Official Title: A Randomized, Double-Blind, Placebo-Controlled, Active Comparator, Multicenter Study to Validate Patient-Reported Outcome Instruments in Adult Subjects With Newly Diagnosed Nontuberculous Mycobacterial (NTM) Lung Infection Caused by Mycobacterium avium Complex (MAC)

NCT Number: NCT04677543

Document Dates: Protocol Version 3: 11-July-2022

Signature Page for INS-415 Global Protocol Amendment 2 Version 3.0

Approve	[redacted] 11-Jul-2022 14:32:29 GMT+0000

CLINICAL STUDY PROTOCOL

ARISE - A Randomized, Double-Blind, Placebo-Controlled, Active Comparator, Multicenter Study to Validate Patient-Reported Outcome Instruments in Adult Subjects with Newly Diagnosed Nontuberculous Mycobacterial (NTM) Lung Infection Caused by *Mycobacterium avium* Complex (MAC)

Study Number: INS-415

IND Number: 108674
EudraCT Number: 2020-002545-42

Insmed Incorporated
700 US Highway 202/206
Bridgewater, NJ 08807-1704

	Version Number	Amendment	Date
Protocol Amendment	3.0	Global Amendment 2	11 JUL 2022

CONFIDENTIALITY STATEMENT

INVESTIGATOR'S AGREEMENT

ARISE - A Randomized, Double-Blind, Placebo-Controlled, Active Comparator, Multicenter Study to Validate Patient-Reported Outcome Instruments in Adult Subjects with Newly Diagnosed Nontuberculous Mycobacterial (NTM) Lung Infection Caused by *Mycobacterium avium* Complex (MAC)

Investigator's Signature:

I have fully discussed the objectives of this study and the contents of this protocol with the Sponsor's representative.

I understand that information contained in or pertaining to this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the study, without written authorization from the Sponsor. It is, however, permissible to provide information to a subject to obtain consent.

I agree to conduct this study per this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with the Declaration of Helsinki, the International Council for Harmonisation Guideline for Good Clinical Practice (ICH E6 [R2] GCP) and applicable local laws, regulations and guidelines.

I agree to make available to Sponsor personnel, their representatives and relevant regulatory authorities, my subjects' study records to verify the data that I have entered into the case report forms. I am aware of my responsibilities as an Investigator as provided by the Sponsor.

I understand that the Sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study, I will communicate my intention immediately in writing to the Sponsor.

Print Name	**Signature**
Title	**Date (DD MMM YYYY)**

IMPORTANT CONTACTS

Insmed Incorporated
700 US Highway 202/206
Bridgewater, NJ 08807-1704

Insmed Incorporated Global Clinical and Regulatory Affairs			
Name	**Title**	**Telephone**	**e-Mail Address**
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[redacted], BS	[redacted]	[redacted]	[redacted]

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

Document History		
Type of Protocol Amendment	**Numbering**	**Date**
Global	Version 3.0, Global Amendment 2	11 JUL 2022
Global	Version 2.0, Global Amendment 1	13 AUG 2021
Country-Specific	Version 1.0, Amendment 1, Denmark, Germany, Spain	08 JAN 2021
Original Protocol	Version 1.0	20 MAY 2020

Global Amendment 2: 11 JUL 2022

This amendment is considered to be nonsubstantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Overall Rationale for the Amendment:

The primary driver for the changes in the protocol amendment is to provide a clarification of the planned analysis.

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

Revision	Rationale	Location of Revision
Added text to Protocol Synopsis and Section 10.1 / Sample Size and Power Considerations	Added text to clarify the number of subjects for the cross-sectional validation analysis	Section 10.1

PROTOCOL SYNOPSIS

EudraCT/IND Number:	EudraCT Number: 2020-002545-42 IND Number: 108674
Protocol Number:	INS-415
Amendment Number:	Global Amendment 2
Investigational Product:	Amikacin Liposome Inhalation Suspension (ALIS)
Active Ingredient(s)/INN:	Amikacin sulfate
Study Title:	ARISE - A Randomized, Double-Blind, Placebo-Controlled, Active Comparator, Multicenter Study to Validate Patient-Reported Outcome Instruments in Adult Subjects with Newly Diagnosed Nontuberculous Mycobacterial (NTM) Lung Infection Caused by *Mycobacterium avium* Complex (MAC)
Study Phase:	3b
Indication Under Investigation:	Adults with non-cavitary lung disease with newly diagnosed (initial or subsequent) MAC lung infections

Objectives/Endpoints:

Primary Objective To generate evidence demonstrating the domain specification (via modern psychometric methods [MPMs]), reliability, validity, and responsiveness (within-subject meaningful change) of the patient-reported outcome (PRO) endpoints	***Primary Endpoint*** Findings on psychometric validation optimized and reported for: 1) Cross-sectional validation (modern psychometrics, internal consistency, concurrent validity, and known-groups validity) at Baseline. 2) Test-retest reliability between Screening and Baseline among subjects reporting no change on Patient Global Impression of Severity (PGI-S) between Screening and Baseline. PGI-S anchors will be PRO specific, with a respiratory and fatigue PGI-S applied to the Quality of Life – Bronchiectasis (QOL-B) respiratory domain and Patient-Reported Outcome Measurement Information System – Fatigue-Short Form 7a (PROMIS F-SF 7a), respectively. 3) Within-subject meaningful change estimated via anchor-based methods and validated via empirical cumulative distribution functions (eCDFs) and probability density functions (ePDFs) between Baseline and End of Study (EOS) (Month 7).

Secondary Objectives: To evaluate the effect of each treatment arm (amikacin liposome inhalation suspension [ALIS] + background regimen (azithromycin [AZI] + ethambutol [ETH]) and empty liposome control (ELC) + background regimen on the following:	***Secondary Endpoints***
1. Culture conversion by Month 6	Proportion of subjects achieving culture conversion by Month 6 (negative cultures for MAC at Month 5 and Month 6)
2. Patient-reported respiratory symptoms at Month 7	Change from Baseline to Month 7 in respiratory symptom score
3. Patient-reported fatigue symptoms at Month 7	Change from Baseline to Month 7 in fatigue symptom score
4. Time to culture conversion	Time to culture conversion (first of 2 consecutive negative cultures) of Baseline to EOT assessments
5. Time to first negative culture	Time to first negative culture of Baseline to EOT assessments
6. MAC isolates with amikacin minimum inhibitory concentration (MIC) ≥ 128 µg/mL	Proportion of subjects who develop a MAC isolate with amikacin MIC ≥ 128 µg/mL at more than 1 visit at any timepoint during the study
7. Recurrence of MAC (relapse)	Proportion of subjects who achieved culture conversion and subsequently have at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits that is the same species and genome as cultured at Screening/Baseline.
8. Recurrence of MAC (new infection)	Proportion of subjects who achieved culture conversion and subsequently have at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits that is different than cultured at Screening/Baseline (different species or same species but different genome).
9. Safety and tolerability of ALIS +background regimen	Incidence and severity of adverse events (AEs) and treatment-emergent adverse events (TEAEs) and other safety variables (eg, vital signs, physical examination, clinical laboratory values) from Baseline through the end of study (EOS)

Exploratory Objective To evaluate the effect of each treatment arm (ALIS + background regimen and ELC + background regimen) on the following:	***Exploratory Endpoint***
1. Patient-reported non-respiratory symptoms at Month 7	Change from Baseline to Month 7 in the QOL-B non-respiratory domains (physical, role, vitality, emotional, social, health perception)
2. Within-subject meaningful change threshold estimated in respiratory symptoms from Baseline to Month 7	Proportion of subjects meeting the within-subject meaningful change threshold as reflected in PRO changes scores computed from Baseline in patient-reported symptoms
3. Mean activity and sleep efficiency over time	Longitudinal summary of mean activity and sleep efficiency as measured by Philips Actiwatch Spectrum PRO

Study Design:

Please refer to Figure 1 for a schematic diagram of the study design and treatment duration.

This is a randomized, double-blind, placebo-controlled, active comparator study in eligible subjects with a new diagnosis (initial or subsequent) of MAC lung infection who have not started treatment for their current infection. Subjects will be randomized at Baseline in a 1:1 ratio to receive one of the two treatment regimens: ALIS + background regimen or ELC + background regimen for 6 months. Note that background regimen in this study is defined as AZI+ETH per randomized treatment.

Randomization will be stratified by region (North America, Europe, and Rest of World) and history of MAC lung infection (initial or subsequent). After Baseline, subjects will return to the study site for in-clinic visits at Months 1, 3, 5, 6/EOT, and 7/ EOS.

Visits at Months 2 and 4 do not require in-clinic appointments. At these non-in clinic visits, AEs and concomitant medications will be assessed. eDiary data will be collected continually for assessment of study drugs intake, and subjects will be required to provide and ship sputum samples for analysis. At the Month 6/EOT visit, subjects will discontinue all study drugs and will be followed for a 1 month off treatment period, during which medical or non-medical therapies for MAC lung infection should not be given.

At Month 7/EOS, subjects will complete all protocol-specified assessments and the final EOS procedures.

The procedures and assessments conducted at each study visit in the study are provided in Table 4.

Study Duration:

Subjects will receive ALIS + background regimen or ELC + background regimen for 6 months and then remain off all study drugs for 1 month. The Screening period will be up to approximately -70 days to -1 day (~2.5 months).
The total duration of the study will be up to 9.5 months from the Screening visit to EOS.

Study Sites and Locations:

This study is planned to be conducted at approximately 150 sites globally.

Sample Size Determination:

Power and Sample Size Determination for the Validation Study

Cross-sectional validation will require baseline data from a maximum of 250 subjects to adequately power the planned MPMs. A total of 100 subjects will be enrolled in this study and will be used for this analysis. Additional select baseline data from up to the first 150 subjects enrolled in a separate study will be used.

Longitudinal validation will require 100 subjects to adequately power the planned within-subject meaningful change methods.

The derivation of these sample sizes and the procedures used to compute power are described in Section 10.1.

Subject Eligibility Criteria:

Inclusion Criteria

Subjects must satisfy all of the following criteria to be included in the study:

1. Male or female ≥ 18 years of age (19 years or older in South Korea)
2. Current diagnosis of MAC lung infection. MAC or mixed infection with MAC as the dominant species is allowed, with MAC as the intended organism for treatment
3. Positive sputum culture for MAC within 6 months prior to Screening
4. Positive sputum culture for MAC at Screening

5. A chest computed tomography (CT) scan, read locally, within 6 months prior to Screening to determine presence and size of pulmonary cavities. Subjects who do not have a chest CT scan within 6 months prior to Screening will be required to obtain a chest CT scan, read locally, during Screening
6. In the Investigator's opinion, documented respiratory signs/symptoms at Screening that are attributable to the current MAC lung infection
7. An average QOL-B respiratory domain score of ≤ 85 based on scores at Screening and on the day of enrollment prior to randomization
8. In the Investigator's opinion, underlying lung disease (eg, chronic obstructive pulmonary disease [COPD], bronchiectasis) have been managed according to best local standard of care, and on stable maintenance therapy for a minimum of 4 weeks prior to randomization
9. Willingness and ability to adhere to prescribed study treatment during the study
10. Ability to produce (spontaneously or with induction) approximately 2 mL of sputum for mycobacteriology at Screening
11. Women of child-bearing potential [WOCBP] (ie, fertile following menarche and until becoming post-menopausal unless permanently sterile) and fertile men (ie, all men after puberty unless permanently sterile by bilateral orchidectomy) agree to practice a highly effective method of birth control from Day 1 to at least 90 days after the last dose. Examples of such birth controls are:
    - true abstinence (refraining from heterosexual intercourse during the entire study),
    - copper intrauterine device [IUD],
    - hormonal methods (levonorgestrel-releasing intrauterine system, progestogen implant, combined oral contraceptive pill [combined with barrier method]),
    - exclusive homosexual relationship, or
    - sole male partner who has undergone surgical sterilization with confirmation of azoospermia at least 3 months post procedure while participating in the study.
12. Provide signed informed consent prior to administration of study drugs or performing any study related procedure
13. Be able to comply with study drugs use, study visits, and study procedures as defined by the protocol
14. Men with partners who are WOCBP (pregnant or non-pregnant) agree to use condoms and non-pregnant partners should practice a highly effective method of birth control.

Exclusion Criteria

Subjects who meet any of the following criteria will be disqualified from entering the study:

1. Diagnosis of cystic fibrosis (CF)
2. History of more than 3 MAC lung infections
3. Received any mycobacterial antibiotic treatment for current MAC lung infection
4. Refractory MAC lung infection, defined as having positive MAC cultures while being treated with a multidrug mycobacterial antibiotic treatment regimen for a minimum of 6 consecutive months and no documented successful treatment, defined as negative sputum culture for MAC and cessation of treatment
5. Relapse of prior MAC lung infection, defined as positive sputum culture for MAC ≤ 6 months of cessation of prior successful treatment
6. MAC isolate with MIC for amikacin ≥ 128 µg/mL at Screening
7. Evidence of any pulmonary cavity ≥ 2 cm in diameter, as determined by chest CT scan, read locally, within 6 months prior to Screening
8. Radiographic finding of new lobar consolidation, atelectasis, significant pleural effusion, or pneumothorax during routine clinical care within 2 months prior to Screening

9. Active pulmonary malignancy (primary or metastatic) or any malignancy requiring chemotherapy or radiation therapy within 1 year prior to Screening or anticipated during the study
10. Active pulmonary tuberculosis requiring treatment during Screening
11. Hospitalization for underlying lung disease during Screening
12. Acute pulmonary exacerbation (eg, COPD or bronchiectasis) requiring treatment with antibiotics, or corticosteroids (IV or oral), within 4 weeks prior to and during Screening
13. Predicted forced expiratory volume in 1 second (FEV_1) < 35%, pre-bronchodilator use
14. Current smoker
15. History of lung transplantation
16. Use of inhaled or systemic aminoglycosides with activity against MAC (eg, amikacin, kanamycin, or streptomycin) during Screening
17. Prior exposure to ALIS (including clinical study)
18. Known hypersensitivity or contraindications to use to ALIS, aminoglycosides, or any of their excipients
19. Disseminated MAC infection
20. Positive pregnancy test or lactation at Screening. All WOCBP will be tested. Women not of child-bearing potential are defined as postmenopausal (ie, amenorrheic for 12 months without an alternative medical cause or confirmed by more than one follicle stimulating hormone [FSH] measurement), or naturally or surgically sterile through bilateral oophorectomy, hysterectomy, or bilateral salpingectomy. For women under the age of 45 years, confirmatory testing with FSH should be considered.
21. Administration of any investigational drug within 8 weeks prior to Screening
22. Known or suspected acquired immunodeficiency syndromes (HIV-positive, regardless of CD4 counts). Other immunodeficiency syndromes that may interfere with study participation in the opinion of the Investigator.
23. Significant (as determined by the Investigator) hearing loss, vestibular dysfunction, neuromuscular weakness or a diagnosis of myasthenia gravis, where the potential risk of aminoglycoside toxicity outweighs the potential benefit
24. Aspartate aminotransferase or alanine aminotransferase ≥ 3 times the upper limit of normal (ULN) or total bilirubin ≥ 1.5 times ULN at Screening
25. Absolute neutrophil count ≤ 500/μL at Screening
26. Serum creatinine > 2 times ULN at Screening
27. Current alcohol, medication, or illicit drug abuse
28. Any condition that, in the opinion of the Investigator, interferes with ability to safely complete the study or adhere to study requirements
29. Known and active COVID-19 infection
30. MAC isolate with MIC for clarithromycin ≥ 32 μg/mL at Screening
31. Known hypersensitivity or contraindications to use to ethambutol, azithromycin (including other macrolides or ketolides), or any of their excipients per local labeling guidance

Study Drugs:
ALIS 590 mg or ELC will be administered once daily (QD) by inhalation via nebulization over approximately 6 minutes to up to 15 minutes. ALIS or ELC should be administered around the same time each day, any time of day, in the fasted or as-fed condition. The background regimen of azithromycin 250 mg tablets and ethambutol 15 mg/kg tablets will be taken QD by mouth, with or without food.
Statistical Analyses:
Statistical Analyses: Cross-sectional validation will be conducted at Baseline and will consist of modern psychometric methods (exploratory factor analysis, item response theory models and corresponding assessments of local dependence and differential item functioning), internal consistency, concurrent validity, and known groups validity. Test-retest reliability between Screening and Baseline among subjects reporting no change on PGI-S between Screening and Baseline. PGI-S anchors will be PRO specific, with a respiratory and fatigue PGI-S applied to the QOL-B respiratory domain and PROMIS F-SF 7a, respectively. Within-subject meaningful change estimated via anchor-based methods and validated via eCDFs and ePDFs between Baseline and EOS (Month 7). Anchors employed will include the PGI-S and culture conversion. All of the summaries pertaining to secondary endpoints will utilize and intent-to-treat (ITT) analysis set on available data according to Section 10.3, unless otherwise stated in Statistical Analysis Plan (SAP). Reporting of categorial secondary endpoints will include basic statistics, estimates derived via logistic regression and will include estimates of proportions, difference of proportions together with corresponding 95% confidence intervals (CIs) resulting, as appropriate. Reporting of continuous secondary endpoints will include basic statistics, estimates derived from analysis of covariance (ANCOVA) model with change from Baseline as response variable and treatment and Baseline as independent variables regression and will include estimates with corresponding 95% CIs as appropriate. Analysis of variance and logistic regression models may not include adjustment for randomization strata due to expected small counts within each combination of strata. Reporting by visit of basic summary statistics will be provided as appropriate. Time to culture conversion will be plotted via Kaplan-Meier method. Detailed data imputation rules require for handling of missing events dates for safety data will be described in related data handling documents. The analysis of safety data will be conducted on the safety analysis set (ie, all randomly assigned subjects who receive at least 1 dose of ALIS, ELC, AZI, or ETH). Safety parameters for each treatment group will include the occurrence of AEs, the use of concomitant medications, and changes in clinical laboratory values (serum chemistry, hematology, and urinalysis), vital signs measurements, and physical examination findings (including body weight) between Baseline and EOT. Adverse events will be coded according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Summaries will be presented for all AEs, AEs determined by the Investigator to be treatment-related, serious adverse events (SAEs), and AEs causing withdrawal from the study. Hematology, chemistry, and urinalysis values will be summarized by treatment group over time and by visit.

Data Monitoring Committee:
A Data Monitoring Committee (DMC) will periodically monitor the safety of the study. Details will be provided in the DMC charter.

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AESI	adverse events of special interest
ALIS	amikacin liposome inhalation suspension
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ARDS	acute respiratory distress syndrome
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
ATS	American Thoracic Society
AZI	azithromycin 250 mg
BP	blood pressure
BMI	body mass index
CF	cystic fibrosis
CFR	Code of Federal Regulation
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRO	Clinical Research Organization
CRP	C-reactive protein
CT	computed tomography
DIF	differential item function
DMC	Data Monitoring Committee
DPPC	dipalmitoylphosphatidylcholine
EC	Ethics Committee
eCDF	empirical cumulative distribution functions
eCRF	electronic case report form
EDC	electronic data capture
EFA	exploratory item factor
ELC	empty liposome control
EMA	European Medicines Agency
EOS	end of study

Abbreviation	Term
EOT	end of treatment
ePDF	empirical probability density functions
ER	emergency room
ETH	ethambutol 15 mg/kg
EXACT	EXAcerbations of Chronic Pulmonary Disease Tool
EXACT-RS	EXAcerbations of Chronic Pulmonary Disease Tool – Respiratory Symptoms
FACIT	Functional Assessment of Chronic Illness Therapy
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 second
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
IB	Investigator Brochure
HIV	human immunodeficiency virus
ICC	interclass correlation
ICFs	Informed Consent Forms
ICH	International Council for Harmonisation
ICU	intensive care unit
IDSA	Infectious Disease Society of America
IND	Investigational New Drug
IRB	Institutional Review Board
IRT	item response theory
ITT	intent-to-treat
IUD	intrauterine device
IV	intravenous
IWRS	Interactive Web Response System
LSMEANS	least squares mean
MAC	*Mycobacterium avium* complex
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MIC	minimum inhibitory concentration
MPM	modern psychometric methods
NTM	nontuberculous Mycobacteria

Abbreviation	Term
PGI-S	Patient Global Impression of Severity
PI	prescribing information
PMR	post-marketing requirement
$ppFEV_1$	percent predicted forced expiratory volume
PRO	Patient-Reported Outcome
PROMIS F-SF 7a	Patient-Reported Outcome Measurement Information System Fatigue-Short Form 7a
PV	pharmacovigilance
QD	once daily
QOL-B	Quality of Life Questionnaire – Bronchiectasis
RMSEA	root mean squared error of approximation
RR	respiratory rate
SAE	serious adverse event
SAP	Statistical Analysis Plan
SGRQ	St. George Respiratory Questionnaire
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-emergent adverse event
TRTR	test-retest reliability
ULN	upper limit of normal
VAP	Validation Analysis Plan
WHO	World Health Organization
WOCBP	women of child-bearing potential
w:w	weight:weight

1. INTRODUCTION AND BACKGROUND INFORMATION

1.1. Nontuberculous Mycobacterial Lung Disease

Nontuberculous mycobacterial lung disease caused by MAC is a potentially life-threatening and progressively destructive disease that is associated with symptoms of productive cough, fatigue, shortness of breath, fever, weight loss, lung function decline, and hemoptysis (Kim et al., 2008; Kobayashi et al., 2018; Park et al., 2016). It often complicates other chronic debilitating underlying lung diseases such as bronchiectasis or COPD. When NTM lung disease occurs in patients without underlying lung comorbidities, it has been implicated in progressive lung disease (Prince et al., 1989). If left untreated, MAC lung disease can be progressive, and has a 5-year mortality rate of 33.3% (Ito et al., 2012). Mycobacterial lung disease carries a MAC related 5-year all-cause mortality rate of up to 42% (Diel et al., 2018). Prevalence has increased globally over the past decades (Chalmers et al., 2018; Khan et al., 2008; Khan et al., 2007; Marras et al., 2007).

Signs and symptoms of NTM lung disease caused by MAC are variable and often attributable to an underlying disease, such as COPD, bronchiectasis, CF, HIV, and pneumoconiosis (Griffith et al., 2007). Evaluation is often complicated by the symptoms of other pulmonary comorbidities. Fatigue and loss of energy were reported as the "most common symptoms" by 80% of meeting attendees in an informal poll while 40% reported chronic cough and coughing up blood and phlegm (US FDA Patient Focused Drug Development Workshop, 2015). Less commonly, malaise, dyspnea, fever, and weight loss can also occur, usually with advanced MAC lung disease.

The current treatment of NTM lung disease is primarily with a multidrug regimen based on the treatment of tuberculosis (Daley et al., 2020). The recommendation for MAC lung disease is a 3-drug antibiotic regimen that includes a macrolide, ethambutol, and a rifamycin, however the optimal drugs, regimens, and duration of therapy are unknown. The recommendation of a 3-drug regiment over a 2-drug regimen is stated with low confidence on a conditional recommendation. Currently clinical trials evaluating the clinical utility of a 2-drug versus a 3-drug regimen are ongoing. The regimen is administered for 12 months beyond culture conversion to negative, although the duration of antimicrobial therapy often exceeds 18 months (Johnson and Odell, 2014). Intravenous amikacin or intramuscular streptomycin are recommended for patients with fibrocavitary disease or severe nodular/bronchiectatic disease; however, the optimal treatment has yet to be established. Intravenous aminoglycoside use is limited by poor penetration into lung tissue, poor uptake by alveolar macrophages (Zhang et al., 2018), and the potential for ototoxicity, loss of balance, and impaired kidney function with high or prolonged systemic exposure (Kovacevic et al., 2016; Rybak et al., 1999).

1.2. Amikacin Liposome Inhalation Suspension

ARIKAYCE® was approved by the US FDA on 28 September 2018 under the Limited Population Pathway for Antibacterial and Antifungal Drugs, established by Congress under the 21st Century Cures Act, and under the Accelerated Approval pathway, for the treatment of MAC lung disease as part of a combination antibacterial drug regimen in subjects who do not achieve negative sputum cultures after a minimum of 6 consecutive months of a multidrug background regimen therapy. On 27 October 2020, ARIKAYCE was granted market authorization by the

European Medicines Agency (EMA) for the treatment of MAC in adults with limited treatment options who do not have cystic fibrosis. On 23 March 2021, ARIKAYCE was approved by Japanese Health Authority of the Ministry of Health, Labour and Welfare (MHLW) for the treatment of patients with NTM lung disease caused by MAC who did not sufficiently respond to prior treatment with a multidrug regimen. Amikacin liposome inhalation suspension is a sterile aqueous liposomal formulation for inhalation via nebulization. ALIS is comprised of amikacin sulfate encapsulated in liposomes composed of DPPC and cholesterol; other inactive ingredients include sodium chloride, sodium hydroxide for pH adjustment, and water for injection. ALIS is supplied in a single-use 10 mL vial to deliver 590 mg amikacin to the nebulizer. ALIS is administered by inhalation via a nebulizer system using the [redacted](LAMIRA) nebulizer system. The nebulizer system delivers medication in the form of aerosols that are inhaled into the lungs.

The pivotal study on which approvals were granted, Study INS-212 (CONVERT), evaluated subjects with refractory NTM lung disease caused by MAC. Over 80% of the subjects in this study had been treated with at least 3 antibiotics prior to enrollment in the study, yet all subjects persistently had prior positive sputum cultures. The addition of ALIS 590 mg QD to a multidrug antimycobacterial regimen statistically significantly increased the proportion of subjects achieving culture conversion (defined as 3 consecutive monthly negative sputum cultures) by Month 6 as compared to a multidrug antimycobacterial regimen alone (29.0% vs 8.9%, respectively, $P < 0.0001$). Treatment with ALIS also statistically significantly increased the proportion of subjects achieving durable culture conversion at 3 months off treatment as compared to a multidrug antimycobacterial regimen alone (16.1% vs 0%, respectively, $P < 0.0001$). The safety profile of ALIS 590 mg QD was consistent with the utilization of an antibiotic via nebulization, and with the class of aminoglycosides, albeit with a low incidence of aminoglycoside related safety events.

1.3. Study Rationale

There remains an unmet medical need for patients with non-cavitary lung disease with newly diagnosed MAC lung infections who have not started treatment. A significant proportion of patients treated with currently available multidrug therapy do not achieve treatment success. A systematic review and meta-analysis showed in a total of 16 studies involving 1,462 subjects, the rate of treatment success was 60% (Kwak et al., 2017). In subjects who were macrolide susceptible and had previously been untreated for MAC, treatment for at least 1 year resulted in sputum culture conversion rate of 65.7% (Diel et al., 2018). There is currently no validated PRO instrument to evaluate symptoms in patients with MAC lung disease. This study aims to generate evidence demonstrating the domain specification (via modern psychometric methods), reliability, validity, and responsiveness (within-subject meaningful change) of the PRO endpoints of ALIS-based regimen within the MAC lung disease population. The instrument validated in this study will be used for the assessment of the clinical benefit in a separate study.

1.3.1. Study Population

Patients with MAC lung disease are heterogeneous in part as a consequence of the signs and symptoms of this infection mirroring the underlying pulmonary disease(s) that predispose patients to the infection. The clinical approach to MAC lung disease is to first exclude more common reasons for worsening respiratory disease, and the decision to treat is often based on the

conclusion that the MAC lung disease is progressing, evidenced by adverse changes in radiologic features (eg development of cavitary disease) or persistent or worsening symptoms attributed to MAC. Additionally, there is no standardized approach to the classification or to the severity of disease by CT imaging. The likelihood of a timelier diagnosis is partially related to the experience of a clinician managing a patient with this uncommon infection. The decision on which antibiotic regimen to administer is typically based upon the patient's underlying lung disease.

The proposed study will utilize eligibility criteria to decrease the heterogeneity of the study population. Importantly, the general approach to the clinical management of individuals of such a population is similar to a reasonable extent. This approach of decreasing heterogeneity will improve the power of the study to detect the efficacy and safety of the ALIS-based regimen. In particular, this is important for measurement of patient symptoms, which are inherently subject to random fluctuation that may obscure the treatment effects. The subject population under investigation for this clinical study will include adults with non-cavitary lung disease with a new (initial or subsequent) MAC lung infection who have not started treatment for their current infection.

1.3.2. Treatment and Duration of Exposure

Patients to be evaluated in this study have mild disease and have not received treatment for their current MAC lung infection. The design and treatment have been extensively discussed and agreed with a global steering committee of experts experienced in the management of NTM pulmonary disease and with regulatory agencies. Subjects will be randomized to ALIS + background regimen or ELC + background regimen. Note that background regimen in this study is defined as AZI+ETH per randomized treatment. Treatment will be administered continuously for 6 months followed by 1 month off treatment period with a final EOS evaluation at Month 7. It was agreed upon with the FDA that an active control that is also used in clinical practice would be used in the study.

Based on extensive consultations with medical experts, in this population of newly diagnosed MAC patients with non-cavitary lung disease, treatment with 2 drug oral regimen, specifically azithromycin and ethambutol has been confirmed as acceptable. This macrolide-based regimen is currently prescribed in clinical practice, and keeping the background regimen simple and tolerable will help subjects remain in the study. The regimen excludes rifabutin or rifampin which are associated with well-described side effects that affect patients with MAC lung disease and patient tolerability for regimens that include one of these 2 drugs. In addition, the chosen companion drugs to ALIS can also be considered to contribute to the therapeutic success by virtue of their ability to prevent overgrowth of organisms that may develop resistance to individual agents in the regimen during the course of treatment.

As stated in both the BTS and ATS/ERS/ESCMID/IDSA guidelines, a 2-drug regimen including a macrolide and ethambutol is the regimen with the fewest possible drugs for treating MAC. Data recently published subsequent to the release of the global guidelines show that the 2-drug regimen of azithromycin and ethambutol may be an option for the treatment of non-cavitary MAC lung disease: 76% of patients were able to achieve sputum culture conversion after 12 months of treatment, and none of those who failed to culture convert developed macrolide resistance (Moon et al., 2019). Ethambutol is likely the key companion drug for preventing the

emergence of macrolide resistance (Haworth et al., 2017; Daley et al., 2020). Literature has shown that patients have a higher risk of developing macrolide resistance if they were not treated with ethambutol (Griffith et al., 2007; Morimoto et al., 2016) drug regimen over a 2-drug regimen over a 2drug regimen:

> "In patients with macrolide-susceptible MAC pulmonary disease, we suggest a treatment regimen with at least 3 drugs (including a macrolide and ethambutol) over a regimen with 2 drugs (a macrolide and ethambutol alone) (conditional recommendation, very low certainty in estimates of effect)."

Additionally, both sets of treatment guidelines note the uncertainty over who to treat and when to start treatment for newly diagnosed NTM pulmonary disease as there are no randomized controlled trial data. In the ATS/ERS/ESCMID/IDSA guidelines it is directly stated:

> "No randomized, controlled trials have been conducted to examine the impact of treatment on either survival or quality of life. Limited retrospective observational data have failed to demonstrate that treatment of NTM pulmonary disease prolongs survival over watchful waiting."

The BTS guidelines have a similar statement on the uncertainty "... fulfilling the criteria [ATS/IDSA] does not necessarily imply that treatment should be started, as patients can remain stable without antibiotic treatment."

Prevention of development of macrolide resistance is a priority in MAC treatment. As stated in the ATS/ERS/ESCMID/IDSA guidelines for MAC pulmonary disease: "The optimal drugs, regimens, and duration of therapy are not known." (Daley et al., 2020). The guidelines do not state that a 2-drug regimen is inferior to 3 drugs with regards to prevention of macrolide resistances, only that there is currently insufficient evidence to support the 2-drug regimen. Miwa et al reported that no isolates in either group developed macrolide resistance, although the study was underpowered (Miwa et al., 2014). Ito et al also evaluated the 2-drug regimen compared to 3 drugs; their results suggest that 2-drug treatment with clarithromycin and ethambutol does not lead to a higher incidence of resistance to clarithromycin than the standard 3-drug treatment (Ito et al., 2020). Regardless, drug susceptibility will be closely monitored and the unblinded data will be regularly reviewed by an independent monitoring committee which will continually evaluate drug susceptibility data during the study. Of note there are other active trials that use a 2-drug comparator arm of a macrolide and ethambutol in the treatment of pulmonary MAC disease, but more importantly the clinical equipoise of using a 2- versus a 3-drug regimen is currently being investigated by a large multicenter study. The current active comparator is an accurate control for this study population as this is a population with milder disease, and a 4-drug regimen (including ALIS) is not warranted. Germane to all clinical research, new treatments and treatment durations are being studied.

The optimal duration of therapy in MAC pulmonary disease is unknown. As stated in the 2020 ATS/ERS/ESCMID/IDSA guidelines, while MAC species are the most common organisms causing NTM pulmonary disease, the optimal treatment duration for MAC pulmonary disease has not been evaluated in a prospective randomized clinical trial and the conditional recommendation for 12 months of therapy is given with very low certainty in estimates of the effect. Given the lack of data on the optimal duration of therapy, the panel voted unanimously to continue to follow the recommendations from the 2007 Guideline. The duration of exposure to

the study regimen (6 months) is less than the maximum duration of exposure of 20 months to the ALIS-based regimens in the subjects who participated in both Study INS-212 and Study INS-312 that evaluated a more difficult-to-treat population of adult patients with treatment refractory MAC lung disease.

1.3.3. Efficacy Endpoints, Placebo Control, and Active Comparator

The primary objective of this study is to generate evidence demonstrating the domain specification (via modern psychometric methods), reliability, validity, and responsiveness (within-subject meaningful change) of the PRO endpoints. Specifically, findings on psychometric validation will be optimized and reported for:

1) Cross-sectional validation (modern psychometrics, internal consistency, concurrent validity, and known-groups validity) at Baseline.

2) Test-retest reliability between Screening and Baseline among subjects reporting no change on PGI-S between screening and baseline. PGI-S anchors will be PRO specific, with a respiratory and fatigue PGI-S applied to the QOL-B respiratory domain and PROMIS F-SF 7a, respectively.

3) Within-subject meaningful change estimated via anchor-based methods and validated via eCDFs and ePDFs between Baseline and EOS (Month 7).

There are no established clinical endpoints to evaluate the clinical benefit of a therapeutic regimen in MAC lung disease. This study aims to validate PRO endpoints that can evaluate benefit of a treatment in MAC lung disease. This study will utilize PRO endpoints that are being used uniquely to determine the clinical meaningfulness of any treatment for MAC lung disease, in addition to microbiological endpoints.

This study will assess data generated from PRO instruments. Therefore, given that this endpoint incorporates subject symptomology and subjectivity, it is critical to conduct the study in a double-blinded fashion to minimize the potential for biased responses. An active comparator allows for the evaluation of the incremental benefit of ALIS and is an appropriate, efficient, and accurate control for this study population. The double-blind study design and blinding to microbiology results allows for an evaluation of the efficacy and safety of an ALIS-based regimen.

1.4. Risks and Benefits for Study Subjects

A favorable benefit/risk profile has been established for the use of ALIS in subjects with refractory MAC lung disease. The safety profile is acceptable given the significant risk of the disease, the inadequacy of current treatment approaches, and early indications of positive clinical outcomes from the use of ALIS.

ALIS has not been studied in adults with non-cavitary lung disease with new (initial or subsequent) MAC lung infections who have not started treatment for their current infection. Current treatment for patients with newly diagnosed MAC lung disease is similar to that for patients with refractory MAC lung disease; it is based on treatment with a multidrug regimen. However, optimal treatment has yet to be established, and current treatment have many adverse effects. A significant proportion of subjects treated with currently available multidrug therapy do not achieve treatment success. Data from previous studies (INS-212 and INS-312) in subjects

with refractory MAC lung disease provide insight on the eventual benefit/risk evaluation for this subject population. This study will evaluate the benefit/risk profile of ALIS in subjects with newly diagnosed MAC lung disease.

1.5. Treatment, Route, Dosage, Treatment Period

Eligible subjects with non-cavitary lung disease with new (initial or subsequent) MAC lung infections who have not started treatment for their current infection (see Section 4) and who have signed the ICFs will be randomized and will receive treatment as follows (see Figure 1)

- ALIS 590 mg QD + AZI 250 mg QD + ETH 15 mg/kg QD for 6 months, or
- ELC (matching placebo for ALIS) + AZI 250 mg QD + ETH 15 mg/kg QD for 6 months

1.6. Randomization and Blinding

Upon meeting all inclusion/exclusion criteria, subjects will be randomly assigned through IWRS in a 1:1 ratio to either of the treatment groups. The randomization will be stratified by region (North America, Europe, and Rest of World) and history of MAC lung infection (initial or subsequent). Subjects, Investigators, and Sponsor will be blinded to treatment group assignments and microbiology results throughout the study. In case of subject emergencies, unblinding of the assigned treatment will occur per established procedure.

1.7. Compliance Statement, Ethics, and Regulatory Compliance

This study will be conducted in compliance with the protocol, the ethical principles derived from international guidelines including the Declaration of Helsinki and the CIOMS International Ethical Guidelines, applicable ICH GCP Guidelines, and applicable local regulatory requirement(s) and laws.

1.7.1. Subject Confidentiality

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

The Sponsor will observe the rules laid down in the European Data Protection Regulation (EU) 2016/679 of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and the free movement of such data.

The Investigator must ensure that the subject's anonymity is maintained. On the eCRF or other documents submitted to Sponsor, subjects should be identified by a unique subject identifier as designated by the Sponsor. Documents that are not for submission to Sponsor (eg, signed ICFs) should 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 EC or IRB direct access to review the subject's original medical records for verification of study-related procedures and data. The Investigator is obligated to inform the subject that his/her study-related records will be reviewed by the above-named representatives without violating the confidentiality of the subject.

1.7.2. Informed Consent Procedure

Before a subject's participation in the study, it is the Investigator's responsibility to obtain freely given consent, in writing, from the subject 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 drugs are administered. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject'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 subject. 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 Informed Consent Form (ICF) should be prepared in the local language(s) of the potential subject population.

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

The subject's informed consent (written or electronic) should be obtained prior to his/her participation in the study, and should be documented in the subject's medical records, as required by applicable regulations. Informed consent may be obtained, where applicable, via a virtual telemedicine visit and signed electronically.

The ICF should be signed and personally dated by the subject or a legally acceptable representative, and by the person who conducted the informed consent discussion (not necessarily the Investigator) however, a medically qualified person must be involved during the consenting process. The signed ICF should be retained in accordance with institutional policy, and a copy of the signed consent form should be provided to the subject or legal representative. The date and time (if applicable) that informed consent was given should be recorded on the eCRF.

1.7.3. Regulatory Compliance

The study protocol, subject information and consent form, the IB, any subject diary card or written instructions to be given to the subject, available safety information, subject recruitment procedures (eg, advertisements), information about payments and compensation available to the subjects and documentation evidencing the Investigator's qualifications should be submitted to the IRB/EC for ethical review and approval according to local regulations, prior to the study start. The written approval should identify all documents reviewed by name and version.

Changes in the conduct of the study or planned analysis will be documented in a protocol amendment and/or the SAP.

All protocol amendments and changes to the ICFs or changes of the investigational site, facilities, or personnel (when applicable) must be submitted to the IRB/EC, and where necessary, approval from the IRB/EC must be obtained. The Investigator should notify the IRB/EC of deviations from the protocol or SAEs occurring at the site and other AE reports received from the Sponsor, in accordance with local procedures.

As required by local regulations, the Sponsor's local Regulatory Affairs group will ensure all legal aspects are covered, and approval of the appropriate regulatory bodies obtained, prior to study initiation, and that implementation of changes to the initial protocol and other relevant study documents happen only after the appropriate notification of or approval by the relevant regulatory bodies.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Primary Objective and Endpoint

Objective	*Endpoint*
To generate evidence demonstrating the domain specification (via modern psychometric methods), reliability, validity, and responsiveness (within-subject meaningful change) of the PRO endpoints	Findings on psychometric validation optimized and reported for: 1) Cross-sectional validation (modern psychometrics, internal consistency, concurrent validity, and known-groups validity) at Baseline. 2) Test-retest reliability between Screening and Baseline among subjects reporting no change on PGI-S between screening and baseline. PGI-S anchors will be PRO specific, with a respiratory and fatigue PGI-S applied to the QOL-B respiratory domain and PROMIS F-SF 7a, respectively. 3) Within-subject meaningful change estimated via anchor-based methods and validated via eCDFs and ePDFs between Baseline and EOS (Month 7).

2.2. Secondary Objectives and Endpoints

Objective	*Endpoint*
To evaluate the effect of each treatment arm (ALIS + background regimen and ELC + background regimen) on the following:	
1. Culture conversion by Month 6	Proportion of subjects achieving culture conversion by Month 6 (negative cultures for MAC at Month 5 and Month 6)
2. Patient-reported respiratory symptoms at Month 7	Change from Baseline to Month 7 in respiratory symptom score
3. Patient-reported fatigue symptoms at Month 7	Change from Baseline to Month 7 in fatigue symptom score
4. Time to culture conversion	Time to culture conversion (first of 2 consecutive negative cultures) of Baseline to EOT assessments
5. Time to first negative culture	Time to first negative culture of Baseline to EOT assessments

6. MAC isolates with amikacin MIC ≥ 128 µg/mL	Proportion of subjects who develop a MAC isolate with amikacin MIC ≥ 128 µg/mL at more than 1 visit at any timepoint during the study
7. Recurrence of MAC (relapse)	Proportion of subjects who achieved culture conversion and subsequently have at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits that is the same species and genome as cultured at Screening/Baseline.
8. Recurrence of MAC (new infection)	Proportion of subjects who achieved culture conversion and subsequently have at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits that is different than cultured at Screening/Baseline (different species or same species but different genome).
9. Safety and tolerability of ALIS + background regimen	Incidence and severity of AEs and TEAEs and other safety variables (eg, vital signs, physical examination, clinical laboratory values) from Baseline through the EOS

2.3. Exploratory Objectives and Endpoints

Objective	***Endpoint***
To evaluate the effect of each treatment arm (ALIS +background regimen and ELC + background regimen) on the following:	
1. Patient-reported non-respiratory symptoms at Month 7	Change from Baseline to Month 7 in the QOL-B non-respiratory domains (physical, role, vitality, emotional, social, health perception)
2. Within-subject meaningful change threshold estimated in respiratory symptoms from Baseline to Month 7	Proportion of subjects meeting the within-subject meaningful change threshold as reflected in PRO changes scores computed from Baseline in patient-reported symptoms
3. Mean activity and sleep efficiency over time	Longitudinal summary of mean activity and sleep efficiency as measured by Philips Actiwatch Spectrum PRO

3. STUDY DESIGN

3.1. Overall Plan

This is a randomized, double-blind, placebo-controlled, active comparator study in eligible subjects with a new diagnosis (initial or subsequent) of MAC lung infection who have not started treatment for their current infection (Figure 1). Subjects will be randomized at Baseline in a 1:1 ratio to receive one of the two treatment regimens: ALIS + background regimen or ELC + background regimen for 6 months. Note that the background regimen in this study is defined as AZI+ETH per randomized treatment.

Randomization will be stratified by region (North America, Europe, and Rest of World) and history of MAC lung infection (initial or subsequent). After Baseline, subjects will return to the study site for in-clinic visits at Months 1, 3, 5, 6/EOT, and 7/EOS.

Visits at Months 2 and 4 do not require in-clinic appointments. At these non-in clinic visits, AEs and concomitant medications will be assessed, eDiary data will be collected continually for assessment of study drugs intake, and subjects will be required to produce and ship sputum samples. At the Month 6/EOT visit, subjects will discontinue all study drugs and will be followed for a 1 month off treatment period, during which medical or non-medical therapies for MAC lung infection should not be given.

At Month 7/EOS, subjects will complete all protocol-specified assessments and the final EOS procedures.

This study aims to validate PRO outcomes from the QOL-B and PROMIS F-SF 7a within the MAC lung disease population.

The validating variables will utilize the following PRO tools:

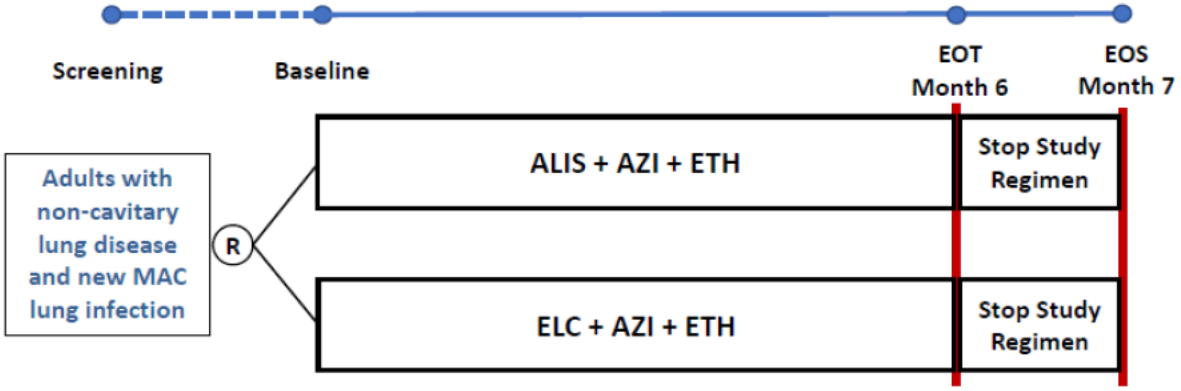
- EXACT
- E-RS
- SGRQ
- FACIT – Fatigue Scale
- PGI-S – Respiratory
- PGI-S – Fatigue

Clinical/Digital validating variables:

- $ppFEV_1$
- Sputum cultures (Section 8.1)
- Assessment of MAC isolates with amikacin MIC ≥ 128 µg/mL
- Actigraphy

The procedures and assessments conducted at each study visit in the study are provided in Table 4. Please refer to Figure 1 for a schematic diagram of the study design.

Figure 1: Study Design



ALIS = amikacin liposome inhalation suspension; AZI = azithromycin; ELC = empty liposome control; EOS = end of study; EOT = end of treatment; ETH = ethambutol; R = randomization

3.2. Data Monitoring Committee

A DMC will provide a centralized review function independent of the Sponsor's clinical team and all other individuals associated with the conduct of the study. The Committee will consist of external experts who are not involved in the study conduct and will comprise at least two physicians with pulmonary expertise, an infectious disease physician, and an experienced statistician. Members of the DMC will be free from conflicts of interest with the Sponsor. The DMC will periodically monitor the safety of the study. Further details are provided in the DMC charter.

3.3. Study Duration

Subjects will receive ALIS + background regimen or ELC + background regimen for 6 months and then remain off all study drugs for 1 month. The Screening period will be up to approximately -70 days to -1 day (~2.5 months).

The total duration of the study will be up to 9.5 months from the Screening visit to EOS.

3.4. End of Treatment

The EOT is defined as the date when the EOT visit occurs. The EOT visit will occur when the subject returns for EOT visit after discontinuation or completion of ALIS/ELC treatment only. Discontinuation or completion of AZI and/or ETH does not constitute EOT.

3.5. End of Study

The EOS is defined as the date of the last visit of the last subject.

4. STUDY POPULATION

4.1. Enrollment

This study is planned to be conducted at approximately 150 sites in North America, Europe, and the Rest of World. Subject eligibility by initial or subsequent MAC lung infection is summarized in Figure 2.

Figure 2: Subject Eligibility Flowchart

Abbreviations: dx – diagnosis; Note: Subjects with a subsequent diagnosis of MAC who were previously successfully treated and now have a positive culture > 6 months from cessation of prior successful treatment would not be considered a relapse and would be eligible for enrollment.

To be eligible for enrollment, subjects must meet all of the following inclusion criteria and none of the following exclusion criteria.

4.1.1. Inclusion Criteria

Subjects must satisfy all of the following criteria to be included in the study:

1. Male or female ≥ 18 years of age (19 years or older in South Korea)
2. Current diagnosis of MAC lung infection. MAC or mixed infection with MAC as the dominant species is allowed, with MAC as the intended organism for treatment
3. Positive sputum culture for MAC within 6 months prior to Screening
4. Positive sputum culture for MAC at Screening
5. A chest CT scan, read locally, within 6 months prior to Screening to determine presence and size of pulmonary cavities. Subjects who do not have a chest CT scan within 6 months prior to Screening will be required to obtain a chest CT scan, read locally, during Screening
6. In the Investigator's opinion, documented respiratory signs/symptoms at Screening that are attributable to the current MAC lung infection
7. An average QOL-B respiratory domain score of ≤ 85 based on scores at Screening and on the day of enrollment prior to randomization
8. In the Investigator's opinion, underlying lung disease (eg, COPD, bronchiectasis) have been managed according to best local standard of care, and on stable maintenance therapy for a minimum of 4 weeks prior to randomization
9. Willingness and ability to adhere to prescribed study treatment during the study
10. Ability to produce (spontaneously or with induction) approximately 2 mL of sputum for mycobacteriology at Screening
11. WOCBP (ie, fertile following menarche and until becoming post-menopausal unless permanently sterile) and fertile men (ie, all men after puberty unless permanently sterile by bilateral orchidectomy) agree to practice a highly effective method of birth control from Day 1 to at least 90 days after the last dose. Examples of such birth controls are:
    - true abstinence (refraining from heterosexual intercourse during the entire study),
    - copper IUD,
    - hormonal methods (levonorgestrel-releasing intrauterine system, progestogen implant, combined oral contraceptive pill [combined with barrier method]),
    - exclusive homosexual relationship, or
    - sole male partner who has undergone surgical sterilization with confirmation of azoospermia at least 3 months post procedure while participating in the study.
12. Provide signed informed consent prior to administration of study drugs or performing any study related procedure
13. Be able to comply with study drugs use, study visits, and study procedures as defined by the protocol
14. Men with partners who are WOCBP (pregnant or non-pregnant) agree to use condoms and non-pregnant partners should practice a highly effective method of birth control.

4.1.2. Exclusion Criteria

Subjects who meet any of the following criteria will be disqualified from entering the study:

1. Diagnosis of CF
2. History of more than 3 MAC lung infections
3. Received any mycobacterial antibiotic treatment for current MAC lung infection
4. Refractory MAC lung infection, defined as having positive MAC cultures while being treated with a multidrug mycobacterial antibiotic treatment regimen for a minimum of 6 consecutive months and no documented successful treatment, defined as negative sputum culture for MAC and cessation of treatment
5. Relapse of prior MAC lung infection, defined as positive sputum culture for MAC ≤ 6 months of cessation of prior successful treatment
6. MAC isolate with MIC for amikacin ≥ 128 µg/mL at Screening
7. Evidence of any pulmonary cavity ≥ 2 cm in diameter, as determined by chest CT scan, read locally, within 6 months prior to Screening
8. Radiographic finding of new lobar consolidation, atelectasis, significant pleural effusion, or pneumothorax during routine clinical care within 2 months prior to Screening
9. Active pulmonary malignancy (primary or metastatic) or any malignancy requiring chemotherapy or radiation therapy within 1 year prior to Screening or anticipated during the study
10. Active pulmonary tuberculosis requiring treatment during Screening
11. Hospitalization for underlying lung disease during Screening
12. Acute pulmonary exacerbation (eg, COPD or bronchiectasis) requiring treatment with antibiotics, or corticosteroids (IV or oral), within 4 weeks prior to and during Screening
13. Predicted FEV_1 < 35%, pre-bronchodilator use
14. Current smoker
15. History of lung transplantation
16. Use of inhaled or systemic aminoglycosides with activity against MAC (eg, amikacin, kanamycin, or streptomycin) during Screening
17. Prior exposure to ALIS (including clinical study)
18. Known hypersensitivity or contraindications to use to ALIS, aminoglycosides, or any of their excipients
19. Disseminated MAC infection
20. Positive pregnancy test or lactation at Screening. All WOCBP will be tested. Women not of child-bearing potential are defined as postmenopausal (ie, amenorrheic for 12 months without an alternative medical cause or confirmed by more than one FSH measurement), or naturally or surgically sterile through bilateral oophorectomy, hysterectomy, or bilateral salpingectomy. For women under the age of 45, confirmatory testing with FSH should be considered.
21. Administration of any investigational drug within 8 weeks prior to Screening
22. Known or suspected acquired immunodeficiency syndromes (HIV-positive, regardless of CD4 counts). Other immunodeficiency syndromes that may interfere with study participation in the opinion of the Investigator.
23. Significant (as determined by the Investigator) hearing loss, vestibular dysfunction, neuromuscular weakness or a diagnosis of myasthenia gravis, where the potential risk of aminoglycoside toxicity outweighs the potential benefit
24. Aspartate aminotransferase or alanine aminotransferase ≥ 3 times the ULN or total bilirubin ≥ 1.5 times ULN at Screening
25. Absolute neutrophil count ≤ 500/µL at Screening

26. Serum creatinine > 2 times ULN at Screening
27. Current alcohol, medication, or illicit drug abuse
28. Any condition that, in the opinion of the Investigator, interferes with ability to safely complete the study or adhere to study requirements
29. Known and active COVID-19 infection
30. MAC isolate with MIC for clarithromycin ≥ 32 µg/mL at Screening
31. Known hypersensitivity or contraindications to use to ethambutol, azithromycin (including other macrolides or ketolides), or any of their excipients per local labeling guidance

4.2. Discontinuation of Study Drugs and Participant Discontinuation/Withdrawal

4.2.1. Discontinuation of Study Drugs

All subjects are encouraged to stay in the study for continued safety monitoring regardless of discontinuation of study drugs. If a subject discontinues one or more study drugs (ALIS/ELC, AZI, and/or ETH), they will remain on the other study drug(s) and continue with the scheduled study visits. For these subjects, it is highly encouraged to stop all MAC treatment at Month 6 (per study design) to evaluate the primary and secondary endpoints during the off treatment period. However, if deemed medically necessary per the Investigator, these subjects who prematurely discontinue one or more study drugs may be allowed to continue treatment during the off treatment period.

If a subject discontinues ALIS or ELC:

- The reason for ALIS or ELC discontinuation will be recorded in eCRF.
- The subject will remain on the other study drugs (AZI and ETH) and continue with the next scheduled study visit.
- At the next scheduled visit, the subject will complete the EOT visit assessments.
- The subject will complete a Month 6 visit when they reach Month 6.

If a subject discontinues AZI and/or ETH, the reason for drug discontinuation should be recorded in the eCRF. The EOT visit should not be completed at this time. The subject will remain on the other study drugs (ALIS/ELC and AZI or ETH) and should initiate therapy consistent with the 2020 ATS/ERS/ESCMID/IDSA treatment guidelines (Daley et al., 2020), and continue with the next scheduled study visit. Any changes to therapy and treatment regimen should aim to prevent the development of macrolide resistance. Changes to treatment should be discussed with the Medical Monitor to ensure compliance with the 2020 ATS/ERS/ESCMID/IDSA treatment guidelines.

For subjects who discontinue one or more study drugs, replacement therapy may be provided at the discretion of the Investigator; this is not considered rescue therapy.

Subjects may be discontinued from one or more study drugs at the discretion of the Investigator if they meet treatment stopping criteria per local labeling guidance.

If a subject withdraws consent from continued safety monitoring, the EOS visit should be completed.

4.2.2. Participant Discontinuation/Withdrawal from the Study

A subject may decide to withdraw from the study at any time, for any reason. The Investigator and/or Sponsor also have the right to withdraw a subject from the study if it is no longer in the interest of the subject to continue in the study, or if the subject's continuation in the study places the scientific outcome of the study at risk (eg, if a subject does not or cannot follow study procedures). All subjects who are withdrawn from the study should complete protocol-specified early discontinuation procedures (Section 4.2.3).

4.2.3. Early Discontinuation Procedures

If a subject prematurely discontinues the study, the subject will do the following:

- Attend the EOT visit
- Attend EOS visit 28 days after the last dose of study drugs
- Complete EOS assessment

If a subject withdraws from the study, the Investigator will complete and report the observations as thoroughly as possible up to the date of withdrawal including the date of last treatment and the reason for withdrawal.

If the subject is discontinued from the study due to an AE, the Investigator will follow the subject until the Investigator deems that the AE has resolved or stabilized. In case of unresolved AEs including significant abnormal laboratory values at the EOS assessment, these events will be followed up until resolution or until they become stable.

4.2.3.1. Lost-to-Follow-up

A subject will be considered lost-to-follow-up if the site is unable to contact the subject after all reasonable efforts have been exhausted.

4.2.4. Subject Replacement

Subjects who prematurely discontinue from the study will not be replaced.

4.2.5. Subject Rescreening Procedures

Upon approval by Sponsor, subjects may be rescreened once, if the following apply:

- The screening culture is not positive for MAC, or
- The subject has a MAC isolate with MIC for amikacin ≥ 128 µg/mL at Screening, and/or
- The average QOL-B respiratory domain score is > 85, and/or
- The subject has active COVID-19 infection.

Subject will only be allowed to be rescreened if all other entry criteria have been satisfied. If a subject's average QOL-B respiratory domain score is > 85, the subject may take the

questionnaire again, once on the next day and again within 7 days to calculate a new average; all other Screening assessments do not have to be repeated. Subjects should be rescreened within the original screening period timeframe (approximately -70 to -1 days).

If a subject is rescreened due to Screening culture that is not positive for MAC or MAC isolate has amikacin MIC ≥ 128 µg/mL, all other Screening assessments must be repeated. The subject will have a new Screening window (approximately -70 to -1 days).

If a subject has active COVID-19 infection, the subject may be rescreened once recovered and the diagnostic tests are negative. All Screening assessments must be repeated. The subject will have a new Screening window (approximately -70 to -1 days). Subjects who experienced COVID-19 related hospitalization, severe disease, and/or ARDS may not be rescreened.

Rescreened subjects will be assigned a new subject number within the EDC system.

4.3. Premature Termination of the Study

The Sponsor may decide to terminate the study prematurely. If this occurs, written notification of the study termination is required to be sent to the site.

Conditions that may warrant study termination may include the following:

- Discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study
- Decision on the part of the Sponsor to suspend or discontinue development of the drug
- Decision by a regulatory authority or the Sponsor to stop the study at any time, where applicable

In the event of study discontinuation, subjects will discontinue study drugs. Refer to Section 4.2.3 for early discontinuation procedures.

Refer to Section 6.3 for considerations during public health emergencies.

The Sponsor will notify the regulatory authorities in all countries where the study is being conducted regarding the reason for terminating the study.

5. STUDY DRUGS

The Investigator must ensure that the study drugs will be used only in accordance with the protocol.

5.1. Amikacin Liposome Inhalation Suspension

ALIS is a white, milky suspension consisting of amikacin sulfate encapsulated in liposomes. The liposomes are composed of DPPC and cholesterol, which are dispersed in [redacted] (w/w) sodium chloride solution with sodium hydroxide for pH adjustment.

The concentration of the active ingredient is expressed in terms of amikacin base and is nominally 70 mg/mL. The drug product is filled into a 10-mL, borosilicate Type I flint glass single-use vial, stoppered with a 20-mm bromobutyl stopper and sealed with an aluminum flip off–tear off cap (Table 1).

5.1.1. Empty Liposome Control

A matching placebo referred to as ELC for the ALIS drug product uses the same excipients as the ALIS drug product in the absence of amikacin drug substance. The formulation composition comparison of the ALIS drug product and its matching placebo (ELC) is shown in Table 1.

Table 1: Composition of ALIS and ELC

Component	Quality Standard	Function	ALIS (mg/mL)	ELC (mg/mL)
Amikacin Sulfate	USP, Ph. Eur.	API	70	NA
DPPC	In-house	Liposome formation	[redacted]	
Cholesterol HP	NF, Ph. Eur., JP	Liposome formation		
Dehydrated Alcohol	USP, Ph. Eur., BP	[redacted]		
Sodium Hydroxide	NF, Ph. Eur., JP	To adjust pH		
Sodium Chloride	USP, Ph. Eur., JP	Tonicity		
Water for Injection	USP, Ph. Eur.	Aqueous vehicle		

ALIS = amikacin liposome inhalation suspension; API = active pharmaceutical ingredient; BP = British Pharmacopeia; DPPC = dipalmitoylphosphatidylcholine; ELC = empty liposome control; g = gram; HP = High Purity; JP = Japanese Pharmacopeia; NA = Not Available; NF = National Formulary; Ph. Eur. = European Pharmacopeia; QS = quantum sufficit; USP = United States Pharmacopeia.

The manufacturing process of the diluted ELC uses the similar liposome formation processing process and the same equipment that is used for ALIS drug product. The manufacturing process for the ELC has been developed to produce liposomes that are comparable to ALIS in which the amikacin sulfate is replaced with a [redacted] sodium chloride during infusion and liposome

formation. Once the liposomes are formed, the defiltration is started using [redacted] sodium chloride to the targeted lipid concentrations, eg [redacted] ELC.

5.1.2. Azithromycin and Ethambutol Tablets

The background regimen that will be used in the study are azithromycin 250 mg tablets and ethambutol 100 mg and ethambutol 400 mg tablets. These drugs are commercially available and will be sourced regionally and provided by the Sponsor.

5.1.3. Methods of Assigning Subjects to Treatments

Upon meeting all inclusion/exclusion criteria, subjects will be randomized through an IWRS vendor in a 1:1 ratio to either of the treatment groups. The randomization will be stratified by region (North America, Europe, and Rest of World) and history of MAC lung infection (initial or subsequent).

Investigators (including independent evaluator/raters and clinicians providing care to the subject), Sponsor, and subjects/caregivers will be blinded to treatment group assignments throughout the study.

Study personnel and subjects are strongly discouraged from discussing any aspect of study drugs that may lead to unintentional unblinding of treatment assignment in order to maintain study integrity.

Selected individuals who are not involved in the conduct of the study may have access to unblinded data as needed for safety review or other data review (see Section 3.2).

Unblinding is only to occur at the conclusion of the study or in the case of subject emergencies.

5.1.4. Study Drugs Administration

ALIS 590 mg or ELC will be administered QD by inhalation via nebulization over approximately 6 minutes to up to 15 minutes. Written directions for preparing and administering ALIS or ELC will be provided to subjects. On the day of study visits, ALIS or ELC administration will be the last procedure to be conducted (see Table 4).

Azithromycin 250 mg tablets dosed 1 tablet QD, and ethambutol tablets dosed at 15 mg/kg will be taken by mouth QD, with or without food. The Investigator should refer to local labeling guidance:

- To monitor AZI and ETH use
- To conduct any necessary monitoring procedures (eg, ECG monitoring, ophthalmic exams, audiology testing)

The Investigator is responsible for recording any AEs and SAEs as appropriate (see Section 9.10).

Timing of Administration

Study drugs should be administered QD around the same time each day, any time of day, in the fasted or as-fed condition.

5.1.4.1. Dose Interruption

It is recommended that all subjects remain on prescribed daily treatment throughout the study. However, experience has shown that dose interruptions may be helpful to avoid discontinuation of ALIS (Swenson et al., 2020). If a subject experiences a nonserious AE that is distressing and an interruption of study drugs dosing (ALIS/ELC, AZI and/or ETH per Investigator discretion) is needed to avoid discontinuation of study drugs, contacting the Medical Monitor to discuss subject management is encouraged.

At the discretion of the Investigator, dose adjustments of azithromycin and/or ethambutol may be allowed if criteria are met per local label recommendation (ie, renal or hepatic insufficiency).

All dose interruptions must be documented in the subject's source documentation and recorded in the eCRF.

5.1.5. Method of Assessing Treatment Compliance

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

Accountability for study drugs administration during the study is the responsibility of the Investigator or designees. Drug accountability will be recorded at each in-clinic study visit by review of electronic diary (eDiary) data (accounting for prescribed dose interruptions/adjustments) and count of returned study drugs.

A subject will be considered noncompliant with use of study drugs if treatment adherence is less than 80% or more than 120% unless instructed by the Investigator to interrupt dosing for safety reasons (Section 5.1.4.1). Subjects who are non-compliant with study drugs should be retrained.

5.1.6. Emergency Unblinding

Unblinding of treatment assignment for a subject may be necessary due to a medical emergency, rescue therapy (Section 5.2.2), or any other significant medical event (eg, pregnancy).

In the case of an emergency, the Investigator has the sole responsibility for determining if unblinding is warranted. The Investigator is required to follow the specific steps outlined in the unblinding procedure in the Study Reference Manual. Prior to unblinding, it is suggested that the Investigator contact the Sponsor and discuss the intended unblinding with the Medical Monitor or appropriate Insmed Incorporated study personnel (if this does not interfere with emergency treatment). In all cases of unblinding, the date, including justification for unblinding, must be fully documented and signed by the Investigator and written notification provided to the Sponsor. The originally signed copy is maintained in the study file at the site, and a copy is provided to the Sponsor or its designee. The date and reason for the unblinding is also entered in the study file and any associated AE reports. The Investigator can obtain the identity of the study drugs dispensed to a subject through IWRS.

5.1.7. Labeling and Packaging

Subjects will receive kits containing vials of ALIS or ELC and the nebulizer system. The nebulizer system is manufactured by PARI Respiratory Equipment, Richmond, VA, a subsidiary of PARI Pharma GmbH, Germany.

AZI tablets and ETH tablets will be sourced commercially. The packaging will vary depending on the manufacturer.

Labels will be prepared in accordance with GMP requirements and local regulatory guidelines. Label text will be translated into local language.

5.1.8. Storage

ALIS and ELC must be stored at 2ºC to 8ºC (36ºF to 46ºF); do not freeze. All sourced commercial drug supply, specifically Azithromycin 250 mg tablets and Ethambutol 100 mg and Ethambutol 400 mg tablets, should be stored according to the temperature ranges indicated on the commercial packaging.

At the study site, all study drugs must be stored in a secured place with restricted access.

5.1.9. Dispensing of Study Drugs

At visits specified in Table 4, subjects will be dispensed adequate study drugs to allow for daily dosing, including extra supply for potential study visit scheduling delays. Study drugs may be dispensed directly to the subject's home by courier if dispensing at the site is not feasible.

5.1.10. Drug Accountability

Drug accountability records will be maintained for all clinical supplies. All transactions will be recorded on the drug accountability records including shipment receipts and study subject doses. All transactions will be recorded on a real-time basis.

The pharmacy or responsible study staff will maintain detailed documentation of the number and identification of vials and dispensing units with copies of these documents to be provided to the Sponsor at the end of the study. All used and unused study drugs will be maintained by the site until inventoried by the Clinical Trial Monitor. Upon completion of the drug inventory by the Study Monitor, the used and any unused vials and tablets will be disposed of in accordance with instructions provided to sites and according to site destruction policies. Documentation of destruction should be provided to the Sponsor.

Accountability of the nebulizer system will be maintained and tracked at the site. Subjects must return the nebulizers to the study site at EOT. Additional instructions for handling the disposal or return of the devices will be provided to the sites and according to site destruction policies.

5.2. Concomitant Medications

Any medications the subject takes other than study drugs, including all vaccinations received, on or after the first dose of study drug are considered concomitant medications and will be collected and documented in the study eCRF.

5.2.1. Bronchodilator Therapy

If a subject is being treated with or started on bronchodilator therapy for management of underlying lung disease, the subject should be pretreated with a bronchodilator prior to ALIS or ELC administration throughout the study. If a subject is not on bronchodilator therapy and experiences a TEAE or SAE that is a respiratory symptom, the Investigator may consider pretreatment with a short acting bronchodilator prior to the ALIS or ELC administration. If a subject is pretreated with a bronchodilator prior to the ALIS or ELC administration, the subject should consistently pretreat moving forward in the study. Data will be collected on the concomitant medication eCRF page.

5.2.2. Rescue Therapy

Rescue therapy is defined as any change from the randomized treatment (ALIS + background regimen or ELC + background regimen) as prescribed in the study (includes drug, dose, frequency, route of administration) due to progression of MAC lung infection. Rescue therapy does not include changes from randomized treatment (eg, removal or replacement of drug) due to intolerance or AEs from study drugs.

If at any time during the study the Investigator believes that a subject has worsened clinically (eg, progressive worsening of signs/symptoms), the Investigator will determine the cause of the clinical deterioration (eg, intercurrent illness, noncompliance with pulmonary therapies, or progression of the MAC lung infection) and should contact the Medical Monitor. The clinical symptoms and cause should be documented in the subject's source documentation. If non-urgent intervention is required, the Investigator will optimize concomitant therapy and will contact the subject within 7 days of presentation via a telephone visit to reassess the subject. Within 14 days of determination of clinical worsening, the subject should make all attempts to return to the study site for an unscheduled visit for in-clinic assessment. However, if the subject is unable to return for the unscheduled visit, the assessment of change in signs/symptoms can be confirmed with a telephone visit. At that visit, the Investigator will then confirm the cause of the worsening signs/symptoms and should contact the Medical Monitor. If the Investigator confirms that the cause of the worsening in signs/symptoms is due to progression of MAC lung infection and rescue therapy is warranted, the Investigator will contact the Sponsor to unblind the subject from treatment assignment (Section 5.1.6). All contacts with the Medical Monitor should be documented in the subject's source documentation.

Subjects who receive rescue therapy will stop randomized treatment (ALIS + background regimen or ELC + background regimen), be treated by local standard of care or as recommended by the Investigator, and will continue with their remaining scheduled study visits.

Data will be collected on the appropriate eCRF pages.

5.3. Prohibited Medications and Medications with Limited Use

Subjects are prohibited from using any inhaled antibiotics or any aminoglycosides with activity against MAC, such as streptomycin or kanamycin at Screening and throughout the study. The use of these drugs should not be initiated during the study period unless as determined necessary by the Investigator. Subjects are also prohibited from using any antibiotics for the treatment of MAC lung infection (besides study drugs), unless required as rescue therapy (Section 5.2.2).

Other prohibited medications include those contraindicated with concomitant use with azithromycin and ethambutol per local labeling guidance. The reason for use must be documented in the eCRF. Some antibiotics used for the treatment of concomitant conditions that may have activity against MAC may be used, but not for more than 14 consecutive days.

Any questions about prohibited medications should be discussed with the Medical Monitor. A list of prohibited medications and medications with limited use for the study is provided in Table 2. Refer to local labeling guidance for other prohibited medications with concomitant use with azithromycin and ethambutol.

Table 2: List of Prohibited Medications and Medications with Limited Use

PROHIBITED Drugs	**LIMITED USE Drugs (not more than 14 consecutive days)**
Aminoglycosides: Amikacin, Kanamycin, Streptomycin	Other Macrolides: Erythromycin, Clarithromycin, etc.
Rifamycins: Rifampin, Rifampicin, Rifabutin, Rifamycin, etc.	Fluoroquinolones: Levofloxacin, Moxifloxacin, Sitafloxacin, Ciprofloxacin, etc.
Bedaquiline	Linezolid, Tedizolid
Clofazimine	Tigecycline, Eravacycline, Omadacycline
Isoniazid	Any other antibiotic with activity against MAC
Ethambutol (only allowed per protocol)	--
Azithromycin (only allowed per protocol)	--

5.4. Precautionary Medications

Chronic anti-inflammatory therapy (eg, high-dose ibuprofen, prednisone ≤ 10 mg/day or the equivalent) is permitted if the regimen remains unaltered for at least 28 days preceding Day 1/Baseline and throughout the study. The doses should not change except for safety reasons or as needed for medical management of the subject.

Although systemic exposure to amikacin is low after ALIS administration, precaution should be taken if subjects require the following systemic medications that may have possible interactions with amikacin, including, but not limited to the following: potent diuretics (such as ethacrynic acid and furosemide), beta lactam antibiotics (such as penicillins and cephalosporins); bisphosphonates; platinum compounds and thiamine.

The following may have possible interactions with azithromycin or ethambutol: digoxin, colchicine, and coumarin anticoagulants. Antacids should not be taken simultaneously with azithromycin or ethambutol. Azithromycin or ethambutol must be administered at least 1 hour before or 2 hours after antacid is taken.

Local labeling guidance for azithromycin and ethambutol must be consulted.

Examples of precautionary medications for ALIS are provided in Table 3.

Table 3: List of Examples of Precautionary Medications for ALIS

Name of Drug	Clinical Symptoms/ Treatment	Mechanism/ Risk Factor(s)
Potentially nephrotoxic blood substitutes such as Dextran Hydroxyethyl starch, etc.	Since there is a risk that nephrotoxicity occurs or is aggravated, concomitant use should be avoided. When nephrotoxicity occurs, drug should be discontinued and appropriate treatment be implemented such as renal dialysis, etc.	The mechanism of action is not completely clear but there are reports that the combination leads to deposits of aminoglycoside antibiotics in blood and to vacuolization of the proximal tubular epithelium.
Loop diuretics Ethacrynic acid Furosemide Azosemide, etc.	Since both drugs may cause or aggravate nephro- and ototoxicity, concomitant use should be avoided.	The mechanism of action is not completely clear but there are reports that the combination leads to increased blood concentration and renal deposits of aminoglycoside antibiotics.
Nephrotoxic and ototoxic drugs such as Vancomycin, Enviomycin, Platinum-containing anticancer drugs (cisplatin, carboplatin, nedaplatin), etc.	Since both drugs may cause or aggravate nephro- and ototoxicity, concomitant use should be avoided.	Both drugs are nephrotoxic/ ototoxic but the mechanism of action of the interaction is not known.
Anesthetics Muscle relaxants Tubocurarine Pancuronium bromide Tolperisone Botulinum Type A toxin products, etc.	Risk of respiratory suppression. If respiratory suppression occurs, choline esterase inhibitor or calcium preparation, etc. should be administered as appropriate.	Both drugs have a neuro-muscular inhibitory effect. Concomitant use will aggravate this effect.
Nephrotoxic drugs Cyclosporine A Amphotericin B, etc.	Risk that renal impairment occurs or is aggravated.	Both drugs are nephrotoxic but the mechanism of the interaction is not known.

6. STUDY PROCEDURES

6.1. Schedule of Assessments

The Schedule of Assessments is presented in Table 4.

COVID-19 restrictions and/or concerns may impact subject attendance at in-clinic visits (Section 6.3). In the event subjects are restricted from attending in-clinic visits or if subjects have concerns regarding travel and attending in-clinic visits (due to potential public health concerns), the site should contact the Sponsor on how to conduct the scheduled assessments, and decisions should be documented in the source documentation.

The PRO instruments will be provided to the subject in electronic format on a computer tablet and should be conducted at approximately the same time of day at the specified timepoints.

All PRO assessments must be completed prior to other study assessments (eg, physical examination, vital signs, laboratory assessments, audiology, etc) with the exception of sputum collection. If the subject produces their best sputum samples first thing in the morning prior to being able to attend a clinic visit, the subject should consistently collect sputum samples first, then proceed with PRO assessments throughout the study.

At each in-clinic visit, the subject will complete the PRO assessments in the order listed below. The approximate time to complete each assessment is provided; these are conservative estimates based on different studies and actual time may be less than indicated.

1. QOL-B (~10-15 minutes)
2. PROMIS F-SF 7a (~8-12 minutes)
3. EXACT (~3 minutes)
4. EXACT-RS (~3 minutes)
5. SGRQ (~10 minutes)
6. FACIT– Fatigue (~8-12 minutes)
7. PGI-S– Respiratory (~1 minute)
8. PGI-S– Fatigue (~1 minute)

All remaining assessments must be completed prior to administration of study drugs. If additional assessments for monitoring are recommended per local labeling guidance, the site should complete unscheduled assessments.

Table 4: Schedule of Assessments

INS-415		**Treatment Phase**						
		In-Clinic		**Non In-Clinic[a]**	**In-Clinic**	**Non In-Clinic[a]**	**In-Clinic**	
	Screening	**Baseline/ Day 1**	**Month 1**	**Month 2**	**Month 3, 5**	**Month 4**	**Month 6 (EOT)[b]**	**Month 7 (EOS)[c]**
	(V1)	**(V2)**	**(V3)**	**(V4)**	**(V5, 7)**	**(V6)**	**(V8)**	**(V9)**
Visit Window	**Approximately -70 days to -1 day**	**--**	**(±7)**	**(±7)**	**(±7)**	**(±7)**	**(±7)**	**(±7)**
Informed consent	X	--	--	--	--	--	--	--
Pregnancy test[d]	X	X	X	X	X	X	X	X
Randomization	--	X	--	--	--	--	--	
QOL-B[e]	X	X	--	--	X	--	X	X
PROMIS F-SF 7a[e]	X	X	--	--	X	--	X	X
EXACT[e]	X	X	--	--	--	--	--	X
EXACT RS[e]	X	X	--	--	--	--	--	X
SGRQ[e]	X	X	--	--	--	--	--	X
FACIT-Fatigue[e]	X	X	--	--	--	--	--	X
PGI-S Respiratory[e]	X	X	--	--	X	--	X	X
PGI-S Fatigue[e]	X	X	--	--	X	--	X	X
Actigraphy[f]		X	X		X	--	X	X
Medical history	X		--	--	--	--	--	--
Physical examination[g]	X	X	--	--	X	--	X	X
Vital signs and pulse oximetry[h]	X	X	X	--	X	--	X	X
FEV_1	X	X	--	--	--	--	--	--
Audiology[j]	X[i]		--	--	--	--	X	--

INS-415		**Treatment Phase**						
		In-Clinic		**Non In-Clinic[a]**	**In-Clinic**	**Non In-Clinic[a]**	**In-Clinic**	
	Screening	**Baseline/ Day 1**	**Month 1**	**Month 2**	**Month 3, 5**	**Month 4**	**Month 6 (EOT)[b]**	**Month 7 (EOS)[c]**
	(V1)	**(V2)**	**(V3)**	**(V4)**	**(V5, 7)**	**(V6)**	**(V8)**	**(V9)**
Visit Window	**Approximately -70 days to -1 day**	**--**	**(±7)**	**(±7)**	**(±7)**	**(±7)**	**(±7)**	**(±7)**
ECG[j]	X							
Ophthalmic Exam[j]	X							
Concomitant medications	X		X	X	X	X	X	X
AE assessment	X[k]	X[l]	X	X	X	X	X	X
Sputum collection for microbiology[m]	X	X	X	X	X	X	X	X
Chemistry[n]	X	X	X	--	X	--	X	X
Hematology [n]	X	X	X	--	X	--	X	X
Urinalysis [n]	X	X	X	--	X	--	X	X
CRP[n]	--	X	X	--	X	--	X	X
CT scan of chest (by local read)[o]	X	--	--	--	--	--	--	--
Send sputum collection containers home	X	X	X	--	X	--	X	--
Administer study drugs at site or at home[p]	--	X	X	--	X	--	--	--
eDiary for study drugs compliance	--	X	X	X	X	X	X	--
Dispense study drugs[q]	--	X	X	--	X	--	--	--
Collection of study drugs	--	--	X	--	X	--	X	--

AE = adverse event; CRP = C-reactive protein; CT = computed tomography; EOS = end of study; EOT = end of treatment; EXACT = EXAcerbations of Chronic Pulmonary Disease Tool; ERS = EXACT Respiratory Symptoms; FACIT-Fatigue = Functional Assessment of Chronic Illness Therapy; FEV1 = forced expiratory volume in 1 second; ICF = informed consent form; MAC = *Mycobacterium avium* complex; PGI-S = Patient Global Impression of Severity; PRO = patient-reported outcomes; PROMIS Fatigue Short Form 7a = Patient-Reported Outcome Measure Information System Fatigue-Short Form 7a; QOL-Bronchiectasis = Quality of Life Questionnaire – Bronchiectasis; SAE = serious adverse event; SGRQ = St George Respiratory Questionnaire; WOCBP = women of child-bearing potential.

a. If applicable; may be converted to clinic visits if needed due to local requirements
b. If a subject discontinues ALIS or ELC, the subject will have reason for ALIS or ELC withdrawal recorded and return at the next scheduled visit to complete the EOT visit assessments. The subject will remain on the other study drugs (AZI and/or ETH) and continue with the next scheduled study visit. The subject will complete a Month 6 visit when they reach Month 6.
c. Subjects will remain off all study drugs and should avoid initiating any new medical or non-medical therapies for MAC lung infection.
d. Serum pregnancy testing will be performed on WOCBP at Screening. A urine pregnancy test (at least 25 mIU/mL sensitivity) must be performed for all WOCBP at all subsequent visits starting at Baseline. Women not of childbearing potential are defined as postmenopausal (ie, amenorrheic for at least 12 months), or surgically or naturally sterile.
e. All PRO assessments will be performed prior to any other assessments (with the exception of sputum collection) and administration of study drugs and should be performed in the following order: QOL-B, PROMIS F-SF 7a, EXACT, EXACT-RS, SGRQ, FACIT-Fatigue, PGI-S-Respiratory, and PGI-S-Fatigue.
f. Collection of data is continuous. Data will be retrieved by study staff at in-clinic visits.
g. The physical examination will also include measurement of body weight; the measurement of height, without shoes, will only be done at Screening. All physical examinations should be performed prior to administration of study drugs.
h. Vital signs and pulse oximetry will be assessed prior to administration of study drugs.
i. The Baseline audiology examination must be performed during Screening or on Day 1 before administration of study drugs and evaluated by the Investigator.
j. Tests may be performed if required by local labeling guidance; Investigators should perform all monitoring according to local label
k. Only SAEs will be collected after the ICF is signed until Baseline.
l. At Baseline, AEs will be assessed only after administration of study drugs. Any AE that has occurred prior to the first dose must be included in the subject's medical history, including any AEs that occur within the Screening period.
m. Sputum cultures will be collected by study personnel at in-clinic study visits prior to administration of study drugs. Subjects will provide and ship sputum cultures at non in-clinic visits. Subjects who are not able to ship sputum samples may bring the samples to the clinic site. Two sputum samples will be obtained from each subject at each assessment; subjects will produce 1 sputum sample on the day prior to the scheduled visit and 1 sputum sample on the day of the visit prior to dosing with study drugs. At Screening, subjects will produce 1 sputum sample on the first day of the visit and 1 sputum 1 week later. The second sputum sample provided at Screening can be produced any time during the week following the Screening visit.
n. All laboratory testing should be performed prior to the administration of study drugs at in-clinic visits. Laboratory testing will **not** be performed at the Month 5 visit.
o. A prior chest CT scan may be used as a subject's Screening measurement if this CT scan was obtained within 6 months from the subject's Screening visit. Subjects who do not have a chest CT scan within 6 months prior to Screening will be required to obtain a scan during Screening.
p. Study drugs may be administered at home during a home care visit (if applicable), if administration at site is not feasible
q. Study drugs may be dispensed directly to the subject's home by a courier if dispensing at site is not feasible

6.2. Protocol Deviations

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

The Investigator should notify the IRB/EC of any deviations from the protocol in accordance with local procedures.

6.3. Public Health Emergencies – COVID-19

During the COVID-19 public health emergency, the Sponsor, IRB/IECs, and Investigators shall follow the most current version of local guidance to assure the safety of study subjects, maintain compliance with GCP, and minimize risks to study integrity.

In anticipation that the COVID-19 pandemic may have an impact on the conduct of clinical studies, the Sponsor does not intend to screen any new subjects in this study unless the impact of the COVID-19 pandemic is deemed manageable and no longer interfering with the conduct of the study at individual sites, and subjects can safely participate in this study.

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 should contact the Sponsor on how and when to implement temporary and/or alternative mechanism of scheduled assessments, and all decisions taken should 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 following suggested guidance should be followed:

Continuation or suspension of the study

The Sponsor, in consultation with clinical Investigators and IRBs/ECs, will determine if the protection of a subject's safety, welfare, and rights are best served by continuing or suspending the study at a 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:

Study recruitment

The Sponsor 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/EC, and its local health authority mandates. If, due to COVID-19, study discontinuation exceeds the assumed rate, the Sponsor may allow enrollment past the assumed sample size (Section 10.1).

Subjects already enrolled in the study

- If a subject 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 subject's safety can be preserved by other means.
- If subjects no longer have access to the investigational product, direct-to-subjects delivery by couriers may be implemented, provided that all safety considerations and applicable health authority requirements have been addressed.
- If a subject has a documented COVID-19 infection while in the study, the event will be reported as an AE or SAE, depending on the criteria. The site will follow the guidance provided by health authorities in the treatment of those subjects.

7. PSYCHOMETRIC VALIDATION

7.1. Variables to be Validated

Psychometric validation will consist of cross-sectional and longitudinal validation analyses. At baseline, cross-sectional validation will consist of provisional modern psychometric methods.

7.1.1. Quality of Life Questionnaire – Bronchiectasis

The QOL-B is a validated, self-administered, reported outcome questionnaire used to assess symptoms, functioning, and health related quality of life in adults with non-CF bronchiectasis (Quittner et al., 2015). It measures outcomes over a recall period of 1 week. The questionnaire contains 37 items on 8 scales (physical, role, vitality, emotional, social, treatment burden, health perception, and respiratory) (Appendix 1).

The QOL-B questionnaire will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically. The QOL-B questionnaire will be the first assessment to be conducted at the specified study visits (with the exception of sputum collection, if applicable).

Each of the 37 items is scored from 1 to 4, and each of the 8 scale scores is standardized on a 0 to 100-point scale, with higher scores representing fewer symptoms or better functioning and quality of life. Scores are calculated for the 8 domains: physical, role, vitality, emotional, social, treatment burden, health perception, and respiratory.

7.1.2. PROMIS Fatigue Short Form 7a

The PROMIS F-SF 7a is a self-administered questionnaire assessing a range of self-reported symptoms over the past 7 days, from mild subjective feelings of tiredness to an overwhelming, debilitating, and sustained sense of exhaustion that likely decreases one's ability to execute daily activities and function normally in family or social roles (Ameringer et al., 2016). Fatigue is divided into the experience of fatigue (frequency, duration, and intensity) and the impact of fatigue on physical, mental, and social activities over 7 items. Examples of items are: "How often did you feel tired," and "How often were you too tired to take a bath/shower" (Appendix 2).

The PROMIS F-SF 7a will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically. The PROMIS F-SF 7a will be the second assessment to be conducted at the specified study visits.

Response options are on a 5-point Likert scale, ranging from 1=never to 5=always. The PROMIS F-SF 7a is universal rather than disease-specific and given its brevity, poses minimal burden to the subject.

7.2. Validating Variables

7.2.1. EXAcerbations of Chronic Pulmonary Disease Tool (EXACT)

The EXACT PRO was developed and qualified for use in characterizing COPD clinical endpoints (Jones et al., 2011; Leidy et al., 2010; Leidy et al., 2011; US FDA, 2013). Several of the concepts assessed by the EXACT are relevant to characterizing the symptomatic phenomena expected within bronchiectasis. These include, but are not limited to, dyspnea and fatigue. The EXACT total score measures acute, sustained, and worsening signs and symptoms exceeding subject-specific expected variability. The EXACT PRO is composed of 14 items from which a total score is generated. Domains assessed by the EXACT PRO include dyspnea, cough and sputum production, chest symptoms, difficulty expectorating, fatigue, sleep disturbance, and fear or concern (Appendix 4).

The EXACT PRO will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically.

7.2.2. Exact Respiratory Symptoms (EXACT-RS)

The E-RS is an 11-item subset of the EXACT PRO assessing exacerbations of respiratory symptoms (Leidy et al., 2014a; Leidy et al., 2014b) (Appendix 4).

The E-RS will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire .

7.2.3. St. George's Respiratory Questionnaire (SGRQ)

The SGRQ is a self-administered instrument for the assessment of overall health, daily life, and perceived well-being among individuals with obstructive airways disease (Jones et al., 1992). The instrument consists of 50 items with 76 weighted responses grouped into a set of 17 questions. The items are divided into 2 parts and 3 categories: symptom, activity, and impact. Part 1 (symptom component) assesses an individuals' perception of their recent respiratory problems. Part 1 evaluates frequency and severity of symptoms including cough, sputum production, wheezing, breathlessness, and the duration and frequency of attacks of breathlessness and wheeze. Part 2 (activity and impact components) addresses individuals' current state. The activity component is composed of a series of items evaluating metabolic equivalence of incrementally increasingly physically demanding activities, ranging from sitting to climbing a flight of stairs. The impact component measures domains of social functioning and psychological disturbances resulting from airway disease (Appendix 5).

The SGRQ will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire .

7.2.4. Functional Assessment of Chronic Illness Therapy (FACIT) – Fatigue Scale

The FACIT-Fatigue Scale is a short, 13-item, easy to administer, tool measuring an individual's level of fatigue during their usual daily activities during the past week (Smith et al., 2010). The level of fatigue is measured on a four-point Likert scale (4 = not at all fatigued to 0 = very much

fatigued). FACIT-Fatigue is routinely administered in studies of respiratory disease populations for accurately evaluating the common sequela of fatigue (Appendix 6).

The FACIT-Fatigue will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically.

7.2.5. PGI-S Respiratory

The PGI-S Respiratory score is a simple categorical rating of symptom severity. The scale is 1 = not at all to 5 = extremely severe (Appendix 3).

The PGI-S Respiratory will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically.

7.2.6. PGI-S Fatigue

The PGI-S Fatigue score is a simple categorical rating of symptom severity. The scale is 1 = not at all to 5 = extremely severe (Appendix 3).

The PGI-S Fatigue will be completed at study visits in the order specified in Section 6.1 and Schedule of Assessments (Table 4). Subjects will self-administer the questionnaire electronically.

8. EFFICACY ASSESSMENTS

8.1. Sputum Culture

Microbiological assessment of sputum specimens will be used for secondary efficacy measurements.

During the study, pre-dose expectorated or induced sputum samples (approximately 2 mL) are required at study visits specified in the Schedule of Assessments (Table 4). To improve the probability of obtaining a good sputum specimen, 2 sputum samples will be obtained from each subject at each assessment; subjects will provide 1 sputum sample on the day prior to the scheduled visit, and 1 sputum sample on the day of the visit prior to dosing with study drugs. At Screening, subjects will provide 1 sputum sample on the day of the visit and 1 sputum during the week following the Screening visit. If a subject is unable to produce sputum spontaneously, sputum may be induced. If after induction, a subject is still unable to produce sputum despite reasonable efforts, this will be recorded as non-productive at that time point. It is recommended to schedule an in-clinic visit as a backup to non in-clinic visits in case the subject is not able to spontaneously produce sputum at home. If a subject is unable to spontaneously produce sputum at home on the day prior to the scheduled visit, the subject should then come into the clinic for sputum induction on the scheduled visit. The induction should be performed within the specified visit window (Table 4). At the scheduled visit, once sputum is collected (spontaneously or by induction), study drugs can be administered. If the subject produces their best sputum samples first thing in the morning prior to being able to attend a clinic visit, the subject should consistently collect sputum samples first, then proceed with PRO assessments and other assessments throughout the study.

Sterile, leak proof, non-wax, disposable plastic containers labeled with 2 subject identifiers will be used to collect specimens aseptically to avoid contamination. Sputum samples should be refrigerated, not frozen, until shipped to the central microbiology laboratory as soon as possible to avoid overgrowth by contaminating normal flora. No fixative or preservatives are to be used with sputum samples. Detailed instructions for collecting, processing, and shipping sputum specimens will be provided in the site laboratory manual. Subjects will be provided with instructions for collecting, processing, and shipping sputum specimens for samples collected at non in-clinic visits.

Sites are strongly discouraged from performing local mycobacterium sputum cultures for the duration of the study, as this may introduce bias to subject assessments. Sputum may be sent to local laboratories to be cultured for organisms other than mycobacterium.

Microbiological Assessment

Sputum specimens will be cultured in broth media (liquid) in addition to agar media (solid), and will be held for up to 6 weeks. A negative culture result will not be reported until after this time has transpired. All contaminated samples will be retreated and re-cultured once.

Growth of other bacteria due to co-infections (eg, *Pseudomonas aeruginosa*, *M. abscessus*, other NTM) will be reported as applicable. Colony counts of MAC growth will be reported. Isolates of MAC will be identified using a commercial RNA probe (AccuProbe, Gen Probe, Inc., GenoType NTM-DR Ver 1.0, Hain Lifescience) and subsequently identified to species using molecular

methodology (Cousins et al., 1996). Standard antibiotic sensitivity testing using MICs will be routinely performed on mycobacterial isolates (CLSI, 2018a; CLSI, 2018b; Forbes et al., 2008).

The MAC species and genotype at the Screening and/or Baseline sputum specimen will be identified by whole genome sequencing (Illumina Nextseq500TM) in subjects who achieve sputum culture conversion and who subsequently have positive sputum culture(s) (broth or agar); typing by whole genome sequencing will also be conducted.

MAC Culture Assessment Definitions

MAC culture negative	No MAC growth on agar media and broth media in all sputum cultures at a visit.
Non-productive	Prior to culture conversion or post recurrence: visits where subjects were recorded as non-productive are considered MAC culture positive. Post culture conversion: visits where subjects were recorded as non-productive are considered MAC culture negative.
Culture conversion	No MAC growth on agar media and broth media in all sputum cultures at 2 consecutive visits. The date of conversion is defined by the date of the first of 2 consecutive negative cultures.
Recurrence: Relapse	Subsequent to culture conversion, at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits. A MAC positive culture is defined as the same species and genome as cultured at Screening/Baseline.
Recurrence: New infection	Subsequent to culture conversion, at least 1 MAC positive culture in agar media or positive cultures in broth media in at least 2 consecutive visits. A MAC positive culture is defined as a culture that is different than cultured at Screening/Baseline (different species, or same species but different genome).

8.2. Actigraphy

The Philips Actiwatch Spectrum PRO actigraphy device resembles a wristwatch and senses the activity of the wearer. It is to be worn continuously by the subject throughout the duration of the study. It is water-resistant and does not need to be removed for bathing. The subject will be provided with charging materials and should be instructed to charge the device once every 30 days (if needed). The data collected should be downloaded at each in-clinic visit or during a home care visit (if applicable).

9. SAFETY ASSESSMENTS

9.1. Vital Signs and Pulse Oximetry

Systolic and diastolic BP, pulse rate, body temperature, RR, and oxygen saturation will be recorded in the eCRF at study visits specified in the Schedule of Assessments (Table 4).

9.2. Physical Examination

A physical examination of the head (external), eyes, ears, nose and throat, lungs, cardiovascular system, abdomen, musculoskeletal system, skin, lymph nodes, central nervous system, and, as appropriate, other body systems will performed at study visits specified in the Schedule of Assessments (Table 4). The physical examination will also include measurement of body weight. The measurement of height, without shoes, will only be done at Screening. Any abnormalities noticed at Screening will be recorded as medical history or as AEs if they occurred after randomization.

9.3. Audiology Test

Audiology testing will be performed at study visits specified in the Schedule of Assessments (Table 4). Additional assessments may be conducted if required per local labeling guidance. Frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz will be evaluated for each ear using air conduction.

9.4. Forced Expiratory Volume in 1 Second (FEV_1)

FEV_1 will be performed by trained personnel at the site and will be based on local assessments. Spirometers will be provided if not available at the study sites. FEV_1 will be performed (prior to bronchodilator use if applicable) at Screening and Day 1/Baseline (Table 4) prior to the administration of study drugs.

9.5. ECG Monitoring

ECG monitoring should be performed if required by local labeling guidance to monitor QT/QTc. In the event of QT/QTc interval prolongation, monitoring should continue until QTcF returns to normal range for the subject. Investigators should manage subject per local labeling guidance.

9.6. Ophthalmologic Test

Ophthalmologic tests (eg, visual acuity, visual field, color vision and fundus) should be performed if required by local labeling guidance. Investigators should manage subjects per local labeling guidance.

9.7. Clinical Laboratory Evaluations

Clinical laboratory tests of hematology, blood chemistry, and urinalysis will be performed via the study designated laboratory at study visits specified in the Schedule of Assessments (Table 4). The clinical laboratory test parameters are listed in Table 5. Laboratory assessments will not be performed at Month 5 (Visit 7).

Mid-stream clean catch urine samples will be collected for urinalysis. Urine dipsticks will be used for urine pregnancy testing. The clinical laboratory test parameters are listed in Table 5.

Instructions for collection, storage, and shipment of clinical laboratory samples will be provided in the clinical laboratory manual provided in the site study binder.

Table 5 Clinical 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, estimated glomerular filtration rate from the Cockcroft-Gault method
Hematology	Hemoglobin, erythrocytes, hematocrit, MCH, MCV, MCHC, leukocytes, differential blood count of neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets
Urinalysis	Qualitative analysis of glucose, ketones, nitrites, protein, pH, leukocytes, blood, bilirubin, specific gravity; microscopic examination for cells, casts, and bacteria

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CO_2 = bicarbonate; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; ULN = upper limit of normal.

C-reactive protein

Blood samples will be drawn at study visits specified in the Schedule of Assessments (Table 4) to measure the concentration of CRP.

Pregnancy Test

A serum pregnancy test will be performed on WOCBP at Screening. A urine pregnancy test (at least 25 mIU/mL sensitivity) will be performed on WOCBP as specified in the Schedule of Assessments (Table 4). Results will be entered into the eCRF. Women not of childbearing potential are defined as postmenopausal (ie, amenorrheic for at least 12 months), or surgically or naturally sterile.

9.8. Chest CT Scan

A chest CT scan will be performed at Screening. A prior chest CT scan may be used if this CT scan was obtained within 6 months from the subject's Screening visit. Chest CT scan will be read locally.

9.9. Adverse Events

The Investigator is responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE as provided in the protocol and remain responsible for following up all AEs.

The Investigator should proactively follow subjects with AEs until the EOS for each subject. At the EOS visit, the Investigator will record the AE status in the eCRF.

9.9.1. Definition of an Adverse Event

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject 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.

Medical occurrences that begin after obtaining informed consent, but before administration of study drugs will be recorded under medical history/Current Medical conditions, not as AEs.

Treatment-emergent adverse events are AEs that occurred on or after the date of first dose of study drugs and within 28 days after the end of treatment.

It is the responsibility of Investigators, based on their knowledge and experience, to determine, those circumstances or abnormal laboratory findings which should be considered adverse events.

9.9.2. Definition of a Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is an important medical event.

Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject 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 (ICH E2A Guideline. Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, October 1994).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples include allergic bronchospasm, convulsions, and blood dyscrasias or development of drug dependency or drug abuse.

Note:

- A procedure is not an AE or SAE, but the reason for the procedure may be an AE or SAE.
- Pre-planned (prior to signing the ICF) procedures or treatment requiring hospitalizations for pre-existing conditions which do not worsen in severity are not SAEs.

- Suspected transmission of an infectious agent via a medicinal product should be considered as a SAE
- Laboratory abnormalities are usually not recorded as AEs or SAEs. However, abnormal laboratory findings (such as clinical chemistry, hematology, or urinalysis) or other abnormal assessments (physiologic tests such as electrocardiograms [ECG], imaging studies, or vital signs) that are associated with signs and/or symptoms, or are considered clinically significant in the judgment of the Investigator, require therapeutic intervention, or lead to discontinuation of the administration of study drugs, must be recorded as AEs or SAEs if they meet the definitions of an AE (or SAE).

9.9.3. Adverse Events of Special Interest

Adverse events of special interest are defined as events known to be attributed to parenteral amikacin and inhalation of antibiotics. Additional information on some AESIs will be collected in the eCRF. The AESI category groups and preferred terms include the following:

- Hypersensitivity pneumonitis (allergic alveolitis, pneumonitis, interstitial lung disease)
- Hemoptysis (hemoptysis)
- Bronchospasm (asthma, bronchial hyperreactivity, bronchospasm, dyspnea, exertional dyspnea, prolonged expiration, throat tightness, and wheezing)
- Exacerbation of underlying pulmonary disease (COPD, infective exacerbation of chronic obstructive pulmonary disease, and infective exacerbation of bronchiectasis)
    - Pulmonary exacerbation will be defined based on the Investigators best clinical judgment. Details of these events should be reported in an additional "pulmonary exacerbation" page of the eCRF.
- Ototoxicity (deafness, neurosensory deafness, unilateral deafness, dizziness, hypoacusis, presyncope, tinnitus, vertigo)
- Nephrotoxicity (nephropathy toxic, azotemia, oliguria, albuminuria, acute kidney injury, anuria, renal impairment)
- Neuromuscular disorders (muscle weakness, peripheral neuropathy, and balance disorder)

9.9.4. Assessment of Severity

The following definitions should be used to assess intensity of AEs:

- Mild: Awareness of sign or symptom, but easily tolerated, ie, does not interfere with subject's usual function.
- Moderate: Discomfort enough to cause interference with usual activity.
- Severe: Incapacitating with inability to work or do usual activity, ie, interferes significantly with subject's usual function.

9.9.5. Assessment of Causality

The Investigator who identifies an AE will determine the causality of each based on the temporal relationship to administration of study drugs and clinical judgment. The degree of certainty about causality will be graded using the categories listed below.

- **Related**: A reaction that follows a reasonable temporal sequence from administration of study drugs; that follows a known or expected response pattern to the study drugs; that disappears or decreases on cessation or reduction in study drugs dose; and/or that reappears or worsens when the study drugs is administered. Could not be reasonably explained by other factors such as underlying disease, complications, concomitant drugs, or concurrent treatments.
- **Not Related**: A reaction for which sufficient data exist to indicate that the etiology is unrelated to the study drugs.

9.9.6. Management of Adverse Events

Initial education of adverse effect expectations is crucial for subjects initiating study drugs in order to maintain retention of subjects in the study. Based on data from the ALIS development program, there is an association with ALIS and respiratory symptoms (eg, cough, dyspnea) reported as AEs, likely from airway irritation by the inhaled antibiotic. Data observed from Study INS-212 (refractory MAC population) show that the majority of subjects treated with ALIS experienced their first AE within 60 days of initiating ALIS. Azithromycin may cause gastrointestinal symptoms. Subjects should be monitored for visual acuity when taking ethambutol.

Data have shown that specific interventions may be helpful in the management of TEAEs (Swenson et al., 2020). Pretreatment with a bronchodilator, move administration of ALIS to the evening, antitussives, post-dose gargle (warm liquid or glycerin), and other interventions may help with management of TEAEs.

9.10. Reporting Requirements

9.10.1. Adverse Events

All AEs will be reported on the Adverse Events Form of the eCRF. Adverse events that occur between the time subject signs the ICF for the study and the time when subject receives his/her first dose of study drugs on Day 1 will be summarized as medical history and not as a TEAE unless the event meets the definition of an SAE as defined above. Additional information on some AEs will be collected in the eCRF.

9.10.2. Serious Adverse Events

All SAEs, regardless of causality, must be reported to organization delegated by the Sponsor on an SAE 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, telephone, and fax number for SAE reporting are presented in the Study Reference Manual.

Unexpected drug related SAEs as assessed by Sponsor or authorized person qualify for expedited reporting and will be reported to the IRB/EC, regulatory authorities, 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, which is suspected to be caused by the investigational medicinal product and which 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.

9.10.3. Pregnancy

Any pregnancy, including the pregnancy of a male subject's female partner, that occurs during any phase of the study must be reported to the Sponsor or designated organization within 24 hours of learning of the pregnancy using a Clinical Study Pregnancy Form.

All study drugs should be discontinued, and the pregnancy should be followed to term. The details of termination must also be reported, including details of birth, the presence or absence of birth defects, congenital abnormalities or maternal and newborn complications, or whether termination was spontaneous or voluntary.

9.10.4. Overdose

An overdose is defined as a dose greater than the dose level evaluated in this study as described in Section 5. An overdose itself is not an AE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses must be reported regardless of sequelae. However, if the overdose results in clinical signs and symptoms, it requires an expedited reporting as if it is an SAE. In the case of a symptomatic overdose, the Investigator should use clinical judgment in treating the overdose and should inform the Sponsor immediately. The Investigators should refer to the relevant documents for detailed information regarding warnings, precautions, contraindications, AEs, and other significant data pertaining to the study drugs used in the study. Such document(s) may include, but not limited to, the IB for ALIS and approved product labeling for ARIKAYCE, or approved product labeling for azithromycin and ethambutol.

9.11. Follow-up of Adverse and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 4.2.3.1). If the Investigator receives a report of any SAE, including a death, at any time after a subject has been discharged from the study, and the Investigator considers the event to be reasonably related to the study drugs or study participation, the Investigator must promptly notify the Sponsor.

9.12. Regulatory Aspects

The Sponsor has a legal responsibility to notify the FDA, National Competent Authorities and Central Ethics Committees of the European Union, and any other foreign regulatory competent agency, as well as all sites, about the safety of the drug. The Investigator has the responsibility to notify the local EC about SUSARs.

The Investigator(s)/institution(s) will permit study related monitoring, audits, IRB/EC review and regulatory inspection(s), providing direct access to source data/documents. Copies of the notification to the EC must be sent to the Sponsor.

10. STATISTICAL METHODS

Statistical analyses methods pertaining to validation of PRO will be included in VAP, and all other analyses will be described in SAP. The SAPs will include detailed description of methods and will be finalized prior to the database lock.

10.1. Sample Size and Power Considerations

Power and Sample Size Determination for the Validation Study

Cross-sectional validation will require baseline data from a maximum of 250 subjects to adequately power the planned MPMs. A total of 100 subjects will be enrolled in this study and will be used for this analysis. Additional baseline data from up to the first 150 subjects enrolled in a separate study will be used.

Longitudinal validation will require 100 subjects to adequately power the planned within-subject meaningful change methods.

The derivation of these sample sizes and the procedures used to compute power are described below.

Cross-sectional Validation Power and Sample Size

The estimated sample size required to conduct cross-sectional validation of the PRO endpoints within the NTM-MAC population was obtained from a power analysis assuming the PRO will be composed of 10 total items analyzed with 5 response categories each. This power procedure determines the sample size required to detect a domain specification that fits the data acceptably well. (Serrano and Iaconangelo, 2019; Serrano and Iaconangelo, 2017) The model fit statistic for which power was computed is the RMSEA. The RMSEA is the primary fit index used in modern psychometric methods for determining the optimal domain structure. This fit assessment is the logical test to power, as the modern psychometric methods require the largest sample sizes of all validation analyses. Therefore, adequately powering modern psychometric methods is key to validation study success. In addition, the precision of all validation analyses subsequent to modern psychometric methods rely on correct domain specification, which itself relies on the RMSEA.

For a 10-item questionnaire, approximately n=250 is required to achieve a power of 0.80 for the RMSEA-based test of acceptable MPM model fit. Therefore, a maximum of 250 subjects are planned for the study cross-sectional validation analyses. These subjects will contribute data at both screening and baseline for the purposes of estimated test-retest reliability as well. Longitudinal validation analyses will be conducted on the subset of 100 subjects enrolled into this study.

Longitudinal Validation Power and Sample Size

An empirical power analysis simulation was conducted to evaluate the optimal sample size to adequately characterize culture converter anchor-based within-subject meaningful change in this study.

Inputs to the power analysis simulations were obtained from the MAC subset of the ALIS treated subjects in Insmed Study TR02-112, and simulation conditions considered reflected conservative variation around observed estimates (larger dispersion and smaller magnitudes of effect). The

following conditions were used to compute power: magnitudes of effect in change from baseline (6pt, 7pt, 8pt, 9pt, and 12pt change from baseline in converters, and 3 point change from baseline in non-converters); culture converter proportions (50%, 60%, and 70%); proportion data missing at random (MAR, 0%, 5%, 20%, 25%, and 30%); and sample size (n=100, n=120, and n=130). The polyserial correlation between simulated change from baseline in QOL-B respiratory domain and culture conversion varied around the observed estimate of 0.47, ranging from 0.3 to 0.6. Within each unique simulation condition r=1000 replications were generated. Empirical power was computed as the number of replications for which statistically significant differences between converters and non-converters in change from baseline in QOL-B respiratory domain score were observed. For example, if 900 replications demonstrated significant differences using the 2-sided test conducted at α=0.05, then power was 90%.

A total sample size of 100 subjects (50 subject per treatment arm) will provide at least 80% power for detecting 7 points mean difference in QOL-B respiratory domain response at 1-month post treatment between subjects achieving culture conversion during the study and those who did not via ANOVA testing. This derivation assumes 50%, 60%, or 70% culture conversion rate, at least 40% polyserial correlation between culture conversion and QOL-B respiratory domain response, and missing data of 0%, 5%, or 20%.

10.2. Analysis Sets

10.2.1. Intent-to-Treat Analysis Set

The ITT analysis set comprises all subjects who were randomized. This set will be analyzed using the treatment to which the subject was randomized.

10.2.2. Safety Analysis Set

The safety analysis set comprises all subjects who were randomized and received at least 1 dose of ALIS, ELC, AZI, or ETH.

10.2.3. Additional Analysis Sets

Additional analysis sets may be defined in the SAP as appropriate.

10.3. Data Handling Conventions

For all variables, only observed data from subjects will be used in the statistical analyses, ie, there is no plan to estimate missing data, unless otherwise stated. Detailed data imputation rules required for handling of missing events dates for safety data will be described in related data handling documents.

Unless otherwise stated, for subjects who discontinue ALIS or ELC and remain in the study for safety monitoring, data post-EOT will be programmatically relabeled to post-treatment visits; details will be included in SAP.

10.4. Multiplicity

All summaries will be descriptive without multiplicity adjustment. Confidence intervals for estimation of treatment differences will not be adjusted for multiplicity, p-values if provide will be nominal.

10.5. Study Population

The ITT analysis set will be used for all study population summaries unless otherwise stated. Summaries will be presented by treatment group and for all subjects based on the data available.

10.5.1. Study Population Disposition, Demographics and Baseline Characteristics

Subject demographic and baseline characteristics, including medical history, prior medications and therapies will be summarized using descriptive statistics.

For continuous variables, descriptive statistics (number [n], mean, standard deviation, median, minimum, and maximum) will be provided. For categorical variables, subject counts and percentages will be provided. Categories for missing data will be presented if appropriate.

The total number of subjects who were screened, randomized, completed the study, discontinued study drugs (including the reason), and withdrew consent from the study (including the reason) will be summarized by treatment group. The reason for discontinuing study drugs or withdrawal of consent from the study will be listed by subject.

Major protocol deviation will be summarized.

Assessments collected exclusively prior to therapy will be listed and summarized as appropriate (including data for FEV_1).

10.5.2. Primary Objective

The primary objective of this study is psychometric validation of PRO instruments.

Psychometric validation will consist of cross-sectional and longitudinal validation analyses. At Baseline, cross-sectional validation will consist of modern psychometric methods (exploratory factor analysis, item response theory models and corresponding assessments of local dependence and differential item functioning), internal consistency, concurrent validity, and known-groups validity. Longitudinal validation analyses conducted between Screening and Baseline will consist of test-retest reliability. The test-retest reliability population will consist of subjects reporting no change on PGI-S (respiratory for QOL-B and fatigue for PROMIS F-SF 7a) between Screening and Baseline. Longitudinal validation analyses conducted between Baseline and EOS (Month 7) will consist of the estimation of within-subject meaningful change. The within-subject meaningful change anchor definition will be derived from the change in PGI-S anchors (respiratory for QOL-B and fatigue for PROMIS F-SF 7a) between Baseline and EOS. In addition, culture converter status will be used as an additional anchor variable. Point estimates of within-subject meaningful change will consist of the mean change score conditioned on the PGI-S anchor definition. In addition, the median estimate will be examined in sensitivity analyses. Point estimates will be validated through the use of eCDFs and ePDFs.

10.5.3. Cross-sectional Validation

10.5.3.1. Modern Psychometric Methods

Evidence associated with these methods will be estimated at baseline. The main function of the modern psychometric methods employed will be the detection of any demonstrably irrelevant, redundant, or biased items. Full-information EFA and IRT models will be estimated in the service of this goal. Irrelevant items will be identified by item loadings (EFA) and slopes (IRT) approaching zero. Redundant items will be identified by local dependence statistics. Biased items will be detected through DIF assessments, including effect-size estimates to discriminate between statistically significant but meaningless DIF.

10.5.3.2. Internal Consistency

Internal consistency will be estimated at baseline via Cronbach's α and McDonald's ω, the latter being the unbiased model-based analog of α. Whereas α assumes item exchangeability, ω does not.

10.5.3.3. Concurrent Validity

Concurrent validity will be estimated based on Pearson correlations for scores between the QOL-B respiratory domain and PROMIS F-SF 7a and the following concurrent validators: EXACT and EXACT RS, SGRQ, and FACIT-Fatigue.

10.5.3.4. Known-Groups Validation

Known-groups validity will be estimated at baseline only. Known-groups validators will consist of the PGI-S (both respiratory and fatigue for the QOL-B respiratory and PROMIS F-SF7a , respectively) and a median split on reported ppFEV$_1$. Known-groups validity will be estimated by fitting a linear model to the PRO score(s) at baseline and contrasting marginal means LSMEANS across known-group validator levels. Known-groups contrasts will consist of comparing the low-severity PGI-S group (reference) against all other PGI-S groups (effect) and the ppFEV$_1$ < median group (reference) against the ppFEV$_1$ $\geq$ median group (effect) in pairwise contrasts.

10.5.4. Longitudinal Validation

Longitudinal validation will consist of both test-retest reliability (conducted pre-exposure between screening and baseline) and within-subject meaningful change (conducted between baseline and EOS (Month 7).

10.5.4.1. Test-retest Reliability

Test-retest reliability correlations will be based on the 2-way random ICC (Shrout and Fleiss, 1979). Test-retest reliability estimates of 0.7 and above will indicate satisfactory retest reliability.

Test-retest reliability will be assessed in a group of subjects reporting no change over the retest interval. In this case, a 30-day retest interval occurring between screening and baseline will be used for TRTR. A symptomatically stable retest sample will be defined among subjects

experiencing no change on the PGI-S between Baseline and follow-up (PGI-S change score is zero).

10.5.4.2. Within-Subject Meaningful Change

Meaningful within-subject change will be estimated via change scores computed between Baseline and EOS (Month 7). Meaningful within-subject change will be characterized via point estimates obtained from the mean and median change score associated with the minimal improvement anchor group.

The point estimates will be examined by generating eCDFs and ePDFs stratified on PGI-S change and culture conversion anchor groups. The separation of eCDFs and ePDFs at the meaningful change point estimates will be contrasted to assess separation between the minimal improvement and no-change anchor groups.

10.5.5. Item-level Validation

Item-level analogs of the analyses outlined above will also be conducted to provide a comprehensive set of validation evidence for FDA review.

10.6. Other Efficacy Analyses

10.6.1. Secondary Efficacy Endpoint Analyses

Summaries of efficacy data will be presented by treatment group and by visit, as appropriate.

Reporting of categorial secondary endpoints will include basic statistics, estimates derived via logistic regression and will include estimates of proportions, and difference of proportions, together with corresponding 95% CIs resulting, as appropriate.

Reporting of continuous secondary endpoints will include basic statistics, estimates derived from ANCOVA model with change from baseline as response variable and treatment and baseline as independent variables, and will include estimates with corresponding 95% CIs as appropriate.

ANCOVA and logistic regression models may not include adjustment for randomization strata due to expected small counts within each combination of strata.

Time to culture conversion will be plotted via Kaplan-Meier method.

10.6.2. Exploratory Efficacy Analyses

All of the summaries pertaining to exploratory endpoints will utilize available data, unless otherwise stated in the SAP. Summaries will be presented by treatment group and by visit, as appropriate.

Difference between treatment groups in change from baseline to Month 7 in the QOL-B non-respiratory domains (physical, role, vitality, emotional, social, health perception) will be summarized. The data will be reported by timepoint and as change from baseline. Summary statistics estimates for means and difference between treatments together with corresponding 95% CIs will be provided.

Difference in proportion of subjects meeting that within-subject meeting meaningful change from baseline criterion from Baseline to EOT and Month 7 in PRO (as assessed QOL-B and PROMIS F-SF 7a) will be evaluated.

Note: The threshold for the criterion will be estimated via quantitative longitudinal analyses and will be available at the time of the analysis (post-unblinding).

Actigraphy data will be collected continuously throughout the study. For the purpose of analysis, mean activity and sleep efficiency will be derived at the protocol specified visits. The data will be reported by timepoint over time. Summary statistics estimates for means and difference between treatments together with corresponding 95% CIs will be provided.

Correlation between PRO respiratory domain mean responses and colony microbiology data will be explored.

Exploratory analyses may be conducted in selected subgroups of the population or pertaining to an exploratory question of interest defined in the SAP.

10.7. Safety Analyses

Safety summaries will utilize safety analysis data set and will be provided by treatment group on available data. Descriptive statistics will be provided.

10.7.1. Extent of Exposure

The exposure data will be summarized descriptively by treatment group (separately for each medication, total as randomized) and will include total number of doses of each medication received (ALIS/ELC, AZI, ETH) and duration of exposure. The amount of medication taken and used for these summaries will be based on the pill count of returned medication.

10.7.2. Concomitant Medications and Prior Therapy

Concomitant medications are those medications taken on or after the first dose of study drugs. Prior medications are those medications taken before the first dose of study drugs. A medication that starts prior to first dose but continues after the first dose of study drugs is classified both in prior and concomitant medications. Any medications taken for MAC lung infection or COVID-19 prior to Screening will be recorded. Non-study drugs taken 1 month prior to Screening and during the study will be recorded. Prior and concomitant medications will be summarized separately and will be mapped to an ATC class and Preferred Name using the WHO Drug Dictionary. These summaries will present the number and percentage of subjects using each medication. Subjects may have more than one medication per ATC class and Preferred Name. At each level of subject summarization, a subject is counted once if he/she reported one or more medications at that level. Each summary will be ordered by descending order of incidence of ATC class and preferred name within each ATC class.

Prohibited medication will be reported as part of concomitant summaries and as protocol deviation summaries if appropriate.

Data will be summarized descriptively by treatment over study duration.

10.7.3. Adverse Event Analyses

Adverse events will be coded using MedDRA by system organ class and preferred term. The severity of AEs will be graded to either mild, moderate, or severe.

The number and percent of subjects with AEs will be presented by treatment group. The severity of AEs, the relationship to study drugs (ALIS/ELC, AZI, ETH), AEs leading to study discontinuation, TEAEs, AESIs, and SAEs will be similarly presented. Treatment-emergent and treatment-related AEs will be tabulated by system organ class and preferred term.

10.7.4. Clinical Laboratory Evaluation Analyses

The results of hematology, blood chemistry, and urinalysis will be tabulated by treatment group. Laboratory values outside the normal ranges will be flagged. Subjects experiencing ALT or AST ≥ 3* ULN and total bilirubin > 2* ULN according to Hy's Law will be listed.

Summary statistics (mean, median, range, change from Baseline) by treatment will be provided for each timepoint, as appropriate.

Baseline value is defined by laboratory results collected prior to first study drugs administration.

10.7.5. Vital Sign Analyses

Systolic and diastolic BP, pulse rate, RR, oxygen saturation, and body temperature will be listed for each subject. If applicable, summary statistics (mean change, percentage change from Baseline, and absolute change from Baseline) by treatment at each time point will be provided. Baseline value is defined as vital signs collected prior to the first dose of study drugs on Day 1/Baseline.

10.7.6. Physical Examination Analyses

Postbaseline abnormal findings of the physical examinations that meet the criteria of AEs will be included within AE reporting.

10.7.7. Audiology Analysis

Audiology test results and the change from Baseline will be summarized.

10.7.8. Other Safety Analyses

C-reactive protein value will be summarized descriptively by visit including changes from baseline.

Additional safety analyses may be specified in the SAP as appropriate.

10.7.9. Planned Interim Analysis

No interim analysis is planned for this study.

11. DATA INTEGRITY AND QUALITY ASSURANCE

The Investigator/investigational site will permit study-related monitoring, audits, IRB/EC 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.

11.1. Source Documents

Study data will be collected on source documents. The Investigator is responsible for assuring that collected data are complete and accurate. Source documentation (the point of initial recording of a piece of data) should support data collected on the eCRF. 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.

11.2. Data Collection

All data obtained for this study will be entered into a local regulation (ie, 21 CFR Part 11 in the USA)-compliant Data Management System provided by the Sponsor or its designee. These data will be recorded with an EDC system using eCRFs. The Investigator will ensure the accuracy and completeness of the data reported to the Sponsor. 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 the Sponsor to verify the proper transcription of data. Data reported in the eCRFs should be consistent with and substantiated by the subject's medical record and original source documents. The eCRF data will be monitored by the Sponsor or designee. The final, completed eCRF Casebook for each subject must be electronically signed and dated by the PI within the EDC system to signify that the Investigator has reviewed the eCRF and certifies it to be complete and accurate.

The Sponsor will retain the final eCRF data and audit trail. A copy of all completed eCRFs will be provided to the Investigator.

11.3. Study Records Retention

Study documents should 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 should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

12. FINANCING AND INSURANCE

12.1. Finances

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

12.2. Reimbursement, Indemnity, and Insurance

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

13. STUDY ADMINISTRATIVE INFORMATION

13.1. Financial Disclosure by the Investigator

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

13.2. Study Registration and Results Disclosure

The Sponsor will provide study information for inclusion in national registries according to local regulatory requirements.

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

13.3. 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 the Sponsor (or designee) and the Investigator. An Investigator Site File prepared by the Sponsor (or designee), 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 Investigators at each site will be included in the Investigator Site File. The respective files will be kept and updated by the Sponsor (or designee) and the Investigator, as applicable.

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

The study may be audited by qualified designees from the Sponsor or a competent regulatory authority (Section 13.11).

13.4. Use of Stored Samples and Data

Mycobacterial isolates will be stored for a period of up to 2 years after the completion (termination) of the study, or longer if required by the institution participating in the study and used for future selective susceptibility testing for correlation with microbiologic and clinical response and for molecular typing of serial isolates to look for multiple strains within individuals that might influence treatment outcome. Stored samples will be labeled with study and subject information and kept in a locked room with limited access. Electronic data will be kept in password-protected computers at the laboratory and then transferred to the Sponsor or CRO, as applicable, for data analysis. Samples and corresponding data will be tracked using the laboratory's specimen tracking system.

Prior Sponsor and IRB/EC approval are required before using or sharing study samples or data in ways not specified in the study protocol.

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 the Sponsor and the IRB/EC.

At any time, subjects may inform the Investigator 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 subjects and the IRB/EC.

13.5. Disposition of Stored Samples and Data

Access to stored samples will be limited by using a locked room. Samples stored by the central laboratories will be labeled with the subject'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, other Investigators may wish to study these samples and/or data. In that case, IRB/EC approval and Sponsor approval must be obtained before any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior Sponsor and IRB/EC approval.

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 the Sponsor and the IRB/EC.

Additionally, subjects may decide at any point not to have their samples stored for a period of up to 2 years beyond the duration of the study. In this case, the PI will request the destruction of all known remaining samples and report what was done to both the subject and to the IRB/EC. This decision will not affect the subject's participation in this protocol.

13.6. Initiation of Study

Before the start of the study at each study site, the Sponsor's study monitor (or designee) will ensure adequacy of the facilities and to discuss responsibilities regarding study protocol adherence with the Investigator and other personnel involved in the study.

The Investigator may not enroll any subject into the study before the Sponsor has received written approval or a favorable opinion from the EC or IRB for conducting the study and a formal meeting has been conducted by the Sponsor's study monitor (or designee) to initiate the study. This meeting will include a detailed review of the study plan and completion of the eCRF.

13.7. Subject 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).

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

If required by local law, subjects 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).

13.8. Subject Identification and Confidentiality

Subject names will not be supplied to the Sponsor. A subject number will be recorded in the eCRF, and if the subject name appears on any other document (eg, laboratory report), it must be obliterated on the copy of the document to be supplied to the Sponsor. All records will be kept confidential to the extent provided by federal, state, and local laws. Subjects will be informed that representatives of the Sponsor, IRB/EC, 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 subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

13.9. Study Monitoring

Before study initiation, at a site initiation visit or at an Investigator's meeting, a Sponsor representative will review the protocol, eCRF, IB, and any study related materials with the Investigators and their staff. During the study, the Study Monitor or a designee will visit the site regularly to check the completeness of subject records, the accuracy of entries on the eCRFs, adherence to the protocol, adherence to ICH GCP and applicable regulatory requirements, the progress of enrollment, and also to ensure that study drugs are being stored, dispensed, and accounted for according to specifications.

The Investigator must give the Study Monitor access to relevant hospital or clinical records to confirm their consistency with the eCRF entries. No information in these records about the identity of the subjects will leave the study center. The monitoring standards of the study 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 eCRFs are performed according to the study specific monitoring plan.

13.10. Protocol Amendments

Any substantial change or addition to this protocol requires a written protocol amendment that must be approved by the Sponsor, before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation, or the scientific quality of the study, require additional approval by the applicable regulatory authority(ies) and IRBs/ECs. Copies of the applicable written approvals must be given to the site monitor or their designee.

The requirements for approval should in no way prevent any immediate action from being taken by the Investigator or by the Sponsor in the interests of preserving the safety of all subjects 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, the Sponsor or its agent should be notified and the applicable regulatory authority(ies)/IRBs/ECs should be informed within 10 working days. 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/EC approval, but the regulatory authority(ies)/IRBs/ECs must be kept informed of such administrative changes in accordance with country-specific requirements.

13.11. Audits and Inspections

Domestic and foreign regulatory authorities, the IRB/EC, and an auditor authorized by the Sponsor may request access to all source documents, eCRFs, 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 subject names are obliterated on the copies to ensure confidentiality.

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

13.12. 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 the Sponsor. Authorship will be determined by mutual agreement. For multicenter studies, it is mandatory that the first publication be based on data from all centers, analyzed as stipulated in the protocol by the Sponsor and statisticians, and not by the Investigators themselves. Investigators participating in multicenter 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 by all other Investigators and the Sponsor.

The Sponsor 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). The Sponsor 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 Sponsor personnel.

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

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APPENDIX 1. QOL-B QUALITY OF LIFE QUESTIONNAIRE

APPENDIX 2. PROMIS FATIGUE SHORT FORM 7A

APPENDIX 3. PGI-S INSTRUMENT

APPENDIX 4. EXACT & EXACT-RS INSTRUMENTS

APPENDIX 5. ST. GEORGE'S RESPIRATORY QUESTIONNAIRE

APPENDIX 6. FACIT FATIGUE SCALE