 2 and 6 hours postdose), on Day 14 (predose and 2 hours postdose), on Day 28 (predose, and at 2 and 6 hours postdose), and at the E/D Visit, if applicable, at which only 1 ECG will be performed. The ECG must be conducted within a ±10-minute window relative to the PK sampling time. ECG should be collected in triplicates with 10 second recording each.

ECG = electrocardiogram, E/D = Early Discontinuation, h = hour, min = minute, PK = pharmacokinetic, PD = pharmacodynamic.

2. INTRODUCTION

Cystic fibrosis, an autosomal recessive disorder, is a disease of exocrine gland function that involves multiple organ systems, but primarily results in chronic respiratory infections, pancreatic enzyme insufficiency, and associated complications. Defects in the CF transmembrane conductance regulator gene cause abnormalities of cyclic adenosine monophosphate-regulated chloride transport across epithelial cells on mucosal surfaces. Defective CFTR results in decreased secretion of chloride and increased reabsorption of sodium and water across epithelial cells. The resultant decreased hydration of mucus results in viscous mucus, which promotes infection and inflammation. Secretions in the respiratory tract, pancreas, gastrointestinal tract, sweat glands, and other exocrine tissues have increased viscosity, making them difficult to clear (Ramsey et al., 2019).

Worldwide incidence of CF varies from 1 per 377 live births (England) to 1 per 90,000 Asian live births in Hawaii (Sharma GD, 2020). It is the most common lethal hereditary disease among Caucasians in the US (1 case per 2,500 - 3,500 births), with lower incidence among African Americans (1 in 17,000 births) and Asian Americans (1 in 31,000 births) (CF News Today, 2020).

In 2019, there were 31,199 persons diagnosed with CF in the United States, and of these, the majority were Caucasian (93.4%) and male (51.8%). The annual mortality rate (per 100) was 1.2%, with a median age at death of 32.4 years. However, because of improvements in the treatment for CF, the median predicted survival age has continued to increase since 1988: from approximately 29 years to 48.4 years (95% CI: 45.9 – 51.5 years) in 2019(CF Foundation Registry, 2019).

Pulmonary outcomes is a key measure of CF health. Pulmonary exacerbations that require intravenous antibiotic treatment in the hospital or at home, are associated with morbidity, mortality, and decreased quality of life. Uncontrolled PEs often result in prolong hospitalization and consequently permanent damage to the lung which manifests as lung function decline (CF FoundationRegistry, 2019). The most severe manifestation of CF (and the most frequent cause of death or lung transplant) is chronic lung disease with the presence of bilateral disseminated bronchiectasis, characterized by chronic lung infection, particularly with *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and excessive inflammation, declining lung function, and eventually respiratory insufficiency.

Currently available pharmacologic treatment for CF PEs include inhaled antibiotics (tobramycin, aztreonam, and colistin), inhaled corticosteroids, leukotriene modifiers, and inhaled beta agonists. In spite of improvement in pulmonary function over the years, there has not been a marked change in the proportion of individuals with CF who are treated with antibiotics for PEs. Approximately 45% of patients 18 years and older were treated with IV antibiotics for pulmonary exacerbation from 2005 to 2019 (CF Foundation Registry, 2019).

2.1. Background

Brensocatib is a reversible, competitive inhibitor of DPP1, with high potency as measured *in vitro* using isolated human DPP1 enzyme and a hematopoietic cell line known to express DPP1.

DPP1 activates NSPs, which when released from neutrophils, are proinflammatory and destructive to surrounding tissues. Neutrophil elastase is a major product of activated neutrophils, and has been identified as a key risk factor in the onset and progression of bronchiectasis and lung function decline in patients with CF (Chalmers, 2017; Chalmers et al., 2017; Sly et al., 2013). Neutrophil elastase has been implicated in the pathogenesis of mucus hypersecretion (Fahy and Dickey, 2010; Voynow et al., 2004), airway inflammation (Owen, 2008; Pham, 2006), and impaired defenses against *Pseudomonas aeruginosa* infection (Hirche et al., 2008; Taggart et al., 2005; Weldon et al., 2009). In addition, NE has been shown to exploit basic defects in anion secretion and sodium ion absorption in CF by disabling CFTR chloride channels and activating epithelial sodium channels (Le Gars et al., 2013; Mall and Hartl, 2014). While NE has gained the most visibility, the other NSPs (ie, CatG and PR3) are expected to similarly contribute to the development of bronchiectasis and be associated with increased exacerbations. Thus, a pharmaceutical agent that inhibits all NSPs may be superior to one that inhibits only NE.

Brensocatib significantly inhibited the activation of NSPs, resulting in decreased elastolytic ability of the cells *in vitro*. Furthermore, brensocatib produced concentration-dependent reductions in both the percentage of neutrophils with membrane-bound PR3 and the overall surface expression of PR3 in differentiating human neutrophils *in vitro*. Studies with naïve and lipopolysaccharide-induced pulmonary inflammation models in rats showed that DPP1 inhibition with oral brensocatib translates well *in vivo,* as significant decreases in the activities of NSPs were observed.

Safety pharmacology studies of brensocatib showed no concerning cardiovascular, respiratory, or central nervous system effects at a notable safety margin to the systemic exposure in humans at a dose level up to 65 mg (single dose).

In vitro evaluation showed that brensocatib is not a significant inhibitor for CYP isozymes (IC_{50} >30 µM for CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2E1, and 3A4/5) or a significant inducer for CYP1A2, 2B6, and 3A4. In addition, it is not a significant inhibitor of transporters, including Pgp, BCRP, OATPs, OATs, OCTs, and MATEs (IC_{50} > 11 µM). Therefore, at a clinically relevant concentration range (≤ 1 µM), drug-drug interactions between brensocatib and CYPs and transporters modulators are not expected.

In a Phase 1 study in healthy volunteers (N=81 male participants), who were randomized to receive: (a) single doses of placebo or brensocatib (5, 15, 35, 50, or 65 mg), or (b) multiple doses of placebo or brensocatib (10, 25, 40 mg QD), daily dosing of brensocatib led to an exposure-related reduction in NE activity with a delayed onset of effect consistent with neutrophil maturation rates (Palmer et al., 2018). In the presence of potent CYP3A4 and Pgp inhibitors (verapamil 240 mg QD or itraconazole 200 mg BID), brensocatib C_{max} and AUC were altered slightly (14%-32% for AUC and -40%-53% for C_{max}). The impact of the CYP3A4 and Pgp inhibitors on brensocatib exposure are considered not clinically significant since the altered C_{max} and AUC were similar to the range of inter-participant variability when brensocatib is dosed alone (ca. 20%-50%). Food intake (high-fat meal) showed a minor effect on brensocatib AUC (<10% reduction) but moderately delayed the oral absorption (t_{max} increased by 0-3 hours and C_{max} decreased by ≤35%). The elimination $t_{1/2}$ of brensocatib in healthy participants was 20-30 hours.

In the Phase 2b Study INS1007-201, a total of 256 participants with NCFBE who had at least 2 PEs in the previous year received brensocatib 10 mg QD (N=82), brensocatib 25 mg QD (N=87), or placebo (N=87). Brensocatib treatment prolonged the time to the first PE as compared with placebo (P = 0.03 and 0.04 for brensocatib 10 mg vs placebo and brensocatib 25 mg vs placebo, respectively). The adjusted hazard ratio for exacerbation (brensocatib versus placebo) was 0.58 (95% CI: 0.35 - 0.95; P = 0.03) in the 10 mg group and 0.62 (95% CI: 0.38 - 0.99; P = 0.046) in the 25 mg group. Sputum NE activity was reduced from Baseline over the 24-week treatment period for both doses of brensocatib (Chalmers et al., 2020).

Both the brensocatib 10 and 25 mg doses were well tolerated in Study INS1007-201. TEAEs were reported by more participants in the brensocatib treatment groups (75 [92.6%] and 74 [83.1%] for 10 mg and 25 mg, respectively), than in the placebo group (67 [78.8%]). One participant ([redacted]-year-old [redacted] with comorbidities; brensocatib 25 mg) had a TEAE of respiratory failure (attributed to bronchiectasis progression; not related to study drug) with an outcome of death. No clinically meaningful trends or changes in ECGs, clinical laboratory values, vital signs, physical examination findings, or postbronchodilator PFTs were observed.

A large, pivotal, confirmatory, randomized, placebo-controlled study in patients with NCFBE is currently ongoing.

A detailed description of the chemistry, pharmacology, efficacy, and safety of brensocatib is provided in the Investigator's Brochure.

2.2. Study Rationale

Cystic fibrosis is caused by abnormalities in the CF transmembrane conductance regulator protein, causing chronic lung infections (particularly with *Pseudomonas aeruginosa*) and excessive inflammation, and leading to bronchiectasis, declining lung function, and respiratory insufficiency. The inflammatory process is dominated by neutrophils that produce NE, as well as other destructive NSPs including CatG and PR3, that directly act upon extracellular matrix proteins and play a role in the host response to inflammation and infection (Dittrich et al., 2018).

Brensocatib is a small-molecule inhibitor of the lysosomal cysteine proteinase DPP1 (also known as cathepsin C) that is being developed for the treatment of NCFBE and CF. Dipeptidyl peptidase 1 activates NSPs, which when released from neutrophils, are proinflammatory and generally destructive to surrounding tissues. A reduction in the concentrations of neutrophil proteases by means of DPP1 antagonism by brensocatib significantly reduced PEs in patients with NCFBE (Study INS1007-201). As bronchiectasis in CF share many common aspects of the pathophysiological mechanisms that lead to the development of disease for the population in Study INS1007-201, it is reasonable to believe that brensocatib might offer a positive clinical response in terms of reduction of exacerbations in patients with CF. Brensocatib administered over 4 weeks is expected to produce a decrease in NE, PR3, and CatG concentrations in the blood and sputum of participants with CF. The selected dose range, dosing regimen, and treatment duration of the current study are based on the biomarker response (blood and/or sputum NSP activity) in healthy volunteers, and participants with NCFBE from Studies D6190C00001 and INS1007-201 (Willow), respectively. The healthy volunteers received brensocatib 10 to 40 mg QD for up to 28 days and had significantly reduced NE activity in blood in a dose-dependent manner starting on Day16, reaching maximum PD effect in blood

toward the end of the 28-day treatment period. The findings were expanded upon in the Phase 2 Study INS1007-201 (Willow) by demonstrating mean reductions in sputum samples not only for NE activity, but also for the activity of CatG and PR3, for participants on brensocatib compared with those on placebo. Activity of NE, PR3, and CatG in sputum returned to baseline levels 4 weeks after the end of the treatment period (Chalmers et al., 2020).

In the current study, the selection of the doses (10 to 65 mg) is based on the dose-response profile of blood NE activity observed in healthy volunteers (Study D6190C00001) and on the sputum NE, PR3, and CatG responses in participants with NCFBE (Study INS1007-201).

In healthy volunteers, dosing with brensocatib 10 to 40 mg QD inhibited blood NE activity in a dose-dependent fashion. In participants with NCFBE, sputum NE, CatG, and PR3 concentrations were reduced with daily doses of brensocatib 10 and 25 mg. The appropriate dose for brensocatib in patients with CF must take into consideration that the exposure for many drugs is reduced when administered to participants with this condition (De Sutter et al., 2020); therefore, a potential for reduced exposure to brensocatib may occur, necessitating a higher brensocatib dose to achieve a similar response in NSP activity.

Pathological changes in patients with CF can lead to alterations in drug absorption and disposition, including significantly increased renal clearance of orally-administered drugs, leading to lower exposure levels (C_{max} and AUC). Based on the AUC in healthy volunteers who received a single dose of brensocatib 65 mg, the plasma AUC of brensocatib 65 mg QD in participants with CF is projected to be ≤10 µg*h/mL, which is approximately a 2-fold margin compared to the NOAEL exposure in dogs following 9-month QD dosing at 8 mg/kg (AUC of 21.6 µg*h/mL).

Due to differences in nutritional status and fat composition, selecting the optimal dosage in patients with CF is a significant challenge. For example, the PK of many antibiotics differ between patients with CF and those with other diseases (Bulitta et al., 2007; Zobell et al., 2013). Establishing the PK and PD effects of brensocatib in participants with CF is a necessary step in the development process.

The main objective of this study is to determine whether the PK of brensocatib in participants with CF differ from those parameters already described in participants with NCFBE. The outcomes of this study, including the safety and PK data, and the PK-PD relationships, will provide the dose selection rationale for future clinical trials of brensocatib for the CF indication in adults.

2.3. Benefit/Risk Assessment

Detailed information about the known and expected benefits and risks and reasonably-expected TEAEs for brensocatib from clinical and nonclinical studies may be found in the Investigator's Brochure.

2.3.1. Benefits

The current study (Study INS1007-211) is not designed to demonstrate clinical benefit for participants. However, the results of this study are necessary for determining the clinical benefit to and the correct dosing of brensocatib in patients with CF. This study will be undertaken in support of the development of a safe and efficacious treatment for CF.

The characteristics of brensocatib that make it a promising candidate for treatment of CF are the following, as observed in the Phase 2 study of participants with NCFBE:

- Both doses of brensocatib (10 and 25 mg QD for 24 weeks) in Study INS1007-201 provided statistically significant and clinically relevant prolongation of time to PE compared with placebo
- In Study INS1007-201, brensocatib 10 mg QD for 24 weeks provided a statistically significant and clinically relevant treatment effect on the annualized rate of PEs compared with placebo, and brensocatib 25 mg QD for 24 weeks showed a numerically lower rate of PEs than with placebo
- Brensocatib reduced sputum NE concentrations in a dose-dependent manner, supporting the proposed mechanism of action
- Achievement of sputum NE concentrations below the limit of quantification postbaseline provided a clinically relevant and statistically significant effect on time to PE

2.3.2. Risks

Key risks of treatment with brensocatib are primarily those effects related to DPP1 inhibition, similar to those effects experienced by patients with PLS, a rare genetic condition in which DPP1 activity is severely reduced (Guarino et al., 2017); (Sorensen et al., 2014):

- Palmoplantar keratosis, characterized by redness and thickening of the soles and palms
- Periodontitis (can be severe enough to cause tooth loss by the second or third decade of life) (Toomes et al., 1999)
- Increased incidence of infections (including severe infections)
- As in the Studies INS1007-101 (healthy volunteers; single and multiple doses [10, 25, 40 mg]) and INS1007-201 (patients with NCFBE; brensocatib 10 and 25 mg QD vs placebo QD for 24 weeks), hyperkeratosis, gingivitis/periodontitis, and infections will be monitored as AESIs.

2.3.3. Overall Benefit: Risk Conclusions

Although there is no clinical experience with brensocatib in patients with CF, the information available to date from the clinical program in patients with NCFBE is supportive of a positive benefit-risk ratio in CF patients as well.

In the Phase 2 study, treatment with brensocatib for 24 weeks significantly prolonged time to first exacerbation (both the 10 mg and 25 mg doses) and lowered annualized rates of exacerbation compared with placebo (10 mg dose). Results were consistent among subgroups based on age, baseline NE concentrations, prior exacerbation history, bronchiectasis severity index, and lung function. The reduction in sputum NE concentration appeared to be greater with the 25 mg dose.

The overall safety profile was similar across treatment groups in both the Phase 1 (Study INS1007-101) and Phase 2 (Study INS1007-201) trials. Although treatment-emergent skin and dental-related AESIs were numerically higher with active treatment, the events resolved or were recovering at the end of study. The skin events reported had no resemblance to lesions in PLS and did not lead to treatment discontinuation. Infections were similar across treatment groups, and fewer participants in the active treatment groups reported serious adverse events (SAEs) of infective exacerbation of bronchiectasis. No new safety signals were identified. Detailed information is provided in the Investigator's Brochure.

Based on the collective information from the healthy volunteers and patients with NCFBE, and given the common aspects of the pathophysiology between NCFBE and CFBE, it is reasonable to hypothesize that a positive treatment effect of brensocatib in patients with CF could be expected. In conclusion, brensocatib offers a positive benefit-risk ratio to support its dosing in patients with CF.

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the PK of brensocatib in participants with CF following once daily oral administration of study drug	C_{max}, t_{max}, AUC_{0-24}, and $t_{½}$ on Day 1 and Day 28
To evaluate the safety of brensocatib compared to placebo in participants with CF over the 4-week treatment period	Frequency of treatment-emergent adverse events[a]
Secondary	
To evaluate the dose-dependency of brensocatib exposure	C_{max}, AUC_{0-24}, and AUC_{last} on Day 1 and Day 28
Exploratory	
To evaluate the effect of brensocatib compared with placebo on the concentration of NE, CatG, and PR3 in sputum over the 28 day treatment period	Change from Baseline to Day 14, Day 28, and over the 28 day treatment period for concentration of NE, CatG, and PR3 in sputum
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	Change from Baseline to Day 14, Day 28, and over the 28 day treatment period for concentration of NE, CatG, and PR3 in blood
To assess the effect of brensocatib compared with placebo on lung function, as measured by change in pulmonary function parameters, including $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$ at Day 28	Change from Baseline to Day 28 in $ppFEV_1$, FEV_1, FVC, and $FEF_{(25-75\%)}$
To evaluate the effect of brensocatib compared with placebo on quality of life as measured by CFQ-R through Week 4	Change from Baseline to Day 14 and Day 28 for the CFQ-R Respiratory Domain score

Objectives	Endpoints
To evaluate the PK/PD relationship between AUC/dose and biomarker activity (blood and/or sputum)	NE, CatG, and PR3 concentrations between Day 14 and Day 28 in blood and sputum vs. brensocatib exposure at steady state
To evaluate the PK/PD relationship between systemic exposure and safety (eg, AESIs of hyperkeratosis, periodontitis/gingivitis, and infections)	AESIs during the 28-day treatment period and their relationship to brensocatib exposure at steady state
To evaluate the PK/PD relationship between systemic exposure and clinical efficacy (ie, $ppFEV_1$ and CFQ-R)	$ppFEV_1$ and CFQ-R during the 28-day treatment period and their relationships with brensocatib exposure at steady state
To evaluate the effect of brensocatib on sputum microbiology	Change from Baseline to Day 28 in number of colony forming units for microbial species.

[a] Frequency of TEAEs includes clinically significant vital signs and laboratory abnormalities.

AESI = adverse events of special interest, AUC = area under the concentration-time curve, AUC_{last} = AUC from time 0 to the last timepoint with measurable concentration, AUC_{0-24} = AUC from time 0 to 24 h postdose, CatG = cathepsin G, CFQ-R = Cystic Fibrosis Questionnaire-Revised, C_{max} = maximum plasma concentration, $FEF_{(25-75\%)}$ = forced expiratory flow between 25% and 75% of forced vital capacity, FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity, NE = neutrophil elastase, PD = pharmacodynamic, PK = pharmacokinetic(s), $ppFEV_1$ = percent predicted forced expiratory volume in 1 second, PR3 = proteinase 3, $t_{½}$ = elimination half-life, t_{max} = time to maximum plasma concentration

4. STUDY DESIGN

4.1. Overall 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 safety and PK data by the SRC and provided that the SRC deems it appropriate to administer the next higher dose, an additional 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 randomly assigned to the 65 mg dose cohort will receive the following:

- 1 tablet of brensocatib 25 mg QD plus 1 tablet of brensocatib 40 mg QD × 28 days

OR

- 2 tablets of matching placebo QD × 28 days

Section 6.3 describes the procedures for blinding the study drug.

Overall, the study will enroll approximately 25 to 34 participants. The first three brensocatib dose cohorts (10 mg, 25 mg, and 40 mg QD) will enroll approximately 7 participants each, and the placebo cohort will enroll approximately 4 participants.

For many patients with CF, CFTR modulators are the cornerstone of their treatment (van der Meer et al., 2021). The proportion of patients prescribed a CFTR modulator has expanded over time, with approximately only 14% of the eligible population not prescribed a CFTR modulator (CF Foundation Registry, 2020). For the active treatment dose cohorts, there will be 2 strata: approximately 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 cohort, approximately 3 participants will be randomized into the CFTR modulator stratum and 1 participant in the non-modulator stratum.

As described in Table 1, 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 receive active treatment (10 mg, 25 mg, and 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.

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

The table illustrates the prospective numbers 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).

	Active (Brensocatib)				Placebo	Total number of participants**
CFTR Modulator Status	**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.

This study is designed to investigate the following:

1. The PK effects of brensocatib 10, 25, and 40 mg QD (and possibly 65 mg QD) after 28 days of administration in participants with CF
2. The PD effects of brensocatib in participants with CF
3. The safety and tolerability of brensocatib in participants with CF

For individual participants, the study will consist of the following:

- Screening Period (28 days)
- Treatment Period (28 days)
- Follow-Up Period (4 weeks), including the following:
  - Day 29 and Day 35 in-clinic visits
  - EOS Visit (Study Day 56; 4 weeks after the last dose of study drug) for a safety follow-up telephone call

Including the Screening, Treatment, and Follow-Up Period, the study participation duration is expected to be 84 days or less. Any study-related safety issues or serious events that extend past Study Day 56 (EOS) will be followed to resolution.

Study drug will be administered to the participant by the study center staff on Day 1, 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 study drug at home.

An SRC (Section 4.1.5) will be set up to review safety and PK data as part of the decision-making process to determine whether to implement the brensocatib 65 mg dose.

4.1.1. Screening Period

The Screening Period will be up to 28 days. Required screening assessments are shown in the SoA, Section 1.3. Participants who fail 1 or more screening assessments may be rescreened at the Investigator's discretion. Section 5.4 contains additional information regarding participants who fail screening and/or are rescreened.

4.1.2. Treatment Period

The Treatment Period is a 28-day period comprising 4 in-clinic visits on Days 1, 2, 14, and 28. Participants must enter the study at the start of either their on-treatment or off-treatment cycled antibiotic regimen. All Treatment Period assessments and procedures are presented in the SoA, Section 1.3.

4.1.2.1. Day 1 (Baseline)

Participants will return to the study center after an 8-hour fast on Day 1 (Baseline) and undergo assessments (inclusion/exclusion criteria, concomitant medications, AEs), and procedures (physical examination, 12-lead ECG, vital signs, clinical laboratory tests, spirometry). Additional procedures and assessments are listed in the SoA, Section 1.3. After assessments and procedures are completed, eligible participants will be randomized to receive brensocatib 10, 25, or 40 mg or placebo QD, orally for 4 weeks. The study center staff will administer the study drug to participants for days of in-clinic visits. On Days 1 and 28, the study center staff will administer the study drug to the participant after 8 hours of fasting on Days 1 and 28. Participants will receive a 28-day supply of blinded study drug on Days 1 and 14. On days when there is no in-clinic visit, the participant will self-administer the study drug with or without food at the same time of day each day.

4.1.2.2. Day 2, Day 14, and Day 28

Participants will return to the study center on Days 2, 14, and 28 (End of Treatment [EOT]), and report all concomitant medications taken and any AEs that have occurred since the last study visit. All Treatment Period assessments and procedures are listed in the SoA, Section 1.3. Participants will return to the study center after an 8-hour fast on Days 1 and 28. Participants will bring study drug bottles and any unused medication for study drug accountability to the Day 14 Visit and return the study drug bottles and any unused medication for study drug accountability on the Day 28 (EOT) Visit.

4.1.3. Follow-Up Period

The Follow-Up Period will extend from Day 29 to Day 56 (EOS) and include visits to the study center on Day 29 and Day 35 for blood sampling for PK analysis, and for blood and sputum sampling for PD analysis (Section 1.3). On Day 56 (EOS) a telephone call will be placed from the study center to the participant to check on well-being and to assess the status of any new and/or ongoing AEs.

4.1.4. Decision to Dose Brensocatib 65 mg

A decision to dose a fourth cohort with brensocatib 65 mg or placebo will be made after the first 3 cohorts have completed the study. Section 4.1.5 describes information on the SRC and its role in determination of the fourth dosing cohort.

If a cohort dosed with brensocatib 65 mg is approved, participants in the cohort will be supplied with bottles of study drug sufficient to dose with 2 tablets QD for 28 days on Days 1 and 14, following the same procedures as the previous cohorts.

As there is currently no 65 mg tablet of brensocatib, each participant in the cohort will take one 25 mg tablet and one 40 mg tablet, or 2 placebo tablets at the same time each day for 28 days, to maintain the blind. Detailed instructions on handling the investigational product will be described in the pharmacy manual and medication card.

4.1.5. Safety Review Committee

Safety monitoring of the study will be conducted by an SRC. After the first 3 cohorts (brensocatib 10, 25, and 40 mg QD) have completed the study, the SRC will review available data (PK and safety data [vital signs, clinical laboratory test values, ECGs, 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 MTD has been reached will be based on the DLT. The MTD is defined in this study as the highest single dose tested without incurring DLT. 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.

The structure of the SRC is described in Section 10.1.8.

4.2. Scientific Rationale for Study Design

Study INS1007-211 is a randomized single-blind, placebo-controlled study. Placebo control eliminates bias and ensures that any differences among treatment groups are due to the active drug. The use of multiple doses of study drug to assess the PK and PD of brensocatib in a specific patient population (in this case, participants with CF), is standard practice in order to determine the dose and dosing regimen that achieves target drug exposures in all relevant populations.

Safety parameters are standard and meet the ICH requirements for safety reporting. As a single-blind study, participants are blinded to their assigned study drug and dose, as are the study center staff and the Investigator. Select Sponsor personnel are not blinded, including the Independent Clinical Pharmacologist and SRC members. Blinding is maintained by means of an IWRS system for assigning treatment.

Efficacy measures include spirometry, which is a validated method for assessing respiratory function, and the CFQ-R, a validated instrument for assessing quality of life for patients with CF.

4.3. Justification for Dose

Brensocatib has been studied using solution and tablet formulations in healthy volunteers, with dosing strengths ranging from 5 to 65 mg, and repeated administrations of 10 mg, 25 mg, and 40 mg across the formulations that support similar conclusions of dose proportionality.

A Phase 2 Study (INS1007-201) that randomized 256 participants with NCFBE demonstrated the efficacy of brensocatib in reducing PEs when administered at 10 mg and 25 mg in comparison to placebo. Furthermore, both doses demonstrated an acceptable safety profile as most TEAEs were mild to moderate in intensity across groups; those reported in ≥10% of brensocatib-treated participants were cough, headache, increased sputum, and dyspnea, with some potential indication of dose dependency for events that may reflect DPP1 inhibition. Modeling and simulations using data from the Phase 2 study support that a reduction of sputum NE below quantification levels is associated with a reduction of exacerbations, and that such reduction is dose dependent.

In patients with CF, the airway inflammation is dominated by neutrophils that produce reactive oxygen species, neutrophil extracellular traps, proteases such as NE, PR3, and CatG, and proinflammatory mediators. Neutrophils are the source of the massive amounts of free elastase in the CF airway (Nichols and Chmiel, 2015). Elastase possesses several deleterious characteristics that account for much of the lung damage seen in CF, and the amount of free elastase in the airway highly correlates with lung function (FEV_1) (Sagel et al., 2012).

Two main factors were taken into consideration regarding dosing for the current study:

- Levels of NE in patients with CF have been described to be higher than in patients with NCFBE (Oriano et al., 2019).
- Patients with CF are often reported to have higher clearances and larger volumes of distribution for a given medication per kilogram of total body weight than healthy volunteers (Bulitta et al., 2011), and many patients with CF report some level of chronic malabsorption.

Based on the findings listed above, it seems reasonable to assess doses higher than those included in the Phase 2 study previously described. Accordingly, the first 3 cohorts (brensocatib 10, 25, and 40 mg) will be dosed concurrently, and an SRC will review safety and PK data from these cohorts. After review, the SRC will decide whether exploring brensocatib 65 mg is appropriate.

4.4. End of Treatment Definition

A participant is considered to have completed treatment if they have completed Day 28 of the study, including the scheduled procedures shown in the SoA (Section 1.3).

4.5. End of Study Definition

The end of the study is defined as the date of the last assessment of the last participant in the study (Section 1.3). A participant reaches EOS if they have completed Day 56 of the study, including the scheduled procedures shown in the SoA (Section 1.3).

5. STUDY POPULATION

The study is expected to enroll approximately 25 to 34 participants at 1 or more centers in the US.

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

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participants must be ≥18 years of age at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Male or female participants with a confirmed diagnosis of CF related lung disease
    a. ppFEV1 between 40% to 90% (inclusive) at Screening Visit and at Baseline
    b. Stable CF treatment for at least 30 days before Screening and willing to remain on a stable regimen throughout the treatment period

Weight

3. Has a body mass index ≥18 kg/m^2

Sex and Contraceptive/Barrier Requirements

4. Male and female participants must use contraceptives that are consistent with local regulations regarding the methods of contraception for those participating in clinical studies.
    a. Male participants:
    Male participants, who are not sterile, with female partners of childbearing potential must be using effective contraception from Day 1 to at least 90 days after the last dose. Such methods include true abstinence (refraining from heterosexual intercourse during the study), combined (estrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine devices, intrauterine hormone-releasing systems.

    b. Female participants:

    Women must be postmenopausal (defined as no menses for 12 months without an alternative medical cause), surgically sterile, or using highly effective contraception methods (ie, methods that alone or in combination achieve <1% unintended pregnancy rates per year when used consistently

and correctly) from Day 1 to at least 90 days after the last dose. Such methods include true abstinence (refraining from heterosexual intercourse during the study), combined (estrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation and supplemented with a double barrier (preferably male condom), intrauterine devices, intrauterine hormone-releasing systems, or vasectomized partner. For WOCBP ≤45 years, an additional confirmatory testing of FSH level with a threshold of >40 mIU/mL should be performed to be considered infertile.

Note: Abstinence is only considered to be a highly effective method of contraception when this is the preferred and usual lifestyle of a participant. Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method are not acceptable methods of contraception.

5. Female participants of childbearing potential must have a negative serum pregnancy test at Screening.
6. Male participants with pregnant or nonpregnant WOCBP partners must use a condom.

Informed Consent

7. Participant is capable of giving signed informed consent as described in Section 10.1.5, which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
8. Able to understand and comply with protocol requirements, restrictions, and instructions and likely to complete the study as planned, as judged by the Investigator.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Severe or unstable CF as per Investigator's judgement.
2. Oxygen saturation (SpO_2) on room air ≤92% at the Screening Visit and at Baseline
3. Currently being treated for allergic bronchopulmonary aspergillosis or nontuberculous mycobacteria or TB.
4. Active and current infection by SARS-CoV-2.

5. Screening test results (hematology, clinical chemistry, coagulation, and/or urinalysis) with clinically significant abnormalities that would interfere with the study assessments, as judged by the Investigator, including the following:
    - Hemoglobin <10 g/dL at Screening.
    - An ANC <1,000/mm^3 at Screening.
    - Clinically significant hepatobiliary disease defined as alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) values >3 × upper limit of normal (ULN) and total bilirubin >2 × ULN, excluding confirmed Gilbert's Syndrome
    - Abnormal renal function test result at Screening, defined as eGFR <30 mL/min (by the Chronic Kidney Disease – Epidemiology Collaboration equation formula) (Levey et al., 2009) for participants 18 years and older.
6. History of malignancy in the past 5 years, except completely treated *in situ* carcinoma of the cervix and completely treated non-metastatic squamous or basal cell carcinoma of the skin.
7. History of any illness or condition that, in the opinion of the Investigator, might confound the results of the study or pose an additional risk in administering study drug to the participant.
8. Established diagnosis of hepatitis B viral infection or positive for HBsAg at Screening:
    – Participants who have gained immunity for hepatitis B virus infection after vaccination (participants who are HBsAg-negative, HBsAb-positive, and HBcAb-negative) are eligible for the study.
    – Participants with positive HBcAb are eligible for the study only if hepatitis B virus DNA level is undetectable.
9. Established diagnosis of HCV infection at Screening. Participants positive for hepatitis C antibody are eligible only if HCV RNA is negative.
10. History of HIV infection or positive HIV serology at Screening.
11. Acute upper or lower respiratory tract infection, pulmonary exacerbation, or changes in therapy (including IV and oral antibiotics) for pulmonary disease within 4 weeks prior to Day 1 (administration of the first dose of study drug). Participants meeting this criterion could be rescreened 4 weeks after resolution of symptoms.
12. History of prolonged QT/QTc interval with QTcF >480 msec at Screening.
13. History of solid organ or hematological transplantation.

14. Any clinically significant abnormal laboratory values at Screening or diseases or disorders (eg, survivors of severe COVID-19 disease including ARDS, cardiovascular, pulmonary, gastrointestinal, liver, kidney, neurological, musculoskeletal, endocrine, metabolic, psychiatric, physical impairment, or a lung transplantation) that, in the opinion of the Investigator, may put the participant at risk by participating in the study, or interfere with the participant's treatment, assessment, or influence the results of the study, or have compliance issues with the study or have a planned or anticipated major surgical procedure during the study.
15. Unstable disease due to colonization with *Burkholderia cenocepacia*, *Burkholderia dolosa*, and *Mycobacterium abscessus* as judged by the Investigator.
16. Any "non-CF-related" acute illness within 2 weeks prior to Day 1 (first dose of study drug). "Illness" is defined as an acute (serious or nonserious) condition (eg, gastroenteritis).
17. Have diagnosed periodontal disease and are either:
    - Currently treated by a dentist for this condition or
    - Expected to have periodontal disease-related procedures within the study period.
18. Received any live attenuated vaccine within 4 weeks prior Screening. If a live vaccine has been administered, the participant should wait 4 weeks prior to rescreening. During the study, participants may not receive any live attenuated vaccine.

Prior/Concurrent Clinical Study Experience

19. Ongoing participation in another therapeutic clinical study or prior participation in an investigational drug study within 90 days prior to Screening. A washout period of ≥5 terminal half-lives of the previous investigational study drug or 90 days, whichever is longer, must elapse prior to Screening.
20. Known history of hypersensitivity to brensocatib or any of its excipients
21. Use of any immunomodulatory agents within 4 weeks before the Screening Visit is prohibited during the study through Visit 8 (EOS) (including, but not limited to: bortezomib, ixazomib, thalidomide, cyclophosphamide, mycophenolate, Janus kinase inhibitors, IFN-γ, and azathioprine).
22. Continuous use of high-dose NSAIDs is prohibited during the study through Visit 8 (EOS) (Section 10.6.1).

Alcohol and Tobacco

23. History of alcohol, medication, or illicit drug abuse.

24. Current smoker, as defined by Centers for Disease Control and Prevention: An adult who has smoked 100 cigarettes in his or her lifetime and who currently smokes cigarettes.

5.3. Lifestyle Considerations

Not applicable.

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized to receive study drug. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened after consultation with the study Medical Monitor. Participants who meet all inclusion criteria but fail screening due to active PE or ongoing standard periodontal care can be rescreened up to 2 times with the Sponsor's Medical Director approval. If a participant is approved for rescreening, the Medical Director will determine whether some or all of the screening assessments must be repeated.

NOTE: Rescreened participants should be assigned a new participant number ("subject ID") by the IWRS system for every screening/rescreening event.

5.5. Criteria for Temporarily Delaying Administration of Investigational Product

Not applicable.

6. INVESTIGATIONAL PRODUCT AND CONCOMMITANT THERAPY

Investigational product is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol. In this protocol, the terms "investigational product" and "study drug" are interchangeable.

Brensocatib film-coated tablets are round, biconvex, brown, film-coated tablets containing the equivalent of 10 mg, 25 mg, and 40 mg of brensocatib (INS1007) drug substance and to be administered orally QD. Each film-coated tablet contains active ingredient of brensocatib drug substance and compendial ingredients: microcrystalline cellulose, dibasic calcium phosphate dihydrate, sodium starch glycolate, silicon dioxide, and glyceryl behenate.

Participants will be assigned to receive brensocatib 10, 25, or 40 mg QD or placebo QD for 28 days. They will be randomly assigned to a dosing cohort with 10 participants receiving active drug and 2 participants receiving placebo (12 participants per cohort). Insmed will provide sites with the investigational products as described in Table 2.

Participants will receive the same number of tablets each day to maintain the blind.

If the 65 mg is to be assessed, participants in this cohort will receive 65 mg QD (25 mg + 40 mg tablets) or matching placebo tablets on the same schedule as the previous cohorts.

6.1. Investigational Product Administration

Participants will be instructed to take 1 tablet of the study drug once a day, with water and with or without food, at approximately the same time of day each day in the morning. On the days of in-clinic visits the study drug will be administered by the study center staff. On Day 1 and Day 28 visits, participants will visit the study center and receive study drug after an 8-hour overnight fast. Except for Days 1 and 28, the study drug can be administered with or without food.

Details about the study drug are presented in Table 2.

Table 2: Investigational Product for Study INS1007-211

Arm Name:	**Placebo**	**Brensocatib**			
Unit Dose:		**10 mg**	**25 mg**	**40 mg**	**65 mg**
Type:	Placebo	Drug	Drug	Drug	Drug
Formulation:	Tablet	Tablet	Tablet	Tablet	N/A[a]
Route:	Oral	Oral	Oral	Oral	Oral
Use:	Experimental	Experimental	Experimental	Experimental	Experimental
Manufacturer:	Insmed	Insmed	Insmed	Insmed	Insmed
Packaging and Labeling:	Study drug will be provided in HDPE bottles	Study drug will be provided in HDPE bottles	Study drug will be provided in HDPE bottles	Study drug will be provided in HDPE bottles	Study drug will be provided in HDPE bottles
Administered As:	One placebo tablet[b]	One 10-mg brensocatib tablet	One 25-mg brensocatib tablet	One 40-mg brensocatib tablet	One 25-mg brensocatib tablet AND one 40-mg brensocatib tablet

[a] Currently no 65 mg tablet exists. Participants who are randomized to brensocatib 65 mg will receive 2 bottles of study drug with different color labels on the bottles (1 bottle with brensocatib 25 mg tablets, 1 bottle with 40 mg tablets) and will take 1 tablet from each bottle on each dosing day.

[b] In the 65 mg dose cohort, participants randomly assigned to placebo will receive 2 bottles of study drug, both containing placebo tablets. To preserve the blind, participants will take 1 tablet from each bottle on each dosing day.

HDPE = high-density polyethylene, N/A = not applicable.

6.2. Preparation/Handling/Storage/Accountability

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug. Study drug will be stored at temperatures as indicated on the label.

Only participants enrolled in the study may receive study drug and only authorized site staff may supply or administer study drug. All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study drugs are provided in the Study Pharmacy Manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

After meeting all inclusion criteria and none of the exclusion criteria, participants in each stratum (CFTR modulator and non-modulator) will be randomized by means of an 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 dose 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 study drug to which they are randomly assigned, as are the study center staff and the Investigator. Select Sponsor personnel comprising the SRC members and the Independent Clinical Pharmacologist are not blinded. The site will contact the IWRS prior to the start of study drug administration for each participant. The site will record the assigned study drug label number on the applicable electronic case report form (eCRF), if required.

Study personnel and participants are strongly discouraged from discussing any aspect of study treatment, with the exception of safety issues, to maintain treatment blinding and study integrity.

The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the Investigator has the sole responsibility for determining whether unblinding of a participant's study drug assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the Investigator decides that unblinding is warranted, the Investigator should make every effort to contact the Sponsor prior to unblinding a participant's study drug assignment unless this could delay emergency treatment of the participant. If a participant's study drug assignment is unblinded, the Sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation, as applicable.

6.4. Study Drug Compliance

When participants are dosed at the site, they will receive study drug directly from the Investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study drug and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study drug. Study site staff will examine each participant's mouth to ensure that the study drug was ingested.

When participants self-administer study drug, compliance will be assessed at each visit. Drug accountability will be assessed by counting returned tablets during the site visits on Days 14 and 28 and documenting in the source documents and relevant forms. Deviation(s) from the prescribed dosage regimen should be recorded.

A record of the quantity of brensocatib tablets dispensed to and administered by each participant must be maintained and reconciled with study drug and compliance records. Start and stop dates, including dates for delays will also be recorded.

6.5. Dose Modification

Not applicable.

6.6. Continued Access to Investigational Product After the End of the Study

Not applicable.

6.7. Overdose

An overdose is defined as a dose greater than the dose level(s) evaluated in this study. There is no known antidote to the study treatment.

An overdose itself is not an AE. However, any clinical signs and symptoms associated with the overdose should be reported via the SAE form within 24 hours of awareness, even if no seriousness criteria for the events are met.

The Investigator should also:

- Contact the Medical Monitor in case of symptomatic overdose within 24 hours
- In consultation with the Medical Monitor, determine whether the study drug should be interrupted or discontinued
- Closely monitor the participant for any AE/SAE and laboratory abnormalities and follow them up until they resolve
- Fully document the overdose (eg, intentional or accidental, the dose, duration of the overdose) and associated signs and symptoms in the participant's source documents and the appropriate case report form (CRF) pages.

6.8. Concomitant Therapy

Any medications the participant takes other than the study drug from Day 1 (Baseline) to Day 56 (EOS) are considered concomitant medications and will be collected and documented in the study eCRF.

Prior medications are those medications taken before the first dose of study drug. Concomitant medications are those medications taken after the first dose of study drug. A medication that starts prior to first dose but continues after the first dose of study drug is classified both in prior and concomitant medications. Prior procedures are procedures performed before the first dose of study drug. Any procedures performed from Day 1 to Day 56 (EOS) are considered concomitant procedures and will be collected and documented in the study eCRF.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Participants must begin the study at the start of either their on-treatment or off-treatment cycled antibiotic regimen.

As stated in the Section 2, brensocatib interactions with substrates or modulators of CYP3A4 and Pgp are unlikely at the dose levels of 10 mg to 65 mg.

6.8.1. Rescue Medicine

Not applicable.

6.8.2. Dose Interruption

The Investigator may interrupt dosing for safety reasons.

6.8.3. Prohibited Medications

Prohibited medications are as follows:

- Use of any immunomodulatory agents within 4 weeks before the Screening Visit is prohibited during the study through Visit 8 (EOS) (including, but not limited to: bortezomib, ixazomib, thalidomide, cyclophosphamide, mycophenolate, Janus kinase inhibitors, IFN-γ, and azathioprine)
- Continuous use of high dose NSAIDs is prohibited during the study through Visit 8 (EOS) (Section 10.5.1, Comparable NSAID Dose Levels)
- Long-term use of systemic steroids (>4 weeks, regardless of dose) for any chronic condition
- Live attenuated vaccines are prohibited during the study from 4 weeks prior to Screening until 4 weeks after the last dose of the study drug
- Use of investigational drugs within 90 days or ≥5 terminal half-lives of the previous investigational study drug (whichever is longer) of the Screening Visit

The following procedures are prohibited during study participation:

- Major elective surgical procedures
- Elective periodontal procedures

Participants who have already scheduled a major surgical procedure or a prescheduled periodontal procedure are not eligible to participate in the study.

7. DISCONTINUATION OF STUDY DRUG AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1. Discontinuation of Study Drug

Participants who discontinue the study drug for any reason may not continue in the study unless discontinuation was due to an AE. Participants who discontinue the study will be asked to attend an Early Discontinuation Visit and undergo the scheduled assessments (SoA, Section 1.3).

7.1.1. QTc Stopping Criteria

If a clinically significant finding is identified (including, but not limited to QTcF >480 msec) after enrollment, the Investigator or qualified designee will determine whether the participant can continue in the study and whether any change in participant management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

7.1.2. Pregnancy

Refer to Section 8.2.5.

7.1.3. Participant Discontinuation/Withdrawal From the Study

A participant may decide to withdraw from the study at any time, for any reason, without prejudice to subsequent care or treatment by the Investigator. When this occurs, the participant should complete all the procedures for the Early Discontinuation Visit. Participants who discontinue the treatment due to AEs should remain under observation until the resolution or stabilization of the AEs. A participant may be discontinued from the study drug treatment before completion for any of the following reasons:

1. Participant death
2. Participant experiences an AE(s)/SAE(s) that warrants discontinuation, as judged by the Investigator and/or Insmed Medical Director
3. A major protocol deviation, which in the opinion/discretion of the Investigator and/or Insmed, compromises the data integrity of the study
4. Participant is noncompliant with the study drug treatment (defined as <80% compliance after at least 2 reeducation efforts)
5. Withdrawal by participant
6. Termination of the study by the Sponsor
7. Investigator discretion
8. Participant lost to follow-up
9. Participant pregnancy
10. Skin events (Section 8.2.7.1)
11. Periodontal criteria met (Section 8.2.7.2)

12. Diagnosis of a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
13. Serious infection (Section 8.2.7.3), defined as any infection (other than associated with a PE) that:
    - Requires parenteral treatment with an antibiotic requiring admission to the hospital or parenteral treatment with an antifungal, antiviral, antiparasitic, or antiprotozoal agent
    - Is an opportunistic infection, such as TB or other infection whose nature or course may suggest an immunocompromised status
    - If a participant requires oral antibiotics to treat acute pulmonary symptoms, pneumonia, or any other infection, discontinuation/withdrawal from the study will be determined by the Investigator
14. Participants who develop neutropenia defined as ANC <1000/mm^3
15. Participants who develop any of the stopping rules for liver safety defined in Section 10.6.

7.2. Lost to Follow up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

Before a participant is deemed lost to follow up, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

"Lost-to-follow-up" should be marked only in an exceptional case when all documented attempts to reach the participant by the Investigator or other staff members were unsuccessful.

8. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study drug.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 6 mL.

Sputum sample collection will be attempted on all participants able to provide a sample either spontaneously, with chest physiotherapy, or by induction. Although sputum collection is an important exploratory PD analysis, in light of the potential challenges obtaining an adequate sample due to the concomitant administration of CFTR modulators, sputum collection will be attempted on all participants per local practice, however, sputum collection will not be required for participants who cannot provide sputum. Sputum sample collection will be prioritized for PD analyses, and if there is sample remaining, microbiology will be assessed.

The study centers may use their own sputum induction protocols or the sputum induction guidelines provided in Appendix Section 10.5.

8.1. Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA, Section 1.3.

8.1.1. Physical Examinations

The complete physical examination includes assessment of the head (external), eyes, ears, nose, throat, mouth (including gums and teeth), lungs, cardiovascular system, abdomen, musculoskeletal system, skin, lymph nodes, and central nervous system. Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.1.2. Vital Signs

Vital signs will be taken before blood collection for laboratory tests. Vital signs include body temperature (°C), pulse (bpm), respiratory rate (breaths/min), blood pressure (systolic over diastolic [mm Hg]), and oxygen saturation (SpO_2). Blood pressure will be assessed after the participant has been seated for 5 minutes. Clinically significant abnormal values of vital signs will be recorded as an AE.

8.1.3. 12-Lead Electrocardiograms

A 12-lead ECG will be performed as indicated in the SoA, Section 1.3.

Refer to Section 7.1.1 for QTc withdrawal criteria and any additional QTc readings that may be necessary.

8.1.4. Clinical Safety Laboratory Assessments

Clinical safety laboratory assessment parameters are provided in Table 3.

Table 3: Clinical Laboratory Parameters

Category	Laboratory Parameters
Clinical Chemistry	Sodium, chloride, potassium, CO_2, magnesium, calcium, glucose, phosphate, total bilirubin (with direct and indirect fractionation, if total bilirubin is elevated >2 ULN), alkaline phosphatase, LDH, AST, ALT, albumin, total protein, creatinine, urea-nitrogen, uric acid, eGFR from the Chronic Kidney Disease - Epidemiology Collaboration equation formula (Section 8.1.5)
Hematology	Hemoglobin, erythrocytes, hematocrit, MCH, MCV, MCHC, leukocytes, differential blood count of neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets
Coagulation	Prothrombin time, PTT, aPTT, and INR
Urinalysis	Qualitative analysis of glucose, ketones, nitrites, protein, pH, leukocytes, blood, bilirubin, specific gravity; microscopic examination for cells, casts, and bacteria
Special Tests	For women ≤45 years of childbearing potential, an additional confirmatory testing of FSH level with a threshold of >40 mIU/mL is required at Screening

ALT=alanine aminotransferase, aPTT=activated partial thromboplastin time, AST=aspartate aminotransferase, CO_2=carbon dioxide, eGFR = estimated glomerular filtration rate, FSH=follicle-stimulating hormone, INR= international normalized ratio, LDH=lactate dehydrogenase, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration, MCV=mean corpuscular volume, PTT=partial thromboplastin time, ULN=upper limit of normal.

The Investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents.

Laboratory abnormalities are usually not recorded as AEs or SAEs. Abnormal laboratory findings (such as clinical chemistry, hematology, or urinalysis) or other abnormal assessments (physiologic tests such as ECGs or vital signs) that are associated with signs and/or symptoms, are considered clinically significant in the judgment of the Investigator, or require therapeutic intervention, or lead to discontinuation of the administration of study drug, must be recorded as AEs or SAEs if they meet the definitions of an AE (or SAE).

All laboratory tests with values considered clinically significantly abnormal during participation in the study should be repeated until the values return to normal or baseline, or are no longer considered clinically significant by the Investigator or Medical Monitor.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.

All protocol-required laboratory tests, as defined in Table 3, must be conducted in accordance with the Laboratory Manual and the SoA (Section 1.3).

If laboratory values from non-protocol-specified laboratory tests performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the Investigator (eg, SAE or AE or dose modification), then the results must be recorded.

8.1.5. Estimated Glomerular Filtration Rate

The eGFR calculation per CKD-EPI equation will be analyzed for each participant who is ≥18 years at Visit 1 (Screening), at Day 1 (Baseline), and Day 28 (EOT).

The CKD-EPI equation described below will be used to calculate the eGFR of creatinine clearance in mL/min:

GFR = 141 × min (Scr/κ, 1) α × max (Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] × 1.159 [if Black]

where:

Scr is the serum creatinine in mg/dL,
κ is 0.7 for females and 0.9 for males,
α is -0.329 for females and -0.411 for males,
min indicates the minimum of Scr/κ or 1,
max indicates the maximum of Scr/κ or 1.

8.1.6. Pregnancy Testing and Contraception

Serum pregnancy test will be conducted for WOCBP at Screening; all WOCBP must have a negative serum pregnancy test before randomization.

Urine pregnancy tests will be conducted on Day 1, Day 14, Day 28, and Day 35 and at the Early Discontinuation Visit (if applicable).

For all participants of childbearing potential, all methods of contraception, including abstinence, must be in use from Day 1 through the Day 56 EOS Visit, and for at least 90 days after the last dose of study drug.

Participants who are not sexually active during the Screening Period must agree to the contraceptive requirements if they become sexually active with a partner of the opposite sex during the study and for at least 90 days after the last dose of study drug. The contraceptive requirements for participants are as follows:

Male participants who can father a child must agree to use 1 highly effective method of contraception from Day 1 to at least 90 days after the last dose. Acceptable methods include true abstinence (refraining from heterosexual intercourse during the study), combined (estrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine devices, intrauterine hormone releasing systems.

Male participants with pregnant or non-pregnant WOCBP partners must use a condom.

Female participants must be postmenopausal (defined as no menses for 12 months without an alternative medical cause), surgically sterile, or using highly effective contraception methods (ie, methods that alone or in combination achieve <1% unintended pregnancy rates per year when used consistently and correctly) from Day 1 to at least 90 days after the last dose. Such

methods include true abstinence (refraining from heterosexual intercourse during the study), combined (estrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation and supplemented with a double barrier (preferably male condom), intrauterine devices, intrauterine hormone-releasing systems, or vasectomized partner. For women ≤45 years of childbearing potential, an additional confirmatory testing of FSH level with a threshold of >40 mIU/mL should be performed to be considered infertile.

Note: Abstinence is only considered to be a highly effective method of contraception when this is the preferred and usual lifestyle of a participant. Periodic abstinence (calendar, symptothermal, postovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method are not acceptable methods of contraception.

8.2. Adverse Events, Serious Adverse Events, and Other Safety Reporting

The definitions of AEs, TEAEs, and SAEs can be found in Section 10.3.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative). Adverse events may be solicited or unsolicited. Solicited and unsolicited AEs are defined in Section 10.3.

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3.

8.2.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected continuously from the signing of the ICF until the Day 56 EOS Visit as specified in the SoA (Section 1.3). Medical occurrences that begin after obtaining informed consent but before study drug administration will be recorded as medical history/current medical conditions, not as AEs; however, SAEs that occur after signing the ICF and before the first dose of study drug is administered, must be recorded and reported as SAEs.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.3. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study drug or study participation, the Investigator must promptly notify the Sponsor.

8.2.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.2.3. Follow-Up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, and AESIs (as defined in Section 8.2.7), will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.2).

At the EOS, all ongoing AEs will be assessed by the investigators to determine whether they are resolved and stable or not clinically significant and reported in the appropriate eCRF page. Any study-related safety issues or serious events that extend past Study Day 56 (EOS) will be followed to resolution.

Further information on follow-up procedures is provided in Section 10.3.

8.2.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the Investigator to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study drug under clinical investigation are met.

Insmed has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study investigational medicinal product (IMP) under clinical investigation. This study is being conducted in the US only; regulatory requirements relating to safety reporting to the US FDA, IRBs, and Investigators will be followed.

An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure, or other documents and will notify the IRB, if appropriate according to local requirements.

Investigator safety reports must be prepared for SUSARs according to local regulatory requirements and Insmed policy and forwarded to Investigators as necessary. No serious adverse reactions are considered expected for brensocatib. Please refer to the Reference Safety Information in the Investigator's Brochure.

8.2.5. Pregnancy

Details of all pregnancies that occur in WOCBP participants within 90 days after study drug administration will be collected. Male participants with WOCBP partners must continue to use contraception for at least 90 days after study drug administration and report any pregnancies that occur within that time period.

If a pregnancy is reported, the Investigator will record pregnancy information on the appropriate form and submit it to Insmed within 24 hours of learning of the pregnancy in a female participant or female partner of a male participant and after obtaining the necessary signed informed consent from the female partner.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.

The pregnant participant/pregnant female partner of a male participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the pregnant participant/pregnant female partner of a male participant and the neonate and the information will be forwarded to Insmed.

Any poststudy pregnancy-related SAE considered reasonably related to the study drug by the Investigator will be reported to Insmed as described in Section 10.3.4. While the Investigator is not obligated to actively seek this information in former study participants who are pregnant or pregnant female partners of former male participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating in the study, prior to study drug administration, will be discontinued from the study.

8.2.6. Cardiovascular and Death Events

Cardiovascular and death events must be reported as AEs/SAEs per the reporting instructions in Section 10.3.

8.2.7. Adverse Events of Special Interest

Adverse events of special interest (AESI) are defined as events known as related to treatment with DPP1 inhibitors. The AESI category groups and preferred terms include the following: hyperkeratosis (Section 8.2.7.1), periodontal/gingival events (Section 8.2.7.2), and infection (Section 8.2.7.3).

8.2.7.1. Hyperkeratosis

At each study visit the Investigator (or designated healthcare professional in case of a public health emergency) will perform a careful skin evaluation, especially of the palmar and plantar surfaces, the dorsal surfaces of the hands and feet, the Achilles tendon area, knees, and elbows. If there are any signs or symptoms of hyperkeratosis or erythema or deterioration of the preexisting conditions that warrant further evaluation upon Investigator discretion, the participant will be referred to a dermatologist for further assessment. The skin evaluation by the dermatologist for the participant should then be assessed thereafter on an interval per the dermatologist discretion until the end of the study.

Skin exfoliation or signs of skin thickening in the palms, soles, dorsum of the hands and feet, Achilles tendon area, knees, or elbows should warrant evaluation by the dermatologist.

Decisions to discontinue the participant from the study will be made by the Investigator after consulting with the dermatologist. Upon receiving the dermatologist's evaluation, the Investigator should decide whether to continue the participant in the study or initiate early discontinuation of the participant.

8.2.7.2. Periodontitis/Gingivitis

Participants will be advised to conduct self-monitoring of their oral soft tissue, gingiva, and tooth mobility and to report any findings. Occurrence of periodontitis/gingivitis during the study will be considered an AESI.

In the case of suspected periodontitis/gingivitis, participants will be referred to a dentist/periodontist for evaluation, diagnosis, and treatment.

Participants will be discontinued from the trial if they develop severe periodontal disease as defined by:

- Pocket depth measurement and attachment loss ≥6 mm on 2 or more teeth
- Have Class 3 mobility or Class 3 furcation involvement

8.2.7.3. Other Infections

Participants will be monitored throughout the study for potential infections. Severe infections, such as any infection requiring treatment with parenteral antibiotics/antiviral/antifungal agent, any clinical endoparasitosis, any opportunistic infection, should be reported as AESIs. Generally, all uncommon, atypical, peculiar, or unusually persistent infections should be reported as AESIs.

Pneumonia will be a specific subcategory that will be monitored through the study.

8.3. Pharmacokinetic Assessments

The schedule for PK assessments and sampling windows is shown in the SoA, Section 1.3.

8.3.1. PK Sample Collection

Blood samples of approximately 6 mL each will be collected for measurement of plasma concentrations of brensocatib and for PK analysis. The total blood volume collected per participant (plasma drug concentration for PK analysis) will be approximately 114 mL or ~ 8 tablespoons.

A maximum of 2 samples may be collected at additional timepoints during the study if an SAE occurs or at early termination.

Each PK plasma sample will be divided into 2 aliquots (1 for PK analysis and the other for backup). All samples will be stored frozen (–70°C) until shipment and then stored at –70°C until analysis. If a –70°C freezer is unavailable, PK samples can be stored at –20°C. The actual date and time (24-hour clock time) of each sample will be recorded.

Full instructions for the collection and handling of PK samples will be provided by Insmed in a separate Laboratory Manual.

8.3.2. PK Analysis

Individual PK parameters of brensocatib will be determined using noncompartmental analysis for the following parameters: C_{max}, t_{max}, C_{min}, $AUC_{0\text{-}24}$, AUC_{last}, $AUC_{0-\infty}$, CL/F, Vd/F, $t_{½}$, $R_{ac(Cmax)}$, and $R_{ac(AUC)}$ on Day 1 and Day 28, as appropriate. C_{trough} on Day 2, Day 14, Day 28, and Day 29 will be evaluated by statistical descriptive values (Section 9.4.2).

Among the PK parameters, the primary endpoints for PK evaluation will be C_{max}, T_{max}, AUC_{0-24} and $t_{1/2}$ on Days 1 and 28. All PK parameters are described in Table 4.

Table 4: Pharmacokinetic Parameters

Parameter	Definition
$AUC_{0-\infty}$	Area under the concentration-time curve from time 0 to infinity
AUC_{0-24}	Area under the concentration-time curve from time 0 to 24 hours postdose
AUC_{last}	Area under the concentration-time curve from time 0 to the last timepoint with measurable concentration
CL/F	Apparent total clearance of drug from plasma after extravascular administration
C_{max}	Maximum plasma concentration
C_{min}	minimum plasma concentration
C_{trough}	plasma concentration before the next dose
$R_{ac(AUC)}$	Accumulation ratio based on AUC at steady state (AUC_{0-24} ratio between Day 28 and Day 1)
$R_{ac(Cmax)}$	Accumulation ratio based on C_{max} at steady state (C_{max} ratio between Day 28 and Day 1)
$t_{½}$	Elimination half-life
t_{max}	Time to maximum plasma concentration
V_d/F	Apparent volume of distribution

8.3.3. Pharmacokinetic and Pharmacodynamic Evaluations

Relationships between PK (brensocatib dose and exposure) and PD effects (eg, NE concentrations in blood and sputum, $ppFEV_1$) and safety (eg, AESI, including hyperkeratosis, periodontitis/gingivitis, and infections) will be explored and the results may be reported separately.

8.4. Pharmacogenomics

Pharmacogenomic analyses are not included in this study.

8.5. Biomarkers

Collection of biological samples for biomarker research is also part of this study. The following samples for biomarker research are required and will be collected from all participants in this study as specified in the SoA:

- Blood (6 mL sample per PD collection timepoint)
- Sputum (3 mL sample per PD collection timepoint)
- The total blood volume per participant collected over the course of the study for PD analysis will be approximately 42 mL or ~3 tablespoons.

Samples will be tested for the effect of brensocatib compared with placebo on the concentration of NE, CatG, and PR3 over the 4-week treatment period and 1 week after the treatment, to evaluate the relationship between study drug dose, AUC, and biomarker activity.

8.6. Sputum Assessments

Collection of sputum samples for microbiology assessment is also part of this study. The following samples will be collected from all participants in this study as specified in the SoA:

- Sputum (3 mL sample per PD collection timepoints; PD assessments will be prioritized, and any remaining sputum sample will then be used for microbiology assessment)

8.7. Medical Resource Utilization and Health Economics

Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

8.8. Efficacy Assessments

The schedule for efficacy assessments is shown in the SoA, Section 1.3

8.8.1. Spirometry

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

Prebronchodilator PFT by spirometry (FEV_1, ppFEV_1, FVC, PEFR, and $FEF_{[25-75\%]}$) will be performed per the ATS/ERS criteria (Miller et al., 2005). Participants will be provided with detailed instruction on how to conduct the FVC maneuver per ATS/ERS spirometry standardization before performing the test. Time of the last bronchodilator medication use before the procedure will be recorded.

The administration of a bronchodilator as part of a chronic and stable regimen is not prohibited.

Spirometry should be performed preferably in the morning (AM) at approximately the same time each visit. The same spirometer and standard spirometric techniques, including calibration, will be used to perform spirometry at all visits and, whenever possible, the same person should perform the measurements. Pulmonary function tests will be performed with the participant in a sitting position; however, if necessary to undertake the testing with the participant standing or in another position, this should be noted on the spirometry report. For any participant, the position should be consistent throughout the study. Three (3) measurements fulfilling the ATS acceptability and repeatability criteria should be obtained at every visit. The acceptability criteria must be applied before the repeatability criteria. Unacceptable maneuvers must be discarded before applying the repeatability criteria. If a participant fails to provide repeatable maneuvers, an explanation should be recorded in the source documentation. At least 2 acceptable curves must be obtained. The largest FEV_1 and largest FVC should be recorded after the data are examined from all of the acceptable curves, even if they do not come from the same curve. The $FEF_{(25-75\%)}$ should be obtained from the single curve that meets the acceptability criteria and gives the largest sum of FVC plus FEV_1 (best test). Automated best efforts, which combine FEV_1 and FVC are not acceptable. The spirometer must be calibrated following the principles of the ATS/ERS guidelines every day that a study participant is assessed and spirometry is carried

out. The calibration records should be kept in a reviewable log. It is preferred that the calibration equipment (ie, 3-L syringe) that is used to calibrate the spirometer be subjected to a validated calibration according to the manufacturer's specifications.

Participants should be advised to rest at least 30 minutes and not to eat a large meal for at least 2 hours prior to the test. If a participant is scheduled to have pulmonary rehabilitation on the day of their visit, they should be advised to have the PFT done before the rehabilitation on that day.

Detailed instruction on how to conduct the spirometry and document the results are provided in a separate Spirometry Manual.

8.8.2. Cystic Fibrosis Questionnaire – Revised

The CFQ-R (Henry et al., 2003) is a disease-specific HRQOL measure for children, adolescents, and adults with CF. It is a profile measure of HRQOL with 9 QOL domains (Physical Functioning, Vitality, Emotional State, Social Limitations, Role Limitations/School Performance, Embarrassment, Body Image, Eating Disturbances, Treatment Constraints) 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. The CFQ-R questionnaire should be performed before any other study procedures.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

No statistical hypotheses will be evaluated in this study.

Primary, secondary, and exploratory objectives and corresponding endpoints are described in Section 3.

9.2. Sample Size Determination

Overall, the study will enroll approximately 25 to 34 participants. The 10 mg, 25 mg, and 40 mg cohorts will enroll approximately 7 participants each, and the placebo cohort will enroll approximately 4 participants. For the active treatment cohorts, 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 cohort, approximately 3 participants will be randomized into the CFTR modulator stratum and 1 participant in the non-modulator stratum.

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

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 doses study designs and is considered sufficient to meet the study objectives.

$AUC_{0\text{-}24}$ 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 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 CV(%) of the GM. Table 5 presents participants treated with brensocatib per dose group (N=10 and N=7), and Table 6 presents participants treated with brensocatib per stratum (N=5) (CFTR modulator) within dose group.

Table 5: 95% CIs for Values of Inter-Participant CV(%) of the AUC_{0-24} and C_{max} GM in Each Actively Treated Cohort

N	Geometric CV(%)	Geometric Mean 95% CI LL	Geometric Mean 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.
CV = percent coefficient of variation, CI = confidence interval, GM = geometric mean, LL = lower limit, UL = upper limit.

Table 6: 95% CIs for Values of Inter-Participant CV(%) of the AUC_{0-24} and C_{max} GM for Each Stratum Within Each Actively Treated Cohort

Geometric CV(%)	Geometric Mean 95% CI LL	Geometric Mean 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).
CV = percent coefficient of variation, CI = confidence interval, GM = geometric mean, LL = lower limit, UL = upper limit.

The range of values for GM CV(%) is based on the results of Day 29 AUC_{last} and C_{max} in a study of patients with 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 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%.

9.3. Analysis Sets

The following analysis populations will be applied to this study.

- Randomized Population: All randomized participants.
- Intent-to-Treat Population: All randomized participants who receive at least 1 dose of study drug with at least 1 predose and 1 postdose efficacy measure.
- Safety Population: All randomized participants who receive at least one dose of study drug. Participants are to be included in the analysis according to the dose and study drug received.
- PK Population: All randomized participants who receive at least 1 dose of brensocatib and have sufficient data to calculate at least 1 PK parameter.

- PD Population: All randomized participants who receive at least 1 dose of study drug or placebo with at least 1 predose and 1 postdose PD measure.

9.4. Statistical Analyses

The SAP will be finalized prior to database lock, and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints.

9.4.1. General Considerations

There is no hypothesis-testing in this study, and no inferential statistical analyses will be conducted. There will be no proxy values for missing data. PK, PD, and efficacy data will be presented in tables, listings, and graphs as appropriate for each variable. All data will be summarized by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within strata (CFTR modulator and non-modulator) if data warrant. The C_{max} and AUC data may be pooled data across all dose levels within each stratum if the systemic exposure is approximately dose proportional, and the exposure difference between CFTR and non-CFTR participants will be compared based on dose-normalized C_{max} and AUC. Data from placebo participants will be excluded from PK evaluation.

PK parameters, such as C_{max}, t_{max}, C_{min}, $AUC_{0\text{-}24}$, AUC_{last}, $AUC_{0-\infty}$, CL/F, Vd/F, $t_{½}$, $R_{ac(Cmax)}$, and $R_{ac(AUC)}$, will be derived using non-compartmental analysis, as appropriate. C_{trough}, the predose levels on Day 2, Day 14, Day 28, and Day 29, will be listed and summarized using descriptive statistics based on nominal timepoints.

The frequency of TEAEs is the primary safety endpoint for this study. All safety analyses will be performed on the safety population. AE listings will be produced. TEAEs, SAEs, AEs leading to treatment and/or study withdrawal, and AESIs will be listed for each participant along with outcome, severity, and relatedness to study IMP. Ad hoc summaries of TEAEs may be prepared if the volume of data allows.

Exploratory endpoints include PD markers (NE, CatG, and PR3 in sputum and in blood), FEV_1, and CFQ-R Respiratory Domain Score. In addition, the relationship between brensocatib exposure and NE activity will be explored.

9.4.2. Primary Endpoint

Plasma concentrations of brensocatib will be listed and summarized by active dose group and by active dose group within strata over each scheduled sampling time, using descriptive statistics (including arithmetic mean, SD, median, minimum and maximum, GM with 95% CI, and CV(%) of the GM, as appropriate). Individual plasma concentration data versus time will be presented in data listings, along with graphical plots of individual and GM plasma concentration-time plots presented in linear and semi-logarithmic scales.

The main PK endpoints (C_{max}, t_{max}, $AUC_{0\text{-}24}$, and $t_{½}$ on Day 1 and Day 28) will be determined using non-compartmental analysis.

All plasma PK parameters, including the ones that are not included in the primary endpoint, will be listed and summarized using descriptive statistics. All concentrations will be listed and summarized using descriptive statistics based on nominal timepoints.

A detailed SAP will be developed. The SAP will be finalized and approved by signature prior to the database lock to preserve the integrity of the statistical analysis and study conclusions.

The frequency of TEAEs is a primary endpoint of this trial. Section 9.4.5.1 discusses the analyses of AEs.

9.4.3. Secondary Endpoints

Analysis of dose dependency for brensocatib $AUC_{0\text{-}24}$, AUC_{last}, and C_{max} after single- and multiple-dose administration will be performed using a power law model. The log-transformed PK parameters will be regressed onto log-transformed dose. An estimate of the slope and corresponding 95% CI will be reported. In addition, pairwise comparisons of dose dependency among brensocatib dose levels may be performed based on dose-normalized $AUC_{0\text{-}24}$, AUC_{last}, and C_{max} for Day 1 and Day 28. The details of the analysis will be included in the SAP.

9.4.4. Exploratory Endpoints

NE, CatG, and PR3 (in sputum and in blood) will be exploratory endpoints. The results of these parameters will be listed by participant and timepoint and summarized by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within strata (CFTR modulator and non-modulator, pooled data across all dose levels). Where sufficient sample sizes are achieved (ie, adequate sample obtained and minimum number of quantifiable concentrations), a linear model with appropriate covariance pattern will be fit to the exploratory endpoints and between-group comparisons with associated 95% CIs will be reported.

Individual figures of NE, CatG, and PR3 versus time will be presented with all participants overlaid on the same plot for each dose level (spaghetti plots). Mean plots versus time will also be presented by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within each stratum (CFTR modulator and non-modulator).

FEV_1 and CFQ-R Respiratory Domain are also exploratory endpoints. These variables will be listed and summarized in tables by brensocatib dose levels and placebo.

The relationship between dose and the 3 PD parameters (NE, CatG, and PR3) will be displayed graphically.

Additional PK-PD evaluations will be performed in separate analyses, in which the relationship between brensocatib exposure (dose or AUC) and clinical measurements (PD biomarkers, $ppFEV_1$ and AESI) will be explored. The results from these PK-PD analyses will be reported separately.

Microbial species and colony forming units from sputum assessments will be listed and summarized by brensocatib dose levels and placebo.

9.4.5. Safety Analysis

All safety analyses will be performed on the Safety Population.

All safety data will be summarized by treatment received (which may differ from the randomized treatment arm) and for the entire safety population, using descriptive statistics.

Participant disposition will be listed and summarized including the number of withdrawals and the primary reason for withdrawal. Participants excluded from any analysis sets will be listed including the reasons for exclusions.

All safety data will be listed for each participant and summarized appropriately. Adverse events will be coded using MedDRA and summarized by SOC and PT. Additional summaries by severity (NCI CTCAE) and relationship to study treatment will be presented.

All clinical safety laboratory data and vital signs will be listed and summarized including changes from Baseline. Any out-of-range laboratory measurements will be flagged in the listings.

The analysis of the safety endpoints and their reporting will be described in more detail in the SAP.

9.4.5.1. Adverse Events

Adverse events will be coded using MedDRA SOC and PT. The severity of AEs will be graded per the NCI CTCAE v4.0.

The number and percent of participants with AEs including those collected at early discontinuation will be presented by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within each stratum (CFTR modulator and non-modulator). The severity of AEs, the relationship to study drug, AEs causing study discontinuation, and SAEs will be similarly presented. All AEs reported during the study will be listed. Treatment-emergent and treatment-related AEs will be tabulated by SOC body system and organ class. Placebo from all treatments will be pooled and described.

9.4.5.2. Clinical Laboratory Tests

The results of hematology, blood chemistry, and urinalysis will be listed for each participant. Laboratory values outside the normal ranges will be flagged. If applicable, summary statistics (mean, range, change from Baseline) by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within each stratum (CFTR modulator and non-modulator) will be provided for each timepoint including EOT, which will summarize the last measurement for participants irrespective of whether they complete, discontinue early, or are lost to follow-up. Baseline values are defined as laboratory values from blood and urine samples collected prior to first dose on Day 1 (Baseline). Summary statistics of clinical laboratory values will be tabulated by treatment and dose.

9.4.5.3. Vital Signs

Systolic and diastolic blood pressure, pulse rate, respiratory rate, body temperature, and oxygen saturation will be listed for each participant. If applicable, summary statistics (mean change, percentage change from Baseline, and absolute change from Baseline) by treatment group (dose levels of brensocatib and pooled placebo) and by treatment group within each stratum (CFTR modulator and non-modulator) at each timepoint and EOT will be provided. Baseline values are

defined as vital signs collected prior to the first dose on Day 1. The AEs reported in association with the clinically significant abnormalities in vital signs will be presented in the relevant AE listings.

9.4.5.4. Physical Examination

Physical examination postbaseline findings that meet the criteria for an AE will be listed in the relevant AE listings.

9.4.5.5. Electrocardiogram

The results of the 12-lead ECG tracings will be listed for each participant. Postbaseline ECG results that meet the criteria for an AE will be listed in the relevant AE listings. If applicable, summary statistics of ECG results will be provided.

9.5. Interim Analysis

No formal interim analysis is planned for this study.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents must be submitted to an IRB by the Investigator and reviewed and approved by the IRB before the study is initiated.

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

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

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB
- Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, and all other applicable local regulations

10.1.2. Protocol Deviations

The Investigator must conduct the study in compliance with the protocol as agreed to by Insmed and, if required, by the regulatory authority(ies), and which was given approval/favorable opinion by the IRB.

The Investigator must notify the IRB of deviations from the protocol in accordance with IRB reporting requirements.

As of May 2020, due to concern regarding COVID-19, there may be restrictions to participant attendance at in-clinic visits or elective inpatient admissions. In the event participants are restricted to attend in-clinic visits, or if the participant has concern regarding travel and attending in-clinic visits (due to potential public health concerns), the site must contact Insmed on how to conduct the scheduled assessments, and decisions must be documented in the source documentation. Where necessary, in-clinic visits may be conducted via telemedicine link and home health care visits.

10.1.3. Public Health Emergency Situations

During the COVID-19 public health emergency, Insmed, IRBs, and Investigators shall follow the most current version of local guidance to assure the safety of study participants, maintain compliance with GCP, and minimize risks to study integrity. The continuity of clinical study conduct and oversight may require implementation of temporary or alternative mechanisms, which will remain in effect only for the duration of the local public health emergency. Examples of such mechanisms may include, but are not limited to, any of the following: telephone contact, virtual visits, telemedicine visits, online meetings, non-invasive remote monitoring devices, use of local clinic or laboratory locations, and home visits by skilled staff. The site must contact Insmed on how and when to implement temporary and/or alternative mechanism of scheduled assessments, and all decisions taken must be documented in the source documentation. Additionally, all temporary mechanisms utilized, and the resulting deviations from planned study procedures are to be documented as being related to COVID-19.

In a situation where local health authorities declare a public health emergency while the study is ongoing, the suggested guidance in the following subsections must be followed.

10.1.3.1. Continuation or Suspension of the Study

Ensuring the safety of trial participants is paramount. Insmed, in consultation with the clinical investigators and IRB, will determine whether the protection of a participant's safety, welfare, and rights are best served by continuing or stopping the trial at the specific site. Such decision will depend on specific circumstances, including the ability to conduct appropriate safety monitoring, the potential impact on the investigational product supply chain, and other considerations.

If the decision is to continue the study, the following considerations will be taken into account:

10.1.3.1.1. Study Recruitment

The Insmed will communicate to the sites the decision on whether to continue or suspend recruitment after considering the specific circumstances of each site, recommendation by the IRB, and its local health authority mandates. If, due to COVID-19, study discontinuation exceeds the assumed rate, Insmed may allow enrollment past the assumed sample size.

10.1.3.1.2. Participants Already Enrolled in the Study

If the decision is to continue the participation of participants already enrolled, the following steps must be taken:

- If a participant is not able to complete a protocol specified study visit, it may be necessary to adjust the visit schedule, convert in-clinic visits to telemedicine visits, and/or postpone study procedures until the next available in-clinic study visit. If there is information available from previous visits (ie, laboratory assessments) that requires follow-up procedures or other safety assessments, the Investigator will decide if an on-site visit or home healthcare visit is required or whether the participant's safety can be preserved by other means.

10.1.3.1.3. Participants Infected by COVID-19

If a participant has had a past documented mild to moderate COVID-19 infection prior to enrollment in the trial but the participant has recovered and the current diagnostic tests are negative (negative antigen by any diagnostic laboratory kit), the participant can be screened, at the Investigator's discretion. Participants who experienced hospitalization, severe disease, and/or COVID-19 ARDS must be excluded.

If a participant has a documented infection by COVID-19 while in the trial, the event will be reported as an AE or SAE, depending on the criteria. The Investigator will follow the guidance provided by health authorities in the treatment of those participants.

10.1.4. Financial Disclosure

The disclosed financial interest of the Investigator must be collected before Screening of the first participant, following study completion at the Investigator site and 1 year following overall study completion. The Investigator must promptly update this information if any relevant changes occur during this period.

10.1.5. Informed Consent Process

Before a participant's participation in the study, it is the Investigator's responsibility to obtain freely given consent, in writing, from the participant or legally acceptable representative after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any study IMPs are administered. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective participant, to the participant's participation in the clinical study. An informed consent document that includes both information about the study and the consent form will be prepared and given to the participant. This document will contain all the elements required by the ICH E6 (R2) Guideline for GCP and any additional elements required by local regulations. The written ICF must be prepared in the local language(s) of the potential participant population.

In obtaining and documenting informed consent, the Investigator must comply with the applicable regulatory requirements and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form and any revision(s) must be approved by the IRB prior to being provided to potential participants.

The participant's written informed consent (written or electronic) must be obtained prior to his/her participation in the study, and must be documented in the participant's medical records, as required by applicable regulations. The ICF must be signed and personally dated by the

participant or a legally acceptable representative and by the person who conducted the informed consent discussion (not necessarily the Investigator). The signed ICF must be retained in accordance with institutional policy, and a copy of the signed consent form must be provided to the participant or legal representative. The date and time that informed consent was given must be recorded in the CRF.

Participants who are rescreened are required to sign a new ICF.

Informed consent must be obtained prior to participation in the study, including procedures to be performed during Screening. For example, after the participant has provided the informed consent, they will be instructed to report bronchodilator use upon to returning to the clinic for the Screening spirometry assessment.

10.1.6. Protection of Participant Identification and Confidentiality

The Investigators and Insmed will preserve the confidentiality of all participants taking part in the study, in accordance with GCP and local regulations.

The Investigator must ensure that the participant's anonymity is maintained. On the CRF or other documents submitted to Insmed, participants must be identified by a unique participant identifier as designated by Insmed. Documents that are not for submission to Insmed (eg, signed ICFs) must be kept in strict confidence by the Investigator.

In compliance with ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB direct access to review the participant's original medical records for verification of study-related procedures and data. The Investigator is obligated to inform the participant that his/her study-related records will be reviewed by the above-named representatives without violating the confidentiality of the participant.

Participant names will not be supplied to Insmed. A participant number will be recorded in the CRF, and if the participant name appears on any other document (eg, laboratory report), it must be obliterated on the copy of the document to be supplied to Insmed. All records will be kept confidential to the extent provided by federal, state, and local laws. The participants will be informed that representatives of Insmed, IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The Investigator will maintain a personal participant identification list (participant numbers with the corresponding participant names) to enable records to be identified.

10.1.7. Data Protection

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

The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be

explained to the participant who will be required to give consent for their data to be used as described in the informed consent

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

10.1.8. Committees Structure

An SRC will be chartered, comprising:

- Insmed Vice President of Pipeline Programs
- Insmed Medical Monitor for Brensocatib Clinical Development Program
- Insmed Clinical Pharmacology Expert
- Vice President, Drug Safety and Pharmacovigilance (or designee)
- Principal Investigator for the study
- And possible external expert consultants

Participant safety will be continuously monitored by the Sponsor, which includes safety signal detection at any time during the study. After the first 3 cohorts (brensocatib 10, 25, and 40 mg QD) have completed the study, the SRC will review available data (PK and safety data [vital signs, clinical laboratory test values, ECGs, and AEs]) 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.

In particular, the SRC will review the following events:

- Any deaths, regardless of causality
- AESIs, including hyperkeratosis, periodontal/gingival events, and infection

Complete details can be found in the SRC charter.

10.1.9. Dissemination of Clinical Study Data

Insmed will provide study information for inclusion in national registries according to national/local regulatory requirements.

Results of this study will be disclosed according to the relevant national regulatory requirements.

10.1.10. Data Quality Assurance

The Investigator/investigational site will permit study-related monitoring, audits, IRB review and regulatory inspections by providing direct access to source data/documents. Direct access includes permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of a clinical study.

10.1.10.1. Data Collection

All data obtained for this study will be entered into a 21 CFR Part 11 compliant Data Management System provided by Insmed or its designee. These data will be recorded with an EDC system using CRFs. The Investigator will ensure the accuracy and completeness of the data reported to Insmed. All data entry, modification or deletion will be recorded automatically in an electronic audit trail.

The Investigator will provide access to his/her original records to permit a representative from Insmed to verify the proper transcription of data. Data reported in the CRFs must be consistent with and substantiated by the participant's medical record and original source documents. The CRF data will be monitored by Insmed or designee. The final, completed CRF Casebook for each participant must be electronically signed and dated by the Principal Investigator within the EDC system to signify that the Investigator has reviewed the CRF and certifies it to be complete and accurate.

The Insmed will retain the final CRF data and audit trail. A copy of all completed CRFs will be provided to the Investigator.

10.1.10.2. Study Monitoring

Before study initiation, at a site initiation visit or at an Investigator's meeting, a Insmed representative will review the protocol, CRF, Investigator's Brochure, and any study related materials with the Investigators and their staff. During the study, the Insmed study monitor or its designee will visit the site regularly to check the completeness of participant records, the accuracy of entries on the CRFs, adherence to the protocol, adherence to ICH GCP and applicable regulatory requirements, the progress of enrollment, and to ensure that study IMP is being stored, dispensed, and accounted for according to specifications.

The Investigator must give the monitor access to relevant hospital or clinical records to confirm their consistency with the CRF entries. Participant confidentiality will be maintained by the study center. The study Insmed monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of primary activity and safety variables. Additional checks of the consistency of the source data with the CRFs are performed according to the study specific monitoring plan.

10.1.10.3. Audits and Inspections

Domestic and foreign regulatory authorities, the IRB, and an auditor authorized by Insmed may request access to all source documents, CRFs, and other study documentation for onsite audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that participant names are obliterated on the copies to ensure confidentiality.

If an inspection is requested by a regulatory authority, the Investigator will inform Insmed, immediately that this request has been made.

10.1.10.4. Study Record Retention

Study documents must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents must be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of Insmed. It is the responsibility of Insmed to inform the Investigator when these documents no longer need to be retained.

10.1.10.5. Source Documents

Source documentation is the point of initial recording of a piece of data. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical study. Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.

Data reported on the CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.11. Study Files and Materials

Before the start of any study related procedures, all initial documents required by ICH GCP, Good Pharmacoepidemiology Practice, and applicable local regulations must be available in the relevant files maintained by Insmed (or delegate) and the Investigator. An Investigator Study File prepared by Insmed (or delegate), containing all applicable documents for use at the study site, will be made available to the Investigator before the start of the study. A list of personnel and organizations responsible for conduct of the study as well as the list of Investigator-delegates (eg, subinvestigator) at each study site will be included in the Investigator Study File. The respective files will be kept and updated by Insmed (or delegate) and the Investigator, as applicable.

All study documentation and materials maintained in the Investigator Study File at the study site must be available for inspection by Insmed's study monitor (or delegate) to determine that all required documentation is present and correct.

The study may be audited by qualified delegates from Insmed or a competent regulatory authority.

10.1.12. Use of Stored Samples and Data

Stored samples will be labeled with study and participant information and secured with limited access and retained per protocol requirements and in accordance with the laboratory's operating procedures. Electronic data will be kept in password protected computers at the laboratory and then transferred to Insmed or CRO, as applicable, for data analysis. Samples and corresponding data will be tracked using the laboratory's specimen tracking system.

Prior Insmed and IRB approval are required before using or sharing study samples or data in ways not specifically specified in the study protocol during conduct of this study.

Any loss or unanticipated destruction of samples (eg, freezer malfunction) or data (eg, loss of a data sheet with individually identifiable information) that violates or compromises the scientific integrity of study data must be reported to Insmed and the IRB.

At any time, participants may inform the Investigator in writing that they do not wish to have their samples stored beyond the completion (termination) of the study. In this case, the Investigator will request that all known remaining samples be destroyed and report the disposition of samples to the requesting participants and the IRB.

10.1.13. Disposition of Stored Samples and Data

Participant samples will be secured with limited access and retained per protocol requirements and in accordance with the laboratory's operating procedures. Samples stored by the central laboratories will be labeled with the participant's study identification information. Data will be kept in password protected computers at the laboratory and then transferred to the vendor for data analysis. Samples and corresponding data acquired will be tracked using the laboratory's specimen tracking system.

In the future, if other Investigators may wish to study these samples and/or data, they need to obtain Insmed approval and participant consent before any sharing of samples and/or data.

Any loss or unanticipated destruction of samples (eg, due to freezer malfunction) or data (eg, loss of a data sheet with individually identifiable information) that results in a violation that compromises the scientific integrity of the data collected for the study will be reported to Insmed and the IRB

Additionally, participants may withdraw authorization in writing to decline their sample storage for a period of up to 2 years beyond the duration of the study. In this case, the Principal Investigator will request the destruction of all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the individual's participation in the study.

10.1.14. Study and Site Start and Closure

Before the start of the study at each study site, Insmed's study monitor (or delegate) shall confirm adequacy of the facilities by study site visit or other acceptable methods.

The Investigator may not enroll any participant into the study before Insmed has received written approval or a favorable opinion from the IRB for conducting the study and a formal meeting has been conducted by Insmed's study monitor (or delegate) to initiate the study.

10.1.14.1. First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants. The first patient screened for participation in the study is considered the first act of recruitment.

10.1.14.2. Study/Site Termination

Insmed or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of Insmed. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by Insmed or the Investigator may include but are not limited to:

- For study termination:
  - Discontinuation of further study IMP development
- For site termination:
  - Failure of the Investigator to comply with the protocol, the requirements of the IRB or local health authorities, Insmed's procedures, or GCP guidelines
  - Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the Investigator
  - Total number of participants included earlier than expected

If the study is prematurely terminated or suspended, Insmed shall promptly inform the Investigators, the IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the participant and must assure appropriate participant therapy and/or follow-up.

10.1.15. Protocol Amendments

Any substantial change or addition to this protocol requires a written protocol amendment that must be approved by Insmed, before implementation. Amendments significantly affecting the safety of participants, the scope of the investigation, or the scientific quality of the study, require additional approval by the applicable regulatory authority(ies) and IRBs. Copies of the applicable written approvals must be filed in Insmed files and Investigator site files.

The requirements for approval must in no way prevent any immediate action from being taken by the Investigator or by Insmed in the interests of preserving the safety of all participants included in the study. If an immediate change to the protocol is felt to be necessary by the Investigator and is implemented by him/her for safety reasons, Insmed or its agent must be notified and the applicable regulatory authority(ies)/IRBs must be informed as soon as possible. Any other regional reporting requirements must be adhered to.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or regulatory authority/IRB approval, but the regulatory authority(ies)/IRBs must be kept informed of such administrative changes in accordance with country-specific requirements.

10.1.16. Financing and Insurance

10.1.16.1. Finances

Prior to starting the study, the Investigator and/or institution will sign a clinical study agreement with Insmed. This agreement will include the financial information agreed upon by the parties.

10.1.16.2. Reimbursement, Indemnity, and Insurance

Reimbursement, indemnity, and insurance shall be addressed in a separate agreement on terms agreed upon by the parties.

10.1.16.3. Participant Reimbursement, Liability and Insurance

The civil liability of the involved parties with respect to financial loss due to personal injury and other damage that may arise as a result of this study being conducted are governed by the applicable legal requirement(s).

Insmed will provide insurance to the Investigator if required by the applicable regulatory and legal requirement(s).

If required by local law, participants taking part in this study will be insured against any injury caused by the study in accordance with the applicable regulatory and legal requirement(s).

10.1.17. Publication Policy

Any formal presentation or publication of data collected from this study will be considered as a joint publication by the Investigator(s) and the appropriate personnel of Insmed. Authorship will be determined by mutual agreement. For multicenter studies, it is mandatory that the first publication be based on data from all centers and analyzed as stipulated in the protocol by the study Insmed and statisticians, not by the Investigators themselves. Investigators participating in multi center studies agree not to present data gathered from a single center or a small group of centers before the full, initial publication, unless formally agreed to by all other Investigators and Insmed.

Insmed must receive copies of any intended communication in advance of publication (at least 15 working days for an abstract or oral presentation and 45 working days for a journal submission). Insmed will review the communications for accuracy (thus avoiding potential discrepancies with submissions to regulatory authorities), verify that confidential information is not being inadvertently divulged, and provide any relevant supplementary information. Authorship of communications arising from pooled data may include members from each of the contributing centers as well as the study Insmed personnel.

At the conclusion of the study, after the data are analyzed, the results of study will be reported in a clinical study report.

10.2. Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 3 will be performed by the central laboratory. Protocol-specific clinical laboratory requirements for the inclusion or exclusion of participants are detailed in Section 5.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Investigators must document their review of each laboratory safety report.

10.3. Appendix 3: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition
• An AE is defined as any untoward medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, October 1994). • A treatment emergent adverse event (TEAE) is defined as any AE that occurs after the first dose of study IMP and within 28 days after the last dose of study IMP
Definition of Unsolicited and Solicited AE
• An unsolicited adverse event is an adverse event that is communicated by a participant or their legal representative who has signed the informed consent. Unsolicited AEs include serious and non-serious AEs. • Potential unsolicited AEs may be medically attended (ie, symptoms or illnesses requiring a hospitalization, or emergency room visit, or visit to/by a health care provider). The participant or their legal representative will be instructed to contact the site as soon as possible to report medically attended event(s), as well as any events that, though not medically attended, are of concern. Detailed information about reported unsolicited AEs will be collected by qualified site personnel and documented in the participant's records. • Unsolicited AEs that are not medically attended nor perceived as a concern by the participant or their legal representative will be collected by interview with the participant/legal representative and by review of available medical records at the next visit. • Solicited AEs are predefined local and systemic events for which the participant is specifically questioned and/or instructed to report.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), that worsen from Baseline, are considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease), that are associated with signs and/or symptoms, require therapeutic intervention, or lead to discontinuation of the administration of study IMP must be reported as an AE
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study IMP administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected IMP- IMP interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study IMP or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses must be reported regardless of sequelae.
- The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE. "Lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE. Lack of expected pharmacological action per se will not be reported as an AE or SAE. Such instances will be captured in the PK and PD assessments.

Events <u>NOT</u> Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2. Definition of SAE

An SAE is defined as any serious adverse event that, at any dose:

a. Results in death

b. Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting.
- Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from Baseline is not considered an AE.

d. Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Is a suspected transmission of any infectious agent via an authorized medicinal product

g. Is an important medical event

h. Other situations:

- Medical or scientific judgment should be exercised by the Investigator in deciding whether SAE reporting is appropriate in other situations such as significant medical events that may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
    - Examples of such events include invasive or malignant cancers, intensive treatment for allergic bronchospasm, blood dyscrasias, convulsions or development of IMP dependency or IMP abuse.

10.3.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information.
- It is **not** acceptable for the Investigator to send photocopies of the participant's medical records to Insmed in lieu of completion of the completed AE reporting form.
- There may be instances when copies of medical records for certain cases are requested by Insmed. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to Insmed.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.
- Life-threatening: An event that places the participant, in the view of the Investigator, at immediate risk of death from the reaction as it occurred (ie, it does not include a reaction that had it occurred in a more severe form, might have caused death).
- Death: Any untoward medical occurrence that at any dose results in death

Assessment of Causality

- The Investigator is obligated to assess the relationship between IMP and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to Insmed. However, it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to Insmed.
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-Up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Insmed to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide Insmed with a copy of any post-mortem findings including histopathology.New or updated information will be recorded in the originally submitted documents.
- The Investigator will submit any updated SAE data to Insmed within 24 hours of receipt of the information.

10.3.4. Reporting of SAEs

All SAEs, regardless of causality, must be reported to the organization delegated by Insmed on a Serious Adverse Event Report Form within 24 hours of becoming aware of the event; corrections and additions are required to be submitted within 24 hours. Study-specific email, phone, and fax number for SAE reporting information will be provided to the study sites.

Unexpected drug related SAEs as assessed by Insmed or authorized person qualify for expedited reporting and will be reported to the IRB, regulatory authorities, and participating Investigators and, if cross reporting is required for SUSARs, in accordance with all applicable global laws and regulations. A SUSAR is a Serious Adverse Reaction that is suspected to be caused by the IMP

and that is unexpected, ie, its nature or severity is not consistent with the information in the relevant Reference Safety Information. SAEs, including those that do not meet requirements for expedited reporting, and all other AEs will be reported to the regulatory agencies as appropriate (ie, for the FDA these are reported in the Investigational New Drug annual report and for the European Medicines Agency, these are reported in the Development Safety Update Report).

SAE Reporting to Insmed via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to Insmed will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by telephone.

SAE Reporting to Insmed via Paper Data Collection Tool

- Facsimile transmission of the SAE paper data collection tool is the preferred method to transmit this information to Insmed.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE data collection tool within the designated reporting time frames.

10.4. Appendix 4: Contraceptive and Barrier Guidance

Contraceptive guidance is presented in Section 8.1.6.

10.5. Appendix 5: Sputum Induction Guidelines

Collection of good sputum samples is critical to this study. Sputum samples will be collected at the clinical site. To facilitate obtaining good sputum samples during the study visit at the study sites, sputum induction may be performed if the participant is unable to expectorate approximately 3.0 mL of sputum.

Purpose

The purpose of this guideline is to provide recommendations to the clinical sites for obtaining a sputum sample by induction if the participant is unable to expectorate a sputum sample on their own or after chest percussion.

Required Equipment

- Standard handheld nebulizer used at the site or the participant can be asked to bring the nebulizer they use at home for pulmonary hygiene
- The nebulizer should be thoroughly disinfected to ensure no cross-contamination
- Sputum specimen containers with label – sputum collection tube provided by central laboratory
- Sodium chloride solution (saline) – 3% and 7%, and possibly 10%
- Standard clinic supplies (eg, disinfectant/germicidal/alcohol wipes, tissues, paper towels)

Procedure

General Instructions:

- At the study site, sputum induction should occur in a private, contained room. Specific processes in place at the clinic to prevent contamination and ensure sterilization before and after sputum induction should be followed.
- Clinic personnel should wear gloves and a mask during the entire procedure.
- Only 1 participant should be induced at a time.
- All collection containers should have a sputum collection label clearly completed with participant identifiers, visit name, date, and time.
- The participant should have been instructed not to eat at least within 1 hour of sputum induction procedure.
- An explanation should be provided to the participant that the purpose of this procedure is to help him/her cough up a sputum sample and that the success of the procedure is dependent on the participant's active participation.

Inhalation and Collection Procedures:

- The induction procedure should start by utilizing either 3% or 7% saline based on the Investigator's preference.
- Approximately 3 to 6 mL of the selected saline should be placed in the nebulizer.

- The participant should be sitting up or in a semi-Fowler position.
- The participant may wear a nose clip during the nebulization.
- The participant should breathe slowly and deeply through the nebulizer mouthpiece inhaling the salt water mist. Remind the participant not to breathe quickly but to have slow, deep breaths pausing at peak inspiration to allow deposition of particles.
- The nebulization time is 10 minutes.
- At the end of this time, the participant should take a few deep breaths, swallow the extra saliva in his/her mouth, and try to cough up a sputum sample.
- The participant should be encouraged to cough forcefully using the deep coughing method and/or "huffing" cough method.
- All sputum should be deposited in the specimen container. The container should not be opened until the specimen is ready to be deposited. The container should be closed immediately after depositing the sample.
- The sputum sample should be approximately 3 mL – slightly below the bottom line (5 mL) on the collection container.
- If a sufficient sputum sample is not collected and the participant appears to be tolerating the induction procedure well, the participant can complete another 10-minute nebulization period.
  - If a second 10-minute nebulization period is required, the recommendation is to increase the sodium chloride concentration (ie, if 3% was used first, then 7% should be used for the subsequent nebulization; if 7% was used first, then 10% should be used for the subsequent nebulization).
  - Closely monitor the participant for tolerability issues or side effects.
  - No more than two 10-minute nebulization periods should be completed.
- The sputum sample should be refrigerated until it is sent to the microbiology laboratory.

Side Effects

- The subject may experience side effects from the sputum induction procedure. The most common side effects include the following:
  - coughing
  - wheezing
  - lightheadedness
  - shortness of breath
  - sore throat
  - nausea
  - headache
  - chest tightness
- Other possible side effects include hyperventilation or bronchospasm. For bronchospasm, ensure the subject receives the necessary medical management.

Miscellaneous

- If the subject needs to expectorate during nebulization, turn off the nebulizer and allow the subject to cough up sputum into the container. If a sufficient specimen is not collected, the subject should then resume the nebulization to complete the 10-minute nebulization duration.

The subject should be encouraged to blow his/her nose as often as needed during the induction procedure to help prevent nasal sections from becoming mixed with sputum specimen.

10.6. Appendix 6: Liver Safety: Suggested Actions and Follow-Up Assessments

Assessing Drug-Induced Liver Injury (DILI)

The Investigator, together with the Sponsor clinical representatives, should perform the ongoing monitoring of standard serum liver test results, as specified in the study protocol, to assess risk of DILI.

In patients with normal serum liver test results at baseline, any of the following laboratory criteria of serum analytes are indicative of DILI once other causes of liver injury have been systematically excluded.

- ALT ≥5 × ULN or
- ALT ≥3 × ULN, and total bilirubin >2 × ULN, and no or minimal elevations in ALP or
- ALP ≥2 × ULN when the source of increased ALP levels is the liver.

In patients with a pre-existing liver disease, laboratory criteria for new-onset DILI should include equivalent fold increases above the patient's pre-treatment baseline levels.

Exclusion of alternative causes of liver injury should include viral serology, serum autoantibodies, and liver imaging studies.

Stopping Rules

Dosing with study treatment should be stopped in participants with unexplained increases in transaminases (ALT or AST) or total bilirubin as follows:

- ALT or AST >8 × ULN
- ALT or AST >5 × ULN for more than 2 weeks
- ALT or AST >3 × ULN and (total bilirubin >2 × ULN or INR >1.5)
- ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

These rules should be adjusted in patients with pre-existing liver disease and abnormal baseline liver tests.

Guidance for the Investigators

- Sponsor representative/Medical Monitor should be notified of potential DILI.
- The Medical Monitor will contact the Investigator to discuss and agree to an approach for the participant's follow-up, that may include subsequent laboratory testing and diagnostic evaluation of alternative causes.
- Monitor the participant until liver function test results and clinical symptoms and signs return to normal or to the baseline levels, regardless of whether the study drug is continued or not.
- Clinically significant laboratory abnormalities suggestive of DILI or the alternative cause of DILI must be reported as AE/SAE.

10.6.1. Prohibited Medications

This appendix is a supplement to Exclusion Criterion #22, which specifies:

- Continuous use of high-dose NSAIDS is prohibited during the study through Visit 8 (EOS)

Comparable NSAID Dose Levels

Nonselective NSAIDs	Low Dose	Medium Dose	High or Max Dose
Diclofenac potassium	50mg bid	50mg tid	50mg qid (in OA/RA only)
Diclofenac sodium	50mg bid	75mg bid	50mg qid or 100mg SR bid (in RA only)
Fenoprofen	200-300mg qid	600mg tid-qid	800mg qid
Flurbiprofen	50mg bid	50mg tid-qid	100mg tid
Ibuprofen	400mg tid	600mg tid-qid	800mg qid
Ketoprofen	25–50mg tid	75mg tid	IR =300mg/day (divide), SR =200mg/day
Naproxen	250mg tid	500mg bid	1250mg/day (divided)
Naproxen sodium	275mg tid	550mg bid	1375mg/day (divided)
Oxaprozin	600mg qd	1,200mg qd	1,200mg qd
Sulindac	150mg bid	200mg bid	200mg bid
Piroxicam	10mg qd	20mg qd	40mg per day (not indicated for OA or RA)
Partially-selective NSAIDs	**Low Dose**	**Medium Dose**	**High or Max Dose**
Etodolac	200mg tid	400mg bid	1,200mg max (IR or SR divided doses)
Meloxicam/Mobic	7.5mg qd	7.5mg qd	15mg qd
Nabumetone	1,000mg qd	1,000mg bid	2,000mg/day (qd or divided bid)
Cox-2 inhibitors	**Low Dose**	**Medium Dose**	**High or Max Dose**
Celecoxib/Celebrex	200mg qd	200mg bid	200mg bid

COX = cyclo-oxygenase; IR = immediate release; NSAID = nonsteroidal antiinflammatory drug; OA = osteoarthritis; RA = rheumatoid arthritis; SR = sustained release
*This table does not represent exact or equivalent dosing conversions. It is based on U.S. Food and Drug Administration approved dosing ranges and comparative doses from clinical trials.

Source: https://www.ncbi.nlm.nih.gov/sites/books/NBK65646/

10.7. Appendix 7: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the table of contents.

Global Amendment 1: 01 JUL 2021

This amendment is considered to be substantial.

Overall Rationale for the Amendment:

The primary driver for the changes in the protocol amendment is to provide clarification for study procedures and address feedback from the Cystic Fibrosis Foundation /Therapeutics Development Network.

Grammatical and typographical errors were corrected throughout the document.

The summary of major changes in this amendment, compared to the original protocol, is shown below:

Description of Change	Brief Rationale	Section Number
Updated synopsis; added AE follow-up information; updated Objectives and Endpoints	To clarify study procedures in overall design and address Cystic Fibrosis Foundation /Therapeutics Development Network feedback	Section 1.1
Updated Schedule of Assessments and Procedures table and footnotes to reflect that the patient related outcome tool CFQ-R is first study procedure to be performed at the scheduled visits, according to HA guidelines; added COVID-19 rapid testing at baseline visit; added drug accountability visit	To clarify study procedures and footnotes, and address Cystic Fibrosis Foundation /Therapeutics Development Network feedback	Section 1.3
Updated PK, PD, and ECG Sampling Schedule; added table for PK, PD, and ECG sample collection	To clarify study procedures	Section 1.3.1
Added background information to elaborate on CYP isozymes and CYP3A4 and Pgp inhibitors in brensocatib exposure	To add to background information	Section 2.1 Section 6.8

Description of Change	Brief Rationale	Section Number
Added and updated objectives and endpoints	To address feedback from Cystic Fibrosis Foundation /Therapeutics Development Network	Section 3
Updated treatment period to indicate when participants should enter the study	To clarify study procedures	Section 4.1.2
Added drug accountability visit on Day 14	Updated to clarify study procedures	Section 4.1.2.2 Section 6.4
Added text referring to pharmacy manual and medication card for IP handling instructions	To clarify study procedures	Section 4.1.4
Definition for EOT added	To clarify study procedures	Section 4.4
Updated inclusion criterion 2	To clarify study procedures	Inclusion Criterion 2
Updated exclusion criterion 1	Updated exclusion criterion 1 to clarify severe or unstable CF as per Investigator's judgement.	Exclusion Criterion 1
Updated exclusion criterion 5	To describe clinically significant abnormalities	Exclusion Criterion 5
Update exclusion criterion 16	To clarify unstable disease due to colonization with specific bacterial species.	Exclusion Criterion 15
Added Investigational Product Administration section to describe study drug administration	To clarify study procedures	Section 6.1
Updated Measures to Minimize Bias: Randomization and Blinding section; updated study team members who may be unblinded	To clarify study procedures	Section 6.3
Updated Prohibited Medications; Clarified that long-term steroid use is >4 weeks	To address feedback from Cystic Fibrosis Foundation	Section 6.8.3

Description of Change	Brief Rationale	Section Number
	/Therapeutics Development Network	
Updated Participant Discontinuation/Withdrawal From Study text on participant discontinuation/withdrawal from study	To address feedback from Cystic Fibrosis Foundation /Therapeutics Development Network	Section 7.1.3
Updated Study Assessments and Procedures to include sample volume and sputum sample collection description	To clarify study procedures	Section 8
Updated text on physical examinations	To clarify study procedures	Section 8.1.1
Added that clinically significant abnormal values will be recorded as an AE	To clarify study procedures	Section 8.1.2
Updated text on PK sample collection, added blood sample volumes	To clarify study procedures	Section 8.3.1
Updated text on biomarkers, added sample types and volumes	To clarify study procedures	Section 8.5
Added section on sputum assessment	To clarify study procedures	Section 8.6
Updated text on spirometry to remove postbronchodilator use	To address feedback from Cystic Fibrosis Foundation /Therapeutics Development Network	Section 8.8.1
Updated text on Cystic Fibrosis Questionnaire – Revised to indicated that questionnaire should be performed prior to any other procedures	To clarify study procedures	Section 8.8.2
Updated PK parameters and definitions of PK and PD populations	To clarify study procedures and statistical methods	Section 9.1 Section 9.3

Description of Change	Brief Rationale	Section Number
Updated sample size determination text	To clarify statistical methods	Section 9.2
Updated primary endpoint text; added primary safety endpoint	To clarify statistical methods and address feedback from Cystic Fibrosis Foundation /Therapeutics Development Network	Section 9.4.2
Updated secondary endpoint text	To address change in secondary endpoints	Section 9.4.3
Updated exploratory endpoint text	To clarify statistical methods and address change to exploratory endpoints	Section 9.4.4
Added sputum induction guidelines	To clarify study procedures	Section 10.5

11. REFERENCES

Bulitta JB, Duffull SB, Kinzig-Schippers M, Holzgrabe U, Stephan U, Drusano GL, Sorgel F. Systematic comparison of the population pharmacokinetics and pharmacodynamics of piperacillin in cystic fibrosis patients and healthy volunteers. *Antimicrob Agents Chemother*. 2007;51(7):2497-507.

Bulitta JB, Kinzig M, Landersdorfer CB, Holzgrabe U, Stephan U, Sorgel F. Comparable population pharmacokinetics and pharmacodynamic breakpoints of cefpirome in cystic fibrosis patients and healthy volunteers. *Antimicrob Agents Chemother*. 2011;55(6):2927-36.

CF News Today. Cystic Fibrosis Statistics. Available from: https://cysticfibrosisnewstoday.com/cystic-fibrosis-statistics/. Accessed on: 10 Nov 2020.

Chalmers JD, Haworth CS, Metersky ML, Loebinger MR, Blasi F, Sibila O, O'Donnell AE, Sullivan EJ, Mange KC, et al. Phase 2 Trial of the DPP-1 Inhibitor Brensocatib in Bronchiectasis. *N Engl J Med*. 2020;383(22):2127-37.

Chalmers JD, Moffitt KL, Suarez-Cuartin G, Sibila O, Finch S, Furrie E, Dicker A, Wrobel K, Elborn JS, et al. Neutrophil Elastase Activity Is Associated with Exacerbations and Lung Function Decline in Bronchiectasis. *Am J Respir Crit Care Med*. 2017;195(10):1384-93.

Chalmers JD, Moffitt KL, Suarez-Cuartin G, Sibila O, Finch S, Furrie E, et al. Neutrophil elastase activity is associated with exacerbations and lung function decline in bronchiectasis. *Am J Respir Crit Care Med*. 2017;195(10):1384-93.

CIOMS. Drug-Induced Liver Injury (DILI): current status and future directions for drug development and the post-market setting. A consensus by a CIOMS Working Group. Available from: https://cioms.ch/wp-content/uploads/2020/06/CIOMS_DILI_Web_16Jun2020.pdf. Accessed on: 09 June 2022.

De Sutter PJ, Gasthuys E, Van Braeckel E, Schelstraete P, Van Biervliet S, Van Bocxlaer J, Vermeulen A. Pharmacokinetics in Patients with Cystic Fibrosis: A Systematic Review of Data Published Between 1999 and 2019. *Clin Pharmacokinet*. 2020;59(12):1551-73.

Dittrich AS, Kuhbandner I, Gehrig S, Rickert-Zacharias V, Twigg M, Wege S, Taggart CC, Herth F, Schultz C, et al. Elastase activity on sputum neutrophils correlates with severity of lung disease in cystic fibrosis. *Eur Respir J*. 2018;51(3).

Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med*. 2010;363(23):2233-47.

Guarino C, Hamon Y, Croix C, Lamort AS, Dallet-Choisy S, Marchand-Adam S, Lesner A, Baranek T, Viaud-Massuard MC, et al. Prolonged pharmacological inhibition of cathepsin C results in elimination of neutrophil serine proteases. *Biochem Pharmacol*. 2017;131:52-67.

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(1):63-76.

Hirche TO, Benabid R, Deslee G, Gangloff S, Achilefu S, Guenounou M, Lebargy F, Hancock RE, Belaaouaj A. Neutrophil elastase mediates innate host protection against Pseudomonas aeruginosa. *J Immunol*. 2008;181(7):4945-54.

Le Gars M, Descamps D, Roussel D, Saussereau E, Guillot L, Ruffin M, Tabary O, Hong SS, Boulanger P, et al. Neutrophil elastase degrades cystic fibrosis transmembrane conductance regulator via calpains and disables channel function in vitro and in vivo. *Am J Respir Crit Care Med*. 2013;187(2):170-9.

Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-12.

Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. *Eur Respir J*. 2014;44(4):1042-54.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-38.

Nichols DP, Chmiel JF. Inflammation and its genesis in cystic fibrosis. *Pediatr Pulmonol*. 2015;50 Suppl 40:S39-56.

Oriano M, Terranova L, Sotgiu G, Saderi L, Bellofiore A, Retucci M, Marotta C, Gramegna A, Miglietta D, et al. Evaluation of active neutrophil elastase in sputum of bronchiectasis and cystic fibrosis patients: A comparison among different techniques. *Pulm Pharmacol Ther*. 2019;59:101856.

Owen CA. Roles for proteinases in the pathogenesis of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2008;3(2):253-68.

Palmer R, Maenpaa J, Jauhiainen A, Larsson B, Mo J, Russell M, Root J, Prothon S, Chialda L, et al. Dipeptidyl Peptidase 1 Inhibitor AZD7986 Induces a Sustained, Exposure-Dependent Reduction in Neutrophil Elastase Activity in Healthy Subjects. *Clin Pharmacol Ther*. 2018;104(6):1155-64.

Pham CT. Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol*. 2006;6(7):541-50.

Ramsey BW, Downey GP, Goss CH. Update in Cystic Fibrosis 2018. *American Journal of Respiratory and Critical Care Medicine*. 2019;199(10):1188-94.

Registry CF. CF Foundation Annual Data Report 2019. Available from: https://www.cff.org/sites/default/files/2021-10/2019-Patient-Registry-Annual-Data-Report.pdf. Accessed on: 10 Nov 2020.

Registry CF. CF Foundation Annual Data Report 2020. Available from: https://www.cff.org/sites/default/files/2021-11/Patient-Registry-Annual-Data-Report.pdf. Accessed on: 01 July 2022.

Sagel SD, Wagner BD, Anthony MM, Emmett P, Zemanick ET. Sputum biomarkers of inflammation and lung function decline in children with cystic fibrosis. *Am J Respir Crit Care Med*. 2012;186(9):857-65.

Sharma GD. Drugs and diseases. Medscape. Available from: https://emedicine.medscape.com/article/1001602-overview#a1. Accessed on: 10 Nov 2020.

Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, Murray CP, Stick SM, Investigators AC. Risk factors for bronchiectasis in children with cystic fibrosis. *N Engl J Med*. 2013;368(21):1963-70.

Sorensen OE, Clemmensen SN, Dahl SL, Ostergaard O, Heegaard NH, Glenthoj A, Nielsen FC, Borregaard N. Papillon-Lefevre syndrome patient reveals species-dependent requirements for neutrophil defenses. *J Clin Invest*. 2014;124(10):4539-48.

Taggart CC, Greene CM, Carroll TP, O'Neill SJ, McElvaney NG. Elastolytic proteases: inflammation resolution and dysregulation in chronic infective lung disease. *Am J Respir Crit Care Med*. 2005;171(10):1070-6.

Toomes C, James J, Wood AJ, Wu CL, McCormick D, Lench N, Hewitt C, Moynihan L, Roberts E, et al. Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nat Genet*. 1999;23(4):421-4.

van der Meer R, Wilms EB, Heijerman HGM. Review: CFTR Modulators: Does One Dose Fit All? *J Pers Med*. 2021;11(6):1-12.

Voynow JA, Fischer BM, Malarkey DE, Burch LH, Wong T, Longphre M, Ho SB, Foster WM. Neutrophil elastase induces mucus cell metaplasia in mouse lung. *Am J Physiol Lung Cell Mol Physiol*. 2004;287(6):L1293-302.

Weldon S, McNally P, McElvaney NG, Elborn JS, McAuley DF, Wartelle J, Belaaouaj A, Levine RL, Taggart CC. Decreased levels of secretory leucoprotease inhibitor in the Pseudomonas-infected cystic fibrosis lung are due to neutrophil elastase degradation. *J Immunol*. 2009;183(12):8148-56.

Zobell JT, Waters CD, Young DC, Stockmann C, Ampofo K, Sherwin CM, Spigarelli MG. Optimization of anti-pseudomonal antibiotics for cystic fibrosis pulmonary exacerbations: II. cephalosporins and penicillins. *Pediatr Pulmonol*. 2013;48(2):107-22.