CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207356Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Microbiology Review
Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	207356
Priority or Standard	Priority
Submit Date(s)	March 28, 2018
Received Date(s)	March 28, 2018
PDUFA Goal Date	September 28, 2018
Division/Office	Division of Anti-Infective Products/Office of Antimicrobial Products
Review Completion Date	September 7, 2018
Established Name	Amikacin liposome inhalation suspension (ALIS)
Proposed Trade Name	ARIKAYCE
Pharmacologic Class	aminoglycoside antibacterial
Code names	Arikace, Liposomal Amikacin for Inhalation (LAI)
Applicant	Insmed Inc.
Formulation	Inhalation suspension
Dosing Regimen	590 mg once daily
Applicant Proposed Indication(s)/Population(s)	Treatment of adult patients with *Mycobacterium avium* complex (MAC) lung disease as part of a combination antibacterial regimen
Regulatory Action	Approval
Indication(s)/Population(s)	LIMITED POPULATION: ARIKAYCE is an aminoglycoside antibacterial indicated in adults who have limited or no alternative treatment options, for the treatment of *Mycobacterium avium* complex (MAC) lung disease as part of a combination antibacterial drug regimen in patients who do not achieve negative sputum cultures after a minimum of 6 consecutive months of a multidrug background regimen therapy. As only limited clinical safety and effectiveness data for ARIKAYCE are currently available, reserve ARIKAYCE for use in adults who have limited or no alternative treatment options. This drug is indicated for use in a limited and specific population of patients. This indication is approved under accelerated approval based on achieving sputum culture conversion (defined as 3 consecutive negative monthly sputum cultures) by Month 6. Clinical benefit has not yet been established. Limitation of Use: ARIKAYCE has only been studied in patients with refractory MAC lung disease defined as patients who did not achieve negative sputum cultures after a minimum of 6 consecutive months of a multidrug background regimen therapy. The use of ARIKAYCE is not recommended for patients with non-refractory MAC lung disease.

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OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DEPI= Division of Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
DPV=Division of Pharmacovigilance
DRISK=Division of Risk Management
CDRH=Center for Devices and Radiological Health
DPMH-Division of Pediatrics and Maternal Health
DMPP= Division of Medical Policy Programs
DCRP=Division of Cardiovascular and Renal Products
DPARP=Division of Pulmonary, Allergy, and Rheumatology Products

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
ADR	adverse drug reaction
AE	adverse event
AEI	adverse event of interest
ALIS	amikacin liposome inhalation suspension
BLQ	below the limit of quantitation
BR	Multi-Drug Background Regimen
CDC	Center for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
DAIP	Division of Anti-infective Products
DMC	data monitoring committee
DPCC	dipalmitoylphosphatidylcholine
ECG	electrocardiogram
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GLP	good laboratory practice
HD	high dose
ICH	International Conference on Harmonization
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent-to-treat
LD	low dose
MD	mid dose
MedDRA	Medical Dictionary for Regulatory Activities
micro-ITT	microbiological intent-to-treat
mITT	modified intent to treat
NA	not applicable
NDA	new drug application
NME	new molecular entity

OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PD	pharmacodynamics
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
REMS	risk evaluation and mitigation strategy
RLD	reference listed drug
SAE	serious adverse event
SAP	statistical analysis plan
SQS	semi-quantitative scale
TEAE	treatment emergent adverse event
TEADR	treatment emergent adverse reaction
TK	toxicokinetics
USPI	US prescribing information

1 Executive Summary Office Level Concurrence

In NDA 207356, the Applicant has provided substantial evidence of effectiveness of ALIS on a surrogate microbiological endpoint of sputum culture conversion in an adequate and well-controlled trial in adult patients with refractory MAC lung disease to support approval under 21 CFR, part 314, subpart H. Sputum culture conversion was defined as 3 consecutive monthly negative sputum cultures by Month 6 of treatment with ALIS plus a background antimycobacterial regimen. Supportive evidence was provided by a Phase 2 trial in patients with refractory NTM lung disease. As the benefit-risk considerations of ALIS only support its use in a limited population of patients with refractory MAC lung disease, the NDA is being approved under the Limited Population Pathway for Antibacterial and Antifungal Drugs (the LPAD pathway).

1.1 Product Introduction

Amikacin liposomal inhalation suspension, ARIKAYCE®, is an orally inhaled product. The active ingredient, amikacin sulfate, belongs to the aminoglycoside class of antibacterial drugs. The recommended dose is one vial (590mg amikacin) administered once daily via oral inhalation using the Lamira™ nebulizer.

1.2 Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness on a surrogate endpoint of sputum culture conversion (a microbiological endpoint) in a Phase 3 trial (INS-212) in adult patients with refractory MAC lung disease (see section 1.3). Supportive evidence is provided by a Phase 2 trial in patients with refractory NTM lung disease, including patients with MAC infection. In the Phase 3 trial, while an improvement in the microbiological endpoint of sputum culture conversion was seen, there was a lack of statistically and/or clinically significant improvement in the clinical endpoints measured (see section 1.3). There was also a higher incidence of respiratory adverse events and more hospitalizations in ALIS-treated patients in the Phase 2 and 3 trials.

However, as pulmonary MAC disease is a serious condition and there is an unmet medical need, the overall benefit-risk assessment was considered acceptable for the limited population of patients with refractory MAC lung disease. As the NDA is being approved under the LPAD pathway, labeling will include appropriate information to reflect the approval in a limited population.

The Applicant will conduct a confirmatory trial that is designed to assess clinical outcomes in patients with NTM lung disease caused by MAC as a requirement of this accelerated approval; the trial will also assess the microbiological endpoint of durability of sputum culture conversion.

1.3 Benefit-Risk Assessment

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Benefit-Risk Summary and Assessment

In NDA 207356, the Applicant is seeking approval of amikacin liposomal inhalation suspension (ALIS) for the treatment of adults with refractory MAC lung disease, under Subpart H (accelerated approval) and the LPAD pathway.

The Applicant has provided substantial evidence of effectiveness on a surrogate endpoint of sputum culture conversion (a microbiological endpoint) in a Phase 3 trial (INS-212) in adult patients with refractory MAC lung disease. Sputum culture conversion was defined as 3 consecutive monthly negative sputum cultures by Month 6 of treatment with ALIS plus a background multi-drug antimycobacterial regimen. The change in sputum culture conversion with ALIS therapy was statistically significant; however, there is uncertainty regarding the relationship of sputum culture conversion with clinical benefit. Persistently positive sputum cultures for NTM is one factor that is used in clinical practice to initiate treatment of NTM. Also, in clinical practice, obtaining negative cultures is considered a marker for successful therapy of NTM infections. There are limited data from retrospective, non-randomized studies or exploratory analyses from non-randomized subgroups to suggest that patients who achieve sputum culture conversion have better outcomes. The design of these studies or analyses does not allow for conclusions to be drawn as to whether treating NTM to achieve a negative culture is causally associated with the observed better outcome or alternatively, that patients who achieve a negative sputum culture with treatment are inherently less ill and the negative sputum culture is a marker for a patient population that will have better outcomes for reasons unrelated to treatment of their NTM (or some combination of these two possible explanations).

In the Phase 2 trial in patients with refractory NTM lung disease, including patients with MAC infection, there was a finding suggestive of a benefit on sputum culture conversion and of clinical benefit based on 6-Minute-Walk Test (6MWT) distance in patients who received ALIS. Given that the trial did not achieve its primary endpoint, additional analyses are considered exploratory. In contrast to the suggestive evidence from the Phase 2 trial, that ALIS was affecting sputum culture conversion and the suggestion of a possible clinical benefit based on 6MWT, in the Phase 3 trial, while there was a statistically significant benefit in the surrogate microbiological endpoint of sputum culture conversion, there was a lack of statistically and/or clinically significant improvement in the clinical endpoints (secondary/exploratory endpoints) measured. There was no improvement in the key secondary clinical outcome of 6MWT distance. In addition, there was no statistically and/or clinically significant improvement in the two patient-reported outcome measures, the Saint Georges Respiratory Questionnaire (SGRQ) and the EuroQol-5 Dimensional-3 Level (EQ-5D-3L). This lack of evidence of clinical benefit from the Phase 3 trial does not help to address the uncertainty regarding the link between clinical benefit and sputum culture conversion. In addition, there was also a higher incidence of respiratory adverse events and more hospitalizations in ALIS-treated patients in the Phase 2 and 3 trials.

However, as pulmonary MAC disease is a serious condition and there is an unmet medical need, the degree of uncertainty regarding the

clearance of sputum culture conversion as a predictor of clinical benefit was considered to be acceptable and the overall benefit-risk assessment was considered acceptable for an accelerated approval for the limited population of patients with refractory MAC lung disease. In this instance, it is imperative that an adequate and well-controlled clinical trial be performed to assess the relationship between treatment with ALIS and whether it in fact provides clinical benefit to patients and the degree of clinical benefit. As the clinical trials have established that use of ALIS is associated with adverse effects, further study to assess clinical benefit will be essential for making more informed benefit/risk assessments regarding the use of ALIS in patients with refractory MAC lung disease. The NDA is being approved under the LPAD pathway reflecting that the benefit-risk for this therapy based on the available evidence and its level of uncertainty is only appropriate for patients with refractory MAC lung disease and not the broader population of patients with NTM lung disease or other patient populations with pulmonary infections or colonization, consistent with section 506(h)(2) of the FD&C Act. The product labeling for ALIS will include appropriate information to reflect the approval in a limited population and also provide information on the observed adverse effects of ALIS therapy.

Safety analyses of the Phase 2 and 3 randomized trials showed a higher incidence of respiratory adverse events (AEs), including hypersensitivity pneumonitis, in ALIS-treated patients compared to those treated with a background regimen alone in the Phase 3 trial or a background regimen plus placebo in the Phase 2 trial. There was also a higher incidence of hospitalizations in the Phase 3 trial among ALIS-treated patients.

As ALIS is being approved under Subpart H, the Applicant will need to conduct an adequate and well-controlled confirmatory trial that is designed to assess clinical outcomes in patients treated with ALIS; the study will address the question of whether the addition of ALIS to a background regimen for MAC lung disease provides clinical benefit to patients with MAC lung disease. The confirmatory trial will also examine the durability of sputum culture conversion. In addition, risks of increased respiratory adverse events and hospitalizations in ALIS-treated patients will be communicated to patients and their healthcare providers who intend to use ALIS.

Using the LPAD pathway, ALIS will be indicated for use in a limited and specific population, namely patients with limited or no alternative treatment options, for the treatment of refractory MAC lung disease as part of a combination antibacterial drug regimen for adult patients. Use of LPAD is necessary to support a positive benefit-risk balance. Additional risk mitigation measures include a Boxed Warning of increased respiratory adverse reactions, including hypersensitivity pneumonitis, hemoptysis, bronchospasm, exacerbation of underlying pulmonary disease that have led to hospitalizations in some cases, Warnings regarding the respiratory AEs, a Limitation of Use to specify the intended population, and a Medication Guide. In addition, the Applicant has agreed to a Postmarketing Commitment (PMC) to provide their communication plan for outlining the risks associated with ARIKAYCE and the fact that clinical benefit has not yet been verified, as well as a PMC to assess drug utilization. The Applicant has also communicated to the Agency that ARIKAYCE will only be available through (b) (4) designated specialty pharmacies. Additional risk mitigation strategies can be considered if safety signals emerge postmarketing.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Lung disease associated with NTM is characterized by progressive, irreversible lung damage and increased mortality. The disease is more prevalent after 60 years of age where the prevalence is estimated at 26.7 per 100,000 persons. • Pulmonary MAC infections are thought to be acquired from the environment (e.g., water, soil) and not transmitted from person to person. • Diagnosis of pulmonary MAC infection requires the presence of respiratory or constitutional symptoms, nodules, bronchiectasis, or cavities on radiological studies, and positive cultures from two sputum samples or one bronchoalveolar lavage. • There is no standard definition for refractory MAC lung disease; failure to convert sputum to culture negative after at least 6 months of treatment with a multidrug antibacterial regimen has been used to define refractory disease.	Patients with refractory pulmonary NTM lung disease may experience a progressive debilitating lung disease with significant morbidity and increased mortality.
Current Treatment Options	• There is no FDA-approved therapy for treatment of MAC lung disease. Guideline-recommended "first line" treatment typically includes intermittent or daily antibacterial combination therapy with a macrolide, a rifamycin and ethambutol; treatment is continued for 12 months after sputum culture conversion to negative. • Treatment of refractory pulmonary MAC disease presents an additional challenge and typically includes switching from intermittent to daily therapy, parenteral administration of amikacin or streptomycin, use of clofazimine, and/or surgical intervention (lung resection).	There is an unmet need for treatment of MAC lung disease. Treatment of refractory pulmonary MAC disease is challenging. ARIKAYCE may provide an additional treatment option for these patients.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• In the Phase 3 Study 212, patients with refractory MAC lung disease treated with ALIS plus a multi-drug background antimycobacterial regimen, as compared to patients treated with a background regimen alone, had a statistically significant improvement in the surrogate endpoint of sputum culture conversion (a microbiological endpoint), defined as 3 consecutively negative monthly sputum cultures by Month 6: ALIS plus background regimen (65/224, 29.0%) compared to background regimen alone (10/112, 8.9%), ($p<0.0001$). • There is a lack of data from adequate and well-controlled trials in the literature that demonstrates sputum culture conversion to correlate with a clinically meaningful benefit to patients. Data from a few observational studies suggest that sputum culture conversion may be associated with a clinical benefit. However, in most of these studies it was difficult to discern if the sputum culture conversion was really a marker identifying a group of patients predisposed to better outcomes such as the type of NTM lung disease, ability to tolerate background regimen, good surgical candidates, etc. • Of note, in this trial there was no improvement in the key clinical secondary endpoint of 6MWT distance in ALIS-treated patients compared to the background regimen only arm. In addition, no statistically and/or clinically significant improvement was demonstrated in two patient-reported outcomes, the Saint George Respiratory Questionnaire (SGRQ) and the EuroQol-5Dimensional-3 Level (EQ-5D-3L) • In Study 112, the primary endpoint was change from baseline in mycobacterial density as assessed by the SQS for mycobacterial culture at Day 84. There was no statistically significant difference	Addition of ARIKAYCE to background regimen resulted in statistically significant improvement in sputum culture conversion by Month 6, a surrogate microbiologic endpoint. There is a lack of data from adequate and well-controlled studies to link the microbiological endpoint of sputum culture conversion to meaningful clinical benefit. In the Phase 3 trial, no statistically and/or clinically significant benefit was demonstrated on any of the clinical endpoints with the addition of ARIKAYCE to the background regimen. It is essential that an adequate and well-controlled trial be conducted for ARIKAYCE as part of its accelerated approval based on a microbiological surrogate endpoint to evaluate whether ARIKAYCE can provide clinical benefit to patients. The reasons for the discordant results between the two trials on the 6MWT distance is not clear. It is unclear why in Study 112, the 6MWT distance showed an improvement in ARIKAYCE plus background regimen-treated patients compared to those on placebo plus a background regimen. This result was not replicated in Study 212 where 6MWT distance

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	between treatment groups (p=0.072). As a secondary endpoint, at Day 84, a greater proportion of subjects in the ALIS group (31.8%) achieved a negative culture than subjects in the placebo group (8.9%). Change in 6MWT distance from baseline to Day 84 was assessed as a tertiary endpoint and subjects in the ALIS group had a mean increase from baseline of 20.6 meters compared to a mean decrease of 25.0 meters in the placebo group. • Study INS-312 was primarily designed to evaluate long-term safety of ALIS. There was no primary efficacy endpoint. • In addition, there is a lack of good quality evidence to demonstrate the role of antimicrobial treatment in providing clinical benefit to patients with pulmonary NTM lung disease. Given that patients are receiving protracted courses of antibacterial drugs that can in some cases cause serious adverse effects, quality evidence to better assess the role of antibacterial drug therapy could help to better understand the risks and benefits of antibacterial drug therapies for pulmonary NTM lung disease.	was assessed at 6 months. In Study 112, it is possible that the difference in 6MWT distance between the treatment arms could be a chance finding from a secondary/tertiary endpoint after a failed primary endpoint.
Risk	• In the Phase 2 and 3 trials, there was significantly higher study drug discontinuation in patients on ARIKAYCE compared to the respective control arms. The main reason for subject discontinuations was adverse events. • Treatment emergent adverse events, particularly respiratory adverse events such as hypersensitivity pneumonitis, bronchospasm, exacerbation of underlying lung disease and hemoptysis were higher in ALIS-treated patients compared to patients treated with background regimen alone. • A higher number of patients treated with ALIS in the randomized Phase 2 and Phase 3 trials experienced serious adverse events. • In the Phase 3 trial, hospitalizations were higher in the ALIS plus	In the Phase 2 and 3 trials, ALIS-treated patients had a higher rate of drug discontinuation due to AEs and SAEs. There was also an increased number of hospitalizations in the Phase 3 trial in ALIS-treated patients compared to those treated with the background regimen alone. Respiratory adverse events accounted for most of the treatment emergent adverse events and were seen in both the Phase 2 and 3 trials.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	background regimen vs. background regimen alone. The most common SAEs and reasons for hospitalization in the ALIS plus background regimen arm were related to exacerbation of underlying pulmonary disease and lower respiratory tract infections, such as pneumonia.	
Risk Management	• The increased risk of hypersensitivity pneumonitis, bronchospasm, exacerbation of underlying lung disease, hemoptysis, and ototoxicity will be described in labeling. In addition to information in the Boxed Warning, these adverse reactions are described in the Warnings and Precautions section and adverse reactions sections and in the Medication Guide.	The adverse reactions seen with ARIKAYCE will be communicated in labeling. In addition, labeling includes a Medication Guide that is required to be provided to the patient when ARIKAYCE is dispensed. Additionally, as the NDA is being approved under the LPAD pathway, labeling includes information regarding the limited population. The Applicant has submitted their plans to communicate to inform healthcare providers, such as, Pulmonary and Infectious Diseases specialists and associated professional societies of the risks and limited clinical benefit seen with ARIKAYCE. They also plan to collect use data on patients prescribed ARIKAYCE, and will distribute ARIKAYCE through (b) (4) specialty pharmacies in the U.S. The Applicant has agreed to provide this information as Postmarketing Commitments (PMCs).

1.4 Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

X	The patient experience data that was submitted as part of the application include:			Section where discussed, if applicable
	X	Clinical outcome assessment (COA) data, such as		Section 7
		X	Patient reported outcome (PRO)	
		□	Observer reported outcome (ObsRO)	
		□	Clinician reported outcome (ClinRO)	
		□	Performance outcome (PerfO)	
	□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	X	Patient-focused drug development or other stakeholder meeting summary reports		Immediately following this table
	□	Observational survey studies designed to capture patient experience data		

Two patient-reported outcomes, the Saint George Respiratory Questionnaire (SGRQ) and the EuroQol 5-dimensional (EQ-5D-3L) were used in the Phase 3 trial, Trial 212.

On October 15, 2015, FDA held a Patient-Focused Drug Development Initiative workshop on NTM lung infections to gain insight into the experiences of NTM patients, caregivers, and other patient representatives regarding significant symptoms, impact on daily life, and currently available therapies. The afternoon included a scientific workshop to explore challenges related to drug development for NTM. A link to the report follows: https://www.fda.gov/downloads/ForIndustry/UserFees/PrescriptionDrugUserFee/UCM496941.pdf.

2 Therapeutic Context

2.1 Analysis of Condition

Lung disease associated with nontuberculous mycobacteria (NTM) is characterized by progressive, irreversible lung damage and increased mortality. The disease is more prevalent after age 60 where the prevalence is estimated at 26.7 per 100,000 persons (Prevots et al, 2010). About 80% of pulmonary NTM disease is caused by MAC.

MAC organisms are ubiquitous in the environment with *M. avium* readily isolated from water and *M. intracellulare* in soil. Unlike *M. tuberculosis*, MAC infections are not transmitted person-to-person. In HIV-negative patients, inhalation of environmental MAC-containing aerosols is

believed to be the primary source of transmission, and pulmonary infection is the main manifestation of MAC disease. Though MAC is ubiquitous in the environment, pulmonary MAC disease is not as common. The host factors that predispose to sustained pulmonary MAC infection are not fully elucidated. Some known predisposing factors for pulmonary MAC disease include underlying lung disease (CF, COPD, bronchiectasis, prior TB, restrictive lung disease) and older age. However, older women with pulmonary MAC disease may not have a prior predisposing factor. These women have distinctive features and are often tall and lean with scoliosis and may have pectus excavatum and mitral valve prolapse. This morphotype has been dubbed "Lady Windermere" syndrome. Many of these women are noted to have bronchiectasis; whether bronchiectasis is the predisposing factor, or the result of the infection is not clearly understood. In addition to underlying lung pathology, older age is also associated with a higher incidence of pulmonary MAC disease. Patients with a deficiency in interferon gamma (IFN-γ) production may be predisposed to MAC disease (Daley, 2017).

Over the past 2-3 decades, an increase in pulmonary MAC disease has been reported in the literature. It is not clear whether this increase represents a higher incidence of pulmonary MAC disease or increased awareness and detection.

The diagnosis is generally based on the presence of respiratory or constitutional symptoms, nodules, bronchiectasis, or cavities on radiological studies, and positive cultures from two sputum samples or one bronchoalveolar lavage (Griffith et al, 2007). There are two main radiologic manifestations of pulmonary MAC disease. In older men, MAC mainly presents as cavitary lesions and fibronodular infiltrates with an upper lobe predominance. In older women ("Lady Windermere" syndrome), it may present as nodular/bronchiectatic lesions in the right middle lobe and lingula (Daley, 2017).

2.2 Analysis of Current Treatment Options

There is no FDA-approved therapy for pulmonary MAC disease, highlighting the high unmet need for pulmonary MAC therapy. Treatment guidelines for MAC lung disease typically recommend a combination of a macrolide, a rifamycin, and ethambutol and is continued for 12 months after sputum cultures become negative (Griffith et al, 2007).

The reported rates of treatment success for MAC lung disease have ranged from 20 to 90% (Field et al, 2004). Much of this variability depends on whether treatment success was calculated based on intention-to-treat analyses and whether relapses were included as failures. If patients who discontinued treatment, required surgery, died, or had a relapse are included, the cure rate is approximately 40%.

There is no standard definition for MAC lung disease treatment failure, but failure to convert sputum to culture negative after at least 6 months of treatment has been used to define refractory disease (Griffith et al, 2012). Treatment options for patients who do not respond to first line therapy are limited and may include switching from intermittent to daily therapy,

parenteral administration of amikacin or streptomycin, use of clofazimine, or lung resection. The addition of inhaled amikacin to a multi-drug background regimen (BR) may represent another treatment option.

3 Regulatory Background

3.1 U.S. Regulatory Actions and Marketing History

ARIKAYCE (amikacin liposome inhalation suspension, ALIS) is not currently marketed in the US or outside the US.

3.2 Summary of Presubmission/Submission Regulatory Activity

There have been (b) (4) INDs supporting the development of ALIS. (b) (4) The studies submitted to this IND focused on studying ARIKAYCE for the treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis (CF) and non-CF bronchiectasis. Initial development was led by Transave, Inc. and the current Applicant, Insmed Inc., became the Sponsor on December 2, 2010.

(b) (4) IND, 108674, supporting the development of ARIKAYCE for NTM was submitted February 17, 2011. Of note, there was a rat carcinogenicity study that was reviewed as a special protocol assessment (SPA) in August 2008 submitted by the previous Sponsor. A clinical hold was placed on (b) (4) NTM programs on August 3, 2011, based on the finding of an increased incidence of squamous cell carcinoma observed in 2/60 female rats at the highest dose tested (45 mg/kg/day). The Agency's focus was to better understand the species-specific aspects of the rat findings, achieving a favorable risk-benefit profile in the target patient populations and the need to assure appropriate patient informed consent. The clinical hold was removed for the NTM program in January 2012 (b) (4) taking into consideration the additional toxicology data including the absence of pre-neoplastic findings in the chronic dog study.

(b) (4)

Orphan designation was granted March 25, 2013 for infections caused by NTM. The product was also granted fast track designation (June 28, 2013) and Qualified Infectious Disease Product (QIDP) (June 28, 2013) designation. Based on the findings of improved microbiologic outcomes in Study 112 and the unmet medical need for therapies for pulmonary MAC disease, breakthrough designation was granted on June 16, 2014.

Under IND 108674, Insmed has had several meetings with the Agency regarding the NTM development program. The findings from the Phase 2 Study TR02-112 were discussed in meetings held on July 3, 2014, September 30, 2014, and November 21, 2014. The positive

result for the 6-Minute Walk Test (6MWT) distance, a tertiary endpoint in the Phase 2 study, led to the incorporation of the 6MWT as a key secondary endpoint to assess clinical benefit in the Phase 3 Study INS-212.

Study INS-212 was conducted in patients with refractory MAC lung infections, defined as individuals who remained culture positive after 6 months of a multi-drug background regimen. The NDA was submitted under the accelerated approval pathway (21 CFR part 314, subpart H). The accelerated approval provisions of FDASIA in section 506(c) of the Federal Food, Drug, and Cosmetic (FD&C) Act provide that FDA may grant accelerated approval to: ". . . a product for a serious or life-threatening disease or condition . . . upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit." This application relies on a surrogate endpoint of sputum culture conversion defined as 3 consecutive negative monthly sputum cultures within 6 months of treatment. There are limited data that suggest a higher mortality in patients with NTM lung infections who remain culture positive despite treatment compared to those who are culture negative. Although there are uncertainties with sputum culture conversion predicting clinical benefit in this patient population, the degree of uncertainty in the surrogate endpoint of sputum culture conversion was considered acceptable in Study INS-212, given the unmet need in patients with refractory MAC lung disease and the seriousness of the disease. At the Antimicrobial Drugs Advisory Committee meeting held on August 7, 2018, the Agency presented a review of the literature assessing if the available information supports a relationship between sputum culture conversion and clinical outcomes. An addendum to the FDA briefing document summarizes review of the literature (https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/UCM615722.pdf). Further discussion regarding the advisory committee meeting is in Section 10 of this review.

In a meeting held on September 30, 2014, the Agency expressed that there was an expectation of a finding supportive of efficacy in a clinical outcome such as the 6MWT distance and recommended the 6MWT as a co-primary endpoint along with sputum culture conversion. The Applicant did not agree, but proposed 6MWT be a key secondary endpoint. The Agency also noted that the endpoint for the confirmatory trial should have a clinical component and expressed its willingness to review potential clinical endpoints including patient-reported outcomes (PR0s).

The importance of the clinical endpoint was further communicated to the Applicant in an electronic communication, dated May 14, 2015, as part of the Agency's feedback regarding the Study INS-212 protocol. The communication also outlined that 6MWT would be heavily relied upon to strengthen the microbiologic surrogate endpoint.

A non-CMC pre-NDA meeting was held on November 15, 2017. The Applicant mainly focused on the clinical and nonclinical data presentation, plan for a 4-month safety update for ongoing studies INS-212 and INS-312 after submission of the NDA.

The device component of this NDA, the Lamira® nebulizer handset, is based on an eFlow Nebulizer System provided by PARI Respiratory Equipment, Richmond, VA, a subsidiary of PARI Pharma GmbH, Germany. Human factors aspects of this device were discussed jointly with CDER and CDRH at a Type C meeting on June 23, 2017. The Applicant's Human Factor study was evaluated by the Agency during the NDA review.

Limited Population Pathway for Antibacterial and Antifungal Drugs (the LPAD pathway)

During the NDA review, the Agency identified ALIS as a candidate for approval under the LPAD pathway. As required in section 506(h)(1)(A) of the FD&C Act, the Agency determined ALIS is an antibacterial drug intended to treat a serious infection in a limited population of patients with unmet needs. Refer to the benefit-risk assessment table above for a discussion of the infection, the limited population for whom the drug is intended, and the unmet need for treatment. Additionally, the submitted evidence does not support a favorable benefit-risk profile in a population broader than the identified limited population; therefore, the LPAD pathway and its associated labeling requirements are a reasonable means to communicate that the safety and effectiveness of the drug have been demonstrated only with respect to the limited population.

FDA recommended to the Applicant on August 28, 2018 that ALIS was an appropriate candidate for the LPAD pathway. On September 13, 2018 the Applicant submitted a written request for approval under the LPAD pathway.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1 Office of Scientific Investigations (OSI)

The Office of Scientific Investigations (OSI) inspected two clinical sites (Drs. Griffith and Winthrop) for three protocols (INS-212, INS-312, and TR02-112) that were submitted to support this application. Both clinical sites enrolled a relatively higher number of subjects in all three studies. The study data derived from these clinical sites, based on the inspections, are considered reliable in support of the requested indication under this NDA.

According to the OSI clinical inspection summary, the clinical sites appeared to be in compliance with Good Clinical Practice. Data submitted by these clinical sites appear acceptable in support of this application. Of note, inspection of Dr. Winthrop's site did reveal minor protocol violations. However, these protocol violations appear unlikely to have significant impact on the primary efficacy endpoint and safety results of the study.

4.2 Product Quality

Novel excipients: Yes

The route of administration, inhalation, is new for cholesterol. It is acceptable to use cholesterol in this inhalation product at the proposed level as qualified by repeat dose animal inhalation toxicology studies described below in Section 5, Nonclinical Pharmacology and Toxicology.

Any impurity of concern: Yes

The (b) (4) degradation product, (b) (4) has been associated with a concentration-related increase in bronchospasm.

Initially, the drug product specification acceptance limit on the (b) (4) degradation product, (b) (4) was proposed at NMT (b) (6) %. This limit was not supported by available nonclinical data. Upon request, the Applicant agreed to revise this limit to NMT (b) (6) %. Accordingly, the proposed shelf life for the drug product was revised from (b) (4) months to 24 months for long term (before dispensing) refrigerated storage and the in-use period (after dispensing) from (b) weeks to 4 weeks for room temperature storage so the product under these conditions will meet the revised limit.

Sufficient controls to ensure safety and efficacy of the commercial product: Yes

The proposed product is a combination product consisting of a suspension of amikacin sulfate in a unit dose glass vial of 590 mg/8.4 mL amikacin and an eFlow Nebulizer System. The proposed drug product ARIKAYCE is a sterile, aqueous, liposome suspension for oral inhalation in a unit-dose glass vial containing a nominal dose of 590 mg of amikacin. The white, milky suspension consists of amikacin sulfate encapsulated in liposomes. The liposomes are composed of dipalmitoylphosphatidylcholine (DPPC) and cholesterol, (b) (4) The concentration of the active ingredient is expressed in terms of amikacin (b) (4) and is nominally 70 mg/mL.

Compendial excipients include Cholesterol (b) (4) Sodium Hydroxide, Sodium Chloride, Water for Injection. They will comply with the appropriate monographs and general USP/NF and Ph. Eur. requirements. The non-compendial excipient dipalmitoylphosphatidylcholine (DPPC) has been used in other approved products with a similar route of administration.

The Phase 2 and 3 formulation composition is the same as the commercial formulation. The Quality Control (QC) in vitro release method and in vitro release acceptance criteria shown in the **Table 1** below are found acceptable, on an interim basis, for the routine QC of the drug product.

Table 1: Quality Control (QC) in vitro release method and in vitro release acceptance criteria

Apparatus/Speed	Volume	Medium	Acceptance Criteria	
USP 2 (paddle) small vessel/150 rpm	Volume varies at each sampling time point [30 to 105.7 mL]	Triton/PBS buffer pH 7.2 at 37°C	**Time (hours)**	**% Release**
			0.5	NMT (b) (4) %
			1.5	(b) (4) %
			3.0	NLT (b) (4) %

The Applicant agreed to the following PMC as a Prior Approval Supplement to develop a new in vitro drug release method:

- Conduct further studies to identify an optimal QC in vitro drug release method in which only the drug released from the liposomes (free drug) is sampled and analyzed and propose new acceptance criteria for the drug product.
- Please see section 14 of this review for additional details.

The stability studies results support the following proposed storage conditions: Long-term storage of ALIS for 9 months when stored under refrigerated conditions of 2-8°C. The in-use period of the product is 4 weeks storage at (b) (4) 25°C ((b) (4) 77°F) (b) (4) . ALIS does not need to be protected from light. ALIS cannot be frozen and will be recommended as such in the label. The data presented across the manufacturing sites of Althea and Therapure suggested comparability of the drug product batches produced at these two manufacturing facilities.

This NDA is recommended for Approval from the Product Quality perspective. All Product Quality aspects including facilities are found acceptable.

Table 2: Drug Product Composition

Component	Quality Standard	Function	Amount per mL	Amount per dose (nominal 8.4 mL) [a]	Theoretical amount per vial (8.9 mL)
Amikacin Sulfate	USP, Ph. Eur.	API	70 mg [b]	590 mg [b]	623 mg [b]
DPPC	Non-compendial, In-house	(b) (4)			
Cholesterol (b) (4) (b) (4)	NF, Ph. Eur., JP	(b) (4)			
(b) (4)			NA	NA	NA
Sodium Hydroxide	NF, Ph. Eur., JP	To adjust pH	(b) (4)		
Sodium Chloride	USP, Ph. Eur., JP	(b) (4)			
Water for Injection	USP, Ph. Eur.	(b) (4)			
(b) (4) (b) (4) (b) (4)					

NA = Not applicable

4.3 Devices and Companion Diagnostic Issues

The CDRH reviewer, Dr. Deepika Lakhani, noted that the ALIS eFlow is a modified version of the Altera® K100380, a PARI Respiratory Equipment FDA-cleared electronic nebulizer system. The ALIS eFlow is also similar to two other FDA-cleared PARI Respiratory Equipment Nebulizer Systems: the Trio® K033833 (originally named the eFlow Nebulizer System) and the eRapid® K112859. The ALIS eFlow, Altera, Trio, and eRapid nebulizer systems all use the same micro-perforated vibrating membrane technology to aerosolize liquid medications. While the ALIS eFlow proposed in the NDA has similar operating principles and components, it has undergone modifications to the controller, nebulizer handset and aerosol head. These changes can potentially impact the performance, electrical safety/ EMC, biocompatibility and cleaning/disinfection of the device. The NDA included adequate data to support that the changes do not impact the safety and efficacy of the nebulizer device.

5 Nonclinical Pharmacology/Toxicology

5.1 Executive Summary

Amikacin products given by intravenous or intramuscular routes of administration are marketed in the U.S. for human use. The Applicant is relying on the Agency's findings of safety for amikacin, as well as published literature to support the current NDA, as permitted under section 505(b)(2) of the Food, Drug, and Cosmetic Act. They have conducted inhalation toxicity studies in rats, mice, and dogs, as well as in vitro and in vivo genotoxicity studies to support this new formulation of amikacin.

Clinical signs of amikacin toxicity were not observed in repeat dose animal studies, though tubular nephropathy was more prevalent in rats treated with ALIS compared to controls.

No proliferative changes or necrosis were observed in respiratory tissues of dogs that received ALIS at exposure levels providing inhaled amikacin doses of up to approximately 30 mg/kg/day (pulmonary deposited dose of 6 mg/kg/day, based on a 20% deposition factor for dogs) for 9 months. The amount of lipid (2:1, DPCC:Cholesterol) inhaled at this dose level was approximately 22 mg/kg/day (pulmonary deposited dose of 4.4 mg/kg/day). Foamy macrophages in lungs were observed in all ALIS-treated dogs with a dose-related increase in severity (inhaled amikacin doses of 5, 10, or 30 mg/kg/day). They were also observed in the lungs of dogs that received empty liposomes (amount matching the high ALIS dose) at a greater incidence than saline controls. Basophilic granules were seen in macrophages from the ALIS groups, but not the empty liposome group. These findings appeared related to clearance.

In a 26-week rat study, signs of minimal to moderate irritation were observed in the respiratory and olfactory epithelium (larynx, trachea, nasal turbinates) of ALIS-treated rats with dose-related severity. These findings are not uncommon in rats exposed to a variety of test substances via inhalation and are not considered specific to ALIS. Foamy macrophages in lungs were observed in all ALIS-treated rats with dose-related increases in severity. The high dose group showed signs of particle overload, including inflammation and lung changes secondary to inflammation. The high dose rats received approximately 90 mg/kg/day inhaled amikacin (pulmonary deposited dose of 9 mg/kg/day) and 60 mg/kg/day inhaled lipid (pulmonary deposited dose of 6 mg/kg/day). Lung changes were less severe at the mid dose, approximately 30 mg/kg/day inhaled amikacin and 20 mg/kg/day inhaled lipid. Rats are more sensitive than other mammalian species (including humans) to inflammation and fibrosis secondary to particle overload in the lungs.

A 28-day inhalation toxicology study with ALIS conducted in juvenile rats did not reveal any special sensitivity compared to adult animals. ALIS was administered daily from postnatal days 10-37 and the inhaled amikacin doses achieved were up to 90 mg/kg/day. No signs of systemic toxicity due to amikacin were observed and treatment did not affect growth or the attainment of developmental milestones.

Bronchioalveolar squamous cell carcinoma, a rare lung tumor, was observed at low incidence in rats that received the highest inhaled dose of ALIS used in this study, 45 mg/kg/day (expressed as amikacin). Other findings were consistent with those from previous inhalation toxicity studies conducted in rats. These tumors are of uncertain relevance to humans, as rats are especially sensitive to developing lung neoplasms secondary to particle overload. Additionally, ALIS was negative in a battery of in vitro genotoxicity studies conducted in bacteria (Ames test) and mammalian cells (mouse lymphoma assay, chromosome aberration assay in Chinese hamster ovary cells) and an in vivo rat micronucleus test.

Reproductive toxicity testing was not performed with ALIS, but intraperitoneal doses of amikacin up to 200 mg/kg/day did not impair fertility of male or female rats despite evidence of parental nephrotoxicity at the high dose. Subcutaneous doses of amikacin up to 100 mg/kg/day in rats (given from GD 8-14) and 400 mg/kg/day in mice (given from GD 7-13) did not cause visceral or structural malformations. Postnatal development of the offspring was not adversely affected by amikacin treatment of the dams. Hearing was not adequately assessed in the offspring, so it is not possible to evaluate any effect of amikacin on this endpoint.

The daily inhaled amikacin dose for humans using ALIS/ARIKAYCE as directed with the Lamira™ Nebulizer System is 590 mg once daily. The amounts of DPPC and cholesterol present in this inhaled dose are (b) (4) mg, for a total lipid amount of (b) (4) mg. In a 60 kg human, the amikacin dose would be 9.8 mg/kg/day, assuming 100% deposition. Adult NTM patients receiving this dose regimen of ALIS had a mean serum AUC_{0-24} of 23.5 µg·hr/mL and mean Cmax of 2.8 µg/mL. These are lower than the mean AUC_{0-24} of 154 µg·hr/mL and Cmax of approximately 76 µg/mL observed in healthy adults that received the approved intravenous amikacin sulfate dose of 15 mg/kg once daily.

Another means of comparing exposure to products given via inhalation is to estimate the inhaled or deposited dose per gram of lung tissue. It provides perspective for ARIKAYCE because some of the adverse lung effects observed in rats may be due to particle overload. In humans, assuming 100% deposition (a conservative assumption) and a lung weight of 1000 g, the daily dose of amikacin is 0.59 mg/g of lung tissue and for total lipid is (b) (4) lung tissue. The high dose (30 mg/kg/day) in the 9-month dog study where only clearance-related effects were observed provided 0.53 mg/g of amikacin and 0.40 mg/g of total lipid, similar to the conservative estimate of clinical exposure. In the 26-week rat study, the high dose (90 mg/kg/day, associated with inflammatory changes that appeared secondary to particle overload) provided 1.9 mg/g of amikacin and 1.1 mg/g of total lipid. The mid dose (30 mg/kg/day where less severe effects were observed) provided 0.75 mg/g of amikacin and 0.42 mg/g of total lipid, similar to clinical exposure. The assumptions for estimating the deposited dose per gram of lung tissue in dogs were 20% deposition and lung weight of 100 g and for rats were 10% deposition and 1.5 g lung weight.

The data from nonclinical studies with ALIS show that the product is expected to be reasonably safe for the proposed clinical indication when used as directed. Thus, there is no objection to its approval from the pharmacology/toxicology review team.

5.2 Referenced NDAs, BLAs, DMFs

IND (b) (4) (CF), IND 108674 (NTM lung disease)- Nonclinical studies with ARIKAYCE and its precursor formulation (SLIT™ amikacin) were reviewed under these INDs.

Although the Applicant did not list a specific RLD for this NDA, there are several approved ANDAs for amikacin sulfate injection. There is also information available from published scientific literature.

5.3 Pharmacology

Primary pharmacology
Amikacin exerts its antimicrobial activity by binding to the 30S subunit of bacterial ribosomes, inhibiting protein synthesis.

Secondary Pharmacology
Studies were not performed with the current product.

Safety Pharmacology
Pulmonary function was assessed in dogs exposed for 60 minutes to test atmospheres containing up to approximately 1.2 mg/L of amikacin and 0.9 mg/L of lipid (SLIT™ Amikacin). There were no biologically significant changes in pulmonary parameters as a result of exposure.

5.4 ADME/PK

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>Absorption</td><td></td></tr>
<tr><td>Studies on the pharmacokinetics and biodistribution of inhaled Arikace™ in rats (Study No. AMIK-BRD-2007-04-PK)</td><td>Amikacin is not well absorbed orally by rats (estimated that bioavailability is 0.002-0.3%). >50% of amikacin dose recovered after administration of ALIS or free amikacin was in stomach/intestine, so this fraction was unlikely to be available systemically.

Comparison of ALIS and Free Tobramycin Administered by Inhalation (AUC_{0-48hr}):
<table>
<tr><th rowspan="2">Drug</th><th colspan="4">AUC (µg•hr/g)</th></tr>
<tr><th>Lung</th><th>Serum</th><th>Kidney</th><th>Urine</th></tr>
<tr><td>ALIS (60 mg/kg)</td><td>56233</td><td>281</td><td>1883</td><td>3674</td></tr>
<tr><td>FT (60 mg/kg)</td><td>11504</td><td>198</td><td>4302</td><td>5408</td></tr>
</table>
</td></tr>
<tr><td>Distribution</td><td></td></tr>
</table>

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>Studies on the pharmacokinetics and biodistribution of inhaled Arikace™ in rats (Study No. AMIK-BRD-2007-04-PK)</td><td>Recovery of Amikacin Following Pulmonary Administration of ALIS or Free Amikacin:
<table>
<tr><th>Organ/Fluid</th><th colspan="2">Amikacin from ALIS (µg)</th><th colspan="2">FA (µg)</th></tr>
<tr><th></th><th>IPD</th><th>6 hr PD</th><th>IPD</th><th>6 hr PD</th></tr>
<tr><td>Lung</td><td>1029 ± 182</td><td>590 ± 59</td><td>800 ± 55</td><td>126 ± 34</td></tr>
<tr><td>Kidney</td><td>19 ± 7</td><td>44 ± 5</td><td>27 ± 1</td><td>54 ± 8</td></tr>
<tr><td>Serum</td><td>134 ± 31</td><td>37 ± 10</td><td>304 ± 188</td><td>15 ± 9</td></tr>
<tr><td>Urine</td><td>32 ± 37</td><td>399 ± 283</td><td>21 ± 13</td><td>688 ± 326</td></tr>
<tr><td>Brain</td><td>5 ± 3</td><td>3 ± 2</td><td>5 ± 2</td><td>4 ±4</td></tr>
</table>
Notes to Table 3: IPD = immediately post dosing and PD = post dosing. All amikacin values are expressed as total µg/organ or fluid (n = 3).</td></tr>
<tr><td>Metabolism</td><td></td></tr>
<tr><td></td><td>Amikacin is not metabolized. According to the label used for parenteral products, when given to normal adult volunteers, 98.2% of an intramuscular amikacin dose was recovered unchanged in urine within 24 hours and 94% of an intravenous dose was recovered unchanged in urine over the same time period.</td></tr>
<tr><td>Excretion</td><td></td></tr>
<tr><td></td><td>Renal; see above.</td></tr>
<tr><td>TK data from general toxicology studies
Dog: 9-month inhalation toxicity study (Study No. 11-6400)
Plasma samples collected immediately after exposure and 0.25, 0.5, 1, 2 and 4 hours after exposure on Day 1 and during months 6 and 9.
Lung tissue samples were collected at necropsy (day after last exposure, or after 3-month recovery).
Doses in table are inhaled doses; pulmonary deposited doses in dogs are assumed to be about 20% of the inhaled dose.</td><td><u>Dog</u>
Accumulation: Plasma accumulation not observed
Dose proportionality: Yes
<table>
<tr><th rowspan="2">Nominal dose level (mg/kg/day)</th><th colspan="6">C_{max} (ng/mL)</th></tr>
<tr><th colspan="2">Day 1</th><th colspan="2">Month 6</th><th colspan="2">Month 9</th></tr>
<tr><th></th><th>Males</th><th>Females</th><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr>
<tr><td>5</td><td>939</td><td>995</td><td>996</td><td>747</td><td>1260</td><td>939</td></tr>
<tr><td>10</td><td>1710</td><td>1350</td><td>1670</td><td>1150</td><td>2160</td><td>1320</td></tr>
<tr><td>30</td><td>5220</td><td>6210</td><td>4260</td><td>4070</td><td>4830</td><td>4450</td></tr>
</table>
<table>
<tr><th rowspan="2">Nominal dose level (mg/kg/day)</th><th colspan="6">AUC_{last} (ng.h/mL)</th></tr>
<tr><th colspan="2">Day 1</th><th colspan="2">Month 6</th><th colspan="2">Month 9</th></tr>
<tr><th></th><th>Males</th><th>Females</th><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr>
<tr><td>5</td><td>2010</td><td>2210</td><td>2100</td><td>1810</td><td>2490</td><td>1750</td></tr>
<tr><td>10</td><td>4180</td><td>3430</td><td>4470</td><td>3140</td><td>5340</td><td>3590</td></tr>
<tr><td>30</td><td>11900</td><td>13600</td><td>9160</td><td>9220</td><td>9530</td><td>9250</td></tr>
</table>
<table>
<tr><th rowspan="2">Nominal dose level (mg/kg/day)</th><th colspan="4">Lung tissue concentration (µg/g)</th></tr>
<tr><th colspan="2">Month 9</th><th colspan="2">Recovery[a]</th></tr>
<tr><th></th><th>Males</th><th>Females</th><th>Males</th><th>Females</th></tr>
<tr><td>5</td><td>660</td><td>696</td><td>-</td><td>-</td></tr>
<tr><td>10</td><td>1320</td><td>1220</td><td>-</td><td>-</td></tr>
<tr><td>30</td><td>2120</td><td>3250</td><td>117</td><td>468</td></tr>
</table>
[a] In males, values were 211 and 22.2 µg/g and in females values were 157 and 778 µg/g.</td></tr>
<tr><td>Rat: 26-week inhalation toxicity study with 12-</td><td><u>Rat</u>
Accumulation: Slight; plasma AUC rose only modestly over the dosing period.
Dose proportionality: Dose proportional exposure from 10-30 mg/kg/day, but less</td></tr>
</table>

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>week recovery (Study No. 07-6308)
Serum samples collected within 5 minutes, and 0.25, 0.5, 1, 2 and 4 hours after exposure on Day 24 and during Weeks 13 and 26. Urine was collected on ice 0-4 hours and 4-16 hours after dosing during Weeks 13 and 26 and for a 16-hour period at the end of recovery (n=5/sex). Lungs were collected at terminal and recovery necropsies (n=6/sex).</td><td>than dose proportional exposure between 30 and 90 mg/kg/day.

<table>
<tr><th>Day</th><th>Sex</th><th>Group[a]</th><th>Nominal Dose (mg/kg/day)</th><th>Actual Dose (mg/kg/day)</th><th>AUC_{last} (ng·h/mL)</th><th>AUC_{all} (ng·h/mL)</th><th>C_{max} (ng/mL)</th><th>t_{max} (h)</th></tr>
<tr><td rowspan="6">24</td><td rowspan="3">Male</td><td>2</td><td>10</td><td>9</td><td>1030</td><td>1350</td><td>761</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>31</td><td>3510</td><td>3510</td><td>1700</td><td>0.25</td></tr>
<tr><td>4</td><td>90</td><td>106</td><td>5900</td><td>5900</td><td>4010</td><td>[b]</td></tr>
<tr><td rowspan="3">Female</td><td>2</td><td>10</td><td>9</td><td>1530</td><td>1530</td><td>1020</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>33</td><td>4130</td><td>4130</td><td>1930</td><td>[b]</td></tr>
<tr><td>4</td><td>90</td><td>114</td><td>6980</td><td>6980</td><td>3930</td><td>[b]</td></tr>
<tr><td rowspan="6">86</td><td rowspan="3">Male</td><td>2</td><td>10</td><td>13</td><td>1850</td><td>1850</td><td>834</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>34</td><td>5000</td><td>5000</td><td>2420</td><td>0.5</td></tr>
<tr><td>4</td><td>90</td><td>104</td><td>8730</td><td>8730</td><td>5990</td><td>[b]</td></tr>
<tr><td rowspan="3">Female</td><td>2</td><td>10</td><td>15</td><td>2140</td><td>2140</td><td>986</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>37</td><td>5070</td><td>5070</td><td>2220</td><td>0.25</td></tr>
<tr><td>4</td><td>90</td><td>113</td><td>11000</td><td>11000</td><td>6770</td><td>[b]</td></tr>
<tr><td rowspan="6">177</td><td rowspan="3">Male</td><td>2</td><td>10</td><td>15</td><td>2290</td><td>2290</td><td>851</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>37</td><td>5380</td><td>5380</td><td>2460</td><td>[b]</td></tr>
<tr><td>4</td><td>90</td><td>123</td><td>9680</td><td>9680</td><td>5800</td><td>[b]</td></tr>
<tr><td rowspan="3">Female</td><td>2</td><td>10</td><td>17</td><td>2280</td><td>2280</td><td>1060</td><td>0.5</td></tr>
<tr><td>3</td><td>30</td><td>41</td><td>5460</td><td>5460</td><td>2810</td><td>0.5</td></tr>
<tr><td>4</td><td>90</td><td>133</td><td>7900</td><td>7900</td><td>4960</td><td>[b]</td></tr>
</table>
[a]Animals in Groups 2, 3 and 4 were exposed to inhaled doses of amikacin for 25, 70 and 240 minutes, respectively, on Day 24 and 30, 70 and 240 minutes, respectively, on Days 86 and 177
[b]5 minutes after the end of the inhalation dose period
AUC_{all} = $AUC_{0\text{-}4h}$

Concentrations (ng/mL) of Amikacin in Urine 4-16 hours after Dosing (as applicable)
<table>
<tr><th>Group</th><th>Interim</th><th>Termination</th><th>Recovery</th></tr>
<tr><td>1</td><td>BLQ</td><td>BLQ</td><td>BLQ</td></tr>
<tr><td>2</td><td>18930</td><td>34670</td><td>BLQ</td></tr>
<tr><td>3</td><td>62067</td><td>89289</td><td>202</td></tr>
<tr><td>4</td><td>214170</td><td>151656</td><td>319</td></tr>
</table>

Concentration (µg/g) of Amikacin in Lung Tissue
<table>
<tr><th>Group</th><th>Termination</th><th>Recovery</th></tr>
<tr><td>1</td><td>NA</td><td>NA</td></tr>
<tr><td>2</td><td>1314</td><td>29</td></tr>
<tr><td>3</td><td>2451</td><td>36</td></tr>
<tr><td>4</td><td>2958</td><td>31</td></tr>
</table></td></tr>
<tr><td>TK data from reproductive toxicology studies</td><td>TK analysis was not performed in the reproduction toxicology studies that will be used to label the drug. These studies were published in 1975, when it was not common to include TK.</td></tr>
<tr><td>TK data from Carcinogenicity studies
ARIKACE™: A 2-Year Inhalation Carcinogenicity Study in Rats (Study No. 09-6345)</td><td></td></tr>
</table>

Type of Study	Major Findings
Doses in table are inhaled doses; pulmonary deposited doses are assumed to be about 10% of the inhaled dose in rats.	

Sex	Group	Nominal Dose (mg/kg/day)[a]	Actual Dose (mg/kg/day)[a]	AUC_{last} (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)
Male	3	5	5.7	1340	694	1
	4	15	15.2	2770	1160	0.5
	5	45	40.9	8500	4960	b
Female	3	5	6.3	1240	622	0.5
	4	15	17.5	2760	1200	0.5
	5	45	45.5	6770	3190	0.25

[a]Animals in Groups 3, 4 and 5 were exposed to inhaled doses of amikacin for 25, 70 and 170 minutes, respectively

[b]5 minutes after the end of the inhalation dose period

5.5 Toxicology

5.5.1 General Toxicology

Arikace (a previous name used by the sponsor for ALIS) has the same formulation as ARIKAYCE (the current product for which approval is being requested under this NDA). A previous formulation known as SLIT™ Amikacin was used in earlier nonclinical testing. The formulations contained the same basic components (thus, nonclinical studies conducted with the older formulation are still considered relevant), but the newer formulation planned for marketing contains a (b) (4) concentration of amikacin and a (b) (4) concentration of lipid relative to drug.

ARIKACE™: A 9-Month Inhalation Toxicity Study in Dogs With a 3-Month Recovery Period/Study No. 11-6400

Key Study Findings

- Neither proliferative changes nor necrosis was observed in respiratory tissues of dogs that received inhaled amikacin doses of up to approximately 30 mg/kg/day (pulmonary deposited dose of 6 mg/kg/day) formulated as Arikace. The lipid (2:1, DPCC:Cholesterol) inhaled dose at the high dose level was approximately 22 mg/kg/day (pulmonary deposited dose of 4.4 mg/kg/day).
- Foamy macrophages in lungs were observed in all Arikace-treated dogs with dose-related increase in severity. They were also observed in lungs of dogs that received empty liposomes at a greater incidence than saline controls. Basophilic granules were seen in macrophages from the Arikace groups, but not the empty liposome group. These findings appeared related to clearance.
- No clinical signs of amikacin toxicity were observed. No kidney changes suggestive of amikacin nephrotoxicity were present.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing:	Inhaled doses of amikacin were approximately 5, 10, and 30 mg/kg/day (achieved doses were within 12% of intended). Pulmonary deposited doses in dogs are assumed to be approximately 20% of an inhaled dose. Test articles were administered daily for 15 minutes (low dose), 30 minutes (mid dose) or 70-90 minutes (high dose and controls).
Route of administration:	Inhalation (face mask)
Formulation/Vehicle:	The clinical formulation of amikacin liposomal inhalation suspension (ALIS) was used. It was known as Arikace at the time of this study (but it is the same formulation as ARIKAYCE). There were 2 control groups- one that received nebulized 1.5% NaCl and another that received empty liposomes matched to the high dose.
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group (main study)
Age:	5-6 months at the initiation of dosing
Satellite groups/ unique design:	2/sex/group (saline and liposome control groups and high dose group) for recovery
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	**Major findings**
Mortality	None
Clinical Signs	Nothing that appeared drug-related.
Body Weights	No treatment-related differences between groups.
Ophthalmoscopy	Nothing that appeared related to treatment.
ECG	No treatment-related changes.
Hematology	No treatment-related differences between groups.
Clinical Chemistry	No treatment-related differences between groups.
Urinalysis	No treatment-related differences between groups.
Gross Pathology	No treatment-related observations.
Organ Weights	Increase in absolute and relative mean lung weights at MD (+16-28%) and HD (+25-39%) compared to saline control. Correlated with increased foamy macrophages. Persisted in HD after recovery (+19-33%), but lower.
Histopathology **Adequate battery:** Yes, with expanded analysis of respiratory	Foamy alveolar macrophages were observed in lungs of dogs treated with Arikace with dose-related severity (LD- minimal to slight, MD- slight to moderate, HD- moderate to severe). Basophilic granules were

system tissues (4 levels of nasal turbinates; nasopharynx) Peer reviewed.	present. Foamy alveolar macrophages (minimal) were observed at greater incidence in lungs of dogs treated with empty liposomes compared to saline controls, but no basophilic granules were present. Increased macrophages (foamy and non) were observed in mediastinal and tracheobronchial lymph nodes. These findings appeared related to clearance of test articles. Signs of minimal to moderate irritation (dose-related degeneration-cytoplasmic vacuolation and swelling) were observed in respiratory and olfactory epithelium of Arikace-treated dogs, primarily in the ethmoid turbinates. Not seen in either control group. Basophilic granules were observed in cytoplasm of epithelial cells at apex of tracheal bifurcation of most HD dogs and one MD dog. Likely indicated deposition of test article. No signs of degeneration, necrosis, or inflammation. Minimal to slight increase in brown pigment in renal tubules was observed in some Arikace-treated dogs. Could be related to clearance. After recovery period, foamy alveolar macrophages containing basophilic granules were still present at HD, but severity was reduced (minimal to slight). Changes in respiratory and olfactory epithelium significantly recovered. Incidence and severity of cytoplasmic basophilic granules was reduced at HD. Brown pigment was no longer present in renal tubules.

LD: low dose; MD: mid dose; HD: high dose

ARIKACE™: A 26-Week Inhalation Toxicity Study in Rats with a 13-Week Interim Evaluation and a 12-Week Recovery Period/Study No. 07-6308

Key Study Findings

- Signs of minimal to moderate irritation were observed in the respiratory and olfactory epithelium (larynx, trachea, nasal turbinates) of Arikace-treated rats with dose-related severity. These findings are not uncommon in rats exposed to a variety of test substances via inhalation and are not considered specific to Arikace.
- Foamy macrophages in lungs were observed in all Arikace-treated rats with dose-related increase in severity. The high dose group showed signs of particle overload, including inflammation and lung changes secondary to inflammation. The high dose rats received approximately 90 mg/kg/day inhaled amikacin (pulmonary deposited dose of 9 mg/kg/day) and 60 mg/kg/day inhaled lipid (pulmonary deposited dose of 6 mg/kg/day). Lung changes were less severe at the mid dose, approximately 30 mg/kg/day inhaled amikacin and 20 mg/kg/day inhaled lipid.
- No clinical signs of amikacin toxicity were observed. Minimal to slight focal nephropathy was observed at the high dose, with reduced incidence and severity after the recovery period.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	Inhaled doses of amikacin were approximately 10, 30, and 90 mg/kg/day (achieved doses were slightly higher than intended; within 30% at the LD, 20% at MD, and 10% at HD). Pulmonary deposited doses in rats are assumed to be approximately 10% of an inhaled dose. Test articles were administered daily for 25-40 minutes (low dose), 70-80 minutes (mid dose) or 215-240 minutes (high dose and control).
Route of administration:	Inhalation (nose-only)
Formulation/Vehicle:	The clinical formulation of amikacin liposomal inhalation suspension (ALIS) was used. It was known as Arikace at the time of this study (but it is the same formulation as ARIKAYCE). The control group received nebulized 1.5% NaCl.
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	15/sex/group (main study)
Age:	6.5 weeks at the initiation of dosing
Satellite groups/ unique design:	12/sex/group for TK (4/sex/timepoint), 10/sex/group for interim sacrifice at 13 weeks, 5/sex/group for recovery
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	Major findings
Mortality	None that appeared drug-related.
Clinical Signs	HD (sporadic, low incidence): unkempt fur, piloerection, moist rales
Body Weights	HD males had reduced body weight gain and weighed 17% less than controls at the end of dosing, and 12% less at the end of recovery. No differences between dose groups of female rats.
Ophthalmoscopy	Nothing that appeared related to treatment.
Hematology	No toxicologically relevant changes.
Clinical Chemistry	No toxicologically relevant changes.
Urinalysis	No treatment-related differences between groups.
Gross Pathology	Dose-related incidence of tan/white foci observed in Arikace-treated rats correlated with accumulation of foamy macrophages. Observed at MD, HD at interim sacrifice and in all Arikace groups at main study sacrifice. Still present at recovery sacrifice in MD, HD, but lower incidence.
Organ Weights	Dose-related increases in absolute and relative mean lung weights of all Arikace groups compared to saline control beginning with interim sacrifice. Correlated with increased foamy macrophages. Recovered after 12 weeks without exposure.

Histopathology **Adequate battery:** Yes, with expanded analysis of respiratory system tissues (4 levels of nasal turbinates; nasopharynx) Peer reviewed.	Foamy alveolar macrophages were observed in lungs of rats treated with Arikace with dose-related severity, beginning 13 weeks after dosing, with incidence and severity increasing by Week 26 (LD- minimal to slight; MD- mostly moderate, some marked; HD- moderate to marked, mostly the latter). Dose-related inflammatory foci were observed at the MD and HD beginning at 13 weeks, and in a few LD animals as well by 26 weeks. Alveolar epithelial hyperplasia was seen at MD, HD beginning at 13 weeks and was associated with the inflammatory foci, as were cholesterol clefts at 26 weeks. After the recovery period, the number of foamy macrophages was reduced in the Arikace-treated rat lungs. Cholesterol clefts were observed in the lungs containing macrophage foci, mostly in the HD group but also in a few LD, MD rats. Cell infiltrates and hyperplasia were also associated with these foci and a minimal focus of fibrosis was observed in alveolar epithelium or the pleural capsule of some HD rats, likely due to inflammation associated with the accumulation of alveolar macrophages. No evidence of fibrosis was seen at weeks 13 or 26. The findings in the recovery group were consistent with a chronic reparative response to particle overload of macrophage clearance. Signs of minimal to moderate irritation (dose-related degeneration-cytoplasmic vacuolation) were observed in respiratory (primarily at levels I and II) and olfactory epithelium (especially at level IV) in nasal turbinates of Arikace-treated rats. Seen at MD, HD beginning at interim sacrifice and also in LD at 26 weeks. Not seen in controls. Focal squamous metaplasia of respiratory epithelium also seen at MD, HD. Minimal to moderate hyperplasia of squamous epithelium in the larynx was observed beginning at 13 weeks in the MD and HD groups, but observed in all Arikace groups at the terminal sacrifice. Cells were well differentiated; no evidence of atypia or dysplasia. Severity increased with dose and minimal to moderate keratinization was observed in some rats, especially at HD. Focal erosions and ulcers were more common at HD. There were greater numbers of mixed inflammatory cells observed in laryngeal mucosa of Arikace-treated rats than controls, with a dose-related increase in the number. Degenerative changes (minimal to slight) in tracheal epithelium (loss of cilia, cellular disorganization, cytoplasmic vacuolation) were observed in MD, HD rats beginning at 13 weeks and in all 3 Arikace groups at 26 weeks. Inflammatory cell debris (eosinic material) was more prevalent in tracheal lumen of HD Arikace-treated rats than controls and MD, LD groups. Changes in respiratory and olfactory epithelium in nasal turbinates and the laryngeal and tracheal changes completely recovered at the LD, and partial recovery was seen at the MD and HD. Minimal focal nephropathy in the renal cortex (basophilic convoluted tubules) observed in HD males beginning at interim sacrifice and progressing to slight severity at terminal sacrifice. Observed in HD females at minimal severity at higher incidence than seen in controls at terminal sacrifice. Nephropathy was not observed at LD, MD after recovery and incidence was lower at the HD.

LD: low dose; MD: mid dose; HD: high dose

General toxicology; additional studies

Observations from shorter term studies in rats and dogs and a 13-week study in mice were consistent with the 26-week rat study and the 9-month dog study described above. In the rats, the findings were more severe in the 26-week study than in shorter studies (e.g., 28-30 days), especially at the high dose.

A 28-day study was conducted in juvenile rats exposed to inhaled amikacin doses of up to 90 mg/kg/day as Arikace via nose-only inhalation from PND 10-37. Functional and developmental endpoints were assessed (sexual maturation, open field testing, motor activity, femur length) as well as standard toxicology endpoints. There were no signs of systemic toxicity due to amikacin and respiratory tract findings did not differ from those observed in adult rats exposed to Arikace. Treatment did not affect growth or the attainment of developmental milestones.

5.5.2 Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay with TR02 (SLIT™ Amikacin)/Study No. 7522-100

Key Study Findings:

- SLIT™ Amikacin ((b) (4) mg/ml amikacin (b) (4) mg/ml dipalmitoylphosphatidylcholine, (b) (4) mg/ml cholesterol) was not mutagenic under the conditions of this study.

GLP compliance: Yes

Test system: S. typhimurium TA98, TA100, TA1535, and TA1537 and E. coli WP2*uvr*A.

Concentrations (based on level of amikacin (b) (4) in definitive study up to 500 µg/plate with S9 and 1000 µg/plate without S9 for Salmonella and up to 100 µg/plate ± S9 for E. coli.

Study is valid: Yes

In Vitro Assays in Mammalian Cells

L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay/Study No. 7522-102

Key Study Findings:

- SLIT™ amikacin- (b) (4) (an aqueous liposomal suspension containing (b) (4) mg/mL amikacin, (b) (4) mg/ml dipalmitoylphosphatidylcholine, (b) (4) mg/ml cholesterol), free amikacin, and SLIT™ amikacin placebo (empty liposomes) were not mutagenic under the conditions of this study.

GLP compliance: Yes

Test system: L5178Y mouse lymphoma cells; all compounds were tested at concentrations up to 5000 µg/ml ± S9, as cytotoxicity was not observed.

Study is valid: Yes

Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells/Study No. 7522-101

Key Study Findings:

- SLIT™ Amikacin ((b) (4) mg/ml amikacin (b) (4) mg/ml dipalmitoylphosphatidylcholine, (b) (4) mg/ml cholesterol) was not clastogenic under the conditions of this study.

GLP compliance: Yes

Test system: Chinese hamster ovary (CHO-WBL) cells; concentrations (based on amikacin (b) (4) up to 100 µg/ml ± S9 in definitive study, with chromosome analysis performed up to 100 µg/ml without metabolic activation and 50 µg/ml in the presence of S9.
Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

A 3-Day Inhalation Toxicity Study in Rats with a Micronucleus Evaluation/Study No. 07-6315

Key Study Findings:

- Arikace (b) (4) mg/ml amikacin, (b) (4) mg/ml dipalmitoylphosphatidylcholine, (b) (4) mg/ml cholesterol) did not induce micronucleated erythrocytes in the bone marrow of rats that received inhaled doses of up to 135 mg/kg/day (pulmonary deposited dose of 13.5 mg/kg/day) under the conditions of this study.

GLP compliance: Yes
Test system: Rats ([Crl:CD® BR] IGS BR) were exposed to Arikace (target inhaled doses of 15, 45, and 135 mg/kg/day- actual doses were within 16% of intended) or 1.5% NaCl for up to 360 minutes/day for 3 days. Pulmonary deposited doses in rats are assumed to be 10% of inhaled dose. Positive control was oral cyclophosphamide (single dose of 20 mg/kg). Femoral bone marrow was harvested 24 hours after last dose and evaluated for presence of micronuclei.
Study is valid: Yes

Other Genetic Toxicity Studies

Amikacin Sulfate Bacterial Reverse Mutation Test (Study No. HUD0381)

Two Lots of amikacin sulfate [Lot 081001-X containing Impurity (b) and an unknown impurity at RRT (b) (4) (MW (b) (4)) and Lot 990167 0015 9 containing an unknown impurity at RRT (b) (4) (MW (b) (4))] were assessed in S. typhimurium strains TA1535, TA1537, TA98 and TA100 and E. coli strain WP2 uvrA (pKM101). The Lots were tested at up to 4509.5 and 4480 µg/plate, respectively (expressed as amikacin (b) (4) No increases in the numbers of revertant colonies were observed, regardless of metabolic activation (rat S9). Thus, neither Lot of amikacin sulfate was mutagenic under the conditions of this study.

5.5.3 Carcinogenicity

ARIKACE™: A 2-Year Inhalation Carcinogenicity Study in Rats ((b) (6) (b) (6) No. 09-6345)

Key Study Findings:

- Bronchioalveolar squamous cell carcinoma was observed in the lungs of 2/60 HD female rats; thus, Arikace was considered tumorigenic in rats under the conditions of this study. A keratinizing pulmonary cyst was present in a 3rd HD female rat; these can be part of the continuum of squamous cell carcinoma formation. However, these tumors are of uncertain relevance to humans, as rats are especially sensitive to developing lung neoplasms secondary to particle overload.

- Hyperplasia of alveolar epithelium was observed in Arikace-treated rats (incidence was dose-related, liposome control similar to MD). Squamous metaplasia of this tissue observed in 2/60 HD females.
- Foamy alveolar macrophages (diffuse) were observed in the lungs of most rats treated with Arikace or empty liposomes; dose-related severity. Focal/multifocal alveolar macrophages were seen less frequently in low dose rats.
- Interstitial alveolar inflammation was seen more frequently in MD, HD and liposome control rats than at the LD; severity was dose-related.
- Survival, body weights, and food consumption did not differ between control and Arikace groups. No treatment-related neoplasms were observed in tissues other than the lungs. Systemic exposure to amikacin assessed 6 months after the initiation of dosing showed Cmax of approximately 4 µg/ml and AUC of approximately 7.6 µg·hr/ml at the HD.

GLP Compliance: Yes
Definitive Doses: 5, 15, and 45 mg/kg/day (as inhaled amikacin (b) (4) assuming 100% deposition) of Arikace and 2 control groups- 1.5% NaCl and liposome only (matched to liposome content at Arikace high dose)
FDA Dose Concurrence: Yes (ECAC meeting minutes dated 9/16/08)
NOAEL: 15 mg/kg/day
Safety Margin: <2-fold human clinical dose, using lung weight (1.5 g for rats, 1000 g for humans) for normalization and assuming a 10% deposition factor for rats and a 100% deposition factor in humans

5.5.4 Reproductive and Developmental Toxicology

Studies were not conducted with ALIS, and the label for approved amikacin injection products contains limited information, but the following publications provide data regarding the reproductive/developmental toxicity potential of amikacin after systemic administration.

Fertility and Early Embryonic Development

Effect of Amikacin on Reproduction: Fertility Study in Rats Following Intraperitoneal Administration Seg. I. (Japanese Journal of Antibiotics, August 1982, pp 194-204)
Key Study Findings

- Amikacin did not impair fertility of male or female rats at doses up to 200 mg/kg/day (highest dose tested), despite evidence of parental nephrotoxicity at this dose.
- Although the paper mentions that no drug-related visceral or skeletal malformations were observed in the fetuses, dams were only dosed until GD 7, so the fetuses were not exposed to drug during most of organogenesis.

Conducting laboratory and location: Banyu Pharmaceutical Co.
GLP compliance: Unknown

Methods

Dose and frequency of dosing:	0, 25, 100, 200 mg/kg/day; Males- 60 days prior to mating through mating period; Females- 14 days prior to mating through GD 7
Route of administration:	Intraperitoneal injection
Formulation/Vehicle:	Sterile physiological saline
Species/Strain:	Wistar rats
Number/Sex/Group:	20
Study design:	Rats were mated at 1:1 ratio. Pregnant females were sacrificed on GD 20. One third of fetuses underwent visceral examination and remaining two thirds underwent skeletal examination.

Observations and Results

Parameters	Major findings
Mortality	No unscheduled mortality.
Clinical Signs	Soft stool at MD, HD beginning 4-5 days after administration started.
Body Weights	HD males had significantly reduced mean body weights compared to controls starting 7 days after onset of dosing and persisting throughout the dosing period. In the female rats, body weight gain and mean body weights were similar amongst the dose groups during the mating period. During gestation, HD female mean body weights were significantly less than control on some days, but overall body weight gain during the entire gestation period did not differ significantly from control.
Necropsy findings	No significant differences in mean fertility parameters between control and amikacin-treated groups (number of corpora lutea, pre- or postimplantation loss, number of implantation sites, numbers of dead and live fetuses). MD: Slight kidney enlargement in males. HD: Kidney enlargement and discoloration in males. Observed in some females at lower incidence.

LD: low dose; MD: mid dose; HD: high dose

Embryo-Fetal Development

Teratological Studies of Amikacin (BB-K8): Effects on fetuses and offspring development of mouse and rats (Japanese Journal of Antibiotics, June 1975, pp 372-384)

Key Study Findings

- Doses of amikacin up to 100 mg/kg/day in rats (given from GD 8-14) and 400 mg/kg/day in mice (given from GD 7-13) did not cause visceral or structural malformations.
- The high dose of 400 mg/kg/day was excessively toxic to maternal rats, precluding the evaluation of offspring at this dose.
- Postnatal development of the offspring was not adversely affected by amikacin treatment of the dams.

Conducting laboratory and location: Banyu Pharmaceutical Co.

GLP compliance: Unknown

Methods

Dose and frequency of dosing: 0 (untreated) and 25, 100, and 400 mg/kg/day of amikacin sulfate

Route of administration: Subcutaneous injection

Formulation/Vehicle: Sterile physiological saline

Species/Strain: DDB4 mice and Wistar rats

Number/Group: Mice: 17-22 litters/group for visceral/skeletal examination and 6-7 litters/group for postnatal development
Rats: 18-26 litters/group for visceral/skeletal examination and 6 litters/group for postnatal development

Study design: Pregnant mice were dosed on GD 7-13 and underwent cesarean section/sacrifice on GD 18. Pregnant rats were dosed on GD 8-14 and underwent cesarean section/sacrifice on GD 20. Fetuses were examined using fresh dissection for visceral abnormalities, then treated and stained for skeletal examination.
For both mice and rats allowed to deliver, postnatal development of offspring was observed for 8 weeks.

Observations and Results

Parameters	**Major findings**
Mortality	No unscheduled mortality in pregnant mice, but 4/22 HD pregnant rats died during GD 17-19. Gross kidney changes suggesting nephrotoxicity were observed in these premature decedents, as well as the surviving HD maternal rats.
Clinical Signs	Food intake was noted to decrease in HD rats over the course of dosing and water intake increased over this time. No clinical signs noted in pregnant mice.
Body Weights	Reduced in HD rats compared to control. No differences between treated and control mice.
Necropsy findings Cesarean Section Data	Mice: No significant differences between amikacin groups and control regarding litter size, postimplantation loss. Rats: Mean litter size reduced at MD, HD compared to control (11.4 vs. 12.7). Late stage postimplantation loss was observed at greater frequency at the HD.
Necropsy findings Offspring	Mice: No visceral or skeletal malformations that appeared related to amikacin. Dose-related increase in reduced skeletal ossification that was most apparent at HD and correlated with reduced fetal body weight. Postnatal development (eye opening, auricle development, incisor eruption, vaginal opening, weight gain, reaction to light and pain

	stimuli, presence of sperm in testes) did not appear to have been affected by drug treatment. Rats: Reduced fetal body weights at HD. Reduced ossification was observed in this group, as were skeletal findings such as split vertebral arches, wavy ribs, and 6th sternabrae deformation that have been associated with maternal toxicity. Postnatal development did not appear to have been affected by drug treatment.

LD: low dose; MD: mid dose; HD: high dose

Teratological Studies of Amikacin (BB-K8): Embryo and Fetus Teratogenicity of Amikacin Intramuscularly Injected into Pregnant Rabbits (Japanese Journal of Antibiotics, June 1975, pp 366-371)

Key Study Findings

- Amikacin did not cause visceral or skeletal malformations in offspring of rabbits dosed intramuscularly with up to 160 mg/kg/day from GD 8-16. However, the number of litters in this study is lower than considered adequate for a developmental toxicology study.

Conducting laboratory and location: Banyu Pharmaceutical Co.
GLP compliance: Unknown

Methods

Dose and frequency of dosing: 0 (untreated), 10, 40, and 160 mg/kg/day
Route of administration: Intramuscular injection
Formulation/Vehicle: Sterile saline
Species/Strain: Japanese White rabbits
Number/Group: 7 litters
Study design: Pregnant rabbits were dosed on GD 8-16 and underwent cesarean section/sacrifice on GD 28. Fetuses were examined using fresh dissection for visceral abnormalities, then treated and stained for skeletal examination.

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	No difference between control and treated animals.
Necropsy findings	No drug-related differences in litter size or viability of offspring.
Necropsy findings Offspring	No drug-related visceral or skeletal variations or malformations were observed in the offspring.

LD: low dose; MD: mid dose; HD: high dose

5.5.5 Other Toxicology Studies

Inhalation of SLIT™ Amikacin for 14 Consecutive days: Functional Analysis of Lung Macrophages (Report No. AMIK-BRD-2005-19-TOX)

Phagocytic function of lung macrophages harvested from rats treated with SLIT™ amikacin for 4 hrs/day for 14 days did not appear to have been compromised. LPS-induced secretion of TNFα and NO by these macrophages was similar between untreated or saline controls and those from rats treated with SLIT™ amikacin.

Macrophage Function Study: Effects of Multiple Inhaled Doses of Arikace™ on the Functions of Alveolar Macrophages (Report No. AMIK-BIO-2009-16-MAC)

Phagocytic function of lung macrophages harvested from rats treated with Arikace (90 mg/kg targeted inhaled dose) one month on/one month off for up to a 6-month cycle did not appear compromised. The macrophages retained their ability to kill yeast. LPS-induced secretion of NO in the macrophages from Arikace-treated rats was greater than that by those from saline-treated rats. In contrast, LPS-induced secretion of TNFα was greater in the control macrophages than those from the Arikace-treated rats. Investigators speculated that NO in the cultured macrophages from Arikace-treated rats may have down-regulated production of TNFα. Levels of both cytokines were low in each treatment group in the absence of LPS stimulation.

6 Clinical Pharmacology

6.1 Executive Summary

The Office of Clinical Pharmacology (Division of Clinical Pharmacology IV) reviewed the information contained in NDA 207356. The clinical pharmacology information provided supports the approval of ARIKAYCE for the treatment of refractory NTM lung disease caused by MAC as part of a combination antibacterial drug regimen for adult patients.

Table 3: Summary of OCP's Recommendations and Comments on Key Review Issues

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness/Safety	Primary Evidence: One Phase 3 trial [Study INS-212]. Supportive Evidence: One Phase 2 trial [Study TR02-112], one ongoing open-label, single-arm extension study of INS-212 (Study INS-312), and sputum/systemic exposure results from Phase 2/3 trials [Studies TR02-112, INS-212].

General dosing instructions	Once daily (QD) oral inhalation of the contents of one 590 mg/8.4 mL vial (590 mg of amikacin) using the Lamira™ Nebulizer System.
Dosing in subgroups (intrinsic/extrinsic)	No dose individualization is recommended based on intrinsic and extrinsic factors.
Labeling/Health Communications	Applicant's proposed labeling is generally acceptable.

6.2 Summary of Clinical Pharmacology Assessment

6.2.1 Pharmacology and Clinical Pharmacokinetics

Table 4: Inhaled ALIS Clinical Pharmacokinetics Summary

Absorption	Observed mean amikacin T_{max} was 1.72 hours and ranged from 1.17 to 4.18 hours in serum. Observed maximum amikacin sputum concentrations were reported between a nominal window of 1 to 4 hours. Amikacin exposure in serum was approximately 15% of that observed following intravenous administration of 15 mg/kg/day amikacin sulfate. Sputum concentrations were high and exhibited large inter-subject variability.
Distribution	Plasma protein binding is approximately 10%. Apparent total volume of distribution (V_z/F) is approximately 368.6 L.
Elimination	Apparent total clearance (CL/F) is approximately 26.8 L/h.
	Metabolism Amikacin does not undergo appreciable metabolism.
	Excretion Renal excretion via glomerular filtration is the primary elimination route.

[†]PK from 12 individuals enrolled in the comprehensive PK sub-study (INS-212) and estimates obtained from noncompartmental analysis after 590 mg inhaled ALIS QD for 3 months.
[††]Average weight of PK population (n=12) was 52 kg (12.8% CV).

6.2.2 General Dosing and Therapeutic Individualization

General Dosing

The concentration of amikacin (b) (4) is nominally 70 mg/mL in one ALIS vial. The nominal total volume of the product-specific eFlow® nebulizer (Lamira™ Nebulizer System) is 8.4 mL. Empirically, the average loaded suspension was 8.43 mL for Phase 2 and Phase 3 trials. Therefore, a nominal delivered dose of 590 mg amikacin (b) (4) is achieved when ALIS is completely aerosolized and inhaled. The complete nebulization time (no more mist) ranges from 14 to 20 minutes.

The recommended dosage regimen of ALIS in adults is once daily inhalation of the contents of one 590 mg/8.4 mL vial (590 mg of amikacin) using the Lamira™ Nebulizer System.

Therapeutic Individualization

No dose adjustment is warranted based on the low systemic exposure of inhaled ALIS. However, monitoring of renal function is advised for specific populations such as the elderly and those with renal impairment as amikacin is primarily eliminated via glomerular filtration.

Outstanding Issues

None.

6.3 Comprehensive Clinical Pharmacology Review

6.3.1 General Pharmacology and Pharmacokinetic Characteristics

Table 5: General Pharmacology and Pharmacokinetic (PK) Characteristics

<table>
<tr><th colspan="2">Pharmacology</th></tr>
<tr><td>Mechanism of Action</td><td>Amikacin, an aminoglycoside antibacterial drug, inhibits protein synthesis via drug-induced mRNA misreading by irreversibly binding to the 30S sub-unit of the bacterial ribosome.</td></tr>
<tr><td>Active Moieties</td><td>Amikacin</td></tr>
<tr><td>QT Prolongation</td><td>The amikacin sulfate for injection label does not include a warning.</td></tr>
<tr><th colspan="2">General Information</th></tr>
<tr><td>Drug Exposure Following the Therapeutic Dosing Regimen</td><td>Serum Exposure
Serum exposures of amikacin [mean (CV%)] following inhalation of 590 mg ALIS QD for 3 months in patients with NTM lung infection using data from a comprehensive PK sub-group (Phase 3 Study INS-212) are reported below.
<table>
<tr><th>Pharmacokinetic Parameter</th><th>NTM infected Patients</th></tr>
<tr><td>Serum Amikacin</td><td>N=12</td></tr>
<tr><td>$AUC_{0\text{-}24}$ (µg.h/mL)</td><td>23.5 (46.3)</td></tr>
<tr><td>C_{max} (µg/mL)</td><td>2.8 (43.0)</td></tr>
</table>
†PK estimates are derived from 12 subjects in a comprehensive PK sub-study of INS-212 with rich sampling to allow noncompartmental analysis.
††Source: Adapted from ICPD-00494-1, Clinical Information Amendment; pk_wide_s212_intensive_subs_18jul2018.csv .

Note: A pooled analysis of sparsely sampled subjects (TR02-112 and INS-212) calculated population PK estimates and exposures broadly in line with the above (ICPD-00494). The population PK analyses were</td></tr>
</table>

<table>
<tr><td></td><td>reviewed but not verified by the FDA Clinical Pharmacology reviewers.

Sputum Exposure
Observed sputum concentrations of amikacin [mean (CV%)] following inhalation of 590 mg ALIS QD for 3 months in patients with NTM lung infection from the Phase 3 trial (INS-212) are reported below. Because of high inter-individual variability, no modeling was performed.

<table>
<tr><td>Nominal Sputum Sampling Time</td><td>NTM infected Patients
Sputum Amikacin Concentration (µg/g)</td></tr>
<tr><td>0-1 hr pre-dose[a] (N=13)</td><td>12 (107)</td></tr>
<tr><td>1-4 hr post-dose (N=12)</td><td>884 (152)</td></tr>
</table>
[a]Time since last dose was 72 hr.
[†]Adapted from ICPD-00494-1, Clinical Information Amendment, Table 2.
[††]Accumulation could not be assessed because of high variability in sputum. Only Month 3 is shown however collection times out to Month 6 were captured.</td></tr>
<tr><td>Healthy vs. Patients</td><td>Amikacin exposures following inhalation of ALIS were not evaluated in healthy subjects. Therefore, no drug exposure comparison between healthy subjects and NTM patients could be conducted.

Note: In patients with different lung disease states (i.e., non-CF vs CF), an approximately 2-fold lower total apparent amikacin clearance (CL_t/F) was observed in non-CF patients, suggesting greater systemic amikacin exposures [ICPD 00316] in non-CF patients than CF patients. Increased amikacin absorption from the lungs of non-CF NTM patients likely explains this observation given pathological lung state differences.</td></tr>
<tr><td>Dose Proportionality</td><td>No dose ranging studies were performed in the NTM lung infection population using the Lamira™ nebulizer system to assess dose proportionality of amikacin in serum or sputum.</td></tr>
<tr><td>Variability</td><td>Variability of observed amikacin exposures in patients with NTM lung infection can be found above in the section of “Drug exposure following the therapeutic dosing regimen.”</td></tr>
<tr><td>Accumulation</td><td>Accumulation was approximately 50% and 33% for serum amikacin exposures (C_{max} and AUC_{0-14}) following inhalation of ALIS at 590 mg QD for 3 months in the comprehensive PK sub-group calculated by noncompartmental methods. Amikacin accumulation was not apparent in sputum (Study INS-212).</td></tr>
<tr><td></td><td></td></tr>
<tr><td>T_{max}</td><td>Observed mean amikacin T_{max} was 1.72 hours and ranged from 1.17</td></tr>
</table>

	to 4.18 hours in serum. Observed maximum amikacin sputum concentrations were reported between a nominal window of 1 to 4 hours.
Bioavailability	The bioavailability of amikacin following inhalation of ALIS is expected to vary due to individual differences in respiratory rates and flow patterns, nebulizer performance, as well as airway pathology. On average, approximately 7.42% of the nominal dose was absorbed based on urine amikacin PK data following inhalation of ALIS (Study TR02-112).
Volume of Distribution	Apparent total volume of distribution (V_z/F) of amikacin is approximately 368.6 L.
Plasma Protein Binding	Approximately 10%.
Substrate Transporter Systems [*in vitro*]	None have been reported.
Elimination	
Half-life	Mean apparent half-life of amikacin ranged from 5.9 to 19.5 hours following inhalation of ALIS.
Metabolism	Amikacin does not undergo appreciable metabolism.
Excretion	Renal excretion of amikacin is via glomerular filtration (>90% of parenteral dose recovered as unchanged drug in urine).
In Vitro Interaction Liability (Drug as Perpetrator)	
Inhibition/Induction of Metabolism	None have been reported.
Inhibition/Induction of Transporter Systems	None have been reported.

[†]*Source: NCA calculated PK estimates adapted from ICPD-00494-1, Clinical Information Amendment;* pk_wide_s212_intensive_subs_18jul2018.csv.

6.3.2 Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

The primary evidence of effectiveness (culture conversion defined as 3 consecutive negative monthly sputum cultures within 6 months of treatment) of ALIS in patients with NTM lung infection was supported by one Phase 3 pivotal trial (Study INS-212). Refer to Section 7.2 "Review of Relevant Individual Trials Used to Support Efficacy" for details on the study design and efficacy evaluation.

Supportive evidence of effectiveness was provided by sputum and systemic exposure results from Phase 2 and 3 trials, TR02-112 and INS-212, respectively. Rich serum sampling from a comprehensive PK subset (INS-212) allowed for estimation of PK parameters using noncompartmental analysis in 13 patients on Day 1 and 12 patients at Month 3. In addition, sparse serum and urine sampling from 14 patients in Phase 2 Study TR02-112 and sparse serum only sampling from 39 patients in Phase 3 Study INS-212, together with the data from the comprehensive PK subset, were pooled to determine PK parameter values and exposure estimates of amikacin via population PK analysis. PK parameter estimates were broadly similar between the two methods. In addition, 58 patients with sputum samples from Study INS-212 were included for descriptive analyses of these airway levels over time. Results demonstrate that higher sputum concentrations and lower systemic concentrations/exposure are achieved with inhaled ALIS at 590 mg QD compared with the approved intravenous amikacin sulfate given QD. Systemic amikacin exposure from inhalation was approximately 15% (AUC_{0-24}) and 3% (C_{max}) of the systemic exposure following parenteral administration of amikacin sulfate at the approved dose of 15 mg/kg/day (Garraffo *et al*.). Alternatively, in sputum inhaled amikacin exposure was approximately 168% (C_{max}) of that following once daily parenteral administration of 30 mg/kg amikacin sulfate in CF adult patients (Byl *et al*.).

Because MAC are intracellular pathogens, the interpretation of sputum concentrations to predict drug efficacy is limited. However, the Applicant has provided preclinical studies looking at alveolar macrophage amikacin concentrations and antibacterial responses. *In vitro* work demonstrates greater amikacin uptake with ALIS compared to free amikacin (without liposomes) by approximately 4-fold in uninfected macrophages [Study VV-NC-000181]. In addition, broncho-alveolar cells (>80% macrophages) isolated from uninfected rats exposed to inhaled ALIS demonstrated similar enhanced amikacin levels (4-6 fold) within these isolated pulmonary cells compared with inhaled free amikacin despite a greater deposited dose (977 $\mu g \cdot g^{-1}$ ALIS vs 2283 $\mu g \cdot g^{-1}$ free amikacin [amikacin (b) (4) [Study VV-NC-000474]. Subsequently, an *in vitro* intracellular infection assay in macrophages demonstrated greater effectiveness with ALIS compared with free amikacin against MAC [Study OSU]. In addition, a mouse model of *Mycobacterium avium* lung infection with once daily inhaled ARIKAYCE at 76 mg/kg for 28 days demonstrated an approximately 2.5 Log_{10} reduction in bacterial lung burden that was comparable to that experienced with once daily intraperitoneal injections of amikacin sulfate at 100 mg/kg (mean difference (Log_{10} CFU/lung): 2.5 vs 2 respectively) [Study OSU 4257].

In summary, inhalation of ALIS achieved low systemic exposure, but high sputum concentrations that may support efficacy and use of ALIS in NTM MAC lung infected patients. However, the Clinical Pharmacology review team has the following considerations:

First, sputum exposure should be interpreted with caution due to the following uncertainties: **i)** how much drug is simply residual from oropharyngeal deposition; **ii)** how much drug is insoluble and thus inactive; **iii)** how much drug is freely available to kill bacteria and how much is inactivated via binding to sputum macromolecules (Bataillon *et al*., Mendelman *et al*., Levy *et al*); and **iv)** does the heterogeneity of the data (i.e., >100% inter-individual variability in observed sputum amikacin concentrations) represent different sampling areas of the lung and

thus introducing a biased analysis in the form of regional airway differences. It seems likely that subject effort/technique and duration of induction would influence sampling of large airways compared to peripheral airways based on expectorated and/or induced sputum methodology.

Second, a definitive analysis could not be performed to assess residual drug effects on culture results (i.e., culture positive/negative) for the following reasons: **i)** uncertainty in within-subject sputum heterogeneity (PK and microbiologic samples collected separately) and both expectorated and induced sputum collection methods with potential regional sampling differences limit an analysis of amikacin effects, and **ii**) uncontrolled differences in background antibacterial drugs with distinct lung penetration kinetics and bacterial susceptibilities. With that said, the Applicant has provided supportive *in vitro* data that demonstrate no drug effect on bacillary load in sputum at amikacin concentrations up to 128 $\mu g \cdot mL^{-1}$ incubated for 24 to 72 hours; matching collection to culture time (MAC MIC range: 0 to 128 $\mu g \cdot mL^{-1}$) (Eagle *et al*). These results are not surprising given the historical literature describing sputum antagonism via electrostatic interactions of the cationic aminoglycoside with polyanionic macromolecular components; effectively reducing antimicrobial activity 25- to 50- fold (Bataillon *et al*., Mendelman *et al*., Levy *et al*).

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The proposed dosing regimen for ALIS is once daily inhalation of one vial containing 590 mg amikacin in adult patients with NTM MAC lung disease. ALIS should be administered by oral inhalation using the Lamira™ Nebulizer System. This dosing regimen was evaluated in the following trials: Phase 2 efficacy/safety (TR02-112), Phase 3 efficacy/safety (INS-212), and Phase 3 safety (INS-312). The review team agrees that this is an acceptable dosing regimen in this patient population.

Dose selection for Phase 2 and Phase 3 efficacy trials was based on an earlier development program for ALIS use in CF and non-CF patients with *P. aeruginosa* lung infection. The Applicant found that in addition to approximately dose proportional increases in sputum concentrations, greater reductions in microbial lung burden were noted with 560 mg compared with 280 mg ALIS QD using the PARI eFLOW™ nebulizer system (TR02-107). Therefore, maximization of local exposures in the lung while minimizing systemic drug exposure was optimized with the larger dose. The 590 mg once daily dosage was subsequently selected for Phase 2 and 3 studies in NTM patients. While the Phase 2 (TR02-112) trial failed to meet the endpoint of a semi-quantitative measurement of the change in mycobacterial density, both the Phase 2 (TR02-112) and Phase 3 (INS-212) trials met the endpoint of culture conversion by Month 6. Refer to Section 7.2, “Review of Relevant Individual Trials Used to Support Efficacy,” for details on the efficacy evaluation of the proposed dosing regimen of ALIS in the phase 2 and phase 3 studies.

With the 590 mg QD ALIS dose regimen, systemic amikacin exposure after 3 months in a richly sampled sub-population (n=12) of Study INS-212, was estimated with a mean serum $AUC_{0\text{-}24}$ of 23.5 $mcg \cdot hr \cdot mL^{-1}$ (ranging from 8.0 to 46.5 $mcg \cdot hr \cdot mL^{-1}$) and mean C_{max} of 2.8 $\mu g \cdot mL^{-1}$ (ranging

from 1.0 to 4.4 $\mu g \cdot mL^{-1}$). Importantly, the maximum observed C_{max} and AUC_{0-24} are below the mean C_{max} of approximately 76 $\mu g \cdot mL^{-1}$ and AUC_{0-24} of 154 $\mu g \cdot hr \cdot mL^{-1}$ observed following intravenous administration of amikacin sulfate at the approved 15 mg/kg/day dose in healthy adults. This translates, on average, to an amikacin C_{max} of approximately 4% and AUC_{0-24} of approximately 15% of those following parentally administered amikacin.

Systemic drug exposure (i.e., C_{max} and AUC_{0-24}) from inhaled amikacin appears to minimize the risk of engendering concentration-related nephrotoxicity and ototoxicity associated with intravenous administration of amikacin. This is supported by the following: **i)** minimal (approx.15%) systemic amikacin exposure (i.e., AUC) with inhaled ALIS compared with parenteral amikacin sulfate and an estimated C_{max} following inhaled (2.8 mg/L) that is lower than the clinically accepted C_{trough} (5 to 10 mg/L) following intravenous amikacin (Jenkins *et al*), and **ii)** a low risk of concentration-dependent toxicities based on previous exposure-response relationships (Drusano *et al*. and Mondongo *et al*).

This was supported by the safety results from Phase 2 (TR02-112) and Phase 3 (INS-212) clinical trials demonstrating that nephrotoxicity risk was no greater in the add-on ALIS arm when compared with the multidrug regimen alone. Of note, tinnitus was observed more frequently in patients taking ALIS plus a background regimen (7.6%) compared with those receiving a background regimen alone (0.9%). This incongruity between serum amikacin concentration and tinnitus response may be related to the following predisposing factors: **i)** concomitant use of ototoxic drugs (e.g., additive effects with macrolides), **ii)** history of long-term ototoxic drug use (e.g., intravenous aminoglycoside use), **iii)** mitochondrial dysfunction secondary to disease (e.g., oxidative stress) or excitotoxicity injury (e.g., acoustic overexposure), or **iv)** genetic mutations. Unfortunately, this information is not available to evaluate. Alternatively, we note that ALIS is a liposomal formulation of amikacin and the precise offending agent and mechanism for tinnitus are currently unknown. Therefore, when taken collectively with the lack of patient risk factor information and given the unique amikacin formulation (i.e., ALIS) and method of delivery, available data preclude a complete clinical pharmacology evaluation and an ALIS-induced tinnitus effect should be considered given empirical observations in the clinical studies (Refer to Section 9.2, “Integrated Assessment of Safety”).

Other adverse events of interest (AEIs) were largely local to the lung and based on the route of administration (i.e., allergic alveolitis, dysphonia, cough, etc.). However, even in the Phase 2 trial, TR02-112, in which an inhaled placebo (empty liposomes) was administered, prevalence rates of these AEIs were greater with ALIS. Unfortunately, available data precluded a clinical pharmacology evaluation to relate exposure-response patterns to de-risk safety concerns. Refer to Section 9.2, “Integrated Assessment of Safety,” for details on the safety evaluation of the proposed dosing regimen of ALIS in the phase 2 and phase 3 studies.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

No alternative dosing regimens are required for specific populations based on intrinsic or extrinsic factors. Patients with known or suspected auditory or vestibular dysfunction should be closely monitored when taking ARIKAYCE. Close monitoring of patients with known or suspected renal dysfunction (including elderly) may be needed when prescribing ARIKAYCE.

Renal Impairment.

Amikacin is primarily eliminated via glomerular filtration and is therefore dependent on the kidney's functional capacity (i.e., renal clearance). However, the Applicant did not perform any simulations in patients with varying renal dysfunction; likely because no correlation was found between creatinine clearance and PK parameters in a pooled PK report (Trials TR02-112 and INS-212; estimated creatinine clearance (CL_{cr}) by Cockcroft-Gault > 49 mL·min^{-1}) (**Figure 1**). In the INS-212 Comprehensive PK subgroup, the median AUC_{0-24} of patients with CL_{cr} between 60 to 70 mL·min^{-1} (n=3/12) was 8.6 mg·h·L^{-1}. This lower than expected value is likely the result of the oral inhalation route and deposited dose/bioavailability challenges. Since systemic exposure (i.e., AUC) of amikacin via inhaled ALIS was estimated to be 15% of the approved 15 mg·kg^{-1}·day intravenous dose, the Clinical Pharmacology review team agrees that monitoring of renal function in patients with renal impairment adequately addresses the risk associated with no dose adjustment.

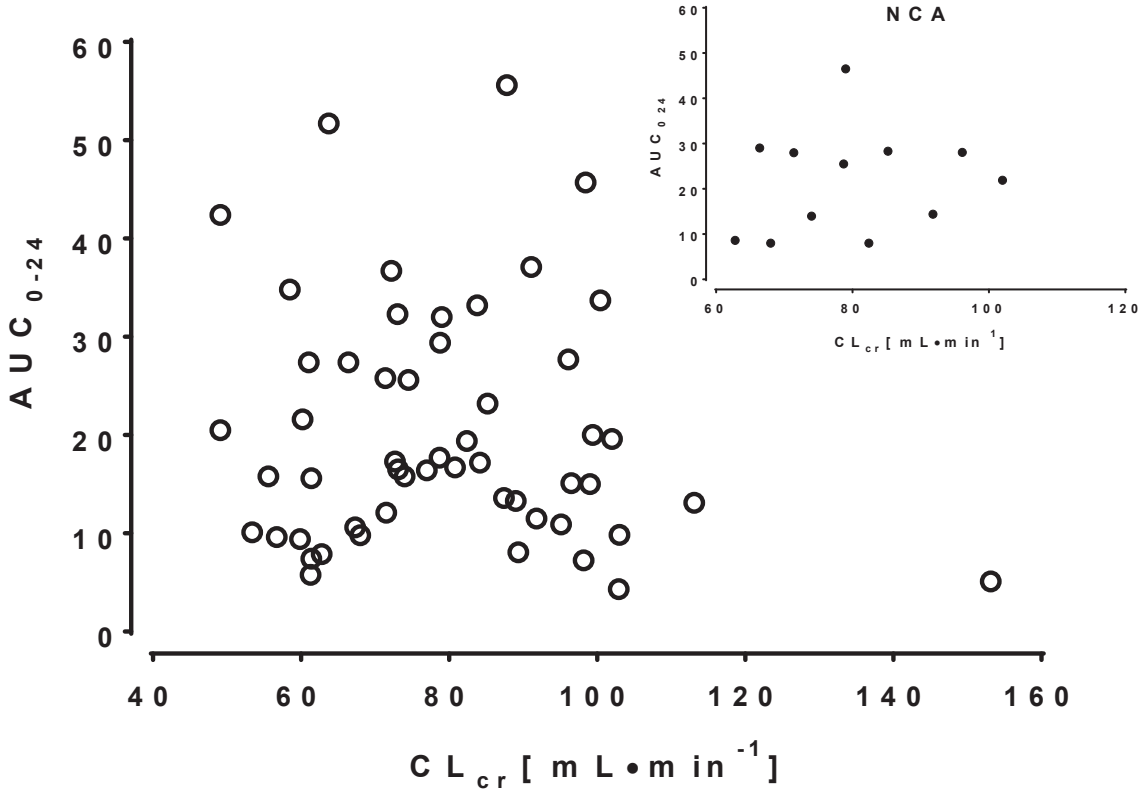


Figure 1: Correlation Between Amikacin Exposure (AUC_{0-24}) and Renal Function (Creatinine Clearance; CL_{cr}). Individual post-hoc estimates of AUC_{0-24} at steady state from Applicant's population PK model are plotted against CL_{cr} determined by Cockcroft and Gault method. The inlet plot is individual estimates by noncompartmental analysis from the 12 comprehensive PK sub-group population richly sampled after 590 mg ALIS QD for 3 months which should approximate steady state.

Source: Reviewer analysis of individual level data obtained from Applicant's PK dataset (ppkin1.xpt and pkexppk.xpt); NCA confirmed by applicant correspondence (ICPD 00494-1; Clinical information amendment; pk_wide_s212_intensive_subs_18jul2018.csv).

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Food Effect

Food intake is not expected to impact amikacin PK following oral inhalation of ALIS. Liposomes should be hydrolyzed and degraded by the harsh environment (gastric acid, bile salts, and lipases) found in intestinal fluids that will disrupt phospholipid and cholesterol composition (Wu *et al*). Furthermore, free amikacin has poor intestinal bioavailability (<1%) given its polar quaternary ammonium group (Deck *et al*.). This is supported by an oral absorption study in rats in which bioavailability was <0.3% after 50 mg/kg ALIS by oral gavage [AMIK-BRD-2007-04-PK]. Therefore, it is reasonable to assume that drug absorption from the gut is insignificant and food would not be expected to impact systemic drug exposures following inhalation of ALIS.

Drug-Drug Interaction

Clinical studies to characterize the drug-drug interaction potential of amikacin following inhalation of ALIS have not been conducted. However, amikacin via systemic administration has been used for more than 40 years, the potential for drug-drug interactions is generally well understood. While systemic amikacin exposure following inhalation of ALIS is approximately 15% of the systemic exposure (i.e., AUC) following intravenous administration of amikacin sulfate, ototoxicity such as tinnitus was more common in those patients receiving ALIS plus a background regimen compared to those only on a background regimen alone. This may suggest that despite lower systemic amikacin exposure co-administered drugs associated with neurotoxicity, nephrotoxicity, and ototoxicity should be avoided as additive pharmacological effects may be possible and unpredictable. Furthermore, while serum concentrations are low, it is unknown what levels are achieved within tissue compartments. Regarding PK drug interactions, diuretics can enhance aminoglycoside concentrations in tissues, concomitant use with ethacrynic acid, furosemide, urea, or intravenous mannitol should be avoided. Therefore, the same drug-drug interaction cautions from amikacin sulfate USP should apply to inhaled ALIS.

7 Statistical and Clinical Evaluation

7.1 Sources of Clinical Data and Review Strategy

7.1.1 Table of Clinical Trials

The efficacy and safety of ALIS in the treatment of MAC lung disease was evaluated in two randomized clinical trials and an open-label extension study. The key study in support of the efficacy of ALIS is Study INS-212, a Phase 3, open-label, randomized trial comparing ALIS added to a multi-drug background regimen versus multi-drug background regimen alone in subjects with refractory MAC lung infections. Supportive information is provided by Studies INS-312 and TR02-112. Study INS-312 is an ongoing open-label, single-arm extension study of patients from either arm of Study INS-212 who did not achieve culture conversion or who had a relapse or recurrence by Month 6 and primarily provides supportive safety data. Study TR02-112, is a Phase 2 study that enrolled subjects with refractory MAC or *M. abscessus* lung infections and included subjects with CF.

Table 6 provides an overview of these studies.

Table 6: Listing of Clinical Studies Relevant to NDA

Trial Identity	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients enrolled	Study Population	No. of Centers and Countries
Controlled Studies to Support Efficacy and Safety							
INS-212	Phase 3, open-label, randomized trial (ongoing)	ALIS 590 mg administered by inhalation QD Multi-drug background regimen composed of at least 2 antibacterial drugs based on ATS/IDSA guidelines	Primary: Sputum culture conversion by 6 months Key Secondary: 6MWT at 6 months	Treatment administered for a minimum of 8 months. Those with culture conversion completed a treatment course of 12 months from the first of 3 consecutive negative cultures. Subjects followed for 12 months off treatment.	ALIS plus multi-drug background regimen/ 224, multi-drug background regimen alone/ 112	Subjects with NTM lung infections caused by MAC that were refractory to treatment	127 sites in 18 countries

				Treatment Phase: baseline up to Month 16 Off-treatment phase: EOT up to Month 28			
TR02-112	Phase 2, randomized, double-blind, placebo-controlled phase followed by open-label extension phase	ALIS 590 mg administered by inhalation QD Placebo: sterile lipid carrier suspension administered by inhalation QD Multi-drug background regimen composed of at least 2 antibacterial drugs based on ATS/IDSA guidelines	Primary: Mycobacterial burden on the SQS Key Secondary: Negative sputum culture at Day 84	Randomized treatment for 84 days. Then open label ALIS plus multi-drug background regimen for all subjects for an additional 84 days. 12 months of follow-up after ALIS discontinuation	ALIS plus multi-drug background regimen/ 45, Placebo plus multi-drug background regimen / 45	Subjects with refractory MAC or *M. abscessus* lung infections and included subjects with cystic fibrosis.	18 sites in the United States and 1 site in Canada
Uncontrolled Safety Extension Study							
INS-312	Open-label extension of INS-212 (ongoing)	Same as INS-212	Primary: Safety Secondary: Sputum culture conversion	Up to 12 months of ALIS 1 month off-ALIS treatment for safety follow-up	ALIS plus multi-drug background regimen/ 133	Subjects from Study INS-212 who did not achieve culture conversion or who had a relapse or recurrence by Month 6	63 sites in 16 countries

QD: once daily; ATS/IDSA: American Thoracic Society/Infectious Diseases Society of America; 6MWT: 6-minute walk test; NTM: non-tuberculous mycobacterial; MAC: *Mycobacterium avium* complex; EOT: end of treatment; SQS: semi-quantitative scale

7.1.2 Review Strategy

Data Sources

This application was submitted in eCTD format. The sources of data used for the evaluation of efficacy and safety for ALIS included the study reports and data sets [Study Data Tabulation Model (SDTM) and Analysis Data Model (ADaM)] submitted by the Applicant which can be found at the following link: \\CDSESUB1\evsprod\NDA207356.

Data and Analysis Quality
The statistical and clinical review teams evaluated the data and analysis quality with assistance from the Office of Computational Science (OCS) through the JumpStart program. In general, the data submitted by the Applicant were acceptable. Minimal programming was needed to reproduce the results presented by the Applicant. Throughout the review, additional analysis datasets were requested from the Applicant to aid in the timeliness of the review.

7.2 Review of Relevant Individual Trials Used to Support Efficacy

7.2.1 Primary Endpoint for Phase 3 Trial (Study INS-212)

In the Phase 3 Study INS-212, the primary endpoint was sputum culture conversion, a microbiologic surrogate endpoint. A successful outcome was defined as 3 consecutive negative monthly sputum cultures at any time within the first 6 months of treatment.

We performed a review of the literature to assess the relationship between sputum culture conversion and clinical outcomes in patients with MAC lung disease. We reviewed the available information to assess whether the information supports a relationship between sputum culture conversion and clinical outcomes. We focused our review on studies that included patients with infections due to MAC only or those that included MAC along with other nontuberculous mycobacterial species.

There are limited data available, based mainly on retrospective, non-randomized studies or exploratory analyses from non-randomized subgroups that evaluate the relationship of sputum culture conversion and clinical outcomes. The main limitation of these studies is the difficulty in assessing if there are differences in patient characteristics between the converters and non-converters that might have an impact on the clinical outcomes. We examined the findings reported in each of 6 publications referenced in this document and also considered the strengths and limitations of the data or publications to address if there is a relationship between sputum culture conversion and clinical benefit.

Griffith et al. (2006) reported on a case series of 51 patients with macrolide-resistant MAC lung disease from a single institution suggesting that 1-year mortality was lower in those who converted to culture negative after salvage therapy that included but was not limited to injectable aminoglycosides and/or lung resection surgery compared to those who remained culture positive. Of note, patients had to be fit enough to undergo surgical resection and compliant enough to tolerate ≥6 months of injectable aminoglycoside therapy. In the group that converted, more patients underwent surgery and received an injectable for ≥6 months.

Thus, the assessment of outcomes is confounded by surgery and selection of patients who were surgical candidates and/or ability to tolerate ≥6 months of injectable aminoglycoside therapy. The report does not allow one to discern if culture negativity is a marker for an intrinsically less ill patient population or if the negative culture is leading to improved outcomes.

In a retrospective, non-randomized study of patients with pulmonary MAC lung disease, Ito et al. (2012) reported in a univariate analysis that 5-year mortality was lower in treated MAC patients who achieved sputum culture conversion; however, the result was not statistically significant. Of note, the authors clarified that some patients were left untreated due to lack of symptoms, patient refusal or severe disease suggesting that these patients were inherently different from those that were treated. The authors also note that ~62% of untreated patients had sputum culture conversion and that patients in the MAC-treated group had more cavitary lesions and positive smears than untreated patients, indicating that the two groups were likely not comparable. Also, if all 117 patients with microbiologic and survival outcome data were included in the analysis, the mortality rates were similar between the treated and untreated groups. The findings from this study suggest that sputum culture conversion may be associated with clinical benefit; however, the inability to convert to a negative sputum culture might reflect more severe disease or be a marker for a worse outcome.

A report on 180 patients from a single center in patients with nodular/bronchiectatic disease suggests an association between early improvement in a semi-quantitative sputum culture scale and subsequent long-term sputum culture conversion and improvement in sputum production and radiologic findings (Griffith 2015). This study was from a single center of a group of patients who were treated with a standard macrolide-containing regimen per the IDSA/ATS treatment guidelines and had 12 months of follow up. As the authors note, some aspects of the study can impact on the generalizability of the findings as the single center is highly experienced in the management of MAC lung disease and the study was limited to nodular/bronchiectatic MAC. As noted by Koh et al. (2017), treatment outcomes, relapse and reinfection may differ based on clinical phenotype of MAC lung disease (nodular/bronchiectatic (NB), fibrocavitary, or cavitary NB) and host factors.

Moon et al. (2016) describe 34 cases of macrolide-resistant MAC from a registry at a single institution. No evidence was provided in the study that achieving culture conversion translates to a clinical benefit/reduction in mortality. The authors note that patients with unfavorable outcomes were more likely to have a sputum smear positive for acid fast bacilli (AFB) at the time of detection of macrolide resistance.

In one randomized, open-label, multicenter trial that assessed two multidrug regimens for the treatment of NTM lung infections (including but not limited to MAC), the authors note that deaths attributed to NTM disease were more frequent in those who were still sputum culture positive for NTM after 12 months of therapy as compared with patients who converted to

sputum culture negative (Jenkins et al. 2008). Determining attributable mortality with any degree of certainty in this patient population can be difficult. The mortality analysis was based on the post-randomization event of sputum culture remaining positive at 12 months and not by the randomized group. Also, no difference was reported in all-cause mortality between patients who remained culture positive and those who became culture negative.

The goals of therapy as outlined by the American Thoracic Society/Infectious Disease Society of America (ATS/IDSA) are symptomatic, radiographic, and microbiologic improvement (Griffith et al. 2007). Though symptomatic improvement is important, differentiating the benefit of MAC treatment is complicated by progression or exacerbation of underlying lung diseases such as bronchiectasis and COPD. Similarly, although radiographic improvement is anticipated and desirable, assessment can be difficult both because of concomitant lung disease and limited potential improvement of MAC-related abnormalities. Additionally, the radiographic evolution of nodular/bronchiectatic MAC lung disease in either treated or untreated patients is not well defined. The microbiological goal is sustained culture conversion, defined as 12 months of consistently negative sputum cultures while on treatment.

In conclusion, there is paucity of high quality data that links the surrogate microbiologic endpoint of sputum culture conversion to clinical benefit, that is, how the patient feels, functions, or survives.

7.2.2 Study INS-212

Study Design and Endpoints

Study 212 is an ongoing randomized, open-label, multicenter study of ALIS in adult subjects with refractory MAC lung infections. Eligible subjects were males or females 18 years of age or older who were positive for MAC on sputum culture while being treated with a multi-drug background regimen (BR) of at least 2 antibacterial drugs for a minimum of 6 months. Multi-drug antibacterial treatment must have been either ongoing or stopped for no more than 12 months prior to screening. All subjects were required to have a MAC-positive culture at screening and at least one other positive culture within 6 months of screening but no less than 1 month apart. Subjects could have had a negative culture at the baseline visit but were still eligible to continue in the study.

Subjects were randomized 2:1 to ALIS administered once a day (QD) plus BR or BR alone. Randomization was stratified by smoking status (current smoker or not) and prior multi-drug regimen at screening (on treatment or off treatment for at least 3 months). Treatment continued for a minimum of 8 months and a maximum of approximately 16 months depending on the timing of culture conversion. Sputum culture results from baseline to Month 6 were made available to the investigative site in time for the Month 8 visit. At that time, subjects were assessed as converters or non-converters. A converter was defined as a subject who had MAC-negative sputum cultures for 3 consecutive months at any time within the first 6 months of the

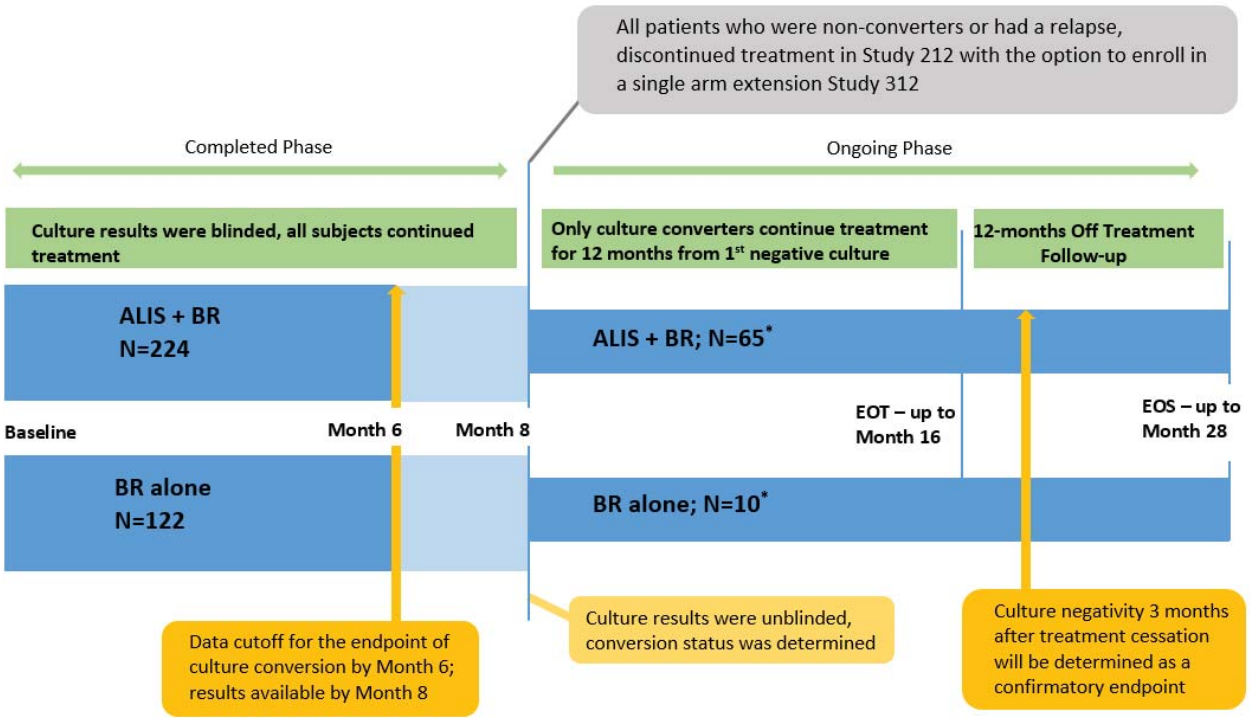
study. A non-converter was defined as a subject who did not have 3 consecutive monthly MAC-negative sputum cultures at any time within the first 6 months of the study. Relapse or recurrence was defined as having MAC-positive sputum cultures in liquid broth media (agar negative) for 3 or more consecutive months or having at least 1 MAC-positive sputum culture on solid media (agar positive) after achieving culture conversion.

If a subject demonstrated culture conversion within the first 6 months, the total duration of treatment was to be 12 months from the first of 3 negative cultures that defined culture conversion. If culture conversion was not achieved with 6 months of treatment, the subject was discontinued from the study at the Month 8 visit. Additionally, subjects who experienced a relapse or recurrence within 6 months of treatment were to be discontinued from the study at Month 8.

Study visits occurred monthly through Month 6, and at Months 8, 10, 12, 14, at end of treatment (EOT), and at 28 days, 3, 6, and 12 months off-treatment. At Month 8, when the culture conversion status was assessed, non-converters and those who experienced a relapse or recurrence within the first 6 months in either study arm were potentially eligible to enter Study INS-312 and initiate or continue ALIS.

If a subject prematurely discontinued the study prior to the Month 12 visit (excluding subjects enrolling in Study 312), the subject had an EOT visit and telephone contact at 28 days after the EOT visit, at Month 12 (counting from Day 1) for safety follow-up, and at 12 months after the EOT visit for safety follow-up and vital status. If a subject prematurely discontinued the study on or after the Month 12 visit, the subject had an EOT visit and telephone contact at 28 days after the EOT visit, and at 12 months after the EOT visit for safety follow-up and vital status.

Figure 2 is schematic representation of the study design.



EOT = End of Treatment; EOS = End of Study; BR = Background Regimen

* 65 and 10 are the number of subjects who were considered converters at 6 months, not all these subjects will be available for the long-term efficacy assessment 12 months off-treatment, due to study discontinuation.

Figure 2: Study INS-212 Study Design

The primary efficacy endpoint is the proportion of subjects achieving culture conversion by Month 6. Secondary efficacy endpoints include the change in six-minute walk test (6MWT) distance at Month 6, the proportion of subjects achieving culture conversion with durability after 3 months off treatment, time to culture conversion, proportion of subjects achieving culture conversion with sustainability at EOT, the change in 6MWT distance at EOT, and the change from baseline at Month 6 in the St. Georges Respiratory Questionnaire (SGRQ). Exploratory endpoints include the change in 6MWT distance at Month 6 for converters versus non-converters (for all subjects and by treatment arm), the change in BMI at Month 6, the change in 6MWT distance at Month 8 and 3 months off-treatment, the proportion of subjects achieving culture conversion with durability after 12 months off-treatment, change from baseline at Month 6 in the SGRQ-Part 2 (Activities of Daily Living), the change from Baseline at Month 6 and EOT in the EQ-5D-3L, the proportion of subjects who develop a new strain of MAC, radiological changes in chest CT scan at EOT, and mortality at the end of study.

During the design of the study, the endpoint of culture conversion by Month 6, which is a surrogate endpoint, was considered acceptable as the primary endpoint for an NDA submission requesting accelerated approval. Although there was a degree of uncertainty in this surrogate endpoint, the high unmet need in patients with refractory MAC lung disease was taken into

consideration. Additionally, there was the expectation of supportive efficacy in a clinical outcome, i.e., 6MWT (a key secondary endpoint). Furthermore, it was expected that durability of culture conversion 3 months after completion of MAC therapy would be established in the long-term.

Key Inclusion and Exclusion Criteria

The inclusion criteria included but were not limited to the following:

1. Male or female, 18 years of age or older.
2. Positive for MAC on culture while being treated with a multi-drug regimen (at least 2 antibacterial drugs) for a minimum duration of 6 consecutive months that was either ongoing or had been stopped no more than 12 months before Screening.
3. Diagnosed with MAC NTM lung infection with evidence of underlying lung disease such as nodular bronchiectasis and/or fibrocavitary disease by chest radiography or chest CT.
4. MAC lung infection documented by at least 2 positive cultures (MAC or mixed infection with MAC as the dominant species), consisting of at least 1 positive culture obtained within 6 months prior to Screening and 1 positive culture at Screening (cultures to be at least 1 month apart). Cultures were obtained from sputum or bronchoscopy.
5. Had a MAC-positive sputum at Screening.

The exclusion criteria included but were not limited to the following:

1. Subjects with cystic fibrosis.
2. Subjects whose MAC NTM infection was resistant to amikacin (as identified by MIC susceptibility > 64 mcg/mL).
3. Subjects who were not able to perform the 6MWT.
4. Prior exposure to ALIS.
5. Disseminated MAC infection.

Statistical Analysis Plan

Analysis Populations

The Intent-to Treat-(ITT) population consisted of the set of all randomized subjects. The primary efficacy analyses were conducted on the ITT population.

The Per-Protocol (PP) population was a subset of the ITT population that received at least 1 dose of randomized treatment, had 6MWT assessments at Baseline and Month 6, had at least 3 consecutive monthly sputum collections after baseline; at least 1 induced or at least 2 spontaneous sputum collections at each month, and compliance ≥60%. Subjects were excluded from the PP if they did not have a MAC-positive sputum at Screening, had an amikacin-resistant MAC NTM infection, or had a major protocol violation. Supportive efficacy analyses were conducted by the Applicant using the PP population. However, the focus of this efficacy review will be the ITT population only.

The Safety population was the set of all subjects who received at least one dose of randomized treatment.

Analysis Methods

There are two separately timed sets of analyses for Study INS-212. The first set of analyses, which is the subject of this NDA, are based on the endpoints assessed at Month 6. These endpoints were assessed after database lock on July 7, 2017 when the last enrolled subject completed their Month 6 visit and data for all subjects who completed Month 6 were available. The second set of analyses will be conducted at study completion on the remaining long-term endpoints.

The primary efficacy endpoint, the proportion of subjects achieving culture conversion by Month 6, was analyzed using a Cochran-Mantel-Haenszel (CMH) test stratified by smoking status and prior multi-drug regimen at a 2-sided significance level of 0.05. Subjects with missing sputum culture results for which culture conversion could not be assessed were considered as non-converters.

The secondary endpoints of 6MWT distance at Month 6, time to culture conversion, and SGRQ at Month 6 are assessed in a hierarchical manner, as listed.

The protocol-stated primary analysis of change from Baseline to Month 6 in 6MWT distance was based on a model under the pattern-mixture model framework. This model assumed that subjects on the ALIS + BR arm with missing data followed the response distribution of the BR alone arm. It involved 3 steps:

1. Using the available non-missing data of the BR alone arm, the posterior mean and covariance estimates were utilized to multiply impute missing data for both treatment arms.
2. The endpoint was then analyzed for each complete dataset with imputed data using an analysis of covariance (ANCOVA) with treatment arm and the randomization strata as factors and the baseline 6MWT distance as a covariate.
3. The treatment least squares mean differences was averaged and the associated standard errors summarized based on within-imputation and between-imputation variances and then yielded a final estimate of treatment difference with associated 95% confidence interval and p-value.

An additional analysis used a mixed model repeated measures (MMRM) analysis. The MMRM model included treatment, month, treatment by month interaction, and randomization strata as fixed factors, the baseline 6MWT distance as a covariate and the baseline 6MWT distance by month interaction. An unstructured covariance matrix was assumed. The between-treatment difference in least squares mean change from baseline to Month 6, the corresponding 95% confidence interval, and p-value were presented.

A supportive ANCOVA analysis of the change from Baseline to Month 6 in 6MWT distance with treatment arm and the randomization strata as factors and the baseline 6MWT distance as a covariate was also conducted. In this analysis, missing data were imputed using a last-observation-carried-forward (LOCF) approach. The LOCF value used was the last post-baseline value.

Time to culture conversion was analyzed using Kaplan-Meier methods as well as a Cox regression model to estimate the hazard ratio along with its 95% confidence interval and p-value.

The SGRQ domain scores and total score were analyzed using an MMRM of the change from baseline in the scores at Month 3 and Month 6 similar to that used in the additional analysis conducted for change from baseline in the 6MWT distance at Month 6.

Sample Size Calculation
The sample size was based on assuming culture conversion rates by Month 6 of 20% in the ALIS plus BR treatment arm and 5% in the BR alone treatment arm. With a 2:1 randomization, a sample size of 261 subjects (174 for ALIS plus BR and 87 for BR alone) would provide at least 90% power for a continuity-corrected Chi-square test at a 2-sided significance level of 0.05.

Sample size was also assessed for the secondary endpoint of change from baseline to Month 6 in 6MWT distance. Assuming a common standard deviation of 100 and a 2:1 randomization, a sample size of 192 subjects (128 for ALIS plus a multi-drug background regimen and 64 for the multi-drug background regimen alone) would provide at least 90% power to detect a between-treatment difference of 50 meters in mean change from Baseline to Month 6 in 6MWT distance using a two-arm t-test at the 2- sided significance level of 0.05.

Additionally, assuming the proportion of subjects achieving culture conversion with durability at 3 months off therapy is 16% for ALIS plus BR and 4% for the BR alone, and a 2:1 randomization ratio, a sample size of 261 subjects would provide at least 83% power for Fisher's exact test at the 2-sided significance level of 0.05.

To ensure that at least 261 evaluable subjects would reach Month 6, it was anticipated that approximately 351 subjects would need to be randomized.

Protocol Amendments
The original protocol was dated November 30, 2014 and was subsequently amended 3 times. The amendments were reviewed and accepted by us and considered to be unlikely to affect the integrity of the study.

Protocol Amendment 1 was dated January 16, 2015, prior to the enrollment of the first subject. The main changes to the protocol were to add comprehensive PK sampling in subjects enrolled in Japan, 6MWT at Month 4 and add pregnancy testing and method (serum or urine) for women of childbearing potential.

Protocol Amendment 2 was dated June 11, 2015. Major changes to the protocol included the following:

- The secondary endpoints were reordered to move up durability of sputum culture conversion at 3 months off all MAC treatment to the second secondary endpoint in

(b) (4)

- Added the exploratory objectives of difference in the 6MWT between converters and non-converters, BMI as a surrogate for clinical outcome, and durability of response after being off ALIS treatment for 12 months.
- Based on FDA recommendation, added that the site staff assessing the 6MWT must be blinded to the subject's open-label treatment assignment.

Protocol Amendment 3 was dated February 22, 2016. Major changes to the protocol included the following:

- Removed "(b) (4)" from the definition of culture conversion for the primary endpoint. This qualifying phrase was dropped from the definition because relapse to a baseline isolate can only be determined by genotyping which will not be assessed until the end of the study and not at the time of the Month 6 analyses.
- Primary and sensitivity analysis methods for the change from baseline to Month 6 6MWT distance were switched based on FDA recommendation.
- Clarified based on FDA recommendation that, "The final analyses of the primary efficacy endpoint, the change from Baseline to Month 6 in 6MWT distance, and time to culture conversion by Month 6 will be performed when the last subject completes Month 6 and data for all subjects who have completed Month 6 are available. These final analyses will be performed prior to the completion of the study."

Compliance with Good Clinical Practices

The Applicant states in the clinical study report that, "This study was designed and monitored in accordance with Sponsor procedures, which comply with the ethical principles of Good Clinical Practice (GCP) as required by the major regulatory authorities, and in accordance with the Declaration of Helsinki."

Financial Disclosure

The Applicant, Insmed Inc, has provided financial certification/disclosure information for all clinical investigators and sub-investigators involved in Study (b) (6). The Financial Disclosure information for Study (b) (6), were compiled through the date of July 7, 2017, the data cut-off day for this Subpart H NDA submission. An FDA Form 3454 "Certification: Financial Interests and Arrangements of Clinical Investigators" was provided for the three NTM trials (b) (6) There were nine investigators and sub-investigators who had disclosable information based on the criteria defined in 21 CFR 54.4(a)(3)(i-v). The nature of the disclosures for these 9 individuals were presented by the Applicant and are summarized below.

Table 7: Financial Disclosures for Investigators/Sub-investigators of Study (b) (6)

Investigator/ sub-investigator	Nature of Disclosure
(b) (6)	Participated on advisory board and in NTM

	symposium; general advisory role
(b) (6)	Consulting; grant work through (b) (6) (b) (6)
	Participated in NTM symposium and NTM microbiologists meeting; was provided speaker fees
	Consulting and grant work through (b) (6) (b) (6)
	Participated in (b) (6) meeting, expert advisory panel, board meeting, and was provided speaker fee
	Speaker fees, grant work through (b) (6) (b) (6)
	Participated in advisory panel meeting
	Consulting; grant work through (b) (6) (b) (6)
	Consulting; steering committee member

(b) (6)

Patient Disposition

A total of 336 subjects were randomized and comprise the ITT population: 224 on ALIS plus BR and 112 on BR alone. The Safety population consists of all but one subject randomized to the ALIS plus BR arm who did not receive treatment.

At the time of the initial analysis supporting this NDA, subjects were on treatment, had completed treatment as defined in the protocol, or had discontinued treatment prematurely. A subject was considered as having completed treatment as defined in the protocol if they: 1) were a converter who successfully completed 12 months of their treatment regimen from the first of 3 negative cultures used to define conversion, or 2) were a non-converter who successfully completed all dosing and protocol requirements up to and including the Month 6 study visit. Of the 336 subjects in the ITT population, 84 discontinued treatment prematurely (75 on ALIS plus BR and 9 on BR alone), 185 completed treatment as defined in the protocol (106 on ALIS plus BR and 79 on BR alone), and 77 were ongoing treatment (43 on ALIS plus BR and 24 on BR alone) at the time of data cutoff.

As noted in Table 8, four times as many subjects discontinued ALIS plus BR as compared to BR alone. The most common reasons for discontinuing treatment prematurely in the ALIS + BR arm were adverse events (17.4%) and withdrawal by subject (9.4%). In the BR alone arm, the most common reason for discontinuing treatment prematurely was withdrawal by subject (5.4%).

Table 8: Subject Disposition- Study INS-212

	ALIS plus BR	BR alone
Randomized (ITT)	224	112
Safety	223	112
Completed treatment as defined in protocol	106 (47.3%)	79 (70.5%)
Ongoing treatment	43 (19.2%)	24 (21.4%)
Discontinued treatment prematurely	75 (33.5%)	9 (8.0%)
Reason for treatment discontinuation		
AE	39 (17.4%)	1 (0.9%)
Withdrawal by subject	21 (9.4%)	6 (5.4%)
Death	5 (2.2%)	2 (1.8%)
Physician decision	3 (1.3%)	0
Rescue medication	3 (1.3%)	0
Other	2 (0.9%)	0
Protocol deviation	1 (0.4%)	0
Non-compliance with study medication	1 (0.4%)	0

Source: Reviewer Table

Protocol Violations/Deviations

Protocol deviations are summarized in Table 9. At least one protocol deviation was reported in a slightly higher percentage of ALIS plus BR subjects (83.9%) compared to BR alone subjects (78.6%). The percentage of major protocol deviations were similar: 21.4% of subjects in the ALIS plus BR regimen arm and 22.3% of subjects in the BR alone arm. The most common reason for a major protocol deviation was related to informed consent procedures (i.e., incorrect version of approved informed consent form signed).

Table 9: Subjects with Protocol Deviations (ITT Population)- Study INS-212

	ALIS plus BR (n=224)	BR alone (n=112)
At Least One Protocol Deviation	188 (83.9%)	88 (78.6%)
Minor	187 (83.5%)	88 (78.6%)
Major	48 (21.4%)	25 (22.3%)
Type of Major Protocol Deviation		
Informed Consent Procedures	26 (11.6%)	14 (12.5%)
Non-compliance with study protocol	14 (6.3%)	5 (4.5%)
Eligibility criteria not met	7 (3.1%)	5 (4.5%)
Safety reporting	4 (1.8%)	5 (4.5%)
Non-compliance with study product	1 (0.4%)	0

Source: Reviewer Table

Note: Subjects could have more than 1 protocol deviation

Baseline and Demographic Characteristics

Baseline and demographic characteristics were generally similar across treatment groups and are summarized in Table 10. The mean age of the subjects was 64 years. Approximately 70% of the subjects were female with a slightly higher percentage of females randomized to the ALIS plus BR arm (73.7%) than to the BR alone arm (60.7%). The majority of the subjects were white (69.9%). Approximately 42% of the subjects were from the United States. Most of the subjects (approximately 90%) were on a multi-drug regimen treatment at Screening and were not a current smoker.

Table 10: Baseline and Demographic Characteristics (ITT Population)- Study INS-212

	ALIS plus BR (n=224)	**BR alone** (n=112)
Age (years)		
mean (sd)	64.6 (9.6)	64.9 (10.2)
median	65	66
min, max	40, 87	32, 85
Sex, n (%)		
Male	59 (26.3)	44 (39.3)
Female	165 (73.7)	68 (60.7)
Race, n (%)		
White	158 (70.5)	77 (68.8)
Asian	58 (25.9)	25 (22.3)
Black	3 (1.3)	3 (2.7)
Other	2 (0.9)	1 (0.9)
Not recorded	3 (1.3)	6 (5.4)
Region, n (%)		
United States	93 (41.5)	48 (42.9)
Asia	48 (21.4)	20 (17.8)
Rest of the world	83 (37.1)	44 (39.3)
Multi-drug regimen at Screening, n (%)		
On treatment	201 (89.7)	101 (90.2)
Off treatment for at least 3 months	23 (10.3)	11 (9.8)
Smoking status, n (%)		
Current smoker	26 (11.6)	10 (8.9)
Not a current smoker	198 (88.4)	102 (91.9)

Source: Reviewer Table

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Treatment Compliance

Table 11 summarizes treatment compliance from baseline to Month 6 and to the Data Cut-off Date for the ALIS plus BR arm of the study.

Table 11: Treatment Compliance from Baseline to Month 6 and up to the Data Cut-off Date for ALIS plus BR arm of Study INS-212

% Compliance[a]	Month 6 N=223	Data Cut-off date* N=223
>120%	1 (0.4%)	1 (0.4%)
80-120% (inclusive)	91 (40.8%)	149 (66.8%)
<80%	131 (58.7%)	73 (32.7%)

Source: Modified from Applicants Table 14.1.9.1 in Study INS-212 Clinical Study Report

[a]Compliance (%) = (Actual number of vials used)/(Prescribed number of vials to be used) × 100%. Prescribed number of vials to be used = Number of days study medication is to be taken (based on the duration of treatment)

*Data cut-off date for Study INS-212 was July 7, 2017 when the last patient in the study completed the Month 6 visit

Reviewer's comment: The Applicant points out that the reason for >50% of patients in the ALIS arm having <80% compliance was due to prescriber-initiated treatment interruptions that were outlined in the protocol in the event of adverse reactions. The main TEAEs that warranted temporary interruption were dysphonia, dyspnea, cough and hemoptysis.

Concomitant Medications

Table **12** summarizes pertinent concomitant medications use by more than 10% of the study population.

Table 12: Pertinent Concomitant Medications Used by More than 10% of Study INS-212 Participants

ATC Class 1/ Preferred Term	**ALIS plus BR N = 224)**	**BR Alone N = 112**
Patients reporting at least 1 Concomitant Medication	223 (99.6%)	111 (99.1%)
Antibacterial drugs	210 (93.8%)	102 (91.1%)
Azithromycin	130 (58.0%)	60 (53.6%)
Clarithromycin	88 (39.3%)	43 (38.4%)
Rifampicin	165 (73.7%)	83 (74.1%)
Rifabutin	32 (14.3%)	14 (12.5%)
Ethambutol	167 (74.6%)	75 (67.0%)
Ethambutol hydrochloride	20 (8.9%)	12 (10.7%)
Clofazimine	31 (13.8%)	19 (17.0%)

Fluoroquinolones	61 (27.2%)	41 (36.6%)
Combinations of penicillins, including beta-lactam-lactamase inhibitors	30 (13.4%)	12 (10.7%)
Selective beta-2 adrenoreceptor agonists	117 (52.2%)	43 (38.4%)
Corticosteroids	24 (10.7%)	12 (10.7%)
Glucocorticoids	62 (27.7%)	21 (18.8%)
Adrenergics in combination with corticosteroids or other drugs excluding anticholinergics	52 (23.2%)	28 (25.0%)
Anticholinergics	55 (24.6%)	25 (22.3%)
Adrenergics in combination with anticholinergics	31 (13.8%)	12 (10.7%)

Source: Modified from Applicant's Table 10 in Study INS-212 Clinical Study Report
ALIS = amikacin liposome inhalation suspension (590 mg); ATC = anatomical therapeutic chemical
Note: At each level of summation (overall, ATC class 1, ATC class 2), patients reporting more than 1 medication are counted only once. Concomitant medications are coded by using WHO Drug Dictionary version September 1, 2014.

Rescue Medication Use

Rescue medications were defined as aminoglycoside drugs with activity against MAC. This includes the intravenous formulation of amikacin used intravenously or nebulized, streptomycin or kanamycin.

Table 13 summarizes the number of patients who required rescue medication between Day 1 and the Month 6 visit (Day 186) by study arm. Patients with a missing start date of rescue medication were counted as being treated between Day 1 and Month 6.

Table 13: Study INS-212 Patients that Required Rescue Medications Between Day 1 to Month 6 Visit

	ALIS plus BR N = 224 n (%)	BR Alone N = 112 n (%)
Patients with at least 1 rescue medication use	32 (14.3)	12 (10.7)
Amikacin	25 (11.2)	10 (8.9)
Kanamycin	4 (1.8)	0
Streptomycin	4 (1.8)	3 (2.7)

Reviewer's comments: Twenty five of the 32 patients in the ALIS plus BR arm, and 11 of the 12 patients in the BR alone arm had missing start date information for the rescue medication; for the review, patients with missing start dates were presumed to have been treated between Day 1 and the Month 6 visit. Of note, the Applicant only reported 3 patients (1.3%) in the ALIS plus BR arm as discontinuing the study due to use of rescue medication. Use of rescue medication was a study discontinuation criterion.

Overall, slightly more ALIS-treated patients received rescue medication compared to the BR alone arm; however, use of rescue medication is unlikely to have altered the conclusions of the study.

Efficacy Results-Primary Endpoint

The primary efficacy endpoint was the proportion of subjects with culture conversion by Month 6 in the ALIS plus BR arm compared to the BR alone arm. These results are summarized in Table 14. Significantly more subjects achieved culture conversion by Month 6 in the ALIS plus BR arm (29.0%) compared to the BR alone arm (8.9%).

The definition of culture conversion required 3 consecutive monthly negative sputum cultures during the first 6 months of the study. However, it was possible that after meeting this definition, a subject could have recurrence of MAC by Month 6. Therefore, a sensitivity analysis was conducted by treating a subject who achieved culture conversion but met the protocol definition of recurrence by Month 6 as a failure. Three subjects in each treatment arm met the protocol definition of recurrence by Month 6. Based on the sensitivity analysis, 27.7% of subjects in the ALIS plus BR arm compared to 6.3% of subjects in the BR alone arm achieved culture conversion.

Table 14: Culture Conversion by Month 6 (ITT Population)- Study INS-212

	ALIS plus BR (n=224)	**BR alone** (n=112)
Primary analysis		
Converter	65 (29.0%)	10 (8.9%)
Non-converter	159 (71.0%)	102 (91.1%)
CMH p-value*	p< 0.0001	
Adjusted odds ratio (95% CI)	4.22 (2.08, 8.57)	
Weighted difference (95% CI)**	20.5 (12.2, 28.7)	
Sensitivity Analysis* **		
Converter	62 (27.7%)	7 (6.3%)
Non-converter	162 (72.3%)	105 (93.7%)
CMH p-value*	p< 0.0001	
Adjusted odds ratio (95% CI)	5.71 (2.53, 12.89)	
Weighted difference (95% CI)**	21.7 (14.0, 29.4)	

Source: Reviewer Table

*CMH p-value and adjusted odds ratio (ALIS plus BR/ BR alone) are calculated using the Cochran-Mantel Haenszel test stratified by smoking status and multi-drug regimen at Screening. An adjusted odds ratio >1 indicates a response of conversion is more likely for subjects on ALIS plus BR compared to those on BR alone
** Difference (ALIS plus BR – BR alone) in proportion of subjects with culture conversion weighted by stratification factors of smoking status and multi-drug regimen at Screening.
*** Analysis treats subjects who reached the protocol definition of recurrence by Month 6 as failures. 3 subjects on each treatment arm had a recurrence by Month 6 after achieving culture conversion.

The following figure summarizes the cumulative proportion of subjects achieving culture conversion by the month of the first negative culture of the 3 consecutive negative cultures that was needed to define culture conversion. Data is shown through Month 4 since the first of the 3 negative cultures had to occur by Month 4 for a subject to be considered as having achieved culture conversion by Month 6. Note that approximately 5% of subjects in both arms had their first negative culture at the Baseline visit.

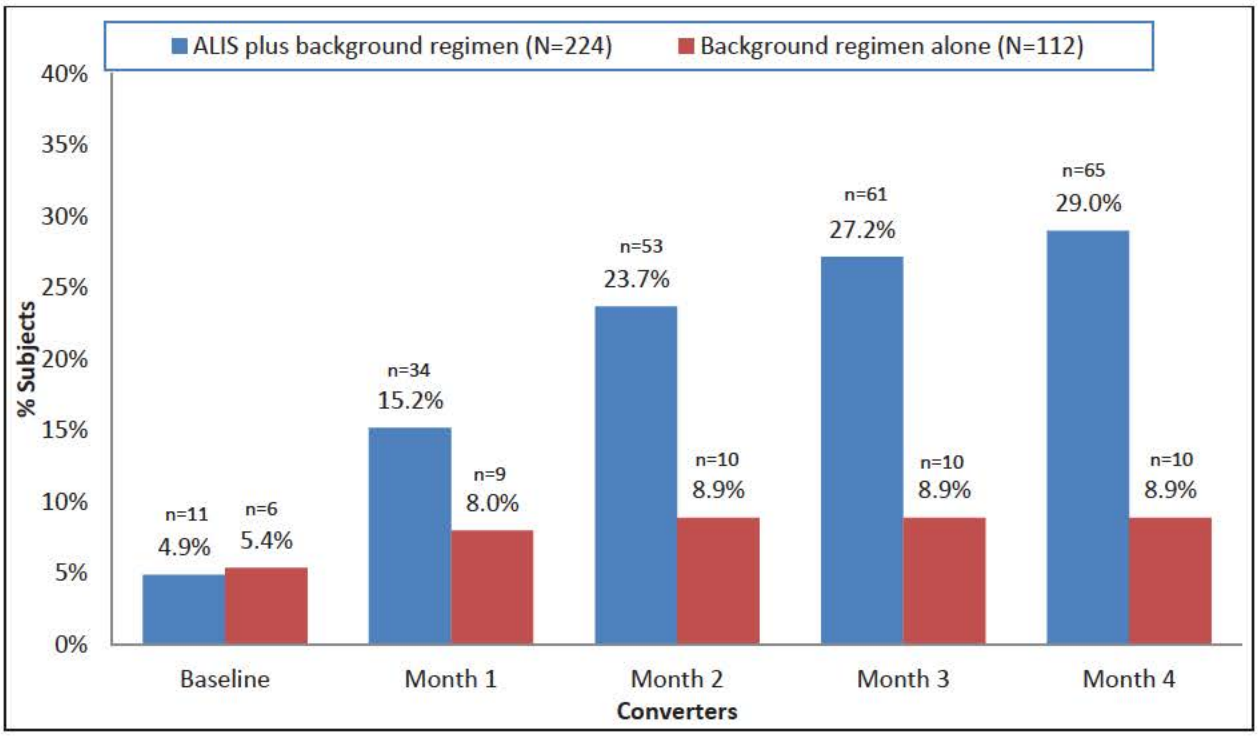


Figure 3: Cumulative Proportion of Subjects Achieving Culture Conversion by Month of First Negative Culture Used to Define Conversion (ITT Population)- Study INS-212

Source: Adapted from Applicant's Figure 3 from Study INS-212 clinical study report

Efficacy Results-Secondary and Other Relevant Endpoints

Change from Baseline to Month 6 in the 6MWT distance is a key secondary endpoint. The protocol-defined primary analysis for this endpoint was to be an ANCOVA with missing data imputed using pattern-mixture modelling. However, the clinical study report presents the primary results using an analysis based on a MMRM with pattern-mixture modeling. Furthermore, upon review of the data used for the analyses presented in the clinical study

report, it was noted that the Applicant only used Month 6 values that were collected while the subject was still on randomized treatment and not all collected 6MWT values. An information request was sent to the Applicant on July 5, 2018 requesting that the analyses for 6MWT distance be redone using any available Month 6 value, before imputation, regardless of whether the subject had discontinued treatment, to submit an updated 6MWT analysis dataset based on this, and to submit the results of the analysis based on the stated final protocol/statistical analysis plan method. The response to the information request was received on July 12, 2018. All 6MWT analyses presented in this review are based on the revised 6MWT analysis dataset (ADSMWT) and presented in the July 12, 2018 response to the information request.

Table 15 summarizes the results of the protocol specified analyses for the 6MWT distance. Regardless of the method used, the LS mean change from baseline at Month 6 was negative for subjects in the ALIS plus BR and numerically worse than subjects on BR alone. The difference (ALIS plus BR minus BR alone) in LS means ranged from -3.0 to -4.8 meters and was not statistically significant.

Table 15: 6MWT distance (ITT Population)- Study INS-212

		ALIS plus BR	BR alone
ANCOVA using pattern-mixture modeling	**Baseline[1]** mean (sd) **Month 6** mean (sd)	n=223 424.2 (128.9) 422.1 (143.1)	n=112 421.0 (125.9) 422.2 (136.3)
	Change from Baseline to Month 6[2] LS mean (se) 95% CI of LS mean	 -1.5 (11.3) (-23.5, 20.6)	 1.5 (12.1) (-22.2, 25.3)
	Difference[3] in LS mean (95% CI) **p-value of LS Mean Difference**	-3.0 (-20.6, 14.7) 0.7394	
MMRM	**Baseline[4]** mean (sd) **Month 6** mean (sd)	n=179 438.6 (118.9) n=167 442.1 (123.5)	n=106 425.2 (122.9) n=103 427.5 (131.4)
	Change from Baseline to Month 6[5] LS mean (se) 95% CI of LS mean	 -9.8 (11.2) (-31.8, 12.3)	 -6.0 (12.2) (-30.0, 18.0)
	Difference in LS mean (95% CI) **p-value of LS Mean Difference**	-3.8 (-21.1, 13.5) 0.6660	
ANCOVA with LOCF	**Baseline[6]** mean (sd) **Month 6** mean (sd)	n=193 434.3 (119.1) 426.3 (135.1)	n=106 425.2 (122.9) 422.8 (132.7)
	Change from Baseline to Month 6[7] LS mean (se)	 -10.4 (12.8)	 -5.6 (13.9)

		ALIS plus BR	BR alone
	95% CI of LS mean	(-35.5, 14.7)	(-32.9 21.6)
	Difference in LS mean (95% CI) **p-value of LS Mean Difference**	-4.8 (-23.0, 13.5) 0.6084	

Source: Adapted from Applicant's Tables 14.2.2.1.PM_6m, 14.2.2.2a, and 14.2.2.3a from response to information request dated 7/12/18

[1] One subject in the ALIS plus BR arm did not have any 6MWT values and was therefore not imputed

[2] Statistics are obtained from a ANCOVA model with pattern-mixture modeling of missing values due to dropout, which includes treatment, and the combination of smoking status and prior multi-drug regimen as fixed factors, and the baseline 6MWT distance as a covariate

[3] Difference is (ALIS plus BR - BR alone)

[4] For baseline, n is number of subjects with a baseline score and either a Month 4 or Month 6 value. For Month 6, n is the number of subjects with a baseline and Month 6 value.

[5] Statistics are obtained from a mixed model repeated measures model which includes treatment, month, the treatment-by-month interaction, and the combination of smoking status and prior multi-drug regimen as fixed factors, the baseline 6MWT distance as a covariate and baseline 6MWT distance-by-month interaction

[6] n is the number of subjects with a baseline and Month 6 score after imputing missing Month 6 score with a last post-baseline observation

[7] Statistics are obtained from a ANCOVA model with LOCF, which includes treatment, and the combination of smoking status and prior multi-drug regimen as fixed factors, and the baseline 6MWT distance as a covariate

Missing 6MWT results at Month 6, however, complicate the interpretation of these results. There was an imbalance between treatment groups in the amount of missing data at Month 6. Approximately 25% of the ALIS plus BR subjects compared to 8% of the BR alone subjects did not have a Month 6 6MWT result. Additionally, most of the missing data is due to discontinuation from the study because of adverse events or withdrawal by the subject. It is not clear how discontinuation due to these various reasons might impact a future 6MWT result. The pattern mixture model via multiple imputation imputes all missing data by the distribution of the non-missing subjects from the BR alone arm. As such, it assumes discontinuation in the ALIS plus BR arm would mimic the average person in the BR alone arm. As many of the subjects who discontinued from the ALIS plus BR arm did so due to pulmonary-associated AEs, this may not be a reasonable assumption.

Given these concerns, additional analyses were conducted trying to account for all missing data with various assumptions. In the first analysis, imputation was as follows: all subjects who died prior to Month 6 and those who discontinued due to an AE or withdrawal by subject and did not have a post-baseline value had an imputed Month 6 value of 0, subjects without a post baseline value had an imputed Month 6 value equal to the baseline value, and otherwise a post-baseline value was carried forward. In the second analysis, imputation was as above with the exception that all subjects who discontinued due to an AE or withdrawal had an imputed Month 6 value of 0. Descriptive results of the mean change from baseline for these analyses are summarized in Table 16. As one would expect given the imbalance in the amount of missing data between treatment groups and a highly conservative assumption of 0 meters walked at Month 6 for much of the missing data particularly in the ALIS plus BR arm, an extreme decrease in the change from baseline is noted and the difference between treatment groups increases.

Table 16: Additional Analyses of Change from Baseline to Month 6 6MWT Distance (ITT Population)- Study INS-212

Change from Baseline to Month 6	ALIS plus BR (n=220)	BR alone (n=111)
Additional Analysis 1 Mean (sd)	-46.1 (143.3)	-18.3 (113.0)
Additional Analysis 2 Mean (sd)	-75.2 (168.5)	-18.3 (113.0)

Source: Reviewer Table
Note: 4 subjects on the ALIS plus BR and 1 subject on the BR alone did not have a baseline value and were therefore not included in the analysis.

An exploratory analysis was an analysis of the change from baseline to Month 6 in 6MWT distance by converter status. This analysis was prespecified by the Applicant because they believed that improvements in 6MWT reflect successful microbiological conversion and not treatment with antibacterial drugs per se. However, converter status is a post-treatment classification and analyses of 6MWT by converter status are not a direct comparison of the effect of treatment. Therefore, the analysis by converter status cannot be fully understood since both converter status and 6MWT distance are outcome variables. Table 17 is a descriptive summary of the mean change from baseline to Month 6 by converter status for each treatment arm. Only subjects who had both baseline and Month 6 6MWT results are included in this analysis. Therefore, interpretation of these results is further clouded by the amount of missing data, particularly, the almost 40% of missing data in the non-converter/ALIS plus BR group. Though based on this analysis it appears that converters have improved 6MWT distance compared to non-converters, treatment with ALIS was not able to show this benefit in the overall population despite having an increased proportion of converters.

Table 17: Change from Baseline to Month 6 6MWT distance by Converter Status (ITT population)- Study INS-212

Change from Baseline to Month 6	ALIS plus BR	BR alone
Converter	(n=63)	(n=9)
Mean (sd)	13.6 (60.5)	27.7 (42.7)
Non-converter	(n=104)	(n=94)
Mean (sd)	-13.4 (68.0)	0.5 (74.9)

Source: Reviewer Table
Note: n is the number of subjects with a baseline and Month 6 6MWT.

The change in total SGRQ score from baseline to Month 6 was an additional secondary endpoint. The results as reported by the Applicant are summarized in Table 18. There was a numerical difference in favor of the ALIS plus BR group as compared to the BR alone. However, given the position of this endpoint in the hierarchical evaluation of secondary endpoints, the difference is not statistically significant. Additionally, the SGRQ has not been validated in

patients with NTM lung disease but a minimally important difference of 4 has been accepted for disease states in which the SGRQ has been validated and this was also not met. More importantly, it is questionable whether these results can be interpreted at all given the amount of and imbalance in missing data at both Baseline (16% vs. 6%, respectively) and Month 6 (28% and 8%, respectively) between ALIS plus BR and BR alone.

Table 18: Change from Baseline to Month 6 in SQRQ (ITT population)- Study INS-212

	ALIS plus BR	BR alone
Baseline[1]	n=189	n=105
mean (sd)	37.9 (21.4)	38.5 (21.5)
Month 6	n=161	n=103
mean (sd)	38.8 (22.6)	37.1 (23.9)
Change from Baseline to Month 6[2]		
LS mean (se)	4.2 (2.0)	0.4 (2.2)
95% CI of LS mean	(0.2, 8.2)	(-4.0, 4.7)
Difference [3] in LS mean (95% CI)	3.8 (0.7, 6.9)	
p-value of LS Mean Difference	0.0177	

Source: Adapted from Applicant's Table 14.2.4 from INS-212 clinical study report
[1] For baseline, n is the number of subjects with a baseline and at least 1 post-baseline score. For Month 6, n is then number of subjects with a baseline and Month 6 score.
[2] Statistics are obtained from a mixed model repeated measures model which includes treatment, month, the treatment-by-month interaction, and the combination of smoking status and prior multi-drug regimen as fixed factors, the baseline 6MWT distance as a covariate and baseline 6MWT distance-by-month interaction
[3] Difference is (ALIS plus BR - BR alone)

Findings in Special/Subgroup Populations

Gender, Race, Age, and Geographical Region

Subgroup analyses of culture conversion by Month 6 were conducted by gender, race, age, and geographic region and are presented in Table 19. In general, the results across subgroups were consistent with that observed for the overall population.

Table 19: Culture Conversion by Month 6 by Gender, Race, Age, and Geographical Region (ITT Population) -Study INS-212

	ALIS plus BR (n=224)	BR alone (n=112)	Difference (95% CI)* p-value**
Gender			
Male	10/59 (17.0%)	4/44 (9.1%)	7.9 (-6.9, 22.7) 0.2408
Female	55/165 (33.3%)	6/68 (8.8%)	24.5 (13.6, 35.4) <0.0001
Race			
White	54/158 (34.2%)	9/77 (11.7%)	22.5 (11.2, 33.8) 0.0001

	ALIS plus BR (n=224)	BR alone (n=112)	Difference (95% CI)* p-value**
Non-White	11/66 (16.7%)	1/35 (2.9%)	13.8 (1.0, 26.6) .0242
Age			
<65 years	31/105 (29.5%)	5/52 (9.6%)	19.9 (6.6, 33.2) 0.0031
≥65 years	34/119 (28.6%)	5/60 (8.3%)	20.3 (8.3, 32.3) 0.0010
Region,			
United States	29/93 (31.2%)	5/48 (10.4%)	20.8 (6.4, 35.2) 0.0040
Asia	10/48 (20.8%)	0/20	20.8 (5.8, 35.8) 0.0056
Rest of the world	26/83 (31.3%)	5/44 (11.4%)	19.9 (4.5, 35.3) 0.0091

Source: Reviewer Table
*Difference of (ALIS plus BR - BR alone) with 95% confidence interval
** p-value based on Chi-square test

Other Subgroups of Interest
Other subgroups of interest include the stratification factors: multi-drug regimen at Screening and smoking status. Table 20 summarizes the results by these stratification factors for culture conversion by Month 6. As 90% of the subjects were on a multi-drug regimen at Screening and not a current smoker, these results are consistent with the overall population. Given the small sample sizes for the off treatment and the current smoker strata, results for these strata must be interpreted with caution.

Table 20: Culture Conversion by Month 6 by Stratification Factors (ITT Population) -Study INS-212

	ALIS plus BR	BR alone	Difference (95% CI)* p-value**
Multi-Drug Regimen at Screening			
On treatment	58/201 (28.9%)	9/101 (8.9%)	20.0 (10.9, 29.1) <0.0001
Off treatment	7/23 (30.4%)	1/11 (9.1%)	21.3 (-10.8, 53.3) 0.1443
Smoking Status			
Current Smoker	2/26 (7.7%)	1/10 (10.0%)	-2.3 (-30.5, 25.9) 0.8254
Not a Current Smoker	63/198 (31.8%)	9/102 (8.8%)	23.0 (13.8, 32.2) <0.0001

Source: Reviewer Table
*Difference is (ALIS plus BR - BR alone) with 95% confidence interval

** p-value based on Chi-square test

7.2.3 Study TR02-112

Study Design and Endpoints
Study TR02-112 was a randomized placebo-controlled Phase 2 trial. It consisted of a double-blind placebo-controlled phase through Day 84 followed by an open-label extension phase for an additional 84 days. In the double-blind phase, patients were randomized (1:1) to receive either ALIS 590 mg daily (qd) in addition to BR or placebo (empty liposomes) in addition to BR. During the open-label phase, all patients received ALIS in addition to BR. The open label extension phase was followed by a 12-month post-treatment observational period.

Eligible subjects were male or female aged 18 to 85 years with a diagnosis of NTM lung disease in accordance with the 2007 ATS/IDSA criteria with evidence of nodular bronchiectasis and/or fibrocavitary disease by chest CT. Subjects must have been receiving an ATS/IDSA guidelines-based treatment regimen for at least 6 months prior to screening with persistently positive mycobacterial cultures for MAC and/or *M. abscessus* and have a positive sputum culture at Screening. At randomization, subjects were stratified based on the presence or absence of CF, and predominant NTM organism at baseline (MAC vs. *M. abscessus*).

Study visits occurred every 28 days throughout the study. Multiple sputum samples were collected at the scheduled study visits. Sputum samples were also to be collected pre-dose at home during the week leading up to the study visit. The 6MWT was conducted at Baseline, Day 84 (end of double-blind phase), and Day 168 (end of open-label phase). All subjects were to have a 28-day post-EOT safety follow-up visit. This visit occurred on Day 112 for those subjects who did not continue in the open-label phase or on Day 196 for those subjects who continued in the open-label phase. Subjects completing the study had the option to enroll in a long-term safety follow-up period and return for a visit 12 months after the last dose of study drug.

The protocol defined primary endpoint was change from baseline in mycobacterial density as assessed by the semi-quantitative scale (SQS) for mycobacterial culture at Day 84. The SQS was a 7-step scale based on mycobacterial burden observed from culture results (Table 21).

Table 21: Semi-quantitative Scale for Mycobacterial Culture

Step	Solid Media Result	Liquid Medium Result	Categorical Result
1	0 colonies	Negative	Culture negative (confirmed with no growth in liquid medium)
2	0 colonies	Positive	Growth in liquid medium only (liquid positive)
3	1-49 colonies	Positive	Agar positive
4	50-100 colonies	Positive	1+
5	>100-200 colonies	Positive	2+

6	>200-500 colonies	Positive	3+
7	>500 colonies	Positive	4+

Source: Table 9-6 of TR02-112 study report.

Secondary endpoints were the proportion of subjects with NTM culture conversion to negative at Day 84; time to NTM culture conversion to negative at Day 84; ordinal, 3-level response from baseline on the SQS for mycobacterial culture at Day 84, where the 3 levels were: (1) improvement, (2) no change, and (3) worsening or death; change from baseline in Respiratory and Systemic Symptoms Questionnaire (RSSQ) at Day 84; change from baseline in Global Rating of Health at Day 84; and time to need for "rescue" anti-mycobacterial and other "rescue" drugs during the 84-day double-blind treatment phase. Tertiary endpoints were the proportion of subjects experiencing improvement from baseline (decrease of 1 step or more) in mycobacterial culture at Day 84; the proportion of subjects experiencing worsening from baseline (increase of 1 step or more) in mycobacterial culture at Day 84; time to improvement from baseline (decrease of 1 step or more) in mycobacterial culture during the 84-day double-blind treatment phase; time to worsening from baseline (increase of 1 step or more) in mycobacterial culture during the 84-day double-blind treatment phase; time to and duration of treatment for acute pulmonary exacerbations during the 84-day double-blind treatment phase (CF subjects only); time to and duration of treatment for acute pulmonary exacerbations during the 84-day double-blind treatment phase (non-CF subjects only); and change in 6MWT distance and post exertion oxygen saturation at Day 84. Exploratory endpoints were the above primary, secondary, and tertiary endpoints assessed through Day 168 and Day 196.

Mycobacterial density as assessed by the SQS had not previously been used in clinical studies for NTM. The Division had concerns regarding the clinical relevance of this endpoint and recommended culture conversion to negative as the primary endpoint during discussions regarding the design of the protocol. The SQS endpoint was maintained as the primary efficacy endpoint by the Applicant because achievement of negative cultures was not expected to occur after only 84 days of treatment in subjects who had already failed to convert with prior treatment. Culture negativity at Day 84 was incorporated as a secondary endpoint.

The focus of this review will be the primary efficacy endpoint, the secondary endpoint of culture negativity at Day 84, and the tertiary endpoint of change in 6MWT distance at Day 84. A discussion of the efficacy results that occurred during the open-label phase when all subjects were receiving ALIS will be limited.

Key Inclusion and Exclusion Criteria

The key inclusion criteria included but were not limited to the following:

- Male or female subjects, ages 18 years to 85 years.
- Diagnosis of pulmonary NTM lung disease in accordance with the 2007 ATS/IDSA criteria with evidence of nodular bronchiectasis and/or fibrocavitary disease by chest CT.
- History of chronic infection defined as at least 2 documented positive cultures in the prior 2 years, of which at least 1 was obtained in the 6 months prior to screening with

either MAC or *M. abscessus* or mixed infection with both species. The cultures could have been obtained from sputum or bronchoscopy.
- Positive sputum culture obtained during the screening period with either MAC or *M. abscessus* or mixed infection with 1 dominant species.
- Receiving ATS/IDSA guidelines-based treatment regimen defined as: adherent to a multidrug regimen for at least 6 months prior to screening with persistently positive mycobacterial cultures.

The key exclusion criteria included but were not limited to the following:
- Forced expiratory volume in 1 second (FEV_1) <30% of predicted at screening.
- Presence of any clinically significant cardiac disease as determined by the Investigator.
- Subjects with hemoptysis of ≥60 mL in a 24-hour period within 4 weeks prior to screening.
- Prior exposure to ALIS.
- Any change in chronic NTM multidrug regimen within 28 days prior to Study Day 1.

Statistical Analysis Plan

Analysis Populations

The primary efficacy analysis population for the double-blind phase was the modified ITT (mITT) population defined as all randomized subjects who received at least 1 dose of study drug. The safety population was defined similarly. Subjects who went on to participate in the open-label phase comprise the open-label mITT (OLmITT) population.

Analysis Methods

Inference testing was planned for only the primary efficacy endpoint. Therefore, type 1 error was not controlled for the multiple secondary, tertiary and exploratory endpoints.

The protocol defined primary endpoint was change from baseline on SQS for mycobacterial culture at Day 84. The SQS was a 7-step scale based on mycobacterial burden observed from culture results. The difference was calculated as the step number at Day 84 subtracted from the step number at baseline. The maximum improvement for a subject could be -6 steps and the maximum deterioration could be +6 steps. For subjects who died prior to Day 84, the change from baseline was imputed as +7 steps. A stratified Wilcoxon rank sum test adjusting for the randomization strata was used to compare the treatment arms at a 2-sided significance level of 0.05.

The proportion of subjects with negative NTM culture at Day 84 was analyzed using a stratified CMH test of treatment arm adjusting for the randomization strata. The analysis of this endpoint conducted by the Applicant excluded subjects with missing culture data. The analysis presented in this review will treat missing culture data as positive and therefore not negative.

Treatment comparisons in the change from baseline in 6MWT distance at Day 84 were made using an ANCOVA with factors for treatment and the randomization strata and the baseline value as a covariate. Missing data were imputed using a LOCF.

Sample Size Calculation
It was assumed that a semi-quantitative reduction of 1 step or more would be considered clinically meaningful. Therefore, with a two-sided significance level of 0.05, 50 subjects per arm would provide 80% power to detect a difference between the treatment arms of at least 0.94 steps on the SQS in the change from baseline in mycobacterial culture, assuming a pooled standard deviation of 1.6, based on a Wilcoxon rank sum test. Additionally, 50 subjects per arm would provide 80% power to detect a difference between the treatment arms of at least 17% in the proportion of subjects with NTM culture conversion to negative, assuming proportions in the placebo and ALIS arms of 3% and 20%, respectively, based on a Mantel-Haenszel test with a two-sided significance level of 0.05.

Protocol Amendments
The original protocol was dated February 1, 2012 and was subsequently amended 3 times. The amendments were not considered to impact the integrity of the trial.

Amendment 1 was dated October 3, 2012. In this amendment, the upper age limit was changed from 75 years to 85 years.

Amendment 2 was dated October 25, 2013. Main changes to the protocol included the following:

- The stated delivered dose per vial of ALIS was changed from 560 mg to 590 mg as this dose more accurately reflected the drug product dose evaluated in the study. This clarification was not due to any product or manufacturing process changes.
- The sample size was changed from 100 randomized subjects to approximately 90 subjects due to difficulties in recruitment of subjects.
- A planned sample size re-estimation after 25% of subjects completed the double-blind phase of the study was removed.

Amendment 3 was dated April 2, 2015. This amendment terminated the continuation of the long-term safety follow-up phase when the last subject reach Month 12. In agreement with the FDA, it was determined that the originally planned 24-month long-term safety follow-up phase was not necessary for the clinical development of ALIS as subjects were not receiving study drug.

Compliance with Good Clinical Practices
The Applicant states in the Clinical Study Report that, “This study was conducted in accordance with the sponsor’s standard operating procedures, which are designed to ensure adherence to Good Clinical Practice (GCP) as denoted in the International Conference on Harmonization (ICH) E6 requirements for GCP and in accordance with the ethical principles outlined in the Declaration of Helsinki.”

Financial Disclosure

The Applicant, Insmed Inc, has provided financial certification/disclosure information for all clinical investigators and sub-investigators involved in Study (b) (6). Please refer to Table 7 in Section 7.2.2 for investigators/sub-investigators who had disclosable information based on the criteria defined in 21CFR 54.4(a)(3)(i-v).

Patient Disposition

A total of 90 subjects were randomized into the double-blind portion of the study. One subject randomized to placebo did not receive study drug. Therefore, the mITT/Safety population consists of 89 subjects: 44 on ALIS and 45 on placebo.

Nine subjects, all in the ALIS group, discontinued treatment prematurely during the double-blind phase. Most discontinued treatment prematurely due to an adverse event. Four subjects, all in the ALIS group, did not complete the double-blind phase. The reasons for discontinuing the study early were due to death, adverse event, withdrawal of consent, and lost to follow-up (one subject each).

Table 22: Subject Disposition in the Double-blind Phase- Study TR02-112

	ALIS	Placebo
Randomized	44	46
mITT/Safety	44	45
Completed treatment	35 (79.5%)	45 (100.0%)
Discontinued treatment prematurely	9 (20.5%)	0
Reason for treatment discontinuation		
AE	7 (15.9%)	0
Other	2 (4.5%)	0
Completed double-blind phase	40 (90.9%)	45 (100.0%)
Discontinued double-blind phase prematurely	4 (9.1%)	0

Source: Reviewer analysis

Of the 80 subjects who completed treatment in the double-blind phase, 78 subjects enrolled in the open-label phase of the study and comprise the OLmITT population. Both subjects who declined participation in the open-label phase were randomized to placebo in the double-blind phase. Approximately 24% of the subjects did not complete treatment in the open label phase. More of these subjects were in the previously treated placebo group than the previously treated ALIS group. Most subjects discontinued study drug due to an adverse event. Only 3 of the 78 subjects did not complete the open-label phase of the study. The reasons for

discontinuing the open label phase were due to an adverse event, death, and subject not wanting to travel to the study site (one subject each).

Table 23: Subject Disposition in the Open-label Phase- Study TR02-112

	ALIS	Placebo	Total
Enrolled (OLmITT)	35	43	78
Completed treatment	28 (80.0%)	31 (72.1%)	59 (75.6%)
Discontinued treatment prematurely	7 (20.0%)	12 (27.9%)	19 (24.4%)
Reason for treatment discontinuation			
AE	5 (14.3%)	11 (25.6%)	16 (20.5%)
Persistent severe cough, study drug related	1 (2.9%)	1 (2.3%)	2 (2.6%)
Other	1 (2.9%)	0	1 (1.3%)
Completed open-label phase	34 (97.1%)	41 (95.3%)	75 (96.2%)
Discontinued open-label phase prematurely	1 (2.9%)	2 (4.7%)	3 (3.8%)

Source: Reviewer analysis

Protocol Violations/Deviations

At least one protocol deviation was recorded during the study for 38 (86.4%) ALIS subjects and 39 (86.7%) placebo subjects. The most common reason for protocol deviations was due to assessment not being performed per the protocol.

Baseline and Demographic Characteristics

Baseline and demographic characteristics were generally similar across treatment groups in the mITT population and are summarized in Table 24. The mean age of the subjects was 58.5 years. Approximately 88% of the subjects were female. The majority of the subjects were white (92%). Approximately 19% of the subjects had CF and two-thirds had predominantly MAC lung infection although some could have been co-infected with other NTM.

Table 24: Baseline and Demographic Characteristics (mITT population)- Study TR02-112

	ALIS (n=44)	Placebo (n=45)
Age (years)		
mean (sd)	58.0 (16.6)	59.1 (15.2)
median	61.5	63.0
min, max	18, 85	19, 80
Sex, n (%)		
Male	6 (13.6)	5 (11.1)
Female	38 (86.4)	40 (88.9)
Race, n (%)		

	ALIS (n=44)	**Placebo** (n=45)
White	42 (95.5)	40 (88.9)
Non-White	2 (4.5)	5 (11.1)
CF strata, n (%)		
Presence of CF	8 (18.2)	9 (20.0)
Absence of CF	36 (81.8)	36 (80.0)
Predominant NTM organism at baseline, n (%)		
MAC	26 (65.9)	28 (62.2)
M. abscessus	15 (34.1)	17 (37.8)

Source: Reviewer analysis

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Treatment Compliance

During the Double-Blind portion of the study, the proportion of patients with greater than or equal to 80% treatment compliance was higher for the Placebo plus BR (89.2%) compared to patients treated with ALIS plus BR (72.8%). Also, the proportion of patients with 100% treatment compliance was higher for patients treated with Placebo plus BR (55.6%) compared to patients treated with ALIS plus BR (31.8%).

Concomitant Medications

Selected pertinent concomitant medications taken during the double-blind portion of the study are summarized in Table 25.

Table 25: Pertinent Concomitant Medications Used by More than 5% of Participants in the Double-Blind Portion of Study TR02-112

ATC Class 1/ Preferred Term	**ALIS N = 44 n, (%)**	**Placebo N = 45 n, (%)**
Patients reporting at least 1 Concomitant Medication	44 (100)	45 (100)
Antibacterial drugs		
Azithromycin	35 (79.5)	34 (75.6)
Clarithromycin	9 (20.5)	4 (8.9)
Ethambutol	21 (47.7)	26 (57.8)
Ethambutol dihydrochloride	3 (6.8)	1 (2.2)
Rifampicin	22 (50.0)	20 (44.4)
Clofazimine	7 (15.9)	11 (24.4)

Ciprofloxacin	9 (20.5)	6 (13.3)
Levofloxacin	10 (22.7)	2 (4.4)
Moxifloxacin	3 (6.8)	7 (15.6)
Moxifloxacin hydrochloride	2 (4.5)	4 (8.9)
Combinations of penicillins, including beta-lactam-lactamase inhibitors	5 (11.4)	2 (4.4)
Selective beta-2 adrenoreceptor agonists	27 (61.4)	24 (53.3)
Glucocorticoids	18 (40.9)	9 (20.0)
Adrenergics and other drugs for obstructive airway disease excluding anticholinergics	13 (29.5)	14 (31.1)
Anticholinergics	8 (18.2)	9 (20.0)

Source: Modified from Applicant's Table 11-4 and 11-5 in Study TR02-112 Clinical Study Report
ALIS = amikacin liposome inhalation suspension (590 mg); ATC = anatomical therapeutic chemical

Rescue Medications
Examination of the concomitant medication dataset for the Double-Blind portion of the study revealed 2 patients (4.5%) in the ALIS plus BR who received IV amikacin, while 3 patients (6.7%) in the Placebo plus BR arm were reported as having concomitant inhaled amikacin use; however, the start and end dates of use of inhaled amikacin were not provided.

Efficacy Results-Primary Endpoint
Table 26 summarizes the baseline SQS category and the change from baseline at Day 84 on the SQS scale for the mITT population. Approximately 11% of subjects in both arms were culture negative at baseline. Additionally, at baseline, a greater proportion of subjects in the ALIS group than in the placebo group were at Step 3 (1-49 colonies) whereas a greater proportion of subjects in the placebo group than in the ALIS group were at Step 7 (> 500 colonies). At Day 84, 52.3% of ALIS subjects and 51.1% of placebo subjects had no change from baseline on the SQS. Overall, the change from baseline was not statistically significant between treatment groups (p=0.072).

Table 26: Baseline and Change from Baseline at Day 84 on the SQS Scale for Mycobacterial Culture (mITT population)- Study TR02-112

	ALIS (n=44)	**Placebo** (n=45)
Baseline		
Step1- Culture negative	5 (11.4%)	5 (11.1%)
Step 2- Growth in liquid medium only	1 (2.3%)	1 (2.2%)

	ALIS (n=44)	**Placebo** (n=45)
Step 3- Agar positive (1-49 colonies)	17 (38.6%)	10 (22.2%)
Step 4- 1+ (50-100 colonies)	2 (4.5%)	4 (8.9%)
Step 5- 2+ (> 100-200 colonies)	2 (4.5%)	2 (4.4%)
Step 6- 3+ (>200-500 colonies)	3 (6.8%)	4 (8.9%)
Step 7- 4+ (>500 colonies)	14 (31.8%)	19 (42.2%)
Change from baseline at Day 84		
-6	1 (2.3%)	0
-5	0	0
-4	1 (2.3%)	0
-3	3 (6.8%)	0
-2	6 (13.6%)	6 (13.3%)
-1	5 (11.4%)	5 (11.1%)
0	23 (52.3%)	23 (51.1%)
+1	2 (4.5%)	5 (11.1%)
+2	0	3 (6.7%)
+3	1 (2.3%)	0
+4	1 (2.3%)	2 (4.4%)
+5	0	1 (2.2%)
+6	0	0
+ 7 (Death)	1 (2.3%)	0
p-value for stratified Wilcoxon rank sum test of treatment arm adjusting for randomization strata	0.072	

Source: Reviewer analysis

Efficacy Results-Secondary and Other Relevant Endpoints

At Day 84, a greater proportion of subjects in the ALIS group (31.8%) achieved a negative culture than subjects in the placebo group (8.9%) (p=0.0057). It should be noted that these results are slightly different from those presented by the Applicant. In the Applicant's presentation, 3 ALIS subjects with missing data at Day 84 were excluded from the analysis. In the analysis presented here, the subjects with missing data are treated as not having a negative culture, i.e., a positive culture. The conclusion drawn, however, is the same.

Table 27: Negative Culture at Day 84 (mITT population)- Study TR02-112

ALIS	**Placebo**
14/44 (31.8%)	4/45 (8.9%)
p= 0.0057*	

Source: Reviewer analysis

*CMH p-value stratified by presence/absence of CF and predominant NTM infection at baseline.

Table 28 summarizes the baseline and the change from baseline to Day 84 in 6MWT distance. Overall, subjects in the ALIS group had a mean increase from baseline of 20.6 meters compared to a mean decrease of 25.0 meters in the placebo group. The difference between treatment groups was in favor of the ALIS group (ANCOVA p=0.0102).

Table 28: 6MWT distance (mITT population)- Study TR02-112

	ALIS	**Placebo**
	n=44	n=45
Baseline (meters)		
Mean (sd)	441.6 (133.6)	441.8 (111.6)
Median	435	457
Min, Max	53, 660	120, 751
Change at Day 84 (meters)*		
Mean (sd)	20.6 (62.4)	-25.0 (100.2)
Median	7	-6
Min, Max	-99, 264	-387, 220
p-value**	0.0102	

*A last observation carried forward was used for 6 subjects in the ALIS arm with missing Day 84 data to calculate the change at Day 84.
**ANCOVA p-value for treatment from model including treatment, presence/absence of CF and predominant NTM infection at baseline strata as factors and baseline value as a covariate.

At the end of the open-label phase, 21/78 subjects (26.9%) achieved a negative NTM culture at Day 168. This corresponds to 13 of the 35 subjects previously treated with ALIS and 8 of the 43 subjects previously treated with placebo in the double-blind phase.

A post-hoc analysis based on culture conversion (3 consecutive negative NTM cultures) by the end of the open-label phase was also conducted by the Applicant. By the end of the open label phase at Day 168, 20 of the 89 subjects achieved culture conversion. Additionally, 14 of these subjects demonstrated durability of this response at the 12-month follow-up visit. Three subjects did not consent to the 12-month follow-up period.

Findings in Special/Subgroup Populations

Gender, Race, Age, and Geographical Region

Subgroup analyses of culture negativity at Day 84 were conducted by gender, race, and age, and are presented in Table 29. Most subjects were female and were white so the results by gender and race should be interpreted with caution. In general, the results for those <65 years and those ≥65 years were consistent with that observed for the overall population. All but 2 subjects were from the United States so an analysis by geographical region was not conducted.

Table 29: Culture Negativity at Day 84 by Gender, Race, and Age (mITT Population) -Study TR02-112

	ALIS (n=44)	**Placebo** (n=45)
Gender		
Male	0/6	2/5 (40.0)
Female	14/38 (36.8)	2/40 (5.0)
Race		
White	13/42 (31.0)	4/40 (10.0)
Non-White	1/2 (50.0)	0/5
Age		
<65 years	10/26 (38.5)	2/28 (7.1)
≥65 years	4/18 (22.2)	2/17 (11.8)

Source: Reviewer Table

Other Subgroups of Interest

Other subgroups of interest include the combination of the stratification factors: predominant NTM infection and presence/absence of cystic fibrosis. Table 30 summarizes the results by these stratification factors for culture negativity at Day 84. Most of the subjects had MAC infection and did not have cystic fibrosis and these results are consistent with the overall population. Given the small sample sizes for the remaining strata, results for these strata must be interpreted with caution.

Table 30: Culture Negativity at Day 84 by Stratification Factors (mITT Population) -Study TR02-112

Predominant NTM/ Presence of CF	**ALIS**	**Placebo**
MAC / absence of CF	12/27 (44.4%)	3/27 (11.1%)
MAC/ presence of CF	0/2	0/1
M. abscessus/ absence of CF	1/9 (11.1%)	0/9
M. abscessus/ presence of CF	1/6 (16.7%)	1/8 (12.5%)

Source: Reviewer Table

The results for the 6MWT distance for the non-cystic fibrosis MAC subgroup (the population studied in INS-212) are summarized in Table 31. At Day 84, subjects in the ALIS group had a mean increase from baseline of 16.3 meters compared to a mean decrease of 13.1 meters in the placebo group.

Table 31: 6MWT distance for non-CF MAC Subjects (mITT Population) -Study TR02-112

	ALIS n=27	**Placebo** n=27
Baseline (meters)		
Mean (sd)	441.6 (147.9)	429.3 (119.1)

Median	435	456
Min, Max	53, 660	120, 618
Change at Day 84 (meters)		
Mean (sd)	16.3 (62.0)	-13.1 (109.2)
Median	7	-2
Min, Max	-99, 264	-387, 220

Source: Reviewer Table

7.2.4 Study INS-312

Study Design and Endpoints

Study INS-312 is an open label extension of Study INS-212. Enrolled patients included patients from Study INS-212 who did not achieve culture conversion or who had experienced a relapse or recurrence by Month 6. All enrolled patients received open label treatment with ALIS 590 mg QD in addition to multi-drug background regimen for up to 12 months. Study INS-312 is currently ongoing. A cutoff date of July 7, 2017 (same as INS-212) was used for the data presented in the initial report of the study.

Subjects had routine visits at Day 1 and monthly through Month 12/EOT. Subjects also returned for a one-month off ALIS treatment safety follow-up visit.

The primary objective of this study is to evaluate long-term safety and tolerability of ALIS for up to 12 months. Secondary efficacy assessments include culture conversion and change in 6MWT distance.

Key Inclusion and Exclusion Criteria

Subjects were eligible to enroll in the study if they successfully completed the Month 6 and EOT visits in Study INS-212 and had not achieved culture conversion by Month 6 in Study INS-212 or had experienced by Month 6 a relapse or recurrence after culture conversion had occurred in Study INS-212 and demonstrated compliance with the treatment regimen in Study INS-212. Subjects were not eligible to enroll if they discontinued Study INS-212 prior to the Month 6 visit.

Statistical Analysis Plan

Analysis Populations

The Safety population consisted of all enrolled subjects who received at least 1 dose of ALIS.

Analysis Methods

Two analyses will be conducted. The initial analyses will assess endpoints up to Month 6 for those patients enrolled as of the data cutoff date. Final analyses will be conducted on all endpoints at the completion of the study.

As the study contains a single arm, all efficacy results are provided as descriptive summaries. The proportion of subjects who achieved negative sputum culture conversion by Month 6 and mean changes in 6MWT distance are summarized overall and by the previous arm in Study INS-212 for the Safety population.

Sample Size Calculation
There was no sample size calculation for this study. The total sample size will be determined based on the number of eligible subjects from Study INS-212 who consent to enroll in this study.

Protocol Amendments
The original protocol was dated September 10, 2015 and was subsequently amended 3 times.

Amendment 1 was dated March 11, 2016. Major changes included the following:

- Switched the order of secondary objectives 1 and 2 to emphasize culture conversion by Month 12 over culture conversion at (b) (6)
- Clarified that assessments done at the Study INS-212 EOT visit counted as baseline for INS-312
- Clarified that the multi-drug background regimen be continued during the 1-month off ALIS follow-up.

Amendment 2 was dated July 13, 2016. A major change included the implementation of a Data Monitoring Committee which was already in place for Study INS-212 and the compassionate use program to enable a more comprehensive review of the safety of ALIS across the entire program.

Amendment 3.1 was dated May 25, 2017. This amendment added an initial analysis for Study INS-312 that would be in alignment with the initial analyses in Study INS-212 to provide supportive data.

Compliance with Good Clinical Practices
The Applicant states in the clinical study report that, "This study was designed and monitored in accordance with Sponsor procedures, which comply with the ethical principles of Good Clinical Practice (GCP) as required by the major regulatory authorities, and in accordance with the Declaration of Helsinki."

Financial Disclosure
The Applicant, Insmed Inc, has provided financial certification/disclosure information for all clinical investigators and sub-investigators involved in Study (b) (6). Please refer to Table 7 in Section **7.2.2** for investigators/sub-investigators with had disclosable information based on the criteria defined in 21CFR 54.4(a)(3)(i-v).

Patient Disposition

At the time of the initial analysis, 133 subjects had enrolled in the study: 59 previously received ALIS plus a BR and 74 previously received a BR alone in Study INS 212. Overall, 24 subjects completed treatment as defined in the protocol, 28 subjects discontinued treatment prior to completion, and 81 were still ongoing treatment. The most common reasons for discontinuing the study were due to adverse event and withdrawal by subject. More subjects who previously received BR alone in Study INS-212 discontinued due to an adverse event than subjects who previously received ALIS plus BR in Study INS-212.

Table 32: Subject Disposition - Study INS-312

	Prior ALIS plus BR in Study INS-212	**Prior BR alone in Study INS-212**	**Total**
Enrolled	59	74	133
	n (%)	n (%)	n (%)
Completed study	9 (15.3)	15 (20.3)	24 (18.0)
Discontinued study prematurely	13 (22.0)	15 (20.3)	28 (21.1)
Ongoing on study	37 (62.7)	44 (59.4)	81 (60.9)
	n (%)	n (%)	n (%)
Reason for study discontinuation			
Death	2 (3.4)	0	2 (1.5)
Adverse event	2 (3.4)	11 (14.9)	13 (9.8)
Lack of efficacy	3 (5.1)	0	3 (2.3)
Withdrawal by subject	5 (8.5)	1 (1.4)	6 (4.5)
Other	1 (1.7)	3 (4.1)	4 (3.0)

Source: Reviewer Analysis

Protocol Violations/Deviations

Protocol deviations were reported in 69.2% of all subjects (59.3% of prior ALIS plus BR subjects and 77% of prior BR alone subjects). The most common protocol deviations were noncompliance with Study Protocol in 43.6% of all subjects, noncompliance with visit schedule in 40.6% of all subjects, and noncompliance with study product in 39.1% of all subjects.

Major protocol deviations were reported in 6% of all subjects (5.1% of prior ALIS plus BR subjects and 6.8% of prior BR alone). The most common major protocol deviations were related to informed consent procedures and reported in 5.3% of all subjects (5.1% of prior ALIS plus BR subjects and 5.4% of prior BR alone).

Baseline and Demographic Characteristics

Baseline and demographic characteristics for the Safety population are summarized in Table 33. The mean age of the subjects was 64 years. Approximately 64% of the subjects were female. The majority of the subjects were white (61.7%). Approximately 39% of the subjects were from the United States. Most of the subjects were not current smokers.

Table 33: Baseline and Demographic Characteristics (Safety population)- Study INS-312

	Prior ALIS plus BR in Study INS-212 (n=59)	Prior BR alone in Study INS-212 (n=74)	Total (n=133)
Age (years)			
mean (sd)	65.1 (8.6)	64.4 (10.3)	64.7 (9.6)
median	65	65.5	65
min, max	46, 85	33, 83	33, 85
Sex, n (%)			
Male	18 (30.5)	30 (40.5)	48 (36.1)
Female	41 (69.5)	44 (59.5)	85 (63.9)
Race, n (%)			
White	33 (55.9)	49 (66.2)	82 (61.7)
Asian	23 (39.0)	17 (23.0)	40 (30.1)
Black	1 (1.7)	3 (4.1)	4 (3.0)
Other	1 (1.7)	0	1 (0.8)
Not recorded	1 (1.7)	5 (6.8)	6 (4.5)
Region, n (%)			
United States	19 (32.2)	33 (44.6)	52 (39.1)
Asia	20 (33.9)	14 (18.9)	34 (25.6)
Rest of the world	20 (33.9)	27 (36.5)	47 (35.3)
Smoking status, n (%)			
Current smoker	8 (13.6)	8 (10.8)	16 (12.0)
Not a current smoker	51 (86.4)	66 (89.2)	117 (88.0)

Source: Reviewer Analysis

Efficacy Results- Primary Endpoint

Study INS-312 was primarily designed to evaluate long-term safety of ALIS. There was no primary efficacy endpoint.

Efficacy Results- Secondary and other Relevant Endpoints

Study INS-312 is currently ongoing and not all subjects had completed the Month 6 visit by the time of the data cutoff date of the report, therefore only a brief summary of the results for culture conversion by Month 6 and 6MWT will be presented and interpretation of these results should be made with caution. While the results are presented by previous treatment arm in Study INS-212, it is important to remember that these groups are not randomized groups in Study INS-312 and are not expected to be similar at the point of enrollment into Study INS-312.

Culture conversion by Month 6 was assessed for those subjects who had at least 3 consecutive months of culture results up to Month 6 available by the data cut-off date. Overall, 3 subjects in the prior ALIS plus BR group in Study INS-212 and 17 subjects in the prior BR alone group in Study INS-212 achieved culture conversion. Approximately 16% of the subjects enrolled by the data cut-off date did not have 3 months of culture results and were therefore not assessed for

culture conversion and an additional 24% of the subjects had not completed Month 6 by the data cut-off date. Therefore, it is possible that more subjects may achieve culture conversion.

Table 34 summarizes the 6MWT results for those subjects who had a baseline and Month 6 value. At the time of data cut-off, only approximately 55% of the subjects had reached Month 6 and contribute to the analysis. Overall, there was a decrease in the distance walked from baseline to Month 6.

Table 34: 6MWT distance (Safety Population) -Study INS-312

	Prior ALIS plus BR in Study INS-212 (n=25)	**Prior BR alone in Study INS-212** (n=36)	**Total** (n=61)
Baseline (meters)			
Mean (sd)	432.4 (124.1)	470.0 (111.7)	454.6 (117.4)
Median	420	464	457
Min, Max	201, 630	231, 735	201, 735
Change at Month 6 (meters)			
Mean (sd)	-28.3 (46.1)	-14.1 (36.2)	-19.9 (40.8)
Median	-33	-7.5	-15
Min, Max	-96, 88	-145, 59	-145, 88

Source: Reviewer Analysis
Note: n is the number of subjects with both a baseline and Month 6 value

Findings in Special/Subgroup Populations
Since Study INS-312 is ongoing, subgroup analysis will not be conducted for this review.

7.3 Integrated Review of Effectiveness

7.3.1 Assessment of Efficacy Across Trials

The assessment of efficacy is primarily based on Studies INS-212 and TR02-112.

The primary assessment of the efficacy of ALIS is based on a microbiological surrogate endpoint in Study 212. The primary endpoint in this study was sputum culture conversion by Month 6. There are limited data from observational studies that suggest sputum culture conversion might correlate with clinical benefit. However, from these studies, it is difficult to determine if the culture conversion is a reflection of patient characteristics rather than a true marker that will likely predict clinical benefit. These uncertainties were considered during the review and were discussed at the Advisory Committee meeting. These uncertainties were considered acceptable, as there are no approved therapies for patients with MAC-lung infection, the indicated population will only be limited to those with refractory disease, and that a confirmatory trial will be conducted to evaluate the clinical benefit. The primary endpoint of TR02-112 was a change in mycobacterial burden in sputum rather than sputum culture

conversion. Culture negativity at Day 84, the end of the double-blind portion of the study, was assessed.

In Study 212, significantly more subjects in the ALIS plus BR group achieved culture conversion by Month 6 compared to the BR alone group (29.0% and 8.9%, respectively). In Study 112, a greater proportion of subjects in the ALIS group (31.8%) achieved a negative culture at Day 84 than subjects in the placebo group (8.9%). This study, however, was unable to show a difference between treatment groups in the change in SQS for mycobacterial culture at Day 84, the primary endpoint.

Since the results for 6MWT distance appeared favorable as a tertiary endpoint in Study 112, it was chosen to provide an endpoint that represented clinical benefit and was incorporated as a key secondary clinical endpoint in Study 212. In Study 112, the change from baseline to Day 84 in 6MWT distance was a mean increase of 20.6 meters in the ALIS group compared to a mean decrease of 25.0 meters in the placebo group. However, the difference in change from baseline to Month 6 in 6MWT distance between treatment groups in Study 212 was not statistically significant. Regardless of the analysis method applied, a decrease in the distance walked at Month 6 was observed for the ALIS plus BR group and the decrease was numerically worse than that observed for the BR alone group. As discussed in Section 7.2.1 under the Efficacy Results-Secondary and Other Relevant Endpoints subheading, interpretation of these results may be complicated by the amount of missing data which is greater in the ALIS plus BR group compared to the BR alone group. Of note, most of the missing data appear to be due to discontinuation from the ALIS plus BR arm because of pulmonary-associated AEs. It is unlikely to expect that such subjects would have an improvement in their 6MWT distance had their results at Month 6 been known and therefore would not be likely to change the conclusion regarding 6MWT in favor of treatment with ALIS.

Due to differences in the duration of the randomized portion of the studies and variations of the microbiological endpoint related to culture conversion/negativity, pooled analyses of the efficacy results have not been reported.

7.4 Summary and Conclusions

7.4.1 Summary and Conclusions – Statistics

The data provided to support the efficacy of ALIS in the treatment of MAC lung disease are primarily based on the results of Study 212, a Phase 3, open-label, randomized trial comparing ALIS added to BR versus BR alone in patients with refractory MAC lung disease. No data are available for the treatment of a broader patient population with MAC lung disease.

In Study 212, significantly more subjects in the ALIS plus BR arm achieved the primary endpoint of culture conversion (three consecutive negative cultures with 6 months of treatment), 65/224 (29%) compared to the BR alone arm, 10/112 (8.9%). This endpoint is a surrogate that was

considered reasonably likely to predict clinical benefit. The long-term endpoint chosen for this trial is durability of culture conversion following cessation of treatment which will be assessed at the completion of the study. The design of the study allowed for the option to switch to ALIS in the BR alone arm after Month 8 by enrolling in Study 312. Culture conversion with durability after 3 months off treatment, by definition, requires subjects to have had culture conversion by Month 6 and then maintain it until 3 months after the completion of treatment making treatment comparisons with this long-term endpoint interpretable. However, assessment of additional long-term outcomes, including possible clinical endpoints, by initial randomized treatment group will be limited.

Supportive efficacy information is provided by Study 112, in patients with refractory MAC/*M. abscessus* lung infections. In this trial, at Day 84, a greater proportion of subjects in the ALIS group (31.8%) achieved a negative culture than subjects in the placebo group (8.9%). This was a secondary endpoint in a study that did not meet its primary endpoint of a change in SQS for mycobacterial culture at Day 84.

The one clinical outcome that has been assessed in these studies thus far is the 6MWT distance, the results for which were not significant in Study INS-212 at the 6-month visit. A trend in favor of ALIS was seen in Study TR02-112 at Day 84.

Efficacy data from Study INS-312 are difficult to interpret at this time as the study is ongoing and not all subjects have completed the Month 6 visit by the time of the data cut-off date. Additionally, any supportive efficacy information provided by this trial will be extremely limited due to lack of randomized comparative arms.

7.4.2 Summary and Conclusions - Clinical

In Study 212, a statistically significant improvement in the microbiological endpoint of sputum culture conversion, defined as 3 monthly negative sputum cultures by Month 6, was observed in patients treated with the addition of ALIS to BR (29%) as compared to patients treated with a BR alone (8.9%). A similar finding was noted in the Phase 2 Study 112, although sputum culture conversion was a secondary endpoint in the study and was assessed at a different timepoint (Day 84) than in the Phase 3 trial. Findings from these two trials provide evidence that the addition of ALIS to BR improved attainment of sputum culture conversion.

Clinical endpoints in Study 212 did not show statistically significant improvement in the ALIS+BR group as compared to the BR alone group. There was no statistically significant improvement in the key secondary clinical outcome of 6MWT distance. This contrasted with the positive trend in improvement of the 6MWT, although not statistically significant, noted in Study 112. In addition, there was no statistically and/or clinically significant improvement in the two patient-reported outcome measures, the SGRQ and EQ-5D-3L.

In the absence of improvement in clinical outcomes in the studies presented in support of this

NDA, we reviewed the current scientific literature to assess the correlation, if any, between improvement in the microbiologic surrogate endpoint of sputum culture conversion to improvement in clinical outcomes, such as, how a patient feels, functions or survives. Currently, there are limited data to suggest that sputum culture conversion correlates with clinical benefit.

Since this application is being reviewed under the accelerated pathway, the Applicant will conduct a confirmatory trial to demonstrate clinical benefit associated with ALIS therapy. Inaddition, the confirmatory trial should also address the long-term durability of the observed improvement in sputum culture conversion.

One of the critical shortcomings in the design of the Phase 3 trial is the limited comparative data beyond Month 8. This is because patients who did not convert to sputum culture negative by Month 6 were discontinued from the study or crossed over to the open label extension Study 312 where all participants received ALIS.

Of note, in both Study 212 and the double-blind portion of Study 112, there was a disproportionate number of patients in the ALIS plus BR arm who discontinued treatment prematurely compared to patients treated with BR alone. The main reason for these discontinuations were discontinuation due to adverse events.

In conclusion, the Applicant has provided substantial evidence of efficacy on a surrogate endpoint of sputum culture conversion in a single adequate and well-controlled trial in a limited population of patients with refractory MAC lung disease. The uncertainties with the surrogate endpoint and the safety profile of ALIS, allow for a favorable risk-benefit assessment only in the limited population of patients with refractory MAC lung disease. The Applicant will conduct a confirmatory trial to demonstrate clinical benefit for patients on ALIS+BR and attempt to validate the microbiologic endpoint of sputum culture conversion by Month 6 as a surrogate for clinical benefit.

8 Clinical Microbiology Review

8.1 Nonclinical Microbiology

8.1.1 Mechanism of Action

The primary mechanism of action of aminoglycosides is the disruption and inhibition of protein synthesis by binding to the 30 S ribosomal subunit. Amikacin enters the bacterial cell by the interaction of its positively charged molecules with the negatively charged bacterial cell surface disrupting the overall architecture of the cell wall and binding to the ribosomes thus preventing the synthesis of proteins resulting in the death of the bacterial cell.

8.1.2 Antibacterial Activity

8.1.2.1. Spectrum of Activity

Amikacin is active against gram-negative bacteria such as *Pseudomonas aeruginosa, E. coli, Proteus* spp*., Klebsiella* spp*., Enterobacter* spp*., Serratia* spp*., Acinetobacter* spp*.,* and gram-positive bacteria such as *Staphylococcus aureus, Streptococcus* spp., *Enterococcus* spp., and species of mycobacteria comprising *MAC and M. tuberculosis.*

8.1.2.2. Intracellular Antimicrobial Concentration Assessment

Activity in vitro

The Applicant conducted a study to determine the in vitro activity of Arikayce against three phagocytized *Mycobacterium avium* strains. The monocytic cell line was ATCC (THP-1) and the mycobacterial strains were ATCC MAC104 and A5 (blood isolates), and MAC3388 (lung isolate) of *M. avium*. THP-1 monolayers were infected with 1 x 10^7 bacteria, incubated, and non-phagocytized bacteria were removed. The Arikayce treatments contained 10, 8, 4, 2, or 1 mcg/mL of amikacin. Amikacin sulfate was used at a concentration of 10 mcg/mL of amikacin. At 10 mcg/mL, Arikayce demonstrated significant bacterial killing compared with a comparable concentration of amikacin sulfate.

8.1.2.3. Antimicrobial Interactions and Fixed Combination Studies

There is a synergistic effect between aminoglycosides and members of the beta-lactam class of antibacterial drugs, as well as, amikacin and rifabutin in combination with other drugs for the treatment of MAC. (Hallander et al., Moellering et al.,1971).

Checkerboard titration was used to assess antimicrobial drug interactions. The results are expressed as Fractional Inhibitory Concentration Index (FICI). The FICI is defined as the MIC of the drug in combination divided by the MIC of the drug used alone. If the FICI is <0.5 the antimicrobial combination is synergistic, 0.5 to 1.0 indicates additivity, and >2 indicates antagonism. For combinations of 3 drugs a score of <0.75 indicates synergy and <1.5 additivity (Berenbaum,1978). Table 33 shows the result of various drug combinations with amikacin. In a 2-drug combination of amikacin, a synergistic or additive affect was observed with 5 of the 6 strains. In a 3-drug combination, amikacin had either a synergistic or additive effect on all strains of MAC tested.

Table 35: FICI Values for Agents in Combination Against MAC

Strain no.	FIC index[a] for the combination:										
	CLA-AMI	CLA-INH	CLA-SPA	CLA-EMB	CLA-CLO	CLA-RFB	CLA-INH-AMI	CLA-SPA-AMI	CLA-EMB-AMI	CLA-CLO-AMI	CLA-RFB-AMI
485	1	1	1	1	1	1	1	1	0.5	0.75	1
486	2	2	1	1	0.5	0.5	1.5	1.5	0.75	0.5	0.5
901	0.5	1	0.5	1	0.5	0.5	1	1	0.75	0.5	1
902	1	1	0.5	0.25	0.5	0.25	1.5	0.75	0.75	0.5	0.5
905	1	1	1	1	0.5	0.25	1	1	0.75	0.5	0.5
911	0.5	2	0.5	0.5	0.25	0.125	1.5	0.5	0.75	0.5	0.5

Source: Fattorini et al, 1995
AMI = amikacin liposome inhalation suspension; CLA = clarithromycin; CLO = clofazimine; EMB = ethambutol; INH = isoniazid; MAC = *Mycobacterium avium* Complex; RFB = rifabutin; SPA = sparfloxacin.

8.1.2.4. Time Kill Assay

A series of time kill assays was conducted with various concentrations of amikacin against *M. avium* IWGMT49, 101 (Serovar 1). At 2 mcg/mL, amikacin showed bactericidal activity with ≥99% killing achieved within 3 days (Figure 4). The bactericidal effect was concentration and time-dependent. It should be noted that the bactericidal activity was diminished when MAC cells were in late log phase of growth. It was reported that at a concentration of 16 mcg/mL and 64 mcg/mL, amikacin resulted in 96% and 98% killing respectively, after 10 days of incubation.

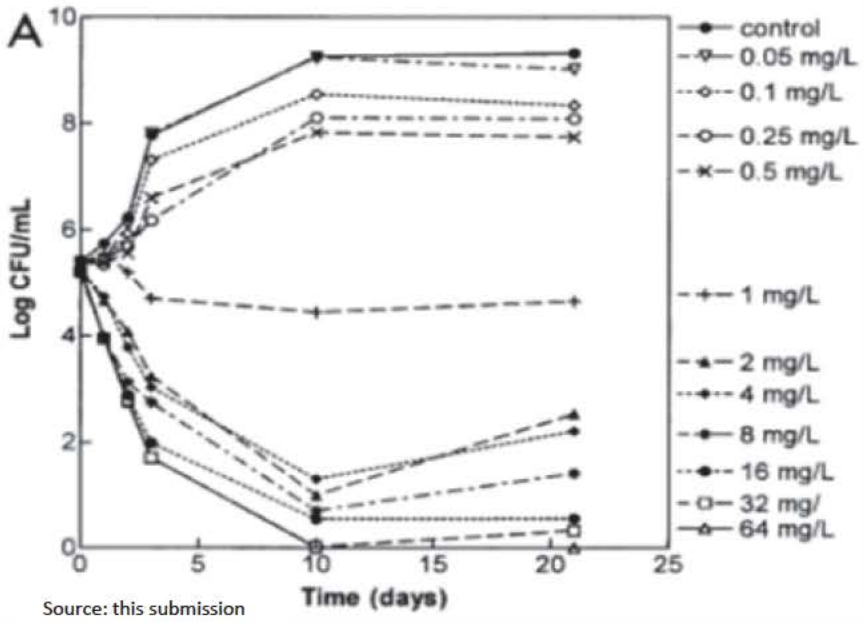


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Figure 4: Time-Kill of Amikacin Against *M avium* 101(serovar1)

8.1.3 Resistance and Cross-Resistance

The main mechanism by which NTM resist the effects of aminoglycosides is by alteration to ribosome binding sites by mutations in, and methylation of, the 16S rRNA. Resistance to amikacin is usually associated with an MIC > 64 mcg/mL. Mutations often occur at position A1408G in the rrs gene and at an MIC > 64 mcg/mL which affects the drug binding to the A site of the 16S rRNA.
Efflux pumps specific for aminoglycosides do not appear to play a major role in resistance. Cross-resistance among other aminoglycosides has been demonstrated in rapidly-growing mycobacterial cells.

8.1.4 Antibacterial Activity in Animal Models

De Steenwinkel et al., investigated the activity of amikacin in a mouse model of disseminated *M. avium* 101 (serovar 1). The MICs of clarithromycin, ethambutol, rifampicin and amikacin for the MAC101 strain were 4, 16, 64 and 128 mcg/mL, respectively. The animals that were used in all experiments were certified as pathogen free, they were female C57BLl6 mice (IFFA Credo, l'Arbresle, France), 10-12 weeks old and weighed 20-25 g.

Dilutions of antimycobacterial agents were prepared according to the manufacturer's instructions. The animals were inoculated intravenously with 2×10^8 cfu MAC (end-log phase) via the tail vein. Treatment was started at 24 hours after inoculation with clarithromycin at 100 mg/kg, as monotherapy or combinations of clarithromycin with 75 mg/kg of ethambutol and 40 mg/kg of rifampicin, liposome-encapsulated amikacin (LE-amikacin) or placebo (LE placebo) intravenously in the tail vein twice-weekly, for only the first 3 weeks of the treatment period.

The course of infection was assessed by quantification of the number of viable bacteria in the lungs, spleen, liver, inguinal lymph nodes, mesenteric lymph nodes, and blood at indicated time points. Treatment of the infection with clarithromycin resulted in a significant decrease in the mycobacterial burden but not total elimination of the bacteria. Total elimination of bacteria was not observed with the clarithromycin/ethambutol combination. However, when LE-amikacin-l (97 mg amikacin liposomes) was added for twice-weekly treatment during the first 3 weeks of clarithromycin/ ethambutol treatment, there was a rapid decrease of MAC cfu's followed by complete elimination of MAC from all organs except the spleen. Within 12 weeks, mesenteric lymph nodes were sterile and within 18 weeks, lungs, liver, and inguinal lymph nodes were sterile. The cultures of MAC in the spleen at 18 and 24 weeks resulted in 1.30 ± 0.49 and 0.45 ± 0.36 $\log_{10}$ cfu respectively. There was no change in susceptibility to clarithromycin and ethambutol.

8.2 Clinical Microbiology

8.2.1 Assay Descriptions and Methodologies

8.2.1.1. Reliability of Sputum Culture Results

The effect of residual amikacin in the sputum was tested to ascertain that cultures would not be affected by drug concentrations in sputum specimens collected for culture. The applicant added 4, 16, 64 and 128 mcg/mL amikacin in banked sputum specimens. No amikacin was added to the control samples. The samples were inoculated with 16 unique *M. avium* and *M. intracellulare* isolates (with known amikacin MIC values), standardized to 0.75 McFarland and 1.0 McFarland turbidity standard (1 McFarland Standard of H37Rv=$1.97X10^6$ cfu/mL at OD_{600}) each sputum specimen was cultured after 24 and 72 hours, so as to simulate the amount of time the specimen would have spent prior to culture (in transportation, etc.). A total 288 combinations of amikacin and bacteria were prepared for this study. Only at the highest concentration of 128 mcg/mL was any amikacin detected in the sputum however, the culture results were not affected by the residual amikacin (Eagle et al., 2018).

8.2.1.2. Culture, Assay and Identification

MAC isolation was generally done using acid fast smears from sputum and growth from solid and liquid cultures. Preliminary identification of MAC complex was done using an RNA probe (AccuProb). However, AccuProbe used for the identification of MAC does not distinguish between *M. avium* and *M. intracellulare*.

The individual isolates were identified by hybridization of the 16S rRNA and sequencing**.** The Clinical and Laboratory Standards Institute Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing (CLSI MM18-A, 2008) was used for reference. Isolates of the *M. abscessus* group were identified as *M. abscessus*, *M. massiliense* or *M. bolletii* using restriction fragment length polymorphisms of the hsp65 gene. (Telenti et al, 1993).

Susceptibility Testing was performed using Clinical and Laboratory Standards Institute protocol (CLSI M24-A2, 2011). As a quality assurance measure the laboratories exchanged isolates for testing, to determine the consistency of MIC results for amikacin and clarithromycin. The quality control strain was *M. avium* ATCC 700898.

8.2.1.3. Microbiological evaluations: Culture

Culture of sputum specimens, identification and susceptibility testing of relevant isolates from Study TR02-112 were performed at a central laboratory (University of Texas at Tyler). For Studies INS-212 and INS-312 a total of (b) (4) central laboratories were utilized.

In the double-blind phase of the study, sputum (expectorated or induced) was evaluated microbiologically at screening, within 4-5 hours after the patient received the first dose of Arikayce ™ or placebo, on Day 1. The patients were then re-evaluated prior to receiving doses on Days 28, 56, and 84 (± 7 days) before starting the open-label dose period.

During the open-label phase of the study, sputum samples for microbiological evaluation were collected on Days 113, 141 and 169 (± 7 days) to determine the efficacy of Arikayce™. Additional follow-up evaluations occurred at 28 days (± 7 days) after the last dose, i.e., Day 197, and during routine care on Day 562. The sample for culture that was obtained at all of these visits was expectorated sputum. The following microbiological procedures were performed: (i) Semi-quantitative smear for AFB and mycobacterial culture. (ii) Gram-stained smear and routine microbiological culture. (iii) Modified AFB smears and culture for *Nocardia spp.* (iv) Wet preparations and fungal cultures. (v) Susceptibility tests on all relevant bacterial isolates. Isolates of *Mycobacterium* spp. were placed in tween albumin broth and stored at
-70^0 C in the mycobacteriology section at NIH Clinical Center, to be used for amikacin susceptibility tests and molecular characterization. Microbiological tests were performed using the routine protocols at the NIH clinical microbiology laboratory.

8.2.2 Clinical Microbiology Analyses of Efficacy (mITT population)

8.2.2.1. Study TRO-112: A Randomized, Double-Blind, Placebo-Controlled Study of Liposomal Amikacin for Inhalation (Arikayce®) in Patients with Recalcitrant Nontuberculous Mycobacterial Lung Disease.

The type of specimens cultured were from either expectorated or induced sputum. Please refer to section 7 for additional details.

Studies performed at the University of Texas at Tyler indicated that the MIC_{50} of a group of MAC against amikacin was 8 mcg/mL, and that 86% of the isolates had a MIC of ≤16mcg/mL (Brown-Elliott et al, 2013). Table 36 shows the MIC of amikacin against MAC isolates at baseline.

Table 36: Baseline MIC (mcg/mL) of Amikacin against MAC Species– Study 112

ITT Population – all Regions

Baseline MAC spp.	MIC mcg/ mL	ALIS + BR			Placebo+BR (N=28) (%)	Overall (N=85) (%)
		<3 mths N=33 %	>3 mths N=24 %	Total N=57 %		
Unspeciated	2	1 (3.3)	0	1 (2.0)	0	1 (1.4)
	4	0	0	0	0	0
	8	2 (6.6)	3 (15.8)	5 (10.2)	3 (13.0)	8 (11.1)
	16	13 (43.3)	9 (47.4)	22 (44.9)	6 (34.8)	30 (41.7)
	32	6 (20.0)	6 (31.6)	12 (24.5)	7 (30.4)	19 (26.4)
	64	5 (16.7)	1 (5.3)	6 (12.2)	4(17.4)	10 (13.9)
	>64	3 (10.0)	0	3 (6.1)	1(4.3)	4 (5.6)

Source: Reviewer's table.

Results

Please refer to section 7 for additional details.

Study INS-212:

Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation in Adult Patients with Nontuberculous Mycobacterial Lung Infections caused by *Mycobacterium avium* Complex that are Refractory to Treatment.

This was a randomized, open-label Phase 3 efficacy study followed by an off-treatment observational phase. The purpose was to evaluate the time to culture conversion in patients treated with Amikacin Liposome Inhalation suspension(ALIS) 590 mg in conjunction with a multi-drug background regimen(BR), compared to the BR alone.

The drug was administered once daily by the Lamira Nebulizer Plus. Patients were randomized 2:1 for treatment of ALIS with BR or BR alone, and were stratified by smoking status and prior multi-drug regimen at screening (on treatment or off treatment for at least 3 months). Conversion was assessed at monthly intervals lasting up to Month 6. Those who culture converted were treated for an additional 12 months from the first month of the 3 consecutive negative specimen cultures. The non-converters and those who relapsed or were re-infected were removed from the study. Figure 5 shows the correlation between Amikacin MIC and culture conversion of MAC species.

Results

Please refer to section 7 for additional details.

Against the 335 MAC isolates, the amikacin MIC_{50} ranged from 16 to 32 mcg/mL and the MIC_{90} ranged from 32 to 64 mcg/mL. The susceptibility results were computed from both the ITT and PP population. These values were similar regardless of the geographical location from which the specimens originated, or the treatment group to which the patients were assigned. The susceptibility results for the ITT population at baseline are shown in Table 37. At baseline, the majority of MICs fell between 16 and 32 mcg/mL. In this study 38 subjects had an increase of ≥ 4 over the baseline MIC and of these, 36 were non-converters.

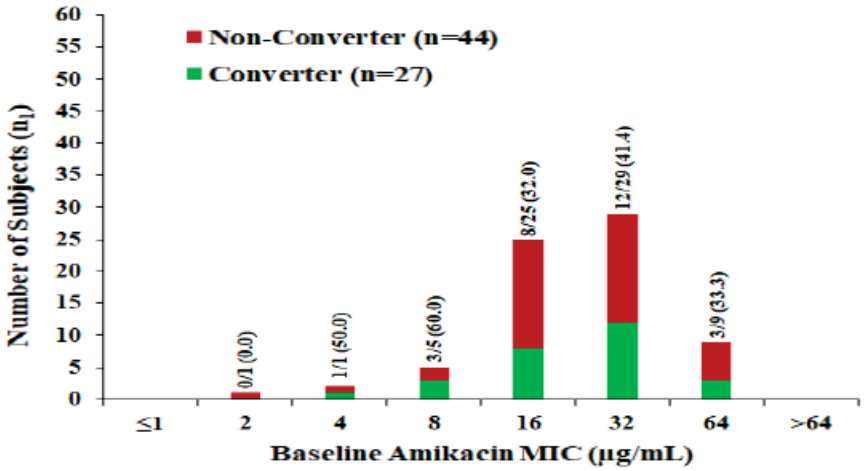


Figure 5: Correlation of Amikacin MIC to Culture Conversion of MAC Species in ALIS + BR-Treated Subjects (Study INS-212, PP)

Table 37: Baseline MIC (mcg/mL) of Amikacin against MAC Species– Study INS-212, ITT Population - All Regions

Baseline MAC species	MIC Mcg/mL	ALIS+MDR (N=224) n (%)	MDR (N=112) n (%)	Overall (N=336) n (%)
	<=1	2 (0.9)	0	2 (0.6)
Any species	2	1 (0.4)	0	1 (0.3)
	4	8 (3.6)	0	8 (2.4)
	8	28 (12.6)	21 (18.8)	49 (14.6)
	16	67(30.0)	33 (29.5)	100 (29.9)
	32	87 (39.0)	45 (40.2)	132 (39.4)
	64	29 (13.0)	12 (10.7)	41 (12.2)
	>64	1 (0.4)	1 (0.9)	2 (0.6)
Total		223	112	225

Source: this study

Study INS-312:

This is a single-arm extension of Study INS 212; it is a safety study that includes patients who had: (1) not achieved conversion by Month 6, and were taken off of the study at Month 8, or (2) patients who had relapsed, or (3) had been re-infected. Relapse or recurrence was defined as growth of MAC on 1 or more agar based medium or growth of more than 2 consecutively positive specimens in broth after having achieved culture conversion.

The primary objective of this study was to determine the long-term safety and tolerability of a once daily dose of ALIS 590 mg in conjunction with a background therapy. The treatment was administered for 12 months to patients who were

refractory to the standard multi-drug treatment for MAC lung disease. A second objective was to evaluate the time to culture conversion and the number of patients who had converted at 6 months, and at the 12-month end-of-treatment period.

All patients were treated with ALIS + BR. At 6 months, approximately 15% of the patients who were previously treated with BR alone in Study 212 had converted. This was a greater number than the number of additional patients who had been treated with ALIS +BR in INS 212 (BR alone vs. ALIS+BR=23.0% vs. 5.1 %). The average time to conversion was 3.2 months. Table 38 shows the baseline results of the ITT population. Figure 19 in appendix 15.5 shows the conversion rates by baseline amikacin MIC, there is no correlation in this study between amikacin baseline MIC and conversion levels for MAC. In this study 13 subjects had a ≥ 4-fold increase over the baseline MIC of amikacin, 9 of whom were non-converters and only one showed resistance to clarithromycin.

Table 38: Baseline MIC (mcg/mL) of Amikacin against MAC Species– Study INS-312

ITT Population - All Regions

Baseline MAC species	MIC Mcg/mL	ALIS+MDR (N=59) n (%)	MDR (N=74) n (%)	Overall (N=133) n (%)
Any species	2	1 (1.7)	1 (1.4)	2 (1.5)
	4	2 (34)	1 (1.4)	3 (2.3)
	8	5 (8.5)	16 (21.6)	21 (15.8)
	16	13 (22.0)	26 (35.1)	39 (29.3)
	32	19 (32.2)	21 (28.4)	40 (30.1)
	64	12 (20.3)	8 (10.8)	20 (15.0)
	>64	7 (11.9)	1 (1.4)	8 (6.0)
Total		59	74	133

Source: this study

Conclusion: The addition of amikacin to a mycobacterial drug regimen in all studies (TR02-112, INS-212 and INS-312) improved both the number of patients who were converted to sputum culture negative and the time to conversion.

At baseline the overall frequency of clarithromycin resistance > 8mcg/mL was 21.8% (73/335). The frequency of clarithromycin resistance for MAC overall was similar across treatment groups. For patients treated with ALIS+BR it was 51/223 (22.9%) and for patients treated with BR alone it was 19.6% (22/112).

8.2.2.2. **Activity of Amikacin against other Bacteria**

Susceptibility of other bacteria to amikacin including NTMs other than MAC, was tested using CLSI M24-A2, 2011 broth micro-dilution and macro-dilution procedures. The MIC_{50} for other NTM ranged from 0.25 to 8 mcg/mL and the MIC_{90} from 2 to 32 mcg/mL. Table 78 in appendix 15.5 shows the susceptibility of select species of NTM other than MAC found in the literature. The susceptibilities were determined by a variety of methods.

The quality control bacterium for susceptibility testing was *M. avium* ATCC 700898 and was performed using CLSI broth micro-dilution test described in (CLSI M24-A2, 2011). The quality control results MIC - 2 to 16 mcg/mL were acceptable.

8.2.2.3.
Interpretive Criteria.
There are no interpretive criteria for inhaled amikacin in liposomes. However, the MIC for amikacin was ≤ 64 mcg/mL.

9 Review of Safety

9.1.1 Safety Review Approach

The refractory NTM population (n=388) was considered the primary safety population. The safety population comprised patients from the Phase 3 Study 212, its single-arm extension Study 312 and the Phase 2 Study 112.

About 91% (352/388) of the primary safety population consisted of non-CF patients with refractory MAC infection; the remaining 9% included non-CF patients predominantly infected with *Mycobacterium abscessus* (4.6%), and CF patients with refractory MAC and *M. abscessus* infections.

There were significant differences in the design of the Phase 3 Study 212 from the Phase 2 Study 112. Study 212 only included non-CF patients with refractory MAC infection while Study 112 had a heterogenous study population which included CF patients and patients with refractory *M. abscessus* infection. Study 212 also had an open label randomized design for the first 8 months that compared ALIS added to background regimen (BR) versus BR alone arm. On the other hand, Study 112 had an initial double-blind, placebo-controlled phase for the first 3 months that compared ALIS added to BR versus BR plus inhaled diluted empty liposomes as a placebo.

Due to these differences in patient population, comparator arms and durations of treatment, safety data from the two studies will be presented separately. Since Study 212 contributed the majority of the safety population, detailed safety assessments and findings will be presented

for Study 212. Pertinent safety findings from Study 112 and Study 312 will be presented separately.

Even though the primary safety population was the refractory NTM population, safety data from patients exposed to multiple doses of ALIS, including patients with CF and non-CF bronchiectasis, were included in the integrated safety dataset to look for low frequency adverse events. The findings from the integrated safety dataset showed a similar adverse event profile as what was seen in the refractory NTM population and will not be covered in the review.

Of note, adverse events of interest (AEIs) were identified based on: (1) the known adverse effects of the aminoglycoside drug class, and (2) the inhalational route of administration and potential for resultant local irritation and inflammation. Table 39 presents the pooled AEI terms along with the MedDRA preferred terms included in the pooled terms.

Table 39: Adverse Events of Interest for ALIS

AEI	Included Preferred Terms
Hypersensitivity pneumonitis*,#	Alveolitis allergic, interstitial lung disease, pneumonitis, allergic reaction to ALIS
Bronchospasm*	Asthma, bronchial hyperreactivity, bronchospasm, dyspnea, dyspnea exertional, prolonged expiration, throat tightness, wheezing
Cough	Cough, productive cough, upper airway cough syndrome
Dysphonia	Aphonia, dysphonia
Exacerbation of underlying lung disease	COPD, infective exacerbation of COPD, infective exacerbation of bronchiectasis
Hemoptysis	Hemoptysis
Nephrotoxicity*	Blood creatinine increased, glomerular filtration rate decreased, hematuria, leukocyturia, proteinuria, renal failure, urinary casts, urinary casts present
Neuromuscular disorder*	Muscular weakness, neuropathy peripheral, balance disorder
Ototoxicity*	Deafness, deafness neurosensory, deafness unilateral, dizziness, hypoacusis, presyncope, tinnitus, vertigo
Pneumonia	Atypical pneumonia, empyema, infectious pleural effusion, lower respiratory tract infection, lung infection, lung infection pseudomonal, pneumonia, pneumonia aspiration, pneumonia pneumococcal, pneumonia pseudomonal, pseudomonal infection, respiratory tract infection
Pneumothorax	Pneumo-mediastinum, pneumothorax, pneumothorax spontaneous
Upper airway irritation	Laryngeal erythema, laryngeal pain, laryngitis, oropharyngeal discomfort, oropharyngeal pain, pharyngeal erythema, pharyngeal edema, pharyngitis, upper respiratory tract inflammation, vocal cord inflammation

*indicate AEIs identified by both the Applicant and the Agency

#Applicant used allergic alveolitis for the pooled terms instead of hypersensitivity pneumonitis

The AEIs based on class effect included clinical and laboratory indicators of nephrotoxicity, clinical signs/symptoms of neuromuscular disorders and clinical signs/symptoms of ototoxicity (both auditory and vestibular). Although the likelihood of these AEIs was deemed low given the local administration of amikacin, the safety database was reviewed for the abovementioned AEIs.

AEIs based on route of administration included allergic alveolitis (referred to as "hypersensitivity pneumonitis" for this review), bronchospasm, cough, dysphonia, exacerbation of underlying lung disease, hemoptysis, pneumonia, pneumothorax, and upper airway irritation. These AEIs were reported while Study 212 was ongoing and this prompted the Agency to request interim reports from the Data Safety Monitoring Committee.

Nephrotoxicity, neuromuscular disorders, ototoxicity, allergic alveolitis (hypersensitivity pneumonitis), bronchospasm, and hemoptysis were identified by both the Applicant and the Agency. The Applicant also labeled exacerbation of COPD as an AEI; the Agency has also added additional AEIs based on potential adverse effects of inhaled products (cough, dysphonia, upper airway irritation, pneumonia, pneumothorax) and expanded exacerbation of COPD to exacerbation of underlying lung disease to include both exacerbation of COPD and bronchiectasis.

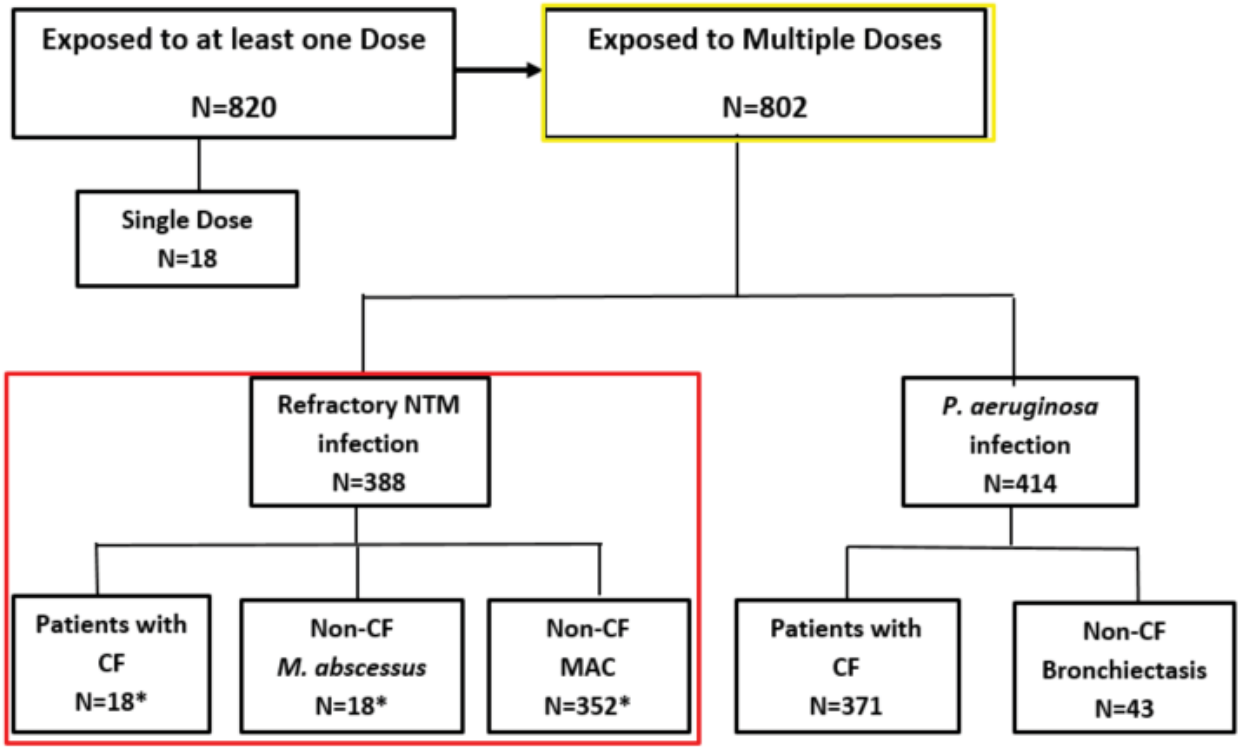
9.1.2 Review of the Safety Database

Overall Exposure

Figure 6 depicts the overall exposure to ALIS throughout the development program. Overall, 820 subjects were exposed to at least one dose of ALIS; 802 of these were exposed to multiple doses of ALIS. The patients with refractory NTM infection, which make up the primary safety population, accounted for 48% (388/802) of multi-dose exposures. The remaining 414 were patients with pulmonary *Pseudomonas aeruginosa* infection, and most had CF.

Within the primary safety population of refractory NTM patients, 91% were non-CF patients with refractory MAC infection while the remaining 9% were comprised of non-CF patients predominantly infected with *M. abscessus (*4.6%), and patients with underlying CF (4.6%).

Figure 6: Overall Exposure to ALIS



NTM: Non-tuberculous mycobacteria; CF: Cystic fibrosis; *P. aeruginosa*: *Pseudomonas aeruginosa*; *M. abscessus: Mycobacterium abscessus*; MAC: *Mycobacterium avium* complex
Yellow box denotes multi-dose exposures; red box denotes the primary safety population
*Included 4 subjects in the scintigraphy substudy of TR02-112; safety data for these subjects was reviewed but not included in the safety presentation

There was heterogeneity in the doses, dosing regimen and durations of exposure among the multi-dose exposures: ALIS dosing in the studies of patients with pulmonary *Pseudomonas aeruginosa* infection were cyclic with 28 days on/28 days off while all patients in the refractory NTM studies were dosed daily. Also, earlier studies in CF patients used doses ranging from 70 mg to 560 mg while later studies, including all 3 NTM studies that make up the primary safety population, were conducted at the proposed dose of 590 mg. Overall, 92% of the multi-dose exposures were at either 560 or 590 mg, including cyclic and daily dosing regimens. All patients in the primary safety population were exposed to ALIS at the proposed dose of 590 mg and at the proposed daily dosing regimen. However, the duration of exposure in the NTM population varied from 3 to 20 months.

The primary safety population is comprised of patients in Study 212, its single-arm extension Study 312, and the Phase 2 Study 112. Table 40 outlines the contribution of each study to the primary safety population along with the comparator arms used in the studies.

Table 40: Safety Database for ALIS for Treatment of Refractory NTM Pulmonary Infection

Safety Database for the Study Drug[1] Individuals exposed to the study drug in this development program for the indication under review N=388 (N is the sum of all available numbers from the columns below)			
Clinical Trial Groups	ALIS + BR* (n= 388)	BR* Alone (n=112)	Inhaled Placebo + BR* (n= 45)
Controlled trials conducted for this indication[2]			
Study 112	44		45
Study 212	223	112	
All other than controlled trials conducted for this indication[3]			
Study 112 open label phase	43**		
Study 112 scintigraphy+ open label Phase	4		
Study 312 (Single arm extension of Study 212)	74**		

[1] *study drug* means the drug being considered for approval.
2 to be used in product's labeling
*BR refers to systemic multi-drug background regimen
**only included ALIS-naive patients in the comparator's arm in the randomized portion of the study

Relevant characteristics of the safety population:

Due to the differences in study design between Study 112 and 212, safety data for the two studies were reviewed separately. Relevant characteristics of the study participants are also presented in detail in the safety results of each study in Section 9.1.4. Overall, participants in the two studies were predominantly non-Hispanic Whites (92% in Study 112 and 70% in Study 212). The majority of the participants were females (87% in Study 112 and 70% in Study 212). The median age of the participants in Study 112 and 212 were 63 and 66 years, respectively. Please refer to Table 10 and Table 24 in Section 7 for additional details.

Reviewer's comment: The predominance of older white women in the trials likely reflects the overall higher prevalence of NTM disease in this population.

Adequacy of the safety database:

Overall, the number of patients exposed to the dose and dosing regimen of ALIS 590 mg daily appears sufficient. The Applicant's proposed duration of treatment is 12 months after the first of three consecutive negative sputum cultures (culture conversion). Both Studies 212 and 312 are ongoing, and more long-term safety and tolerability information will be available once the studies are completed.

9.1.3 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

Overall, the organization of the data submitted by the Applicant was difficult to navigate. This is partly due to the complexity of the study designs. In collaboration with the Office of Computational Science (OCS), a data fitness assessment was performed on April 30, 2018. The results showed some inconsistencies in the data. For example, in the Phase 3 Study 212, 49 duplicate unique subject identification (USUBJID) numbers were noted; these were patients who were initially screen failures but were then enrolled at a later time point. There were 4 separate datasets submitted for Study 112 with similar names (e.g., 4 sets of adverse event datasets).

In some instances, the submitted data were incomplete. For example, due to incomplete hospitalization data submitted for Study 212, the Agency had to send multiple information requests to clarify the submitted data and request additional data.

To assess the quality of the original data and data integrity, the Office of Scientific Investigations (OSI) was consulted and two clinical sites (Drs. Griffith and Winthrop) were selected for inspections for the three protocols (Study 212, 312, and 112) that were submitted to support this application. Both clinical sites enrolled a relatively higher number of patients in Study 212, as well as, Studies 112 and 312. The OSI consult concluded that the study data derived from these clinical sites, based on the inspections, were considered reliable in support of the requested indication under this NDA.

Categorization of Adverse Events

For the Phase 3 Study 212, the Agency did not agree with the Applicant's definition of treatment emergent adverse events (TEAEs). The Applicant defined TEAEs differently for the two study arms. For patients in the ALIS+BR arm, TEAEs were defined as AEs that occurred between Day 1 up to 28 days post the last dose of ALIS while for the BR alone arm, TEAEs were defined as all AEs that occurred between Day 1 and the End of Treatment, which could have been up to Month 16. There was concern that this definition could potentially result in differential follow-up time for the two study arms. In addition, there was also concern that the adverse effects of ALIS may extend beyond 28 days post last dose. Since the optimal

comparative safety data would be obtained from the first 8 months of the study, the Agency defined TEAEs as all AEs that occurred between Day 1 to Day 247, which was the Month 8 visit.

Regarding the Applicant's coding of verbatim reported terms to MedDRA preferred terms, there appeared to be splitting of terms that could minimize a safety signal. For example, there were more than 10 terms describing pneumonia, which included "lung infection", "atypical pneumonia", "lower respiratory tract infection", "lung infection Pseudomonas", "pneumonia", "pneumonia Pseudomonas", "Pseudomonas infection". The term mapping, along with the verbatim reported terms, were closely examined and case report forms, when available, were reviewed to assess the appropriateness of including these terms under one preferred term, that is, pneumonia.

As mentioned in Section 9.1.2, the Applicant had identified AEIs based on route of administration and the adverse effect profile of amikacin; these included clinical and laboratory indications of nephrotoxicity, neuromuscular disorders, ototoxicity, allergic alveolitis, bronchospasm, hemoptysis and exacerbation of COPD. In addition to the AEIs identified by the Applicant, the Agency added additional terms. Please refer to **Table 39** for details of preferred terms included in each AEI.

Routine Clinical Tests

Overall, the Applicant's safety evaluation performed as part of the development program appeared adequate. Evaluations specific to pulmonary function, such as pulmonary function testing and 6-MWT were included. In addition, evaluations based on known systemic adverse effects of aminoglycosides, such as audiometry and renal function testing were also performed in the three NTM studies that make up the safety population.

9.1.4 Safety Results

Overall Deaths during ALIS Development

There were 32 deaths reported during the ALIS development program. All except for one occurred in the three NTM studies. Since the design of Studies 112 and 212 offered ALIS treatment to patients in both treatment arms at the end of the randomized portions of the studies, a mortality comparison between ALIS-treated vs. comparator arm (BR only in Phase 3 Study 212 and Placebo + BR arm in Phase 2 Study 112) is limited only to the randomized portions of both studies. There were 15 deaths during the randomized portion of these two studies, and no significant imbalance in mortality was identified between the ALIS-treated patients vs. those in the respective comparator arms; 13 deaths occurred in single-arm, extension phases of the two studies and subsequent long-term follow-up period. Table 41 summarizes the observed deaths.

Table 41: Overall Deaths during ALIS Development

All deaths during ALIS development	**32**
Deaths during randomized phases of studies	**15**
Study 112 (DB)	**1 (2.3%)** ALIS+BR **0 (0%)** Placebo+BR
Study 212	**9 (4%)** ALIS+BR **5 (4.5%)** BR alone
Deaths during single arm extension studies and long-term follow-up (all exposed to ALIS)	14 (1 in CF StudyTR02-110, 10 in Study 112*, and 3 in Study 312)
Deaths in patients screened but not randomized	3 in Study 212

*Included 1 death in open-label phase, 7 deaths in 12-month follow-up study, and 2 deaths that occurred off-study

Additional details regarding the deaths in the randomized portions of Study 212 and 112 will be discussed in the safety discussions of the respective studies.

INS-212

Baseline Characteristics of Study Participants

Table 42 and **Table 43** summarize the baseline characteristics and pertinent baseline medical history of participants in Study 212, respectively.

Table 42: Baseline Characteristics of Study 212 Participants

	ALIS+BR **N=223** **n (%)**	**BR Alone** **N=112** **n (%)**
Sex		
Male	58 (26)	44 (39.3)
Female	165 (74)	68 (60.7)
Age (Years)		
Median	65	66
Range	40-87	32-85

Race		
American Indian / Alaska Native/Pacific islander/Multiple	2 (0.8)	1 (0.9)
Asian	58 (26)	25 (22.3)
Black or African American	3 (1.3)	3 (2.7)
White	157 (70.4)	77 (68.8)
Ethnicity		
Hispanic or Latino	10 (4.5)	5 (4.5)
Non-Hispanic or Latino	210 (94.2)	102 (91.1)
Not Reported/unknown	3 (1.3)	5 (4.5)
Region of Enrollment		
US	93 (41.7)	48 (42.9)
Ex-US	130 (58.3)	64 (57.1)

Table 43: Pertinent Baseline Medical History of Study 212 Participants

	ALIS+BR N=223 n, %	**BR Alone N=112 n, %**
On BR treatment at baseline	200 (89.7)	101 (90.2)
Prior nebulized inhaled amikacin use	24 (10.8)	15 (13.4)
Current smoker	25 (11.2)	10 (8.9)
Bronchiectasis	168 (75.3)	82 (73.2)
COPD	50 (22.4)	37 (33)
Hemoptysis	28 (12.6)	15 (13.4)
Pulmonary cavitation	26 (11.7)	19 (17)
Pulmonary resection	24 (10.8)	6 (5.4)
Asthma	22 (9.9)	14 (12.5)
Dyspnea	18 (8.1)	15 (13.4)
Emphysema	16 (7.2)	10 (8.9)
Pneumothorax	11 (4.9)	8 (7.1)

Hypoacusis	19 (8.5)	11 (9.8)
Deafness[a]	47 (21.1)	34 (30.4)
Tinnitus	24 (10.8)	13 (11.6)
Vertigo and balance disorders	6 (2.7)	3 (2.8)

[a]Includes deafness, deafness neurosensory, deafness bilateral, deafness unilateral

Reviewer's Comments: Overall, the two study arms were matched for age, race and ethnicity, and region of enrollment. However, there was some imbalance, with a higher percentage of females in the ALIS+BR arm, as females accounted for 74% of subjects in the ALIS+BR arm as compared to 61% in the BR alone arm.

Looking at the pertinent baseline medical history, approximately 90% of patients in each study arm were on BR treatment at time of enrollment. Close to 75% of patients in each study arm had a history of bronchiectasis. Some differences were noted. There were more patients with history of pulmonary resection in the ALIS + BR arm, 10.8% vs. 5.4 % in the BR only arm. There was also a slightly higher percentage of current smokers in the ALIS arm at 11.2% as compared to 8.9% in the BR alone arm. Comorbidities reported at a significantly higher percentage in the BR alone arm included COPD (33% in BR alone arm vs 22.4% in ALIS+BR arm), pulmonary cavitation (17% in BR alone arm vs. 12% in ALIS+BR arm), deafness (30% in BR alone arm vs. 21% in ALIS+BR arm), and dyspnea (13% BR alone arm vs 8% in ALIS+BR arm).

Deaths

There was no imbalance in mortality in Study 212. Of the 17 deaths, 3 occurred prior to randomization and 14 occurred after first dose of study drug; 9 of the 14 deaths occurred in the ALIS-treated arm (4%) while 5 deaths occurred in the BR alone arm (4.5%). Of note, the Applicant classified three of the deaths in the ALIS+BR arm as non-TEAEs based on their previously mentioned definition of TEAEs. Case report forms and narratives of the deaths were reviewed and a summary of the demographics, timing and causes of death for the 14 subjects is presented in **Table 44**.

Table 44: Summary of Deaths in Study 212

Age in yrs	**Sex***	**Study day of death**	**Treatment Duration with study drug**	**Reported Immediate Cause of Death**
ALIS+BR				
71	F	372	189	Acute respiratory failure

70	M	187	143*	COPD exacerbation & acute respiratory failure
60	M	81	70 *	COPD exacerbation & respiratory failure
67	F	61	61	Pulmonary embolism, *Pseudomonas* pneumonia, ongoing MAC infection
79	M	54	54	COPD exacerbation and respiratory failure
61	M	23	23	Respiratory failure and cachexia
60	M	175	43*	COPD
69	F	191	170*	Lung infection
76	M	202	83*	Empyema
BR Alone				
69	M	429	388	Interstitial lung disease
75	F	42	29	MAC infection
70	M	14	14	Cardiogenic shock
63	F	229	217	Pneumonia
74	M	173	143	Respiratory failure

F=Female; M=Male; *ALIS discontinued due to clinical decline;

Reviewer's Comments: As noted in Table 44, pulmonary events (respiratory failure, COPD exacerbation, pneumonia) accounted for the majority of the deaths in both arms. Five patients in the ALIS arm discontinued ALIS due to worsening clinical condition prior to their deaths. Patients in both study arms have underlying co-morbidities that may have contributed to their deaths; however, for those that received ALIS treatment, the contribution of ALIS to the deaths cannot be ruled out.

Serious Adverse Events

Forty-five patients (20.2%) in the ALIS+BR arm, and 18 (16.1%) in the BR alone arm experienced at least one SAE. The majority of these SAEs were in the "Respiratory, thoracic and mediastinal disorders" system organ class (SOC). **Table 45** and **Table 46** below summarize the observed SAEs by MedDRA SOC and preferred term (PT), respectively.

Table 45: Serious Adverse Events Experienced by >1 Patient in Either Treatment Arm in Study 212 by System Organ Class

System Organ Class (SOC)	ALIS+BR N=223 n (%)	BR Alone N=112 n (%)
Patient with at least one SAE	45(20.2)	18 (16.1)
Respiratory, thoracic & mediastinal disorders	29 (13)	10 (8.9)
Infection and infestation	21(9.4)	6 (5.4)
Neoplasm benign, malignant and unspecified	2 (0.9)	1 (0.9)
Psychiatric disorders	2 (0.9)	0
Musculoskeletal & connective tissue disorders	2 (0.9)	0
Vascular disorders	2 (0.9)	0
Gastrointestinal disorders	2 (0.9)	0
Investigations	2 (0.9)	0
Cardiac disorders	1 (0.4)	4 (3.6)

Table 46: Serious Adverse Events Experienced by >1 Patient in Either Treatment Arm by Preferred Term

MedDRA Preferred Term	ALIS + BR N=223 n (%)	BR Alone N=112 n (%)
Patient with at least one SAE	45 (20.2)	18 (16.1)
Pneumonia[a]	12 (5.4)	4 (3.6)
COPD exacerbation[b]	8 (3.6)	2 (1.8)
Hemoptysis	5 (2.2)	4 (3.6)
Hypersensitivity pneumonitis[c]	4 (1.8)	0
Pneumothorax	4 (1.8)	1 (0.9)
Respiratory failure[d]	4 (1.8)	1 (0.9)
Infective exacerbation of bronchiectasis	4 (1.8)	3 (2.7)
Dyspnea	3 (1.3)	0
Anxiety	2 (0.9)	0
Fungal pneumonia[e]	2 (0.9)	1 (0.9)
Acute myocardial infarction	0	2 (1.8)
Pulmonary cavitation	0	2 (1.8)
MAC infection	1 (0.4)	2 (1.8)

[a]Included pneumonia, lung infection, empyema, infectious pleural effusion, lung infection Pseudomonas, pneumonia aspiration, pneumonia pseudomonas
[b]Included COPD and infective exacerbation of COPD
[c] Included allergic alveolitis, pneumonitis, and interstitial lung disease
[d]Included respiratory failure and acute respiratory failure
[e] Includes two subjects in ALIS+BR arm (n=1 bronchopulmonary aspergillosis, n=1 scedosporium infection) and one subject (n=mycotic mycetoma) in the BR alone arm

Hospitalizations in Study INS-212

There were more hospitalization events in ALIS-treated patients as compared to patients treated with BR alone. **Table 47** summarizes the hospitalization events in Study 212.

Table 47: Hospitalization during Study 212

	ALIS+BR N=223	**BR Alone N=112**
All hospitalizations		
Event count*	92	24
Subject count	47 (21.1%)	15 (13.4%)
Excluding unrelated/planned surgical hospitalizations		
Event count*	**82**	**23**
Subject count	**41 (18.4%)**	**15 (13.4%)**

Approximately 60% of patients from either study arm with hospitalizations during the study had one hospitalization event (24 of 41 patients in ALIS+BR arm, 9 of 15 patients in BR alone arm). The remaining 40% had multiple (2-5) hospitalizations; **Table 48** summarizes the frequency of hospitalization in each arm.

Table 48: Frequency of Hospitalization in Study 212

Frequency of Hospitalization	**ALIS+BR N=41 Patients n, %**	**BR Alone N=15 Patients n, %**
1	24 (58.5)	9 (60)

2	7 (17.1)	5 (33.3)
3	3 (7.3)	0
4	5 (12.2)	1 (6.7)
5	1 (2.4)	0
10	1 (2.4)	0

In both study arms, respiratory events were the main reason for hospitalization and are summarized in **Table 49**.

Table 49: Reasons for >1 Hospitalization During Study 212

Reasons for Hospitalization	**ALIS+BR** **N=82 Admissions**	**BR Alone** **N=23 Admissions**
Exacerbation of underlying pulmonary disease	**22**	**5**
Lower respiratory tract infections	**22**	**4**
Pneumonia/respiratory superinfection/bronchitis	19	1
Fungal infections	2	1
MAC infection	1	2
Hemoptysis	7	5
Respiratory failure	**6**	**0**
Dyspnea	**5**	**1**
Pneumothorax	**3**	**1**
Arikayce-induced pneumonitis	**2**	N/A

Reviewer's Comments: Even after accounting for the 2:1 randomization in Study 212, there were more hospitalization events in the ALIS+BR arm (n=82) versus the BR alone arm (n=23). The imbalance in the frequency of hospitalizations persisted even with the exclusion of the one outlier patient in the ALIS+BR arm with 10 admissions. Respiratory conditions were the major causes of hospitalizations. A slightly higher percentage of ALIS-treated patients had at least one hospitalization event compared to patients treated with BR alone (18.4% vs. 13.4%, respectively). This difference is in line with the difference in the percentage of patients experiencing SAEs in the two arms.

Dropouts and/or Discontinuations Due to Adverse Effects

A significantly higher percentage of patients in the ALIS+BR arm (33.5%) discontinued treatment prematurely compared to patients in the BR alone arm (8%). The main reason for premature discontinuation from the ALIS arm was AEs: 39 patients (17.4%) in the ALIS+BR arm discontinued treatment due to AEs compared to 1 (0.9%) in the BR alone arm. Further evaluation of the AEs leading to discontinuation revealed that 31 of the 39 (79.5%) patients discontinued due to pre-defined AEs of interest, further discussed in section 9.1.2. These included bronchospasm (n=9), dysphonia (n=5), exacerbation of underlying lung disease (n=4), ototoxicity (n=4), allergic alveolitis/hypersensitivity pneumonitis (n=3), cough (n=2), hemoptysis (n=2), pneumonia (n=1) and upper airway irritation (n=1).

Reviewer's Comments: It is concerning that a third of ALIS+BR patients discontinued treatment prematurely, mostly due to AEs*.*

Significant Adverse Events

Please refer to the laboratory section for analysis of renal and pulmonary function tests by study arm.

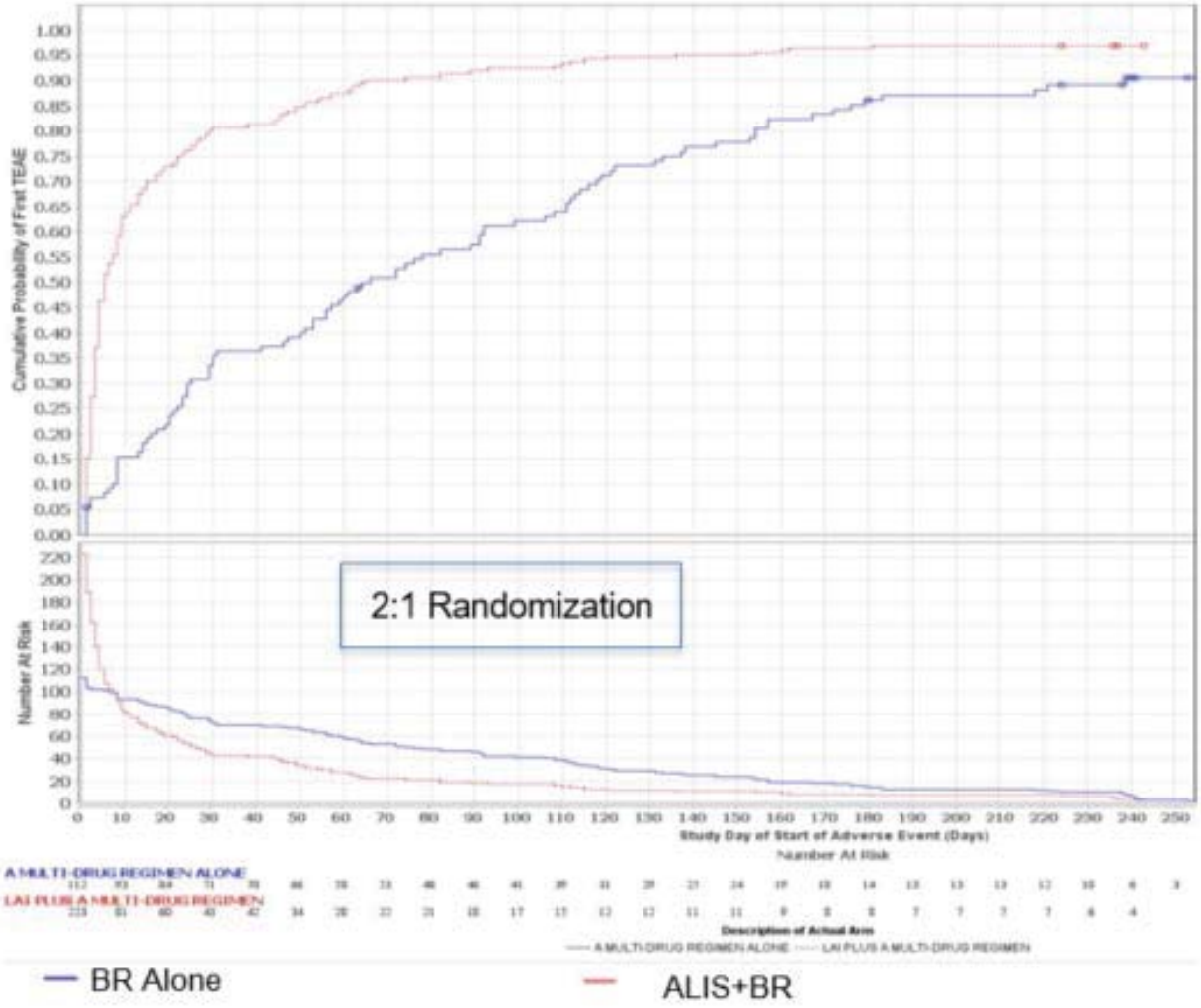
Treatment Emergent Adverse Events (TEAEs) and Adverse Reactions

More patients in the ALIS+BR arm (98.2%) experienced TEAEs compared to the BR alone arm (90.2%). In addition, there was also a significantly higher number of adverse events reported for ALIS+BR-treated patients (1463 events reported in 219 patients) compared to patients treated with BR alone (362 events in 101 patients).

Reviewer's Comments: Even after accounting for the 2:1 randomization, there appears to be an excess number of TEAEs reported in ALIS+BR patients as compared to patients treated with BR alone.

In terms of timing of TEAEs, about 80% of ALIS-treated patients experienced their first TEAE in the first month after initiation of ALIS therapy. In contrast, the probability of experiencing a first TEAE in patients treated with BR alone was more evenly distributed over the course of the study. **Figure 7** shows the cumulative probability of the first TEAE by study day.

Figure 7: Timing of Treatment Emergent Adverse Events in Study 212



Incidence of TEAEs is presented by MedDRA System Organ Class (SOC) and Preferred Term (PT) in

Table **50** and **Table 51**, respectively.

Table 50: Treatment Emergent Adverse Events in Study 212 by System Organ Class

System Organ Class (SOC)	ALIS+BR N=223 n (%)	BR Alone N=112 n (%)

Patient with at least one TEAE	219 (98.2)	101 (90.2)
Respiratory, thoracic and mediastinal disorders	196 (87.9)	57 (50.9)
Infections and infestations	97 (43.5)	46 (41.1)
Gastrointestinal disorders	85 (38.1)	25 (22.3)
General disorders and administration site conditions	75 (33.6)	16 (14.3)
Nervous system disorders	52 (23.3)	10 (8.9)
Musculoskeletal and connective tissue disorder	48 (21.5)	17 (15.1)
Skin and subcutaneous tissue disorders	41 (18.4)	12 (10.7)
Investigations	37 (16.6)	13 (11.6)
Ear and labyrinth disorders	32(14.3)	10 (8.9)
Eye disorders	25 (11.2)	6 (5.4)
Metabolism and nutrition disorders	23 (10.3)	12 (10.7)
Psychiatric disorders	23 (10.3)	5 (4.5)
Injury, poisoning and procedural complications	14 (6.3)	6 (5.4)
Cardiac disorders	13 (5.8)	5 (4.5)
Blood and lymphatic system disorders	10 (4.5)	2 (1.8)
Vascular disorders	9 (4)	4 (3.6)
Renal and urinary disorders	8 (3.6)	5 (4.5)
Hepatobiliary disorder	4 (1.8)	1 (0.9)
Neoplasm benign, malignant and unspecified	4 (1.8)	3 (2.7)
Endocrine	1 (0.4)	1 (0.9)
Reproductive system and breast disorder	1 (0.4)	0 (0)
Immune system disorders	1 (0.4)	2 (1.8)

Table 51: Treatment Emergent Adverse Events in Study 212 in >5% of Participants by Preferred Term in the ALIS+BR arm

MedDRA Preferred Term	ALIS+BR N=223 n (%)	BR Alone N=112 n (%)
Dysphonia[a]	105 (47.1)	1 (0.9)
Cough[b]	87 (39)	20 (17.9)
Dyspnea[c]	48 (21.5)	10 (8.9)
Upper airway inflammation[d]	40 (17.9)	2 (1.8)
Hemoptysis	40 (17.9)	14 (12.5)
Musculoskeletal pain[e]	39 (17.5)	12 (10.7)
Fatigue and asthenia	36 (16.1)	11 (9.8)
Diarrhea	29 (13)	5 (4.5)
Upper respiratory infection[f]	26 (11.7)	20 (17.9)

MedDRA Preferred Term	ALIS+BR N=223 n (%)	BR Alone N=112 n (%)
Nausea	26 (11.7)	4 (3.6)
Pneumonia[g]	22 (9.9)	9 (8)
Headache	22 (9.9)	5 (4.5)
COPD exacerbation[h]	19 (8.5)	4 (3.6)
Tinnitus	17 (7.6)	1 (0.9)
Wheezing	16 (7.2)	2 (1.8)
Pyrexia	16 (7.2)	5 (4.5)
Infective exacerbation of bronchiectasis	15 (6.7)	8 (7.1)
Rash[i]	15 (6.7)	1 (0.9)
Vomiting[j]	15 (6.7)	4 (3.6)
Weight decreased	14 (6.3)	1 (0.9)
Decreased appetite	14 (6.3)	8 (7.1)
Dizziness	14 (6.3)	3 (2.7)
Change in sputum[k]	13 (5.8)	1 (0.9)
Chest discomfort	12 (5.4)	3 (2.7)

[a]Includes "aphonia" and "dysphonia"
[b]Includes "cough", "productive cough" and "upper airway cough syndrome"
[c]Includes "dyspnea" and "dyspnea on exertion"
[d]Includes "oropharyngeal pain", "oropharyngeal discomfort", "throat irritation", "pharyngeal erythema", "upper airway inflammation", "pharyngeal edema", "vocal cord inflammation", "laryngeal pain", "laryngeal erythema", "laryngitis"
[e]Includes "back pain", "arthralgia", "myalgia", "pain/body aches", "muscle spasm" and "musculoskeletal pain"
[f]Includes "nasopharyngitis/cold", "pharyngitis", "sinusitis", and "upper respiratory infection"
[g]Includes "atypical pneumonia", "empyema", "infection pleural effusion", "lower respiratory tract infection", "lung infection", "lung infection pseudomonas", "pneumonia", "pneumonia aspiration", "pneumonia pseudomonas", "pseudomonas infection" and "respiratory tract infection"
[h]Includes "COPD" and "infective exacerbation of COPD"
[i]Includes "rash", "rash maculo-papular", "drug eruption" and "urticaria"
[j]Includes "vomiting" and "post-tussive vomiting"
[m]Includes "increased sputum", "sputum discolored" and "sputum purulent"

Clinically relevant adverse events that occurred in <5% of patients and at higher frequency in the ALIS+BR-treated patients are presented in **Table 52**.

Table 52: Clinically Relevant Adverse Events that Occurred in <5% of Patients and at Higher Frequency in the ALIS plus Background Regimen Arm

	ARIKAYCE + BR N=223 n, (%)	**BR Alone N=112 n, (%)**
Anxiety	10 (4.5)	0 (0)
Oral fungal infection[a]	9 (4)	2 (1.8)
Bronchitis	8 (3.6)	3 (2.7)
Hypersensitivity pneumonitis[b]	7 (3.1)	0 (0)
Dysgeusia	7 (3.1)	0 (0)
Respiratory failure[c]	6 (2.7)	1 (0.9)
Epistaxis	6 (2.7)	1 (0.9)
Neuromuscular disorder[d]	5 (2.2)	0 (0)
Dry mouth	5 (2.2)	0 (0)
Pneumothorax[e]	5 (2.2)	1 (0.9)
Exercise tolerance decreased	3 (1.3)	0 (0)
Balance disorder	3 (1.3)	0 (0)
Pulmonary pain	3 (1.3)	0 (0)

[a]Includes oral candidiasis and oral fungal infection
[b]Includes allergic alveolitis, interstitial lung disease, and pneumonitis
[c]Includes acuter respiratory failure and respiratory failure
[d]Includes muscle weakness, neuropathy peripheral, and balance disorder
[e]Includes pneumonthorax, pneumothorax spontaneous and pneumomediastinum

Treatment Emergent Adverse Events of Interest

Based on the route of administration and the class of the active ingredient, several adverse events of interest (AEI) were identified. Please refer to Section 9.1.5 for detailed discussion of the AEIs.

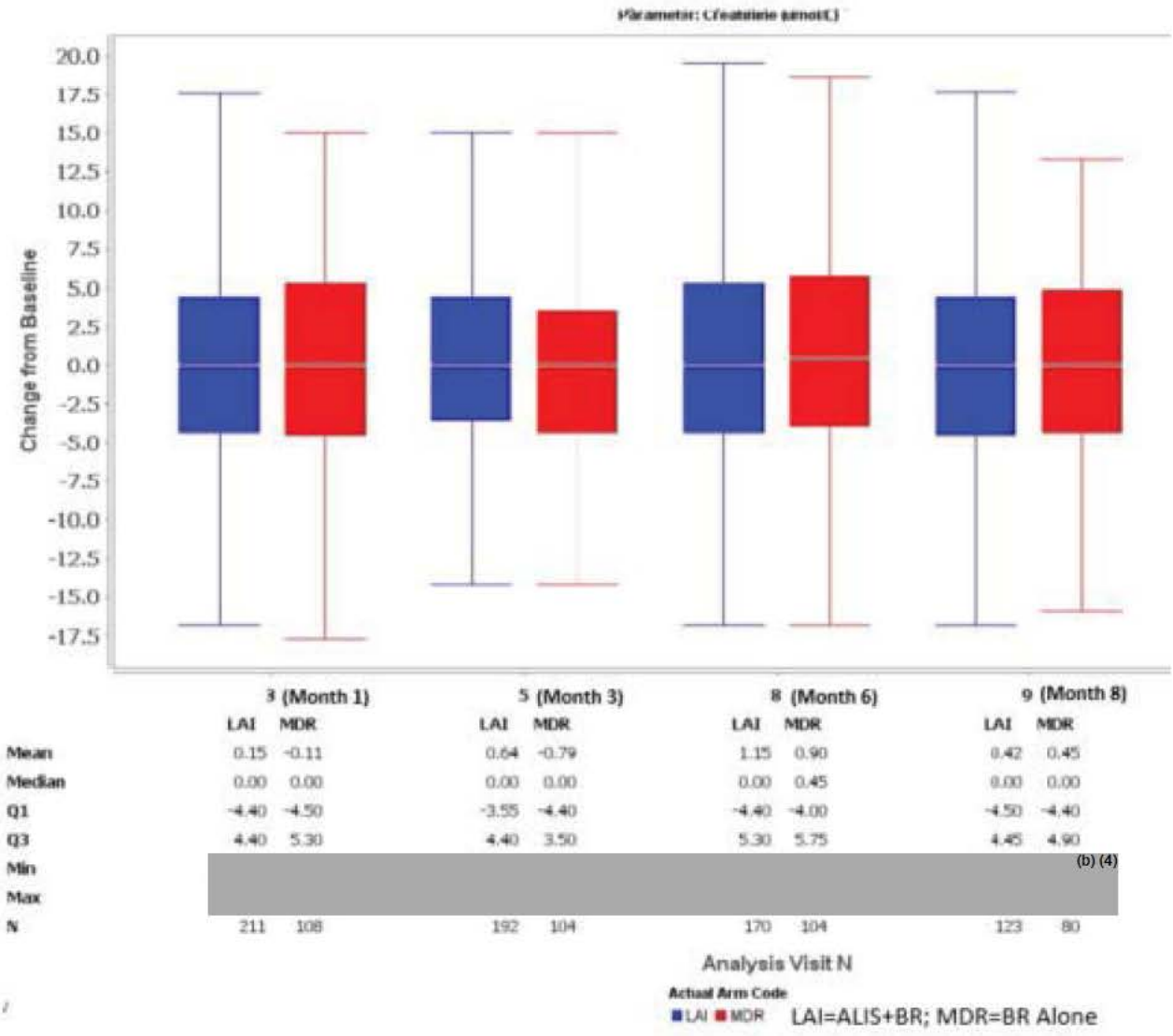
Laboratory Findings

There were no significant changes from baseline in terms of hematology studies (white blood cell count, hematocrit, platelet counts), liver tests (AST, ALT, GGT, bilirubin) and renal function

(serum creatinine levels) in the two study arms.

Figure 8 is presented as an example and depicts changes in serum creatinine levels by study visit for each study arm. Of note, there was a disproportionate loss to follow up among patients in the ALIS+BR arm as compared to the BR alone arm: 78.6% of ALIS+BR patients completed the Month 6 visit as compared to 95% of patients treated with BR alone. Hence, this limits the interpretation of the data.

Figure 8: Changes from Baseline Serum Creatinine



Vital Signs

The "ADVS" dataset was evaluated for changes in respiratory rate, oxygen saturation, body

temperature, heart rate, blood pressure and body weight. There were no significant differences between the study arms in terms of body temperature, systolic blood pressure, diastolic blood pressure and heart rate. Changes in respiratory rate, oxygen saturation and body weight are summarized below.

Changes in Respiratory Rate

The median change from baseline respiratory rate was not significantly different in the two study arms.

Figure 9: Changes from Baseline Respiratory Rate by Study Visit

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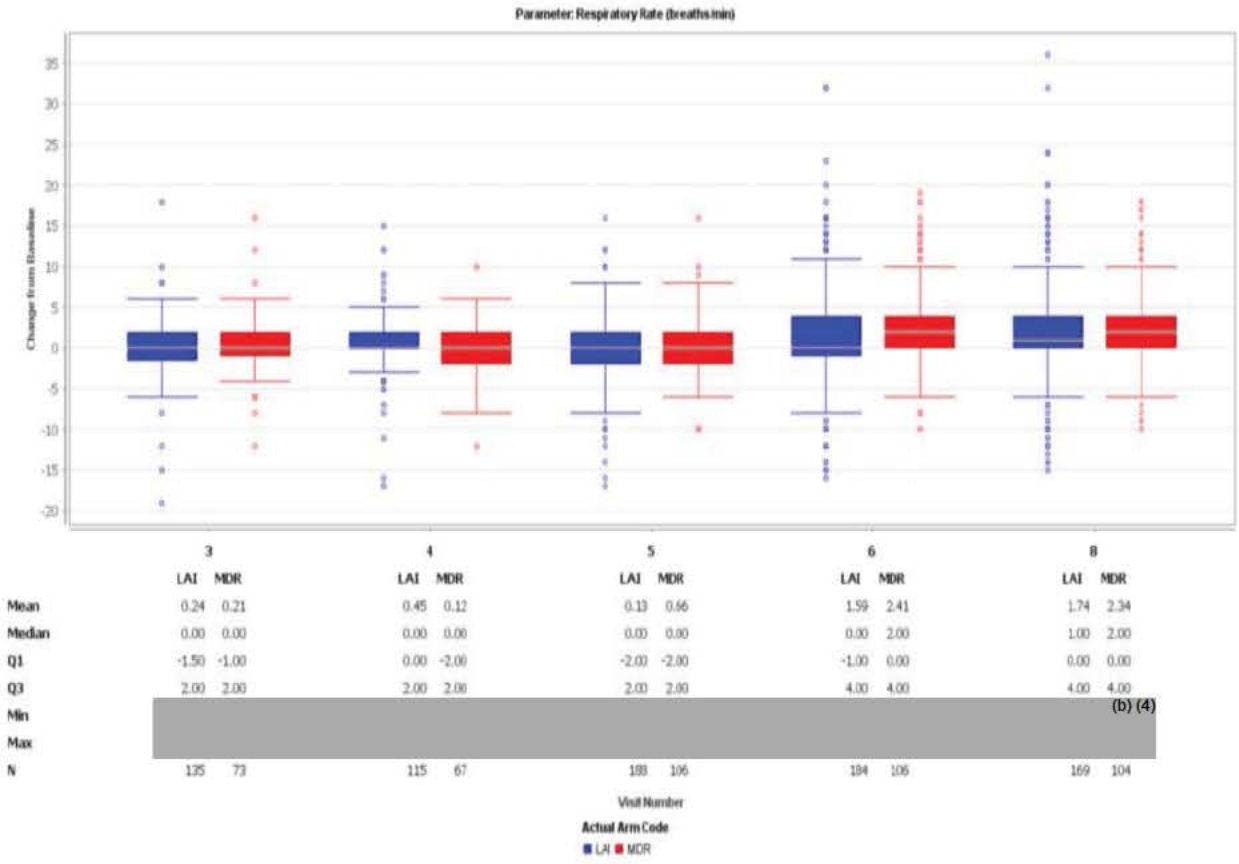


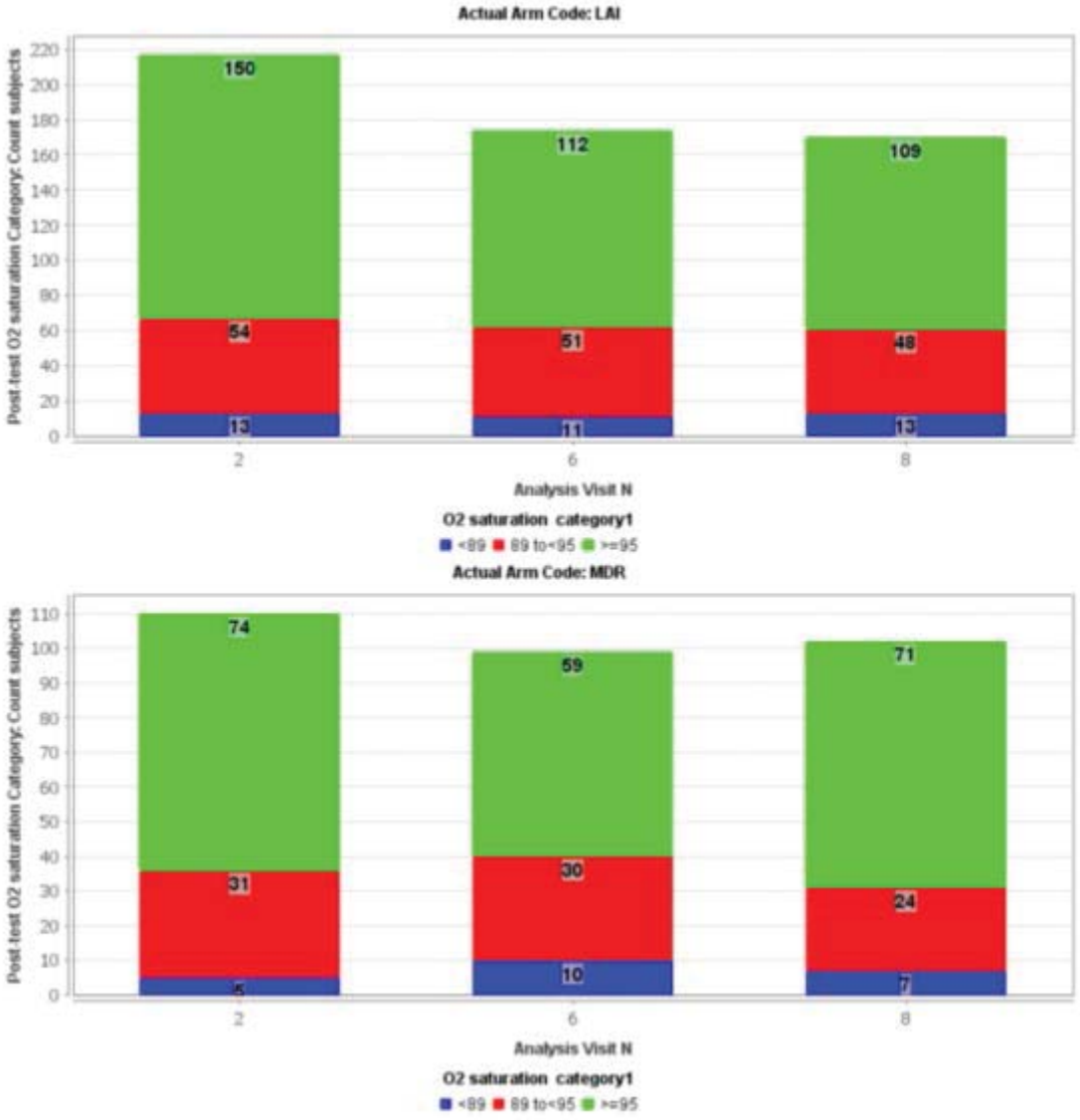
LAI=ALIS plus Background Regimen; MDR=Background Regimen Alone
Visit 3=Month 1; Visit 4=Month 2; Visit 5=Month 3; Visit 6=Month 4; Visit 8=Month 6

Changes in Oxygen Saturation

Overall, the median pulse oximetry at baseline was similar between the two arms with >66% of

patients in either arm having pulse oximetry of >95%. Among the patients that had Month 6 visits, the proportion of patients within each pulse oximetry category (<89%, 89-<95%, >=95%) appears comparable. However, there were disproportionate loss to follow up of patients in the ALIS+BR arm at Month 6 with only 78.6% completing the Month 6 visit as compared to 95% of patients treated with BR alone. Hence, this limits the interpretation of the data.

Figure 10: Percent Oxygen Saturation in Study INS-212 by Study Visit



LAI=ALIS plus Background Regimen; MDR=Background Regimen Alone; Post-test=Post 6-minute walk test
Visit 2=Baseline visit; Visit 6=Month 4 visit; Visit 8=Month 6 Visit

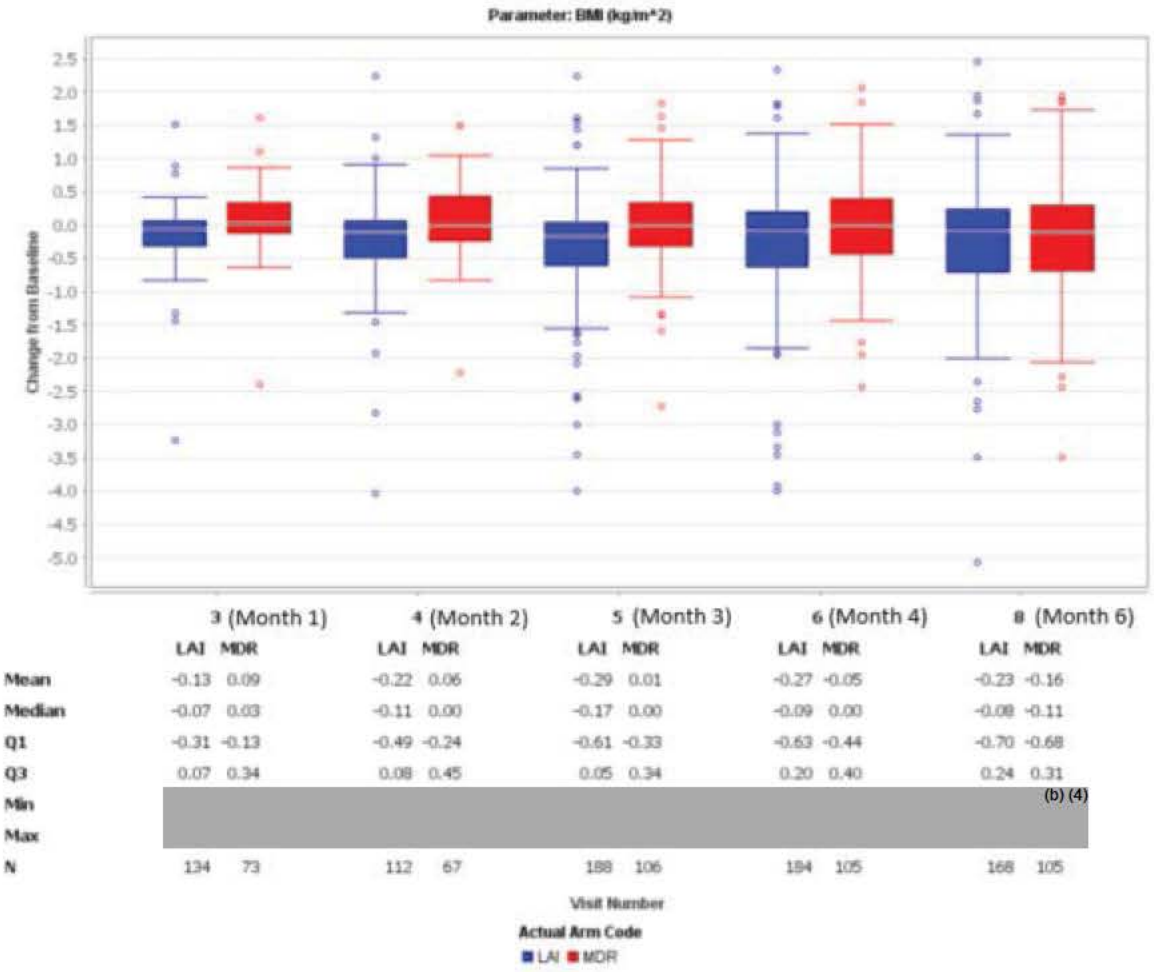
Reviewer's comment: From the available pulse oximetry data, and accounting for the 2:1 randomization, it appears that the percentage of patients with pulse oximetry ≥95% was comparable between the two arms: ALIS+BR arm (109/170) vs. BR alone (71/102). However, as mentioned above, only 78.6% (176 of 223) patients in the ALIS+BR arm completed the Month 6

visit as compared to 95.5% (107 of 112) of patients treated with BR alone. Post-6-minute walk test pulse oximetry was available for the 170 of 176 in the ALIS+BR arm and 102 of 107 in the BR alone arm that had completed the Month 6 study visit. Hence, the significant drop in patients who completed the Month 6 visit in the ALIS+BR arm (most due to discontinuation because of adverse events) may be masking a potential difference in pulse oximetry and other Month 6 visit assessments.

Changes in BMI

Overall, the baseline BMI of the two study arms were comparable. Figure 11 summarizes changes from baseline BMI by study visit for the two arms.

Figure 11: Changes from Baseline Body Mass Index by Study Arm



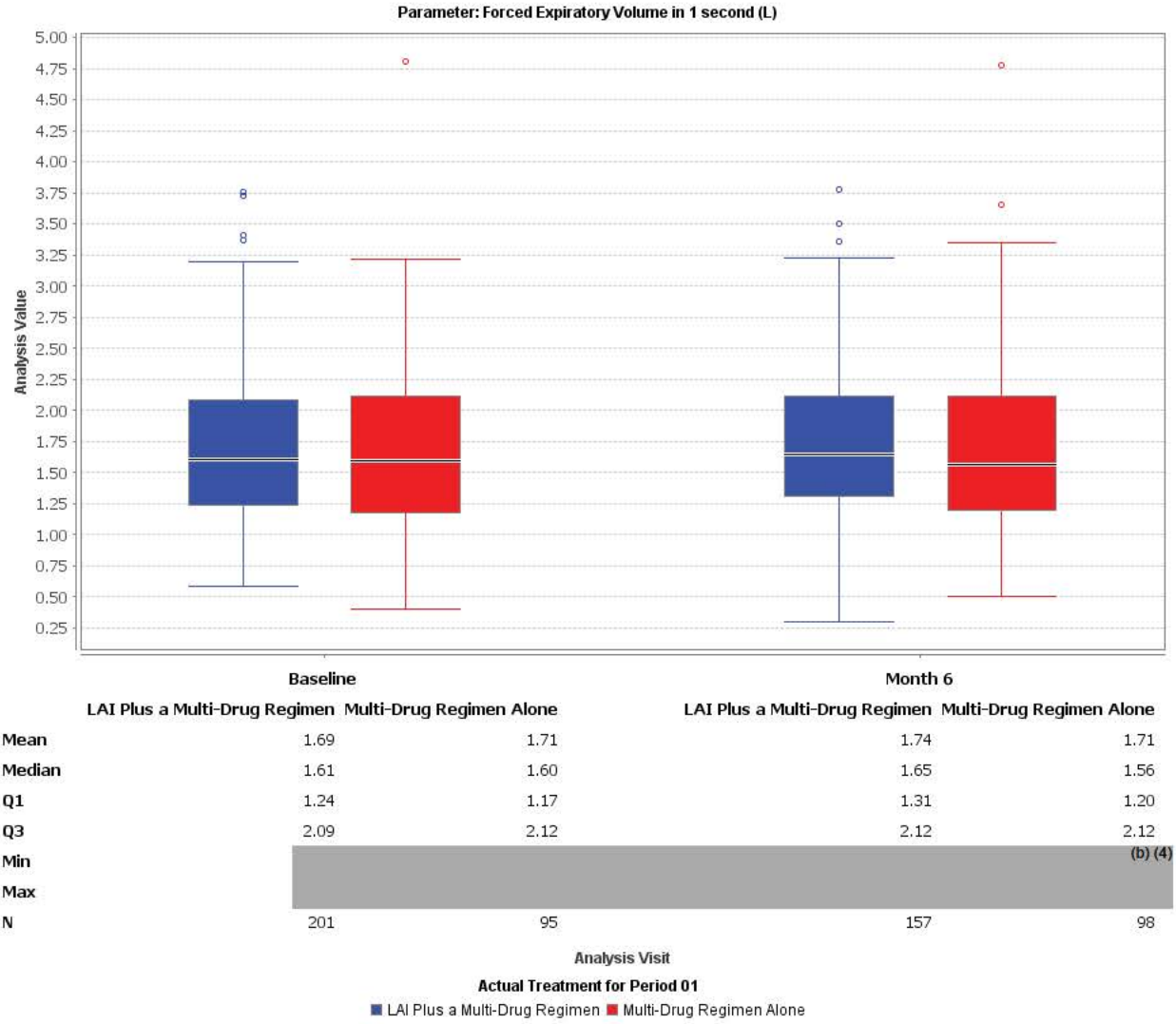
Reviewer's comment: Although the median change in BMI was not significantly different, there were more outliers in terms of decrease in BMI by -2 points in the ALIS+BR arm as compared to patients treated with BR alone. This is consistent with the higher incidence of weight loss reported as TEAEs in the ALIS+BR arm (6.3% in ALIS+BR arm vs. 0.9% in BR alone arm). As previously noted, only 78.6% of patients in the ALIS+BR arm completed Month 6 visit compared

to 95.5% treated with BR alone. This difference in proportion of patients that were able to complete the Month 6 visit could further impact differences in BMI between the two arms.

Pulmonary Function Test

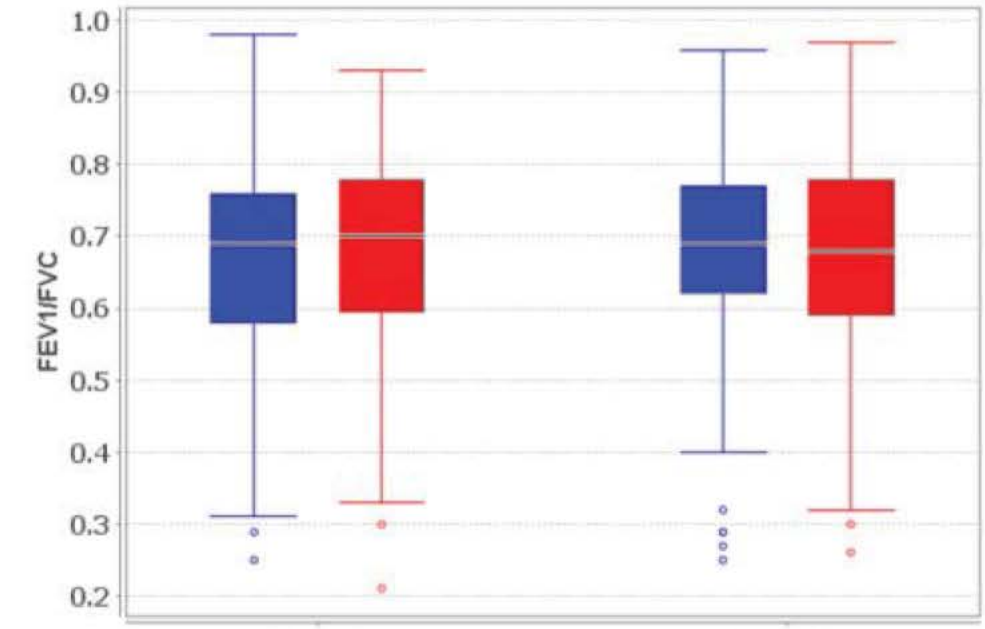
There was no significant change in forced expiratory volume (FEV), forced expiratory volume at 1 second (FEV1) and forced vital capacity (FVC) between baseline and Month 6. **Figure 12** and **Figure 13** summarize the FEV1 and FEV1 to FVC (FEV1/FVC) at baseline and Month 6 for the two study arms. As noted previously, the differential completion of Month 6 visit with significantly less patients in the ALIS-treated arm completing the Month 6 visit could have impacted potential differences in pulmonary function tests between the two arms.

Figure 12: Forced Expiratory Volume in 1 Second (L) at Baseline and Month 6 of Study 212



	Baseline		Month 6	
	LAI Plus a Multi-Drug Regimen	Multi-Drug Regimen Alone	LAI Plus a Multi-Drug Regimen	Multi-Drug Regimen Alone
Mean	1.69	1.71	1.74	1.71
Median	1.61	1.60	1.65	1.56
Q1	1.24	1.17	1.31	1.20
Q3	2.09	2.12	2.12	2.12
Min				(b) (4)
Max				
N	201	95	157	98

Figure 13: FEV1/FVC at Baseline and Month 6 of Study 212



	Baseline		Month 6	
	LAI	MDR	LAI	MDR
Mean	0.67	0.66	0.68	0.66
Median	0.69	0.70	0.69	0.68
Q1	0.58	0.59	0.62	0.59
Q3	0.76	0.78	0.77	0.78
Min	(b) (4)			
Max				
N	201	95	157	98

Analysis Visit

Actual Arm Code

LAI MDR LAI=ALIS+BR; MDR=BR Alone

Reviewer's Comments: *Given the irreversible lung damage and abnormalities in the studied population, demonstration of improvement in pulmonary function may be difficult. As previously noted, only 78.6% of patients in the ALIS+BR arm completed the Month 6 visit compared to 95.5% of patients treated with BR alone. This difference in the proportion of patients who were able to complete the Month 6 visit could have impacted potential differences in pulmonary function tests between the two arms.*

Electrocardiograms (ECGs)

A 12-lead ECG was obtained at screening and at the end of treatment (EOT) visit and was not

part of the monthly assessments.

Reviewer's Comments: Since ALIS is administered locally (via inhalation) and resulted in minimal systemic exposure, the risk of ECG changes related to ALIS is low.

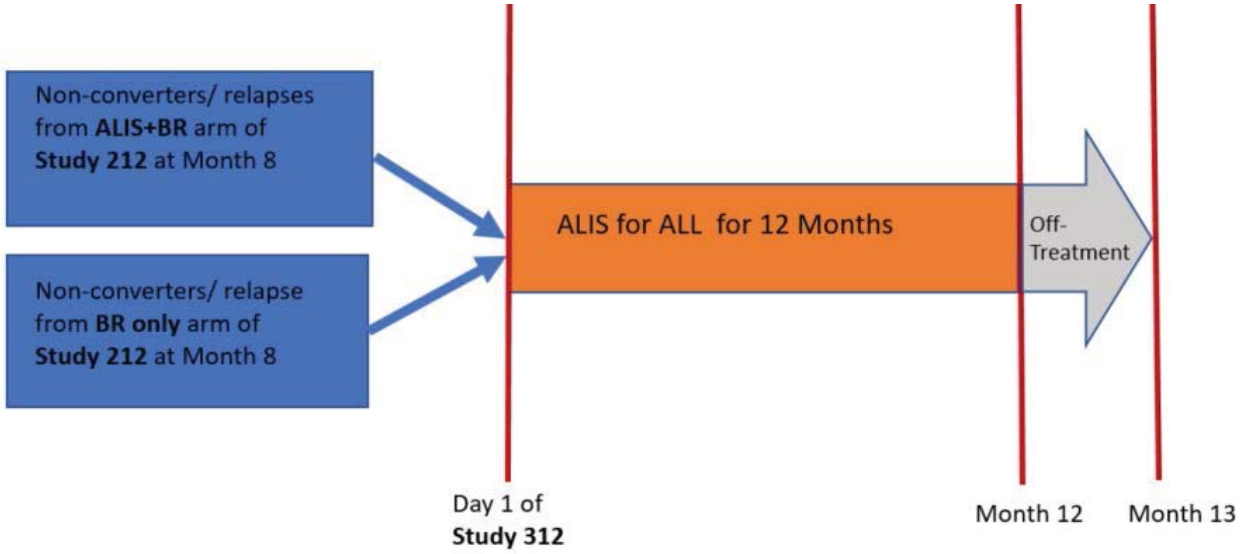
Immunogenicity

This section is not applicable to this NDA.

INS 312

Study 312 is the open-label, single-arm extension study of Study 212; participants included patients from either arm of Study 212 that did not achieve culture conversion or had a relapse after 6 months of therapy. These patients were offered 12 months of ALIS along with their BR. Figure 14 summarizes the study design of Study 312.

Figure 14: Study 312 Study Design



Study 312 is ongoing. Since all participants of Study 312 are receiving ALIS in the study, the safety comparison is mainly evaluating TEAEs occurring early in ALIS treatment and TEAEs that occur with longer use of ALIS.

Deaths

Table 53 summarizes the three deaths that occurred in Study 312 by the time of data cut-off for the NDA submission.

Table 53: Deaths that Occurred in Study 312 (by data cut-off)

Age in years	Sex*	Study day of death	Treatment Duration in Study 312 (Days)	Treatment Duration in Study 212 (Days)	Reported Immediate Cause of Death
63	F	124	124	237	COPD
71	F	112	90	248	Chest infection
79	F	59	31	238	Respiratory failure

*F=Female, M=Male

Reviewer's Comment: The three deaths occurred in patients treated for >7 months with ALIS during Study 212 and were continuing ALIS in Study 312. All three had underlying comorbidities that may have contributed to their deaths; two of the three had a diagnosis of fungal infections: one had a Scedosporium infection, and one had a diagnosis of pulmonary aspergillosis. Given the complexity of their medical conditions, discerning the contribution of ALIS to their deaths is difficult.

Serious Adverse Events

There were no imbalances in SAEs between the two groups, with SAEs reported in 20.3% of patients starting on ALIS vs. 18.6% of patients continuing on ALIS. Summaries of SAEs are presented in Table 54.

Table 54: SAEs that Occurred in >1 Patient Starting on ALIS in Study 312

Preferred Terms	Starting on ALIS N=74 n (%)	Continuing on ALIS N=59 n (%)
Patients with at least 1 SAE	**15 (20.3)**	**11 (18.6)**
Pneumonia	3 (4.1)	1 (1.7)
Infective exacerbation of bronchiectasis	2 (2.7)	1 (1.7)
Atrial fibrillation	2 (2.7)	0 (0)
COPD	2 (2.7)	1 (1.7)
Hemoptysis	2 (2.7)	1 (1.7)

Dropouts and/or Discontinuations Due to Adverse Effects

There were similar rates of premature discontinuation with 20.3% (n=15) of patients starting on ALIS discontinuing prematurely compared to 22% (n=13) of those that were continued on ALIS; however, reasons for premature discontinuation differed between the two groups. In patients

that were newly started on ALIS, discontinuation due to adverse events accounted for 11 of the 15 discontinuations (73%) while discontinuation due withdrawal by subject (5/13) and discontinuation due to lack of efficacy (3/13) were the main reasons for discontinuation for those that were continued on ALIS.

Treatment Emergent Adverse Events (TEAEs) and Adverse Reactions

Table 55 summarizes the TEAEs in Study 312. Similar to what was observed in Study 212, patients starting on ALIS experienced a higher incidence of TEAEs and, in particular, a significantly higher incidence of dysphonia, cough, dyspnea, and upper airway irritation.

Table 55: Treatment Emergent Adverse Events in Study 312

PT Terms	Starting on ALIS N=74 n, (%)	Continuing on ALIS N=59 n, (%)
Subjects with at least 1 TEAE	69 (93.2)	43 (72.9)
Dysphonia[a]	33 (44.6)	3 (5.1)
Cough[b]	30 (40.5)	4 (6.8)
Dyspnea[c]	12 (16.2)	3 (5.1)
Upper airway irritation[d]	10 (13.5)	2 (3.4)
Fatigue and asthenia	10 (13.5)	1 (1.7)
Hemoptysis	9 (12.2)	5 (8.5)
Diarrhea	9 (12.2)	3 (5.1)
Upper respiratory infection[e]	7 (9.5)	7 (11.9)
Exacerbation of bronchiectasis	6 (8.1)	3 (5.1)
Nausea	6 (8.1)	1 (1.7)
COPD exacerbation	6 (8.1)	3 (5.1)
Changes in sputum[f]	6 (8.1)	1 (1.7)
Abdominal pain[g]	6 (8.1)	2 (3.4)
Tinnitus	5 (6.8)	1 (1.7)
Dizziness	5 (6.8)	1 (1.7)
Pneumonia[h]	4 (5.4)	4 (6.8)
Chest discomfort	4 (5.4)	0 (0)
Hematuria	4 (5.4)	4 (6.8)
Headache	4 (5.4)	1 (1.7)
Weight decreased	4 (5.4)	1 (1.7)

[a]Includes dysphonia and aphonia

[b]Includes cough and productive cough
[c]Includes dyspnea, dyspnea at rest and dyspnea on exertion
[d]Includes laryngitis, larynx irritation, oropharyngeal pain, pharyngeal erythema, throat irritation and vocal cord inflammation
[e] Includes upper respiratory tract infection, viral upper respiratory infection, nasopharyngitis
[f]Includes increased sputum, sputum discolored
[g]Includes abdominal pain, abdominal pain, abdominal discomfort, abdominal pain upper and abdominal pain lower

[h]Includes pneumonia, Pseudomonas infection, Klebsiella infection, and lower respiratory infection

Reviewer comment: Compared to patients continuing on ALIS in Study 312, patients starting ALIS experienced a significantly higher incidence of dysphonia, cough, dyspnea and upper airway irritation. Most likely, the difference in these TEAEs reflects that patients previously treated with ALIS in Study 212 who then continued on ALIS in Study 312 were the ones that were either more tolerant or developed tolerance to ALIS, whereas patients who experienced treatment-limiting ALIS-related AEs in Study 212 may not have elected to continue on in Study 312. On the other hand, all of the patients who were previously on BR alone in Study 212 were now starting on ALIS in Study 312 and therefore experienced more TEAEs due to the first-time exposure to ALIS.

Treatment Emergent Adverse Events of Interest

Based on the route of administration and the class of the active ingredient, several adverse events of interest (AEI) were identified. Please refer to Section 9.1.5 for a detailed discussion of the AEIs.

TR02-112

Figure 15 below depicts the study design for Study 112.

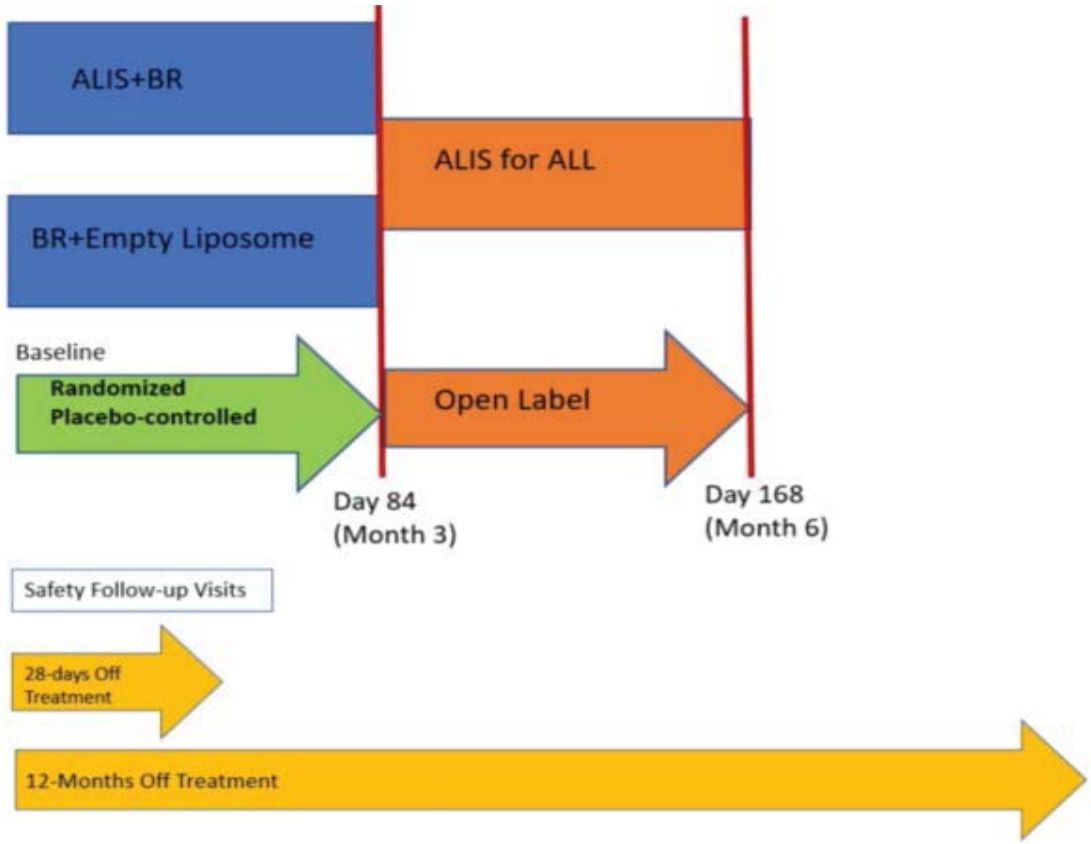


Figure 16: Study 112 Study Design

As previously discussed, Study 112 had a heterogenous study population, with inclusion of non-CF patients infected with *M. abscessus* (n=9 in ALIS+BR, n=9 in BR+inhaled empty liposomes) and patients with CF (n=8 in ALIS+BR, n=9 in BR plus inhaled empty liposomes). The safety analyses included all subjects. Of note, this was the only study that included an inhaled placebo (empty liposomes) plus BR comparator arm. This provided an opportunity to compare the AEs associated with ALIS+BR versus inhaled placebo (diluted empty liposome vehicle)+BR.

Deaths

Overall, there were 9 deaths during the study; two additional deaths (one in each study arm) occurred off-study. In the double-blind (DB) phase, there was one death in the ALIS+BR arm and none in the BR+placebo arm. The death in the ALIS arm was a 64-year-old female with a history of bronchiectasis who had an initial report of pulmonary exacerbation on Day 56. She had ongoing symptoms and was hospitalized on Day 80. Her last dose of ALIS was on Day 79. Her clinical condition progressively worsened despite IV antibacterial therapy and IV methylprednisolone. She died on Day 91. The cause of death was reported as ARDS following septic shock and multifocal pneumonia.

There were additional deaths during the open label (OL) and 12 months-follow up phase of the

study. Due to the design of the study, all 8 deaths that occurred after the DB phase occurred in patients who were treated with ALIS either during the DB phase or in the OL phase. There were two additional deaths that occurred after patients discontinued the study.

Serious Adverse Events

Serious adverse events that occurred in the double-blind phase of Study 112 (the first 84 days) are summarized in Table 56.

Table 56: Study TR02-112 Serious Adverse Events that Occurred During the Double-blind Phase by System Organ Class and Preferred Term

System Organ Class (SOC)	ALIS+BR N=44 n (%)	Placebo*+BR N=45 n (%)
Patient with at least one SAE	**8 (18.2)**	**4 (8.9)**
Infection and infestation	**5 (11.4)**	**3 (6.7)**
Infective exacerbation of bronchiectasis	2 (4.6)	1 (2.2)
Infective exacerbation of cystic fibrosis	1 (2.3)	0 (00
Viral gastroenteritis	1 (2.3)	0 (0)
Pneumonia	1 (2.3)	2 (4.4)
Respiratory, thoracic& mediastinal disorders	**2(4.6)**	**1 (2.2)**
Acute respiratory distress syndrome	1 (2.3)	0(0)
Eosinophilic pneumonia	0 (0)	1 (2.2)
Hemoptysis	1 (2.3)	0 (0)
Respiratory disorder (Hypersensitivity lung reaction)	1(2.3)	0 (0)
Cardiac disorders	**1 (2.3)**	**0 (0)**
Supraventricular tachycardia	1 (2.3)	0 (0)
Injury, poisoning & procedural complications	**1 (2.3)**	**0 (0)**
Fractured left femur and humerus	1 (2.3)	0 (0)
Gastrointestinal disorders	**0**	**1 (2.2)**
Small intestine obstruction	0	1 (2.2)

*Placebo was inhaled empty liposomes

Reviewer's comment: The proportion of patients with SAEs was significantly higher for patients treated with ALIS+BR (18.2%) compared to inhaled placebo+BR (8.9%). Hence, it is possible that addition of the active ingredient, amikacin, to the liposome vehicle contributed to the increased the risk of SAEs.

Dropouts and/or Discontinuations Due to Adverse Effects

Nine patients discontinued study treatment prematurely, and all 9 (20.5%) were in the ALIS+BR arm; 7 of the 9 (78%) discontinued due to AEs. Infective exacerbations of underlying pulmonary disease and dyspnea accounted for most of the discontinuations due to AEs.

All 4 patients that prematurely discontinued from the study were also in the ALIS+BR arm. Death, AE, withdrawal by subject, and loss to follow-up accounted for one discontinuation each.

Reviewer's Comment: Similar to the observation in Study 212, there was a significantly higher incidence of premature study treatment discontinuations in ALIS-treated patients, mostly due to AEs.

Treatment Emergent Adverse Events and Adverse Reactions

TEAEs by System Organ Class (SOC) that occurred in the DB phase are summarized in Table 57.

Table 57: TR02-112 Treatment Emergent Adverse Events by System Organ Class

System Organ Class (SOC)	ALIS+BR N=44 n (%)	Placebo*+ BR N=45 n (%)
Patient with at least one TEAE	**41(93.2)**	**40 (88.9)**
Respiratory, thoracic and mediastinal disorders	35 (79.5)	23 (51.1)
Infections and infestations	32 (72.7)	18 (40)
General disorders and administration site conditions	15 (34.1)	9 (20)
Gastrointestinal disorders	10 (22.7)	11 (24.4)
Nervous system disorders	10 (22.7)	4 (8.9)
Musculoskeletal and connective tissue disorders	8 (18.2)	9 (20)
Ear and labyrinth disorders	6 (13.6)	4 (8.9)
Skin and subcutaneous tissue disorders	6 (13.6)	7 (15.6)
Injury, poisoning and procedural complications	5 (11.4)	0 (0)
Metabolism and nutrition disorders	5(11.4)	3 (6.7)
Psychiatric disorders	5 (11.4)	2 (4.4)
Cardiac disorders	4(9.1)	3 (6.7)
Investigations	4 (9.1)	5 (11.1)
Blood and lymphatic system disorders	2 (4.5)	0 (0)
Eye disorders	1 (2.3)	1 (2.2)
Reproductive systems and breast disorder	1 (2.3)	0 (0)

*Empty liposomes were used as placebo.

TEAEs by preferred term (PT) are summarized in **Table 58**.

Table 58:TR02-112 Treatment Emergent Adverse Events by Preferred Term

MedDRA Preferred Term	ALIS+BRN=44 n (%)	Placebo*+BR N=45 n (%)
Exacerbation of underlying lung disease[a]	22 (50)	10 (22.2)
Infective exacerbation of bronchiectasis	17 (38.6)	9 (20)
Infective exacerbation of cystic fibrosis	4 (9.1)	1 (2.2)
COPD exacerbation	1 (2.3)	0 (0)
Dysphonia[b]	20 (45.5)	4 (8.9)
Cough[c]	16 (36.4)	6 (13.3)
Upper airway inflammation[d]	11 (25)	2 (4.4)
Oropharyngeal pain and throat irritation	10 (22.7)	1 (2.2)
Laryngitis	3 (6.8)	1 (2.2)
Fatigue	7 (15.9)	4 (8.9)
Chest discomfort	5 (11.4)	0 (0)
Hemoptysis	5 (11.4)	5 (11.1)
Musculoskeletal pain[e]	5 (11.4)	4 (8.9)
Nausea	5 (11.4)	4 (8.9)
Abdominal pain/discomfort	4 (9.1)	1 (2.2)
Pneumonia[f]	4 (9.1)	4 (8.9)
Pyrexia	4 (9.1)	3 (6.7)
Upper respiratory infection[g]	4 (9.1)	2 (4.4)
Wheeze	4 (9.1)	1 (2.2)
Dyspnea	3 (6.8)	1 (2.2)
Headache	3 (6.8)	3 (6.7)
Rash[h]	3 (6.8)	0 (0)

*Empty liposomes were used as placebo.
[a]Includes “infective exacerbation of bronchiectasis”, “infective exacerbation of CF” and “COPD exacerbation”
[b]Includes “aphonia” and “dysphonia”
[c]Includes “cough”, “productive cough” and “upper airway cough syndrome”
[d]Includes “oropharyngeal pain”, “throat irritation”, “laryngitis”
[e]Includes “back pain”, “arthralgia”, “myalgia”, “pain/body aches”, “muscle spasm”, “musculoskeletal discomfort” and “musculoskeletal pain”
[f]Includes “pneumonia”, “eosinophilic pneumonia”, and “respiratory tract infection bacterial”
[g]Includes “nasopharyngitis/cold” and “upper respiratory infection”
[h]Includes “rash” and “urticaria”

*Reviewer’s comment: Similar to what was observed in Study 212, TEAEs were predominantly in the “Respiratory, thoracic and mediastinal disorders” and “infection and infestation” system organ class***.**

9.1.5 Analysis of Submission-Specific Safety Issues

Adverse events of interest (AEIs) specific for ALIS were identified based on the adverse effects of systemically administered amikacin and the aminoglycoside antibacterial class, in general, as well as, based on the inhalational route of administration and potential for resultant local irritation and inflammation. Please refer to Table 39 for details on the pooled AEI terms.

The AEIs based on class effect included clinical and laboratory indicators of nephrotoxicity, clinical signs/symptoms of neuromuscular disorders and clinical signs/symptoms of ototoxicity (both auditory and vestibular). Although the likelihood of these AEIs was deemed low given the local (non-systemic) administration of amikacin, the safety database was reviewed for the abovementioned AEIs.

AEIs based on route of administration included allergic alveolitis/hypersensitivity pneumonitis, bronchospasm, cough, dysphonia, exacerbation of underlying lung disease, hemoptysis, pneumonia, pneumothorax, and upper airway irritation. Table 59, Table 60, and Table 61 present the incidence of these AEIs in Studies 212, 312, and double-blind phase of 112, respectively.

Table 59: Incidence of Adverse Events of Interest in Study 212

Adverse Events of Interest	ALIS + BR N=223 n (%)	BR only N=112 n (%)
Dysphonia	105 (45.7)	1 (0.9)
Cough	87 (39)	19 (17)
Bronchospasm	64 (28.7)	12 (10.7)
SAE	**3 (1.3)**	**0 (0)**
Hemoptysis	40 (17.9)	14 (12.5)
Ototoxicity	38 (17)	11 (9.8)
Upper airway irritation	37 (16.6)	2 (1.8)
Exacerbation of underlying lung disease	33 (14.8)	11 (9.8)
SAE	**11 (4.9)**	**4 (3.6)**
Pneumonia	22 (9.9)	9 (8)
SAE	**12 (5.4)**	**2(1.8)**
Hypersensitivity pneumonitis	8 (3.6)	0 (0)
SAE	**4 (1.8)**	**0 (0)**
Pneumothorax	5 (2.2)	1 (0.9)
SAE	**4(1.8)**	**1 (0.9)**

Nephrotoxicity	5 (2.2)	3 (2.7)
Neuromuscular disorder	2 (0.9)	0 (0)

Reviewer's Comment: With the exception of nephrotoxicity, there was a higher incidence of all the other AEIs in the ALIS+BR–treated patients compared to the placebo+BR arm. There was a considerably higher incidence of dysphonia, cough, bronchospasm, hemoptysis, ototoxicity, upper airway inflammation, and exacerbation of underlying lung disease.

Of note, the incidence of ototoxicity was driven mostly by a higher incidence of tinnitus and dizziness in the ALIS+BR-treated arm compared to the placebo+BR arm.

Most of the reported AEIs were not classified as serious. However, there were more cases of pneumonia, hypersensitivity pneumonitis and pneumothorax that were classified as serious in the ALIS+BR arm.

Table 60: Incidence of Adverse Events of Interests in Study 312

	Started on ALIS **N=74** **n (%)**	**Continued on ALIS** **N=59** **n (%)**
Dysphonia	33 (44.6)	3 (5.1)
Cough	30 (40.5)	4 (6.8)
Bronchospasm	15 (20.3)	3 (5.1)
Exacerbation of underlying lung disease	10 (13.5)	4 (6.8)
SAE	**4 (5.4)**	**1 (1.7)**
Hemoptysis	9 (12.2)	5 (8.5)
SAE	**2 (2.7)**	**1 (1.7)**
Upper airway irritation	8 (10.8)	2 (3.4)
SAE	**1 (1.4)**	**0 (0)**
Ototoxicity	10 (13.5)	3 (5.1)
Nephrotoxicity	7 (9.5)	6 (10.2)
Pneumonia	3 (4.1)	4 (6.8)
SAE	**3 (4.1)**	**3 (5.1)**
Pneumothorax	1 (1.4)	0 (0)

SAE	**1 (1.4)**	**0 (0)**
Hypersensitivity pneumonitis	1 (1.4)	1 (1.7)
SAE	**1 (1.4)**	**1 (1.4)**
Neuromuscular disorder	1 (1.4)	1 (1.7)
SAE	**1 (1.4)**	**0 (0)**

Reviewer's Comment: The higher incidence of AEIs in patients newly starting on ALIS in Study 312 versus those continuing on ALIS from Study 212 may reflect that patients who received ALIS in Study 212 and had AEIs may not have elected to continue ALIS in Study 312 and those that continued ALIS may be the ones that were able to better tolerate ALIS therapy. This may partly explain the fewer AEIs noted in those continuing on ALIS vs. starting on ALIS.

Table 61: Adverse Events of Interest in Double Blind Phase of Study 112

AE of Interest Term	**ALIS +BR N=44 n (%)**	**BR + Placebo* N=45 n (%)**
Dysphonia	21 (47.7)	6 (13.3)
Exacerbation of underlying lung disease	18 (40.9)	10 (22.2)
SAE	2 (4.5)	1 (2.2)
Cough	16 (36.4)	9 (20)
Upper airway irritation	10 (22.7)	2 (4.4)
Bronchospasm	7 (15.9)	4 (8.9)
Ototoxicity	5 (11.4)	4 (8.9)
Hemoptysis	5 (11.4)	5 (11.1)
Pneumonia	3 (6.8)	3 (6.7)
SAE	1 (2.3)	2 (4.4)
Hypersensitivity pneumonitis	1 (2.3)	1 (2.2)
SAE	1 (2.3)	1 (2.3)
Nephrotoxicity	1 (2.3)	0 (0)
Neuromuscular Disorder	1 (2.3)	1 (2.2)

*Inhaled liposomes diluted in hypertonic saline were used as placebo

Reviewer's Comment: Similar to Study 212, patients in the ALIS+BR arm of Study 112 experienced a significantly higher incidence of the following AEs compared to those receiving placebo+BR: dysphonia (47.7% vs. 13.3%), exacerbation of underlying lung disease (40.9% vs.

22.2%), cough (36.4% vs. 20%), upper airway irritation (22.7% vs. 4.4%), bronchospasm (15.9% vs. 8.9%) and ototoxicity (11.4% vs. 8.9%). Of note, Study 112 is the only study with inhaled placebo (diluted empty liposomes) and the notable difference in AEs between the two arms suggests that the addition of amikacin, the active ingredient of ALIS, may have contributed to the observed differences in TEAEs.

In addition to the 11 cases of hypersensitivity pneumonitis noted in the ALIS-treated patients in Table 59, Table 60, and Table 61 above, there were 2 additional cases in the open-label phase. Details of the 13 cases of hypersensitivity pneumonitis in patients treated with ALIS across the three NTM trials are summarized below:

<u>INS-212</u>

1. Patient (b) (6) : 67-year-old male with multiple comorbidities including a history of COPD, bronchiectasis, prior 30 pack-year smoking history, remote history of pulmonary TB, and s/p lung resection. ALIS was interrupted on Day 5 due to dyspnea; he was restarted on Day 9. ALIS was interrupted again due to "pneumonia" on Day 16; CXR showed changes NOT typical for pneumonia. He was hospitalized on Day 21 for dyspnea. He was treated with bronchodilators and prednisolone and discharged on Day 23. ALIS was restarted on Day 35; however, on Day 39, the patient experienced prolonged dyspnea (>2hrs), wheezing, nausea, right sided chest pain and "lethargy". The investigator interpreted the symptoms as allergic reaction and ALIS was discontinued. He was then re-challenged on D119 with dose "titration"; on Day 140, ALIS was discontinued permanently due to dyspnea after dosing, increased sputum production, decreased appetite and reduced exercise tolerance. The event was reported as serious and Grade 3 in severity. The investigator deemed the adverse events as definitely related to ALIS.

   Reviewer's comments: The applicant did not classify this case as a case of hypersensitivity pneumonitis/allergic alveolitis. Instead, the patient was coded as having "allergic reaction to ALIS". Based on the clinical presentation of the patient and the recurrence of symptoms with re-introduction of ALIS, a potential irritant, I consider this a case of hypersensitivity pneumonitis. This patient failed multiple attempts of re-challenge with ALIS, with recurrence of respiratory symptoms, such as dyspnea with re-introduction of ALIS. I agree with the investigator's assessment of relatedness of the observed adverse events to ALIS.

2. Patient (b) (6) : 76-year-old female with a history of nodular bronchiectasis. On Day 3, she reported increased cough, hoarseness, and increased sputum after ALIS administration. She presented to a hospital on Day 18 with complaints of cough, hoarseness and fever and was treated with tulobuterol (beta 2 adrenergic agonist). ALIS was discontinued on Day 18 with last dose taken on Day 17. She presented to the hospital again on Day 22 (5 days post last dose of ALIS) with a complaint of ongoing cough, hoarseness, and fever. A chest CT scan showed an upper lung field ground-glass

opacity, with the possibility of drug-induced pneumonitis. On Day 28, the patient was admitted. She discontinued participation in the study due to a concern of drug-induced pneumonitis on Day 31. She was hospitalized from Day 28 to 52. On Day 32, she had a bronchoscopy and the results of the bronchoalveolar lavage fluid (BAL) showed erythrocytes (2+), neutrophils (1+), lymphocytes (1+), eosinophils (2+), macrophages (3+). The transbronchial lung biopsy did not show any pathogens. She was started on prednisolone and continued treatment intermittently until Day 76. The event of pneumonitis was reported resolved on Day 125 after repeat lung CT showed resolution of the ground-glass opacity. The adverse event was reported as serious and Grade 3 in severity. The investigator deemed the adverse event as probably related to ALIS.

Reviewer's comment: The time course, symptoms and radiologic findings are consistent with hypersensitivity pneumonitis. I agree with the investigator's assessment of relatedness of the observed adverse events to ALIS.

3. Patient (b) (6) : 75-year-old female with multiple comorbidities including a history of bronchiectasis, asthma, hemoptysis and atrial fibrillation. On Day 19, she reported symptoms considered consistent with hypersensitivity pneumonitis by the investigator. Chest imaging (chest x-ray and CT) confirmed the diagnosis. ALIS was discontinued on Day 25 and patient was treated with oral prednisone. The event was reported resolved on Day 92 of the study. The event was reported as Grade 3 in severity. The investigator deemed the adverse event as definitely related to ALIS.

   Reviewer's comment: The narrative submitted by the Sponsor had limited information. It did not include a description of the signs/symptoms, and the findings of the clinical and radiological examinations. It appears that the investigator's assessment of the event's relatedness was based on the clinical signs/symptoms and radiologic findings, typical for the diagnosis of hypersensitivity pneumonitis.

4. Patient (b) (6) : 68 year-old-female with a history of nodular bronchiectasis s/p right middle lobe resection. On Day 31, she was noted to have worsening and increased cough, and increased sputum mixed with blood. Her chest CT showed worsening ground glass shadow. ALIS was discontinued on that day. Repeat chest CT on Day 34 showed a predominantly pan-lobular ground glass shadowing in both lungs. She was hospitalized. Bronchoscopy on Day 37 showed a mild inflammatory cell infiltration suggestive of interstitial pneumonia. BAL on Day 37 showed 58% alveolar macrophages, 38% lymphocytes and 4% neutrophils, with an absolute total cell count of $3.5x10^5$ cells/mL. Transbronchial biopsy showed accumulation of intra-alveolar foamy macrophages, hyperplasia of alveolar type II cells, and mild interstitial infiltration with inflammatory cells. She received a protracted course of steroid therapy initially with IV methyl prednisolone and then oral prednisone. The patient withdrew from the study on Day 52. She was discharged from the hospital on Day 85. She was diagnosed with Aspergillosis on Day 91 and started on antifungal therapy. Pneumonitis was reported as resolved on Day 211.

The adverse event was reported as serious and Grade 3 in severity. Th investigator deemed the adverse event as probably related to ALIS.

Reviewer's comment: The signs/symptoms, radiologic and bronchoscopy findings are consistent with evidence of pneumonitis. I agree with the investigator's assessment of relatedness of the observed adverse events to ALIS.

5. Patient (b) (6) : 71-year-old man with multiple comorbidities including COPD, emphysema, prior history of smoking, bronchiectasis and pulmonary cavitation. ALIS was discontinued on Day 143 due to an overall decline in health. On Day 182, 39 days after the last dose of ALIS, he was hospitalized due to respiratory distress with dyspnea, increased productive cough, fever and chills. The patient was diagnosed with acute respiratory failure; chest x-ray showed right lower lobe pneumonitis. The patient's condition deteriorated, and the family opted for comfort care. The patient died on Day 187 due to acute respiratory failure. The event was reported as serious and Grade 5 in severity. The investigator deemed the event as unrelated to ALIS.

   Reviewer's comment: Although the patient had multiple comorbidities, given the overall profile of ALIS with increased risk of respiratory AEs, it is difficult to rule out ALIS's contribution to the patient's declining health status. I consider the events as possibly related to ALIS.

6. Patient (b) (6) : 80-year-old male with multiple comorbidities including a history of interstitial lung disease due to scleroderma. On study Day 210, he had worsening pulmonary fibrosis, and pulmonary function tests (PFTs) showed progressive restriction and deterioration. His adverse reaction was reported as serious and Grade 4 in severity and was treated with prednisone and rituximab. He discontinued ALIS on Day 223 and discontinued from the study the same day. The Sponsor deemed the adverse event not related to study drug.

   Reviewer's comment: Although the patient had underlying pulmonary fibrosis, it is possible that ALIS contributed to a worsening of his condition. In the literature, chronic cases of hypersensitivity pneumonitis can manifest months after exposure to an irritant. Hence, I consider the event as possibly related to ALIS.

7. Patient (b) (6) : 66-year-old female reported to have cough and dyspnea; she was reported as having mild interstitial pneumonitis (Grade 1 on the case report form). There is no report of treatment with steroids, and ALIS was continued. The investigator deemed the event as definitely related to ALIS.

   Reviewer's comment: The narrative submitted by the Applicant had limited information. It appears that the investigator's assessment of the event's relatedness was based on the clinical signs/symptoms.

8. Patient (b) (6) : 68-year-old female with a history of nodular bronchiectasis. On Day 207, she presented with chills, increased cough, shortness of breath, and her chest x-ray showed a new shadow in the right lung. She was hospitalized on Day 218 with a differential diagnosis of worsening MAC lung infection and drug-induced pneumonia. She was treated with antibacterial and antipyretic drugs. Chest CT showed increased right lower lobe shadow and new cavity formation. BAL was positive for MAC by PCR. She was discharged from the hospital on Day 233. She discontinued ALIS on Day 237. There were no records of treatment with steroids. She continued to have ongoing respiratory symptoms and was evaluated with bronchoscopy with biopsy on Day 281 which showed that the alveolar space was obscured by dense inflammatory cell infiltration (mostly lymphocytes), and epithelioid granulomas; serial CTs showed improvement and on Day 424, 191 days after the last dose of ALIS, pneumonitis was reported resolved.

   Reviewer's comment: The clinical signs/symptoms, radiologic findings and BAL lymphocytosis are supportive of the diagnosis of hypersensitivity pneumonitis. Similar to the prior case, the timing of the clinical presentation could be consistent with a chronic presentation of hypersensitivity pneumonitis. It is unclear how much of the patient's signs/symptoms were due to her underlying pulmonary disease vs. treatment with ALIS. The reviewer considers the event as possibly related to ALIS.

INS-312

1. Patient (b) (6) : 77-year-old female with multiple co-morbidities including a remote history of TB, fibrocavitary disease, Hashimoto's Disease and gastric cancer. During Study 212, she participated in the Background Regimen (BR) alone arm. On Day 40 of Study 312, she presented with mild fever, cough, difficulty breathing and malaise. ALIS was discontinued on Day 42. Chest CT showed interstitial pneumonia. She was started on IV methylprednisolone and IV antibacterial therapy. The patient was weaned from steroids on Day 85 and was reported to have recovered from interstitial pneumonia on Day 93. The patient discontinued from the study on Day 94. She was discharged home on Day 158. The event was reported as serious and Grade 4 in severity. The investigator deemed the event as definitely related to ALIS.

   Reviewer's comments: The reviewer agrees with the investigator's conclusion of relatedness of the AE with ALIS treatment.

2. Patient (b) (6) : 70-year-old female with multiple comorbidities including bronchiectasis, s/p bilateral wedge resection, hypothyroidism, and breast cancer. On Day 73, she developed increased shortness of breath and ALIS was temporarily discontinued on Day 73. On Day 99, chest CT showed ground-glass opacities, and the diagnosis of hypersensitivity pneumonitis was made; on Day 108, BAL work-up was reported as "inconclusive". Treatment with oral prednisone was started and continued until Day 135. ALIS was not re-started. On Day 196, the patient discontinued from the study due to the event of hypersensitivity pneumonitis. The event was considered

serious and rated as Grade 2 in severity. The investigator deemed the event as possibly related to ALIS.

Reviewer's comments: The clinical signs/symptoms and chest CT findings support the diagnosis. The reviewer agrees with the investigator's conclusion of relatedness.

TR02-112

1. Patient (b) (6) : 70 year-old-female with multiple comorbidities with a history of breast cancer, burn injury including smoke inhalation with significant lung injury. On Day 5, she presented with body aches, fatigue and increased cough. On Day 13, she was hypoxic and had a low-grade fever and was diagnosed with severe hypersensitivity reaction. She was subsequently hospitalized. A chest CT showed new diffuse multi-lobar ground glass opacities with thickened interlobular septa, most severe in left upper lobe. She had a bronchoscopy and BAL cytology was notable for clusters of atypical epithelial cells, with reactive type II pneumocytes; there were also inflammatory cells including eosinophils which accounted for 19% of the BAL cell count. A transbronchial biopsy showed acute lung injury. Bacterial and fungal organisms were not detected. Treatment with IV methylprednisolone was started and was eventually changed to oral prednisolone. Due to her ongoing symptoms, she was initiated on IV amikacin on Day 35. Hypersensitivity pneumonitis was reported as resolved on Day 97. The event was reported as serious and Grade 3 in severity. The investigator deemed the event as possibly related to ALIS.

   Reviewer's comments: The timing, clinical signs/symptoms, radiologic and BAL findings are supportive of the diagnosis of hypersensitivity pneumonitis. The review considers the events as probably related to ALIS.

2. Patient (b) (6) 72-year-old female with multiple comorbidities including COPD, bronchiectasis, asthma, and chronic sinusitis. During the double-blind portion of Study 112, she was treated with inhaled placebo plus BR. On Day 14 of the open-label portion of the study where all participants were being treated with ALIS, she presented to the ER with a one-week history of shortness of breath, fever, increased non-productive cough, loose stools, and feeling dizzy. She was noted to be hypoxic with an oxygen saturation level of 91% on room air. ALIS was discontinued. Chest CT angiography showed a new hazy ground glass mosaic attenuation involving the central aspect of the lung, most notably involving the upper lobes (R>L) with patchy areas of increase parenchymal opacity, consistent with a multifocal inflammatory process; BAL cultures grew MAC, *Aspergillus fumigatus* and few *Candida albicans*. She was started on oral prednisone for presumed drug-induced pneumonitis, with improvement in her respiratory symptoms with steroid therapy. Pneumonitis was reported resolved with sequalae and prednisone was discontinued after >5 weeks of therapy. The event was reported as serious and Grade 3 in severity. The investigator deemed the event as possibly related to ALIS.

Reviewer's comments: *I agree with the investigator's assessment of relatedness of the observed adverse events to ALIS.*

3. Patient (b) (6) : 28-year-old female with cystic fibrosis who was treated with ALIS+BR in the double-blind portion of Study 112, and continued ALIS in the open label portion. On Day 91, she presented with worsening dyspnea, fatigue and cough. She was diagnosed with moderate pulmonary exacerbation and was treated with prednisone, rifampin and oseltamivir. On Day 99, rifampin was discontinued, and IV vancomycin and ceftazidime were started. On Day 104, she presented for an unscheduled visit and was subsequently hospitalized for persistent fever, increased sputum production, hypoxia and malaise; chest CT was notable for new diffuse ground glass opacities and patchy alveolar densities. She was treated with IV methylprednisolone and IV tigecycline was added to her antibacterial regimen. The patient was discharged from the hospital on oral prednisone. She presented to a scheduled study visit on Day 139 and at the visit, pulmonary exacerbation and pneumonitis were reported resolved. ALIS was restarted on the same day. On Day 145, the patient was reported to have experienced mild pulmonary exacerbation. ALIS was discontinued at the patient' s request due to worsening respiratory symptoms. She was restarted on IV antibacterial treatment along with an increased dose of oral prednisone. The patient presented for the end of study visit on Day 167, and the event of pulmonary exacerbation was reported resolved. The event was reported as serious and Grade 3 in severity. The investigator deemed the events of pneumonitis and pulmonary exacerbation as unlikely to be related to ALIS.

   Reviewer's comment: The reviewer disagrees with the investigator's assessment of relatedness of the adverse events to ALIS. Re-initiation of ALIS was associated with worsening of the patient's status after initial improvement with steroid therapy. Although the patient's underlying comorbidities contributed to the patient's respiratory symptoms, the contribution of ALIS to the pulmonary exacerbations and pneumonitis cannot be ruled out, particularly with worsening of symptoms with ALIS re-challenge.

   Reviewer's overall comments on the hypersensitivity pneumonitis cases:
   1. *Overall, 10 of 13 of the ALIS-treated patients received remedial therapy with corticosteroids; of these, 4 were initially treated with IV methylprednisolone followed by oral prednisone.*
   2. *Eleven of the 13 patients discontinued ALIS. Two patients were re-challenged, and both failed the re-challenge. One patient reportedly had mild pneumonitis and was continued on ALIS.*
   3. *In terms of the timing of reported hypersensitivity pneumonitis in relation to ALIS treatment initiation, 7 patients were diagnosed within the first 2 months of starting ALIS, and the remaining cases were reported in the time interval of 2-4 months (2 patients), 4-6 months (2 patients) and >6 months (2 patients) after initiation of ALIS.*
   4. *There have been reported cases of MAC-induced hypersensitivity pneumonitis in the literature; however, in the three NTM trials, there were 13 reported cases of*

hypersensitivity pneumonitis in ALIS-treated patients as compared to one case in the comparator arm (in the inhaled empty liposome placebo arm of Trial 112). If the main etiology of the observed hypersensitivity was pulmonary MAC infection, one would expect additional cases in the comparator arms of the three trials. Of note, a thorough review of the Applicant's data, including studies in CF and non-CF bronchiectatic patients did not result in any additional cases of hypersensitivity pneumonitis. Although the role of MAC infection in predisposing these patients to hypersensitivity pneumonitis cannot be ruled out, it is presumed that inhalation of ALIS is associated with the increased incidence of hypersensitivity pneumonitis.

9.1.6 Safety Analyses by Demographic Subgroups

The Phase 3 Study 212 enrolled enough participants to allow for a meaningful comparison between the two study arms based on the following demographic sub-groups: age (<65 vs. ≥65), sex, and global region of enrollment. There were no significant differences in incidence of TEAEs by age or global region of enrollment.

There were some differences in incidence of TEAEs and SAEs by sex. Females on BR alone reported fewer TEAEs than any other group. While males treated with ALIS experienced higher incidences of SAEs and deaths compared to females treated with ALIS. Table 62 summarizes these findings.

Table 62: Incidence of Adverse Events in Study 212 by Sex

Adverse Events of Interest	Female		Male	
	ALIS+BR N=165 n, %	BR Alone N=68 n, %	ALIS+BR N=58 n, %	BR Alone N=44 n, %
TEAEs	164 (99.4)	59 (86.8)	55 (94.8)	42 (95.5)
% Difference between arms within each sex	**12.6**		**-0.7**	
SAEs	25 (15.2)	10 (14.7)	20 (34.5)	8 (18.2)
% Difference between arms within each sex	**0.5**		**16.3**	
Deaths	3 (1.8)	2 (2.9)	6 (10.3)	3 (6.8)
% Difference between arms within each sex	**-1.1%**		**3.5%**	

Reviewer's Comments: The M.O. noted that females treated with ALIS had a higher incidence of dysphonia (58.8% vs. 13.8%) and upper airway irritation (19.4% vs. 8.6%) as compared to males treated with ALIS. On the other hand, males treated with ALIS tended to have a higher incidence of exacerbation of underlying lung disease (29.3%) when compared to females treated with ALIS (12.1%). The difference in incidence of exacerbation of underlying disease and differences in SAEs may be partly due to the inherent differences in the manifestations of MAC disease seen in females and males. As previously mentioned, males more frequently develop cavitary disease while females tend to have the nodular/bronchiectatic form of the disease.

9.1.7 Specific Safety Studies/Clinical Trials

There were no specific safety studies conducted for ALIS for treatment of refractory MAC lung disease.

9.1.8 Additional Safety Explorations

Human Carcinogenicity or Tumor Development

There were no pulmonary-related neoplasms reported. In Study 212, 4 patients treated with ALIS had TEAEs in the neoplasm system organ class (SOC) (n=1 each for the following: squamous cell carcinoma of the skin, gastric adenocarcinoma, benign breast neoplasm, and colon adenoma). These neoplasms were deemed unrelated to ALIS. In Study 112, one patient treated with ALIS had a TEAE of lipoma that was deemed unrelated to study drug.

Pediatrics and Assessment of Effects on Growth

ALIS has not been studied in a pediatric population for the proposed indication of treatment of refractory MAC lung disease.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

The section is not applicable to this NDA.

9.1.9 Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

There is no postmarket experience with ALIS as it is not approved in the US or outside the US.

Expectations on Safety in the Postmarket Setting

Safety issues of concern in the postmarket setting include the risk of increased respiratory adverse reactions including, hypersensitivity pneumonitis, hemoptysis, bronchospasm, and exacerbation of underlying pulmonary disease. These safety concerns will be captured in a Boxed Warning and Warnings in product labeling and a Medication Guide will be provided to patients. Additionally, the NDA is being approved for a limited population of patients.

The Applicant has communicated that they intend to inform healthcare providers, such as, Pulmonary and Infectious Diseases specialists and associated professional societies of the risks and limited clinical benefit of ARIKAYCE. They also plan to collect utilization data on patients prescribed ARIKAYCE, and will distribute ARIKAYCE through (b) (4) specialty pharmacies in the U.S. The Applicant has agreed to PMCs to provide their communication plan activities for outlining the risks associated with ARIKAYCE and the fact that clinical benefit has not yet been verified, as well as, to provide an assessment of use.

The goal is to educate patients and health care providers/prescribers regarding the risks and limited evidence of efficacy based on a surrogate microbiologic endpoint and unknown clinical benefit of ARIKAYCE to aid in ensuring safe use in the indicated population (i.e., adult patients with refractory MAC lung disease) and avoid uninformed off-label use in other patient groups in whom safety and efficacy has not been adequately assessed, such as, patients with non-refractory MAC lung disease, patients with pulmonary disease associated with non-MAC NTM, patients with pulmonary TB, CF and non-CF bronchiectasis patients with bacterial exacerbations, those with ventilator-associated and hospital-acquired bacterial pneumonia, and the pediatric population.

9.2 Integrated Assessment of Safety

Overall, there was no imbalance in mortality in the two randomized trials between patients treated with ALIS plus a background regimen (BR) compared to: (1) patients treated with BR alone (Phase 3 Study 212), or (2) patients treated with BR plus inhaled diluted liposome as placebo (Phase 2 Study 112). However, there was a marked difference in premature treatment discontinuations and patient withdrawals among ALIS-treated patients versus the respective comparator groups in Studies 212 and 112. In the Study 212, one-third of patients in the ALIS arm discontinued treatment prematurely mainly due adverse events (17.4%) and withdrawal by subject (9.4%). A similar trend was noted in Study 112 where all 9 patients (20%) who discontinued study treatment prematurely were in the ALIS+BR arm, and most (7/9) discontinued due to adverse events. In Study 312, in patients that were newly started on ALIS (previously on BR alone in Study 212), discontinuation due to adverse events accounted for 11 of the 15 discontinuations (73%) in this group, while discontinuation due withdrawal by subject (5/13) and discontinuation due to lack of efficacy (3/13) were the main reasons for discontinuation for those that were continued on ALIS from Study 212.

Across all three studies in patients with refractory NTM, the highest at-risk time for TEAEs appeared to be the first 4-6 weeks after initiation of ALIS. There was a higher incidence of serious adverse events in ALIS-treated patients compared to the respective control arms (20% in ALIS+BR arm vs. 16% in the BR alone arm in Study 212, and 18.2% in the ALIS+BR arm vs. 8.9% in the BR +placebo arm in Study 112). The incidence of treatment emergent adverse events was also significantly higher in ALIS-treated patients compared to control arms. Most of the TEAEs were in the “Respiratory, mediastinal and thoracic disorders” and “Infections and infestations” system organ classes (SOCs). In Study 212, dysphonia, cough, dyspnea, upper

airway irritation, tinnitus, wheezing and decreased weight occurred at a significantly higher frequency in ALIS+BR-treated patients compared to those on BR alone.

Adverse events of interest (AEIs) were identified for ALIS based on experience with systemically administered amikacin and, more generally, the known class effects of aminoglycoside drugs, as well as, based on potential adverse effects due to the inhalational route of administration. Except for nephrotoxicity and neuromuscular disorders, the rest of the AEIs, including, allergic alveolitis, bronchospasm, hemoptysis, pneumonia, and exacerbation of underlying pulmonary disease, occurred at significantly higher frequencies in the ALIS-treated patients as compared to those in the control arms.

In Study 212, there were more patients in the ALIS+BR-treated arm (18.4%) with at least one hospitalization as compared to those in the BR alone arm (13.4%). More importantly, after accounting for the 2:1 randomization, the frequency of hospitalization was significantly higher in the ALIS-treated patients (82 hospitalizations in 47 patients as compared to 23 hospitalizations in 15 patients). The main reasons for increased hospitalization in ALIS-treated patients were: exacerbations of underlying pulmonary disease and lower respiratory tract infections.

Overall, ALIS-treated patients had a higher incidence of respiratory associated adverse events compared to patients treated with BR alone. When evaluating use of ALIS, its benefit of local administration vs. intravenously administered antibacterial drugs should be weighed against the observed TEAEs noted with use of ALIS. For patients with refractory pulmonary MAC disease with no or limited therapeutic options, ALIS's potential benefit may outweigh the observed TEAEs. To minimize the risk of increased respiratory TEAEs, both patients and physicians should be aware of the key respiratory adverse events including hypersensitivity pneumonitis, bronchospasm, hemoptysis and increased exacerbation of underlying disease. The risk of increased respiratory adverse reactions and hospitalizations will be communicated in the label through a Boxed Warning.

A risk evaluation and mitigation strategy (REMS) was considered to address concerns regarding increased respiratory adverse events in addition to the abovementioned highlights in the product label. Please refer to Section 13 for details of the REMS discussion.

Appears this way on the original

10 Advisory Committee Meeting and Other External Consultations

The NDA was discussed at the Antimicrobial Drugs Advisory Committee (AMDAC) meeting on August 7, 2018. The meetings materials are available at: https://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/ucm587657.htm.

The following three questions were posed to the committee members. The voting results and a brief summary of the committee's responses are presented below:

1. Is the surrogate endpoint of sputum culture conversion based on three consecutive negative sputum cultures reasonably likely to predict clinical benefit?

Vote Result: Yes: 8 No: 6 Abstain: 0

Committee Discussion: *Eight committee members voted "Yes", that the surrogate endpoint of sputum culture conversion based on three consecutive negative sputum cultures is reasonably likely to predict clinical benefit. Some of these members noted that culture conversion is the clinical tenet used by physicians and aligns with the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) 2007 guidelines for the current standard of care treatment strategy of NTM based on sputum culture conversion to negative. Six committee members voted "No", that the surrogate endpoint of sputum culture conversion based on three consecutive negative sputum cultures is not reasonably likely to predict clinical benefit. One member commented that ALIS seems safer than intravenous amikacin, even though sputum culture conversion might not confer a clinical benefit. One member noted that the Phase 3 trial was not blinded, and no benefit was identified based on patient reported outcome measures. Another member noted that the six studies reviewed by the FDA showed that sputum culture conversion does not correlate with reduction/improvement in symptoms, increased functional benefit or decreased mortality. One member who voted "no" stated that the ATS/IDSA guidelines are written for individual patients and not populations and therefore should not be used as a rationale for predicting clinical benefit from sputum culture conversion especially since the recommendations were based on low quality evidence.*

2. Has the applicant provided substantial evidence of the effectiveness and sufficient evidence of the safety of amikacin liposomal inhalation solution (ALIS) for the treatment of nontuberculous mycobacterial lung disease caused by *Mycobacterium avium* complex as part of a combination antibacterial drug regimen for adult patients?

a. If yes, please provide any recommendations regarding labeling and please comment on the design of the trial that will need to be conducted to confirm clinical benefit.

b. If no, please provide recommendations regarding additional studies/analyses that are needed.

Vote Result: Yes: 2 No: 12 Abstain: 0

Committee Discussion: *Most of the committee members disagreed that the applicant provided substantial evidence of the effectiveness and sufficient evidence of the safety of ALIS for the treatment of NTM lung disease caused by Mycobacterium avium complex as part of a combination antibacterial drug regimen for adult patients. The two committee members who voted "Yes" noted that if it works in a harder to treat population, then it should work overall. Those committee members who voted "No" noted that the applicant was seeking a much broader indication than was studied. These members further noted that this product should only be approved in refractory patients with very limited or no other treatment options. Another concern that was stated was that greater than 30% of the patients in the ALIS treatment arm discontinued study therapy prematurely in the Phase 3 clinical trial. There was a suggestion that treatment naïve patients be studied in a clinical trial.*

Has the applicant provided substantial evidence of the effectiveness and sufficient evidence of the safety of ALIS for the treatment of nontuberculous mycobacterial lung disease caused by *Mycobacterium avium* complex as part of a combination antibacterial drug regimen for adult patients with limited or no treatment options?

a. If yes, please provide any recommendations regarding labeling and please comment on the design of the trial that will need to be conducted to confirm clinical benefit.

b. If no, please provide recommendations regarding additional studies/analyses that are needed.

Vote Result: Yes: 12 No: 2 Abstain: 0

Committee Discussion: *The majority of the committee members agreed that the applicant provided substantial evidence of the effectiveness and sufficient evidence of the safety of ALIS for the treatment of NTM lung disease caused by MAC as part of a combination antibacterial drug regimen for adult patients with limited or no treatment options. These members noted that there was sufficient safety data with ALIS. One member stated that*

ALIS should only be used in patients who cannot tolerate IV amikacin such as: the elderly, those with chronic kidney disease and patients who have received a lung transplant. Regarding labeling, the committee members recommended to include that: (1) there are limited data to support this claim and that efficacy was based on a surrogate endpoint, (2) only six months of data were available to support microbiologic cure, and data supporting longer term durability of culture conversion are not currently available, (3) clinical benefit has not been established, and (4) there is an increased risk of hospitalizations with the use of this product. Some committee members who voted "Yes", recommended that future studies be of longer duration to determine if the durable response of sputum culture conversion is sustained and if this confers clinical benefit to the patient. The committee members who voted "No", stated that there was a lack of a benefit in the 6-minute walk test, and that patient outcomes were worse as evidenced by the dropout rate and increased hospitalizations in ALIS-treated patients. One member also noted concern that if ALIS is approved there will be a lot of off-label use in patients such as those infected with other NTM species, cystic fibrosis patients, and those with Pseudomonas aeruginosa infections.

11 Pediatrics

No pediatric patients were evaluated in the three trials submitted in support of the NDA. Of note, the Pediatric Research Act (PREA) does not apply as ALIS has orphan drug designation for this indication.

12 Labeling Recommendations

12.1 Prescribing Information

Summary of Significant Labeling Changes (High level changes and not direct quotations)		
Section	**Proposed Labeling**	**Approved Labeling**
HIGHLIGHTS	(b) (4)	• Boxed warning added to describe the risk of increased respiratory adverse reactions and resultant hospitalizations.
1 INDICATIONS AND USAGE		• Utilizing the LPAD pathway language, added that ARIKAYCE is indicated in a limited population of adult patients with refractory MAC lung disease, and provided the definition of refractory disease based on the clinical studies in a Limitation of Use that also notes that ARIKAYCE is not recommended for patients with non-refractory MAC lung disease. • Noted that only limited clinical safety and effectiveness data for ARIKAYCE are currently available, and that clinical benefit has not yet been established. • Removed the statement suggesting that (b) (4) • Noted that clinical benefit

		has not yet been established.
• *Reviewer comment: The Applicant requested LPAD during the review cycle.* (b) (4) *was* (b) (4) *from the indication as MAC lung disease is sufficient to describe the disease in question. The definition of refractory disease was added to the Limitation of Use since this was the population studied in the three clinical trials, and that ARIKAYCE is not recommended for patients with non-refractory MAC lung disease. Demonstration of durability of culture conversion is a microbiologic surrogate. Clinical benefit has not yet been established and should be noted in labeling.*		
2 DOSAGE AND ADMINISTRATION	• (b) (4)	• This statement was deleted. • Additional information on preparation was moved from section 17 to section 2.
Reviewer comment: (b) (4)		
5 WARNINGS AND PRECAUTIONS	• (b) (4)	• Sub-headings were rearranged and renamed to the following: hypersensitivity pneumonitis (previously allergic alveolitis), hemoptysis, bronchospasm, exacerbation of underlying pulmonary disease (previously chronic obstructive pulmonary disease), ototoxicity, nephrotoxicity, neuromuscular blockage (previously neuromuscular disorders), embryo-fetal toxicity (added), and the sub-section on laboratory tests was deleted.
Reviewer comment: The sub-headings were rearranged and renamed to highlight adverse reactions by order of clinical importance.		
6 ADVERSE REACTIONS	• (b) (4)	• Updated percentages presented in Table 1

	(b) (4)	based on Agency's safety analyses which utilized a different definition of TEAEs from the Applicant's. • Included a second table to summarize selected adverse reactions that occurred in <5% of ARIKYACE-treated subjects and more frequent than those in the BR alone group in Trial 212. • Deleted the (b) (4)
Reviewer comment: The differences in the Agency's definition of TEAEs from the Applicant's has been communicated to the Applicant in multiple occasions during the review. Please refer to Section 9.1.3 of this review for the definitions of TEAEs used by the Agency vs. the Applicant. Incidence rates of cough, dysphonia, and exacerbation of underlying pulmonary disease (which includes chronic obstructive pulmonary disease) are captured in the first table in section 6. Incidence rates for the additional adverse event of special interest, including hypersensitivity pneumonitis, are captured in the new second table.		
7 DRUG INTERACTIONS	• (b) (4) •	• (b) (4) . • Section 7 was augmented with subheadings to discuss aminoglycoside class drug interactions with drugs with neurotoxic, nephrotoxic, or ototoxic potential, as well as, with diuretics.
Reviewer comment: (b) (4) *should generally not be included in section 7. Subdivision of aminoglycoside drug interactions was deemed more informative by the review team.*		
8 USE IN SPECIFIC POPULATIONS	• (b) (4)	• Relevant sub-sections were revised to better conform to PLLR labeling guidance.

Reviewer comment: Revisions to the PLLR sub-sections were made based on input from the review team		
12 CLINICAL PHARMACOLOGY	• (b) (4)	• In section 12.1, the mechanism of action was revised and shortened to note that ARIKAYCE is an antibacterial drug. • Section 12.2 (Pharmacodynamics) was added as per 21 CFR 201.57 (c)(13) to indicate that ARIKAYCE exposure-response relationships and the time course of pharmacodynamic response are unknown. • Sections 12.3 and 12.4 were revised.
Reviewer comment: Revisions made to streamline the label.		
14 CLINICAL STUDIES	• (b) (4) • •	• Study 1 is now referred to as Trial 1. • The definition of refractory MAC lung disease as noted in Section 1 is repeated in Section 14. • Discussion of Study 1 secondary clinical endpoints based on converter status was deleted. • A statement that additional endpoints to assess clinical benefit (6MWT and SGRQ) did not demonstrate clinical benefit by Month 6 was included at the end of Section 14. • (b) (4)

		• (b) (4) was deleted. • (b) (4) was deleted.
Reviewer comment: Providing the definition of refractory MAC lung disease enhances the discussion of the population studied in Study 212, the Phase 3 trial. (b) (4) *A statement that additional endpoints to assess clinical benefit (6MWT and SGRQ) did not demonstrate clinical benefit by Month 6 was included at the end of Section 14.* (b) (4)		
17 PATIENT COUNSELING INFORMATION	• (b) (4) •	• Reference is now made to a Medication Guide and Patient Instructions for Use. • The list of adverse reactions has been revised and ordered based on clinical significance: hypersensitivity pneumonitis, hemoptysis or cough, exacerbation of underlying pulmonary disease, dysphonia, ototoxicity, neuromuscular blockage (previously (b) (4) (b) (4)), embryo-fetal toxicity (previously (b) (4)).
Reviewer comment: This section has been revised and ordered by the relative clinical significance of the information, with the most important topics applicable to the patient appearing first. The Medication Guide has replaced the Patient Information.		

12.2 Patient Labeling

During the course of the review of the NDA, the review team recommended that the Applicant develop a Medication Guide instead of Patient Information to better convey the increased risk of respiratory adverse reactions associated with ARIKAYCE. The Medication Guide, Instructions

for Use and ARIKAYCE Quick Start Guide (both to be included in the ARIKAYCE 28-day kit) and Carton and Container labeling were evaluated by the review team and edits were recommended to the Applicant, such as, notification that ARIKAYCE will be indicated for a limited population for consistency with the LPAD pathway, re-arrangement of text for improved comprehension and to reduce the potential for medication errors. The Instructions for Use of the Lamira Nebulizer System that will be used with ARIKAYCE was reviewed by CDRH and OPQ. See CDRH and OPQ reviews for details.

13 Risk Evaluation and Mitigation Strategies (REMS)

13.1 Safety Issue(s) that Warrant Consideration of a REMS

The safety review of ALIS raised concerns regarding the risk of increased respiratory adverse reactions (hypersensitivity pneumonitis, bronchospasm, exacerbation of underlying pulmonary disease and hemoptysis) noted in the randomized portions of the Phase 2 and Phase 3 trials. There were also more frequent hospitalization events in the ALIS-treated patients compared to patients treated with Background Regimen alone in the Phase 3 trial.

13.2 Conditions of Use to Address Safety Issue(s)

To address these two concerns, the review team considered the potential use of a REMS with elements to assure safe use (ETASU) in addition to labeling (Boxed Warning, Limitations of Use to specify the intended population, limited population language under the LPAD pathway, Warnings and a Medication Guide). The goal of the REMS with ETASU would have been to inform health care providers regarding the evidence of benefit for ALIS on a surrogate endpoint, lack of evidence on a clinical endpoint, the respiratory adverse events and resultant increase in hospitalizations associated with use of ALIS and also to inform healthcare providers about the limited population of patients in whom the benefits outweigh the risks.

While many of the healthcare providers prescribing ALIS would likely be pulmonary and infectious diseases specialists who would be able to identify and remediate the adverse reactions associated with ALIS, it is also possible that there could be additional prescribers who would be less versed in the benefit-risk considerations with ALIS. Therefore, in addition to maximizing product labeling as noted above, it would be important to communicate the risks to the prescribers and assess the use of the product once approved. The Applicant has provided information regarding their efforts to communicate the safety profile and limitations of the data regarding efficacy to likely prescribers and their plans to monitor use, and to distribute ALIS, through a limited number of pharmacies/specialty pharmacies.

13.3 Recommendations on REMS

The REMS proposal from the Division was discussed with the Division of Risk Management (DRISK) and presented to the REMS Oversight Committee (ROC) that was convened on September 24, 2018.

The Division proposed communicating the limitations regarding the benefit demonstrated with ALIS and risks of ALIS through a REMS with ETASU (hereafter referred to as a REMS) which could include prescriber training and certification in addition to maximizing labeling

as noted above. Pharmacist certification would need to be part of the REMS to ensure that prescribers are certified prior to dispensing ALIS.

The DRISK team noted that the proposed REMS may not be able to achieve the intended goals. First, based on their experience, REMS with healthcare provider certification and training have not always been successful in curtailing off-label use despite robust education programs. Second, the potential prescribers for ALIS would mostly be specialized physicians (pulmonologists, infectious diseases specialists) and they would likely be aware of and be able to manage the respiratory adverse reactions associated with ALIS. DRISK also noted that off-label inhaled amikacin is compounded from the intravenous formulation in hospital pharmacies for other disease states, and a restrictive REMS could curtail use even in the intended population. Based on these points, the DRISK team's recommendation was to communicate the risks of ALIS through labeling, which would include a Boxed Warning and Limitations of Use to specify the intended population. Other non-REMS strategies could include safety communications conducted outside of a REMS, such as, the Applicant sending out Dear Healthcare Provider Letters, and targeted emails by the FDA to Pulmonary and Infectious Diseases professional societies to inform stakeholders of the risks and uncertain benefit associated with ALIS.

At the ROC meeting, two of the ROC members who opined that a REMS with ETASU may not be necessary recommended that additional measures such as a postmarketing requirement may be considered to obtain additional data on off-label use and also noted that healthcare provider education through a REMS has not generally been effective in changing behavior. They noted that if post-approval, additional safety signals arise a REMS can be required. It was also noted that since ALIS will mainly be used by Pulmonary and Infectious Diseases specialists, these prescribers would be cognizant of the safety information in the ALIS label and would be able to recognize and remediate ALIS-related adverse reactions. Hence, a REMS for the purpose of instructing the prescriber may not be necessary. However, both also stated that they would not object to the Division requiring a REMS. One member stated that a REMS was not warranted, and the adverse events could be communicated by maximized labeling. Additionally, it was likely that the Limitation of Use would deter payers from reimbursing off-label use. One member noted that a REMS is appropriate and would be important in communicating the uncertainty of clinical benefit, as well as, the increased risk of adverse reactions and hospitalizations associated with ALIS.

In a submission dated September 26, 2018, the Applicant notified the Agency that they intend to inform healthcare providers, such as, Pulmonary and Infectious Diseases specialists and associated professional societies of the risks and limited clinical benefit. They also plan to collect use data on patients prescribed ARIKAYCE, and will distribute ARIKAYCE through (b) (4) specialty pharmacies in the U.S. The Applicant has agreed to PMCs to provide their communication plan activities for outlining the risks associated with ARIKAYCE and the fact that clinical benefit has not yet been verified, as well as, assessment of use.

Based on the communication of risks in labeling, the Division's discussions with DRISK and the additional information submitted by the Applicant outlining their plans for communication to

healthcare providers and distribution of ALIS, we have determined that a REMS with ETASU is not needed at this time. If any additional information regarding risks arise postmarketing or if it appears that there is a need for additional efforts to educate healthcare providers, the need for a REMS will be revisited.

14 Postmarketing Requirements and Commitments

The Applicant has agreed to the following postmarketing requirement and commitments (PMR/PMCs).

PMR:

- Since ALIS is being approved under subpart H (505(b) of the Federal Food, Drug, and Cosmetic Act (the Act)), a confirmatory trial is required to characterize the clinical benefit of ALIS.
    - The proposed confirmatory trial will be a randomized, double-blind, placebo-controlled clinical trial to assess and describe the clinical benefit of ARIKAYCE in patients with NTM lung disease caused by MAC. The trial will evaluate the effect of ARIKAYCE on a clinically meaningful endpoint, as compared to an appropriate control, in the intended patient population of patients with MAC infection.
        - 3/27/2019 Draft Protocol
        - 6/25/2019 Final Protocol
        - 6/24/2020 First Patient in
        - 6/24/2023 Last Patient Last Visit
        - 6/23/2024 Study Report Submitted

Reviewer comment: We communicated to the Applicant during a teleconference on 9/26/18 that although not specifically included in the title of the proposed trial, we strongly recommend that the comparator arm include standard of care (SOC) as the added benefit of ALIS will need to be demonstrated. Therefore, the proposed confirmatory trial would be a randomized, double-blind, placebo-controlled clinical trial to evaluate ALIS plus standard of care (SOC) versus placebo plus SOC to assess and describe the clinical benefit of ALIS in patients with MAC lung disease. The trial would evaluate the effect of ALIS on a clinically meaningful endpoint in the intended patient population of patients with MAC infection. Additional recommendations included that the Applicant consider stratifying at enrollment on (1) refractory vs. non-refractory disease, and (2) fibrocavitary vs. bronchiectatic/nodular disease. Though not discussed during the teleconference, we will recommend that the placebo in the comparator arm (in addition to SOC) be a diluted liposome formulation similar to the one used in Study 112. It is hoped that clinical benefit could be assessed via a patient-reported outcome (PRO) measure. Additional work would be needed to either develop or adapt a pre-existing PRO for the population of patients with MAC lung infection.

PMCs:
The Applicant agreed to the following PMCs:

PMC - 1: Conduct further studies to identify an optimal QC in vitro drug release method in which only the drug released from the liposomes (free drug) is sampled and analyzed and propose new acceptance criteria for the drug product.

- Submission of Interim PMC Report 6 months after NDA approval (as part of a Prior Approval CMC Supplement to the NDA)
- Submission of Final PMC Report 12 months after NDA approval (as part of a Prior Approval CMC Supplement to the NDA)

PMC - 2: Provide and implement an email, standard mail, and facsimile communication plan to include a Dear Healthcare Provider letter as well as targeted educational materials to clinicians and professional societies.

- Draft submission: 10/2018
- Follow-up issue: 12/2018
- Follow-up issue: 3/2019
- Follow-up issue: 9/2019
- Final report submission: 12/2019

PMC-3: Provide results of a drug utilization assessment including ICD-10 code or other information on the indication and patient demographic/clinical characteristics of users of ARIKAYCE through pharmacies that will be distributing ARIKAYCE, and the results of chart reviews of a random subset of patients who are prescribed ARIKAYCE.

- Protocol Submission: 01/2019
- Interim report: 06/2019
- Interim report: 06/2020
- Interim report: 06/2021
- Interim report: 06/2022
- Interim report: 06/2023
- Final Study report: 06/2024

15 Appendices

15.1 References

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15.2 Financial Disclosure

Please refer to Table 7 in Section 7.2.2 for financial disclosures for investigators/sub-investigators of Studies (b) (6).

Covered Clinical Study (Name and/or Number): (b) (6)

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: (b) (6)		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 9		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 9 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☐	No ☒ (Request explanation from Applicant)

15.3 Nonclinical Pharmacology/Toxicology

[Insert carci data as needed. Limit to 2 pages].

15.4 OCP Appendices (Technical documents supporting OCP recommendations)

15.4.1 Summary of Bioanalytical Methods and Validation

The bioanalytical methods used to measure concentrations of total (bound + free) amikacin in human serum, urine, and sputum were validated by (b) (4), (b) (4). After thawing, serum, urine, and sputum samples underwent addition of an internal standard and then protein precipitation. Bioanalytic study results are summarized in *Table 63*. Selectivity was demonstrated as excessive false positives or negatives due to endogenous substance, co-administered analytes, metabolites, or other substances introduced during sample work up were not observed by the method. Specificity, defined as no peak retention time interference or an absolute bias of ≤20% for the spiked lower limit of quantification (LLOQ) sample, was observed from 6 serum and urine samples, as well as 10 sputum samples of different origin, respectively. Analyte stability was demonstrated over sample processing and long-term storage study times. The Clinical Pharmacology Review Team considers the bioanalytical methods and LLOQs used to determine amikacin concentrations and PK parameters acceptable.

Table 63: Summary of Bioanalytical Methods Used to Quantify Amikacin Concentrations

Study No. (analytical report)	TR02-112 (b) (4)) INS-212 (b) (4))	TR02-112 (b) (4))	TR02-112 (b) (4)) INS-212 (b) (4))	RD-201-23924[a] (AMIK-RES-2011-06-PROC)
Biological Matrix	Serum	Urine	Sputum	---
Detection Method	HPLC-MS/MS[b]	HPLC-MS/MS[b]	HPLC-MS/MS[b]	Scintigraphy
Lower Limit of Quantification (µg/mL)	0.15	0.5	0.1	NA
Validation Range (µg/mL)	0.15 to 40	0.5 to 75	0.1 to 30	NA
Model (weighting)	y= mx+b ($1/x^2$)	y= mx+b ($1/x^2$)	y= mx+b ($1/x^2$)	NA
Inter-day Accuracy (absolute bias %)	≤ ± 6.8	≤ ± 6.91	≤ ± 5.5	NA
Inter-day Precision (CV%[b])	≤ 6.2	≤ 10.96	≤ 5.8	NA
Validation Report No.	VTRSA7900E1	VTRSA7900U1	VTRSA7900S1	AMIK-RES-2011-06-PROC

[a]Scintigraphy study with Pari LC nebulizer to investigate lung deposition was only descriptive in nature
[b]HPLC with mass spectrometry / mass spectrometry detection (LC-MS/MS)
[c]co-efficient of variation expressed as percent (CV%)

More detailed method validation parameters are summarized in *Table 64*, *Table 65*, and *Table 66*.

Table 64: Serum Method Validation Parameters for HPLC-MS/MS

	Acceptance Criteria	Method Performance	Reference
Matrix – Human Serum	(b) (4)		
Anticoagulant – NA			
Assay Volume			
Selectivity			
Analyte-Amikacin		Complies (6 lots interference – free)	VTRSA7900E1
Internal Standard – (b) (4)		Complies (6 lots interference – free)	VTRSA7900E1
Matrix Effect (6 spiked matrix lots)		Complies	VTRSA7900E1
Matrix Effect (6 spiked matrix lots)		Complies	VTRSA7900E1 A5
Co-Medication – Albuterol, Beclomethasone, Budesonide, Fluticasone, Ibuprofen, Colistin Sulfate, Azithromycin, Clarithromycin, Erythromycin, Ciprofloxacin and Tobramycin		Complies	VTRSA7900E1 Addendum 2
Hemolytic Effect Evaluation		Complies	VTRSA7900E1 A5
Lipemic Effect Evaluation		Complies	VTRSA7900E1 A5
Carry-Over		Complies	Assessed in every batch containing standards and QCs
Low Curve Range (0.150 – 40.0 mcg/mL)			
Linearity			
Weighting	(b) (4)	$1/x^2$	VTRSA7900E1
Bias at LLOQ		3.02%	VTRSA7900E1
Bias above LLOQ		-5.74 to 6.82%	VTRSA7900E1
Bioanalytical Range		Complies	VTRSA7900E1
Precision			
Intra-assay		2.59 to 5.67 % (LLOQ); 1.98 to 6.45% (above LLOQ)	VTRSA7900E1
Inter-assay		4.05% (LLOQ); 4.21 to 6.15% (above LLOQ)	VTRSA7900E1
Accuracy			

	Acceptance Criteria	Method Performance	Reference
Intra-assay	(b) (4)	-2.27 to -0.86% (LLOQ); -8.03 to 14.37% (above LLOQ)	VTRSA7900E1
Inter-assay		-1.63% (LLOQ); -7.25 to 8.19% (above LLOQ)	VTRSA7900E1
Dilution (2x)			
Precision		-4.01 to -3.00%	VTRSA7900E1
Accuracy		3.53 to 5.11%	VTRSA7900E1
Truncated Curve Range (0.150 to 20.0 mcg/mL)			
Linearity	(b) (4)		
Weighting		$1/x^2$	VTRSA7900E1
Bias at LLOQ		3.7%	VTRSA7900E1 A5
	Acceptance Criteria	**Method Performance**	**Reference**
Bias above LLOQ	(b) (4)	-5.1 to 4.0%	VTRSA7900E1 A5
Bioanalytical Range		Complies	
Precision		1.3% (LLOQ); 1.0 to 2.7% (above LLOQ)	VTRSA7900E1 A5
Accuracy		1.5% (LLOQ); -5.3 to -0.6% (above LLOQ)	VTRSA7900E1 A5
Dilution (2x, 5x)			
Precision		1.8%, 2.6%	VTRSA7900E1 A5
Accuracy		-4.4%, -0.4%	VTRSA7900E1 A5

Source: Validation report VTRSA7900E1

Table 65: Urine Method Validation Parameters for HPLC-MS/MS

	Acceptance Criteria	Method Performance	Reference
Matrix – Human Urine	(b) (4)		
Anticoagulant – NA			
Assay Volume			
Selectivity			
Amikacin – Analyte		Complies (6 lots interference – free)	VTRSA7900U1
Internal Standard – (b) (4)		Complies (6 lots interference – free)	VTRSA7900U1
Matrix Effect (6 spiked matrix lots)		Complies	VTRSA7900U1
Matrix Effect (10 spiked matrix lots)		Complies	VTRSA7900U1 A4
Carry-Over		Complies	Assessed in every batch.
Curve Range (0.500 – 150 mcg/mL)			
Linearity			
Weighting	(b) (4)	$1/x^2$	VTRSA7900U1
Bias at LLOQ		2.58%	VTRSA7900U1
Bias above LLOQ		-5.24 to 5.66%	VTRSA7900U1
Bioanalytical Range		Complies	VTRSA7900U1
Precision			
Intra-assay		1.61 to 12.98% (LLOQ); 3.48 to 14.15% (above LLOQ)	VTRSA7900U1
Inter-assay		10.96% (LLOQ); 6.37 to 8.86% (above LLOQ)	VTRSA7900U1
Accuracy			
Intra-assay		-10.26 to 9.58% (LLOQ); -5.76 to 9.50% (above LLOQ)	VTRSA7900U1
Inter-assay		-0.67% (LLOQ); -2.16 to 6.91% (above LLOQ)	VTRSA7900U1
Dilution (2x)			
Precision		-9.35 to 0.03%	VTRSA7900U1
Accuracy		2.65 to 3.48%	VTRSA7900U1

	Acceptance Criteria	Method Performance	Reference
Dilution (5x, 10x)			
Precision	(b) (4)	-5.28%, 7.98%	VTRSA7900U1 Addendum 1
Accuracy		4.28%, 3.50%	VTRSA7900U1 Addendum 1

Truncated Curve Range (0.500 to 75.0 mcg/mL)			
Linearity			
Weighting		$1/x^2$	VTRSA7900U1
Bias at LLOQ	(b) (4)	-0.4%	VTRSA7900U1 A4
Bias above LLOQ		-3.4 to 5.1%	VTRSA7900U1 A4
Bioanalytical Range		Complies	
	Acceptance Criteria	**Method Performance**	**Reference**
Precision	(b) (4)	6.9% (LLOQ); 1.0 to 3.0% (above LLOQ)	VTRSA7900U1 A4
Accuracy		1.1% (LLOQ); -4.8 to -0.1% (above LLOQ)	VTRSA7900U1 A4
Dilution (2x, 5x)			
Precision	(b) (4)	2.6%, 1.8%	VTRSA7900U1 A4
Accuracy		-4.0%, 3.4%	VTRSA7900U1 A4

Source: Validation report VTRSA7900U1

Table 66: Sputum Method Validation Parameters for HPLC-MS/MS

	Acceptance Criteria	Method Performance	Reference
Matrix –Human Sputum/Serum Homogenate. Calibration standards were prepared with serum and QCs were prepared using human sputum/serum homogenate.			
Anticoagulant – NA			
Assay Volume	(b) (4)		
Selectivity – Using (b) (4) as IS			
Analyte – Amikacin	(b) (4)	Complies (10 lots interference – free)	VTRSA7900S1
Internal Standard – (b) (4)		Complies (10 lots interference – free)	VTRSA7900S1
Matrix Effect (6 spiked matrix lots)		Complies	VTRSA7900S1
Carry-Over		Complies	VTRSA7900S1
Low Curve Range (0.100 – 12.8 mcg/mL) – Using (b) (4) as IS			
Linearity			
Weighting		$1/x^2$	VTRSA7900S1
Bias at LLOQ	(b) (4)	7.3%	VTRSA7900S1
Bias above LLOQ		-10.5 to 8.9%	VTRSA7900S1
Bioanalytical Range		Complies	VTRSA7900S1
Precision			
Intra-assay	(b) (4)	1.9 to 4.2% (LLOQ)*; 1.0 to 5.6% (above LLOQ)*	VTRSA7900S1
Inter-assay		4.2% (LLOQ); 4.3 to 4.9% (above LLOQ)	VTRSA7900S1
Accuracy			
Intra-assay	(b) (4)	3.9 to 11.2% (LLOQ); -13.9 to 1.8% (above LLOQ)	VTRSA7900S1
Inter-assay		8.5% (LLOQ); -9.5 to -3.6% (above LLOQ)	VTRSA7900S1
*Excludes extrapolated data (Batch 4)			
Dilution (2x, 5x, 10x, 100x, 200x)			
Precision	(b) (4)	-3.2 to 2.4%	VTRSA7900S1
Accuracy		1.1 to 5.6%	VTRSA7900S1
Dilution (500x)			
Precision	(b) (4)	-7.5%	VTRSA7900S1

	Acceptance Criteria	Method Performance	Reference
Accuracy	(b) (4)	3.1%	VTRSA7900S1

Extraction Recovery			
Amikacin	(b) (4)	124%	VTRSA7900E1
Gentamicin (IS)		124%	VTRSA7900E1
Higher Curve Range (0.100 to 30.0 mcg/mL) (b) (4) and Amikacin-d_5 Internal Standard			
Matrix –Human Sputum/Serum Homogenate. Both standards and QCs prepared in Sputum/Serum Homogenate			
Selectivity – Using (b) (4) as IS			
Analyte –Amikacin	(b) (4)	Complies (6 lots interference – free)	VTRSA7900S1 A2
Internal Standard – (b) (4)		Complies (6 lots interference – free)	VTRSA7900S1 A2
Internal Standard – Amikacin-d_5		Complies (6 lots interference – free)	VTRSA7900S1 A2
Matrix Effect – (b) (4) IS (6 spiked matrix lots)		Complies	VTRSA7900S1 A2
Carry-Over		Complies	Assessed in every batch
Linearity – (b) (4) IS			
Weighting		$1/x^2$	VTRSA7900S1 A2
Bias at LLOQ	(b) (4)	1.1%	VTRSA7900S1 A2
Bias above LLOQ		-3.5 to 5.5%	VTRSA7900S1 A2
	Acceptance Criteria	**Method Performance**	**Reference**
Bioanalytical Range	(b) (4)	Complies	
Precision (b) (4) IS			
Intra-assay	(b) (4)	4.1 to 4.7 % (LLOQ); 2.0 to 5.0% (above LLOQ)	VTRSA7900S1 A2
Inter-assay		5.8% (LLOQ); 2.8 to 4.0% (above LLOQ)	VTRSA7900S1 A2
Accuracy – (b) (4) IS			
Intra-assay	(b) (4)	-4.1 to 5.7% (LLOQ); -7.5 to 2.3% (above LLOQ)	VTRSA7900S1 A2
Inter-assay		0.9% (LLOQ); -5.5 to 1.6% (above LLOQ)	VTRSA7900S1 A2
Dilution – (b) (4) IS			
Precision (2x, 5x, 100x)	(b) (4)	2.1% (2x); 1.8 to 3.5% (5x) 2.2 to 4.8% (100x)	VTRSA7900S1 A2
Accuracy (2x, 5x, 100x)		-1.9% (x2) -1.6 to 1.3% (5x); -4.8 to 2.4% (100x)	VTRSA7900S1 A2
Using Amikacin-d5 as Internal Standard			
Analyte – Amikacin	(b) (4)	Complies (6 lots interference – free)	VTRSA7900S1 A2
Internal Standard – (b) (4)		Complies (6 lots interference – free)	VTRSA7900S1 A2

	Acceptance Criteria	Method Performance	Reference
Precision and Accuracy			
Precision	(b) (4)	2.9 (LLOQ); 1.7 to 4.6% (above LLOQ)	VTRSA7900S1 A2
Accuracy		3.0% (LLOQ); -2.8 to 0.3% (above LLOQ)	VTRSA7900S1 A2
Dilution – Amikacin-d_5 IS			
Precision (5x, 100x)	(b) (4)	3.4% (5x); 3.6% (100x)	VTRSA7900S1 A2
Accuracy (5x, 100x)		1.4% (5x), -4.7% (100x)	VTRSA7900S1 A2
Precision (200x)		1.3%	VTRSA7900S1 A2
Accuracy (200x)		2.0%	VTRSA7900S1 A2

Source: Validation Report VTRSA7900S1

15.4.2 Clinical Pharmacology Review of Individual Study Reports

Table 67: Individual Study Evaluations Included in the Integrated Clinical Pharmacology Review

Study No.	Study Description
TR02-112	NTM single dose lung deposition (adsorption, distribution, clearance) (590 mg);
ICPD 00494-1	Population PK analysis for ALIS using data from studies TR02-112 and INS-212

15.4.2.1 Scintigraphy Sub-Study TR02-112

Title: A Randomized, Double-Blind, Placebo-Controlled Study of Liposomal Amikacin for Inhalation (ARIKAYCE) in Patients with Recalcitrant Nontuberculous Mycobacterial Lung Disease: A Scintigraphy Sub-study

PROTOCOL NO	TR02-112
Date(s)	August 2013 to September 2014
Principle Investigator(s)	Dr. K. Olivier
Study Center(s)	National Institute of Allergy and Infectious Disease, Bethesda MD., U.S.A.
Analytical Site(s)	NA
Dosage Strength/Formulation	590 mg ^{99m}Tc pertechnetate labeled amikacin liposomes[a] with PARI investigational eFlow® nebulizer[a]
Active substance	Amikacin

[a] ^{99m}Tc was attached to liposomes not free amikacin.

Objectives:

Primary. Determine the intra- and extra-pulmonary deposition and clearance of ALIS.
Secondary. Determine the pattern of radiolabel distribution of ALIS in the lungs in terms of the ratio of radiolabel in the central airways compared to that in the periphery of the lung.

Methods:

Study Population and Design. Following a blinded observation and assessment of drug safety in 10 NTM adult subjects who had completed a 3-month phase of the main study (TR02-112), 4 volunteers underwent gamma scintigraphy the first 2 min of each 10 min period over a 120 min period. An additional drug retention scan (18-30 hr post dose) was conducted on Study Day 2. Volunteers received a single dose of radiolabeled drug via nebulization prior to the start of scintigraphy.

Gamma Scintigraphy. Deposition and clearance of inhaled radiotracer was performed by imaging two-dimensional digital information. The percent of emitted/inhaled dose was used to estimate the deposition. Images over time allowed for clearance rates. Estimates of reginal lung deposition were performed by drawing 'regions of interest' on the scintigraphic image. Once obtained, a ratio of central to peripheral lung deposition was calculated.

Results:

Participants. Study baseline characteristics and anthropometric measures are described in *Table 68.*

Table 68: Scintigraphy Sub-Study Baseline Characteristics

Subject No.	Gender/Race/Age (years)	Chest X-ray	NTM Infection	CF Diagnosis	Baseline FEV1 (L)	Baseline FEV1 Percent Predicted
(b) (6)	Male/white/62	Nodular	Mab	No	2.82	80.6
	Female/white/67	Nodular and cavitary	MAC	No	1.64	65.9
	Female/white/51	Nodular and cavitary	MAC	Yes	1.02	36.4
	Female/white/19	Nodular	Mab	Yes	0.82	29.5

Note: Baseline was defined as the measurement prior and closest to the administration of study drug.

Abbreviations: CF = cystic fibrosis; FEV_1,= forced expiratory volume in 1 second; NTM = nontuberculous mycobacteria; Mab = *mycobacterium abscessus*; MAC = *mycobacterium avium* complex

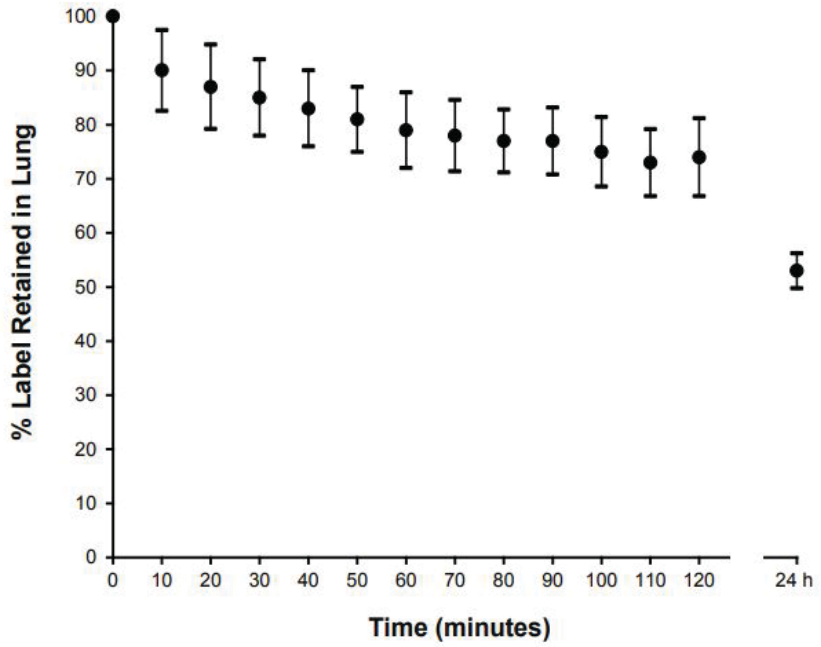
Source: Listing 16.2.11.1, Listing 16.2.12.1, Listing 16.2.4.1, Listing 16.2.6.1

Source: Applicant's Appendix 16, Table 11-1

Lung Deposition and Clearance. The dose deposited in the lung was, on average, 42.8 ± 5.6% of the loaded dose (590 mg amikacin) or 74.8 ± 7.7% of the total emitted dose. Retained in the nebulizer was 12.8 ± 5.4% of the dose. With respect to lung clearance, drug retention at times

0, 1, and 24 hr was 100 ± 0%, 79 ± 7%, and 53 ± 3% respectively. *Figure 17* displays the full radiolabel versus time plot for the right lung. The radiotracer did not appear to distribute well to areas with significant structural changes such as cavities or regions with air trapping.

Figure 17: Amikacin Liposome Retention Over Time in Lung



Source: Applicant's Appendix 6, Figure 11-1

Central to peripheral lung deposition (C/P ratio). Initial deposition of radiotracer was regionally located proximally in the lung. The C/P ratio was 2.05 ± 0.68 suggesting that twice as much drug is deposited centrally compared to peripherally.

Reviewer Comments:

- *The Applicant did not state if the estimates provided in the text were mean ± SD. That is this reviewer's assumption.*
- *Radiotracer was incorporated into the liposome; not amikacin.*
- *Previously, in a healthy adult study using a PARI LC star jet nebulizer on average 14% of the loaded dose and 32.3% of the emitted dose were deposited in the lungs. With respect to lung clearance, drug retention over time following inhaled ^{99m}Tc liposomal amikacin at 0, 1, and 24 hr was 100 ± 0%, 87 ± 9%, and 60 ± 7%. The C/P ratio was 1.63 ± 0.48.*
- *The slightly larger C/P ratio of 2.05 suggests an increase in inertial impaction, central lung deposition and thus decrease in lung retention via cough/mucociliary escalation mechanisms.*
- *Amikacin is not systemically available via the GI tract. Therefore, characterization of this fraction is not required.*

Applicant's Conclusions: Almost half of the loaded dose was deposited to the lungs corresponding to 252 mg of ^{99m}Tc-ALIS. More than half of the deposited dose was retained at 24 hr. ^{99m}Tc-ALIS distributed to both central and peripheral compartments of the lung. However, in areas of cavitation and air trapping this was not apparent.

Reviewer's Assessment: The reviewer agrees broadly with the Applicant's conclusion. However, reginal distribution and disease characteristics suggest that the central lung will be favored. This would benefit disease of the large airways with minimal cavitation and air trapping.

15.4.2.2 PK Report of Pooled Studies TR02-112 and INS-212

Title: Population PK Analysis for Amikacin Liposome Inhalation Suspension (ALIS) Using Data from Studies TR02-112 and INS-212.

PROTOCOL NO	TR02-112 and INS-212
PK REPORT NO	ICPD 00494-1
Date(s)	April 2012 to June 2015 (TR02-112) and May 2015 to July 2017 (INS-212)
Principle Investigator(s)	K. Olivier (TR02-112), D. Griffith (INS-212); N. Onufrak and C. Rubino (ICPD 00494-1)
Study Center(s)	United States of America, Canada, Australia, New Zealand, Austria, Germany, United Kingdom, Netherlands, Italy, France, Spain, Poland, Japan, Israel, Taiwan, Thailand, South Korea, and Sweden
Analytical Site(s)	(b) (4)
Dosage Strength/Formulation	590 mg liposomal amikacin once daily with a PARI investigational eFlow nebulizer[a]
Active substance	Amikacin

[a]Lamira™ is a modified version of the PARI eFlow.

Objectives:

Aims of analyses included:

1. Characterization of systemic amikacin exposure after ALIS administration using a population PK model describing drug disposition in serum and urine.
2. Compare systemic amikacin exposure in Japanese subjects enrolled in INS-212 to that in white subjects enrolled in either study (TR02-112, INS-212).
3. Evaluate amikacin sputum concentrations after ALIS administration at various periods on- and off-therapy.

Methods:

Study Population and Design. Adult subjects with recalcitrant NTM lung disease (*Mycobacterium avium* complex and/or *M. abscessus*) who participated in TR02-112 and INS-212 PK sub-studies receiving repeat daily doses of ALIS (590 mg amikacin) via the Lamira™ nebulizer. TR02-112 was a Phase 2, randomized, double-blind, placebo-controlled study. INS-212 was a Phase 3, randomized, open-label, multicenter and multinational study.

Sampling Scheme. A sparse-sampling design was performed for both TR02-112 and INS-212. With respect to INS-212 there were three PK sub-studies: A Population PK subset (US and Japan Only), Sputum-Only PK Sub-set, and Comprehensive Pharmacokinetic Sub-set (Japan Only).

TR02-112 blood, sputum and urine. PK sampling occurred on Days 1, 2, 28, 56, 84, 112, and 168. For blood, a minimum of two samples and a maximum of three samples (Day 1) were to be collected depending on the day. All days had a pre-dose level (0-4 hr) and then sampling between 0 and 4 hr post-dose. Sputum samples were to be collected pre-dose (0-4 hr) and 0-1 hr post-dose on each day. Twenty-four-hour urine volume collections were performed on Days 1, 84, and 168 and started immediately after dose administration.

INS-212 blood and sputum. In the Population PK sub-set blood and sputum samples were collected 0-1 hr before and 1-4 hr after drug administration daily at Months 1, 3, and 6. In the Sputum-Only PK sub-set sputum was collected at baseline, 72 hr after dose interruption on Months 4,5, and 6, as well as 28 days and 3 months upon discontinuation of treatment. In the Comprehensive Pharmacokinetics sub-set rich blood sampling occurred at Day 1 and Month 3 with 8 draw times from 0 to 24 hr post dose. Sparse sampling occurred on Months 1, and 6 at 0-1 hr pre-dose and 1-4 hr post-dose. Sputum samples were collected pre-dose (0-1 hr), 8 hr (optional), and 24 hr post-dose on Day 1. Then at Months 1, 3, and 6, sputum samples were collected at pre-dose (0-1 hr) and 1-4 hr post-dose.

Pharmacokinetic(PK) Analyses. A pooled population PK approach was utilized for sparse data and candidate PK models fit to serum concentration and urine amount data simultaneously using a Monte Carlo Parametric Expectation Maximization algorithm in S-ADAPT (version 1.57). A 3-compartment mamillary PK model was used for the pooled analysis based on previous reports. The structural model consisted of one absorption site (the lung), one central compartment, and one elimination compartment (the bladder). Furthermore, a zero-order drug input rate and first order elimination rate were assumed. One covariate model incorporating the relationship between body weight and renal drug clearance through a fixed exponent (0.75) allometric model based on previous work. Standard model selection criteria were not performed. Individual estimates of amikacin exposure on Day 1 and steady state were performed by simulation of individual *post-hoc* PK parameters obtained from the final PopPK model. Noncompartmental analyses was performed for the Comprehensive Pharmacokinetic Sub-set. Box-and-whisker plots evaluated PK parameter distribution differences with respect to race (Japanese, Non-Japanese). Descriptive statistics were computed for sputum concentrations. Regarding data imputation, serum amikacin concentrations below the limit of quantification (BLQ) were included if an assay estimated value was reported otherwise the Beal M3 method was used for post-dose levels. For sputum, samples assayed as BLQ were assigned a value equal to one-half the lower limit of quantification (LLOQ), equal to LLOQ, or equal to 0 µg/mL.

Analytical Method and Quantification. PK samples were quantified for amikacin by a validated LC-MS/MS method at a central site (See Bioanalytical Methods Summary). In-study validation

data demonstrated satisfactory linearity, precision, and accuracy over the bioanalytic range of 0.15 to 20 µg/mL with respect to FDA Bioanalytical Method Validation Guidance. All samples were analyzed within stability parameters established during validation of the method.

Results:

Participants. In this pooled population ca. 85% were female, ca. 47% were Caucasian, ca. 53% were Japanese. Study baseline characteristics and anthropometric measures for the Population PK sub-set and Sputum Only sub-set are listed in *Table 69* and *Table 70* respectively.

Table 69: Baseline demographics for the Population PK analysis Sub-set

Variable	Study 112 (n=14)	Study 212[a] (n=39)	PK Population (n=53)
Age (yr)[b]	55.8 (27.9%) 57 (20 – 77)	64.4 (13.8%) 66 (47 – 84)	62.1 (18.5%) 63 (20 – 84)
Weight (kg)	63 (17.2%) 65.2 (43.9 – 80)	51.1 (19.4%) 50 (33.8 – 77.1)	54.2 (21%) 52.6 (33.8 – 80)
Height (cm)	165 (4.7%) 165 (149 – 182)	160 (5.2%) 158 (145 – 180)	161 (5.2%) 160 (145 – 182)
BMI (kg/m^2)	23.2 (15.7%) 21.6 (18.6 – 29.3)	19.9 (13.2%) 20.1 (13.5 – 24.9)	20.8 (15.6%) 20.6 (13.5 – 29.3)
Ideal body weight (kg)	56.5 (12.4%) 57.1 (42.4 – 72.3)	52.9 (16.3%) 50.6 (38.6 – 75)	53.8 (15.5%) 52.8 (38.6 – 75)
BSA (m^2)	1.69 (9.45%) 1.75 (1.35 – 1.91)	1.51 (11.5%) 1.48 (1.25 – 1.97)	1.56 (11.9%) 1.54 (1.25 – 1.97)
Creatinine clearance ($mL/min/1.73m^2$)	87.7 (21.4%) 86.3 (63.3 – 140)	88.9 (19.4%) 88.4 (57.4 – 124)	88.5 (19.7%) 88.3 (57.4 – 140)

Note: Abbreviations are provided in the Abbreviation Listing.
Note: Summary statistics presented as mean (%CV) and median (min. – max.)
a. This all subjects included in the analysis, which includes the population PK and comprehensive PK subsets
b. 23 of 53 subjects were ≥65 years of age (3 in TR02-112 and 20 in INS-212)

Source: Applicant's Table 1

Table 70: Baseline Demographics for the Sputum Only Sub-set

Variable	Sputum Only subset (n=19)	Serum PK subjects[a] (n=39)	All subjects with sputum samples (n=58)
Age (yr)	61.75 (14.2%) 63 (44 - 85)	64.64 (13.53%) 66 (47 - 84)	63.81 (13.86%) 64 (44 - 85)
BMI (kg/m^2)	22.06 (18.21%) 21.1 (17 - 30.2)	19.98 (12.31%) 20.4 (13.5 - 24.9)	20.57 (15.22%) 20.5 (13.5 - 30.2)
BSA (m^2)	1.61 (8.75%) 1.61 (1.4 - 1.91)	1.5 (10.35%) 1.48 (1.25 - 1.97)	1.53 (10.46%) 1.5 (1.25 - 1.97)
Height (cm)	162.63 (4.16%) 161 (150 - 183)	158.78 (4.79%) 158 (144.8 - 180)	159.88 (4.74%) 159 (145 - 183)
Creatinine clearance ($mL/min/1.73m^2$)	77.57 (16.18%) 74.8 (57.6 - 120.3)	88.64 (18.99%) 88.4 (57.4 - 123.6)	85.48 (19.3%) 83.4 (57.4 - 124)
Weight (kg)	58.25 (17.8%) 54 (43 - 80)	50.64 (17.61%) 50 (33.8 - 77.1)	52.81 (18.86%) 50.8 (33.8 - 80)
Ideal body weight (kg)	55.63 (12.62%) 54.2 (47.8 - 77.7)	52.09 (15.14%) 50.5 (38.6 - 75)	53.1 (14.71%) 51.5 (38.6 - 77.7)
Gender			
Male	4/19 (21.1%)	8/39 (20.5%)	12/58 (20.7%)
Female	15/19 (78.9%)	31/39 (79.5%)	46/58 (79.3%)
Race			
White	16/19 (84.2%)	11/39 (28.2%)	27/58 (46.6%)
Asian	3/19 (15.8%)	28/39 (71.8%)	31/58 (53.4%)

Note: Abbreviations are provided in the Abbreviation Listing.
Note: Summary statistics presented as mean (%CV) and median (min. – max.)
a. This all subjects included in the analysis, which includes the population PK and comprehensive PK subsets

Source: Applicant's Table 2

Data Disposition/Imputation/Outliers of Serum and Urine Data.

TR02-112. A total of 238 serum amikacin concentrations and 47 urine samples were collected. After evaluation, 111 serum amikacin concentrations and 23 urine samples from 14 subjects were available for analysis.

INS-212. A total of 395 serum concentrations were collected from 39 subjects. After evaluation, 307 serum samples from 39 subjects were available for final analysis.

Reviewer comments:

- *The Applicant states that variability in absorption plus the sparse sampling scheme did not allow for outlier identification. TR02-112 and INS-212 samples were collected over 6 or 3 occasions, respectively. As few as 1 sample was drawn on some occasions.*

PK Analysis of Serum and Urine Data.

Population Approach. Amikacin population parameter estimates with associated precision are found in *Table 71*.

Table 71: Population PK Parameter Estimates for Amikacin

Parameter	Population mean		Inter-individual variability (%CV)	
	Final estimate	%SEM	Final estimate	%SEM
CLt/F (L/hr)	34.29	16.36	71.82	45.25
Vc/F (L)	272.6	11.41	65.09	31.2
ka (hr^{-1})	1.866	24.86	40.30	122.1
CLr (L/hr)	1.990	---	30.24	142.3
CLr coefficient (L/hr)	1.931	14.29	appears this way on the original	
CLr WTKG power	0.75	---		
Residual error for serum	0.615	3.941		
Residual error for urine	14.0	27.30		
Minimum value of the objective function = 225.3				

Note: Abbreviations are provided in the Abbreviation Listing.
Note: The demographics of the population PK analysis population (n = 53) are provided in **Table 1**.
Population mean CLr = $1.990 \cdot (WTKG/51)^{0.75}$

Source: Applicant's Table 6

Reviewer's Comments:

- *With respect to the goodness-of-fit plots, the individual model predictions vs observations suggest that the model is adequate for post-hoc exposure estimation (C_{max}, AUC). However, some caution is warranted since ε and η-shrinkage was not reported to evaluate the degree to which individual predictions collapse to the individual observation or population parameter estimate given the sparse nature of data (as few as 1 sample on occasion). In general, inter-subject variability was large, however the reliability of these values is acceptable for CL_t/F and V_c as the relative standard errors were <50%. This is apparent when comparing the goodness-of-fit plots between population and individual predictions where incorporation of inter-subject variability improves the fitting. The uncertainty in K_a and CL_r is not unexpected given the drug delivery route, limited sampling, and lack of intravenous data.*
- *The Applicant notes that, CL_t/F and CL_r were higher (ca. 60 to 70 L/h and 2.58 to 2.73 L/h respectively) in the CF and bronchiectasis populations than in the NTM population (34.2 L/h and 1.99 L/h) and thus systemic exposure is higher in NTM (i.e., AUC_{0-24}). While this model does not allow us to determine F, it is likely that the dose deposited/absorbed via the lung differs between these populations (disease state differences) and explains this discrepancy.*
- *Taken collectively, in this reviewer's opinion the model is fit-for-purpose (i.e., post-hoc exposure estimation to explore their distribution/extremes). However, the model parameter estimates were not verified.*

Serum Amikacin Exposure. Descriptive summary statistics for amikacin serum exposure estimates on Day 1 and steady state are provided in *Table 72*.

Table 72: Amikacin Serum Day-1 and Steady-State Exposure Estimates

Parameter	Study 112 (n=14) Mean (CV%) Median (Min-Max)	Study 212 (n=39) Mean (CV%) Median (Min-Max)	PK Population (n=53) Mean (CV%) Median (Min-Max)
C_{max}, Day 1	1.91 (75.0%) 1.31 (0.621 – 5.48)	2.21 (60.4%) 1.75 (0.465 – 6.61)	2.13 (63.6%) 1.70 (0.465 – 6.61)
AUC_{0-24}, Day 1	19.0 (68.4%) 15.1 (4.99 – 44.3)	19.0 (55.7%) 15.8 (4.16 – 53.5)	19.0 (58.6%) 15.8 (4.16 – 53.5)
C_{max}, steady-state	2.12 (71.8%) 1.65 (0.636 – 5.65)	2.32 (59.7%) 1.85 (0.482 – 6.87)	2.27 (62.2%) 1.81 (0.482 – 6.87)
AUC_{0-24}, steady-state	21.6 (69.9%) 16.1 (5.11 – 51.7)	20.0 (55.2%) 16.7 (4.31 – 55.6)	20.4 (59.3%) 16.7 (4.31 – 55.6)
$T_{1/2}$[a]	6.71 (45.7%) 5.48 (4.32 – 14.0)	5.34 (14.8%) 5.48 (3.29 – 7.37)	5.70 (31.3%) 5.48 (3.29 – 14.0)

Note: Abbreviations are provided in the Abbreviation Listing.
Note: The PK parameters reported above take BLQ concentration observations into account given that they are derived from the fit of the model to the dataset in which BLQ observations have been retained (as detailed in **Section 3.2.1.4**)
a. $T_{1/2}$ estimates are derived from the post-hoc CLt/F and Vc/F and are independent of study day

Source: Applicant's Table 7

Urine Amikacin Exposure. Individual estimates of the cumulative amikacin amount excreted in urine over a 24-hour collection period are provided in *Table 73*. Data are from Study TR02-112.

Table 73: Amount of Amikacin Excreted Expressed as a Percentage (Ae%) in Urine

	Ae (%)		
ID	Day 1	Day 84	Day 168
(b) (6)	3.26	1.55	---
	---	17.25	13.25
	---	---	22.61
	8.95	---	---
	2.94	2.22	0.72
	---	---	8.06
	---	---	8.42
	---	---	10.31
	3.24	11.67	14.14
	---	---	3.20
	5.64	8.24	---
	---	---	4.21
	2.71	5.52	8.88
	---	---	3.57

Source: Adopted with modification from Applicant's Report ICPD 00316, Appendix 6

Reviewer Comments:

- *The reviewer also compared the actual observed amount excreted in the urine as a percent of the nominal dose (Ae%). Model estimates were in close agreement with the observed 24-hour Ae% with a mean of 7.42% (ranging from 0.72 to 22.60%; n=14). We note that the data analysis definitions report is incorrect for the dependent variable (DV). It should be mg/L for serum PK samples and mg for urine PK samples (Sponsor confirmed).*
- *While only Study TR02-112 collected urine samples, serum PK parameter estimates in the current pooled analysis (TR02-112 and INS-212) are similar to estimates in Report ICPD 00316 (TR02-112 data alone) suggesting systemic exposure was similar and urine data from TR02-112 are representative of the entire pooled population.*

Comparison of Amikacin PK Parameters and Serum Exposures by Calculation Method. The Applicant evaluated the correlation between exposure estimates from the population PK and a noncompartmental analyses (NCA). The Applicant noted that the AUC_{0-24} was influenced by three patients in whom exposure was underpredicted by 30-34% in the population PK model; median bias (PPK relative to NCA) was -8.5%. With respect to the C_{max}, the population PK model underpredicted by 23-30% in 3 patients; median bias (PPK relative to NCA) was -10.4%. Summary level data for PK parameter estimates and systemic exposure are listed in *Table 74 and Table 75.*

Table 74: Amikacin serum exposure and PK parameters after single dose 590 mg ALIS by NCA[a,b]

	Cmax (mg/L)	AUC_{0-24} (Hr*mg/L)	CL/F (L/Hr)	Vz/F (L)	$T_{1/2\_z}$ (hr)
Mean	**1.4**	**14.2**	**40.4**	**487.1**	**7.9**
CV%	**38.7**	**37.0**	**35.7**	**57.4**	**31.1**
Min	**0.7**	**6.7**	**24.7**	**201.0**	**5.3**
Max	**2.3**	**21.0**	**66.0**	**971.8**	**12.2**
N	**13**	**13**	**13**	**13**	**13**

[a]Reviewer analysis of individual level data obtained from Applicant's PK dataset (ppkin1.xpt).
[b]Applicant correspondence has confirmed NCA exposure and PK estimates (dataset pk_wide_s212_intensive_subs_18jul2018.csv.
[#]extravascular dose option and linear Up Log Down
[##]2 individuals excluded based on λ_z r^2 <0.7

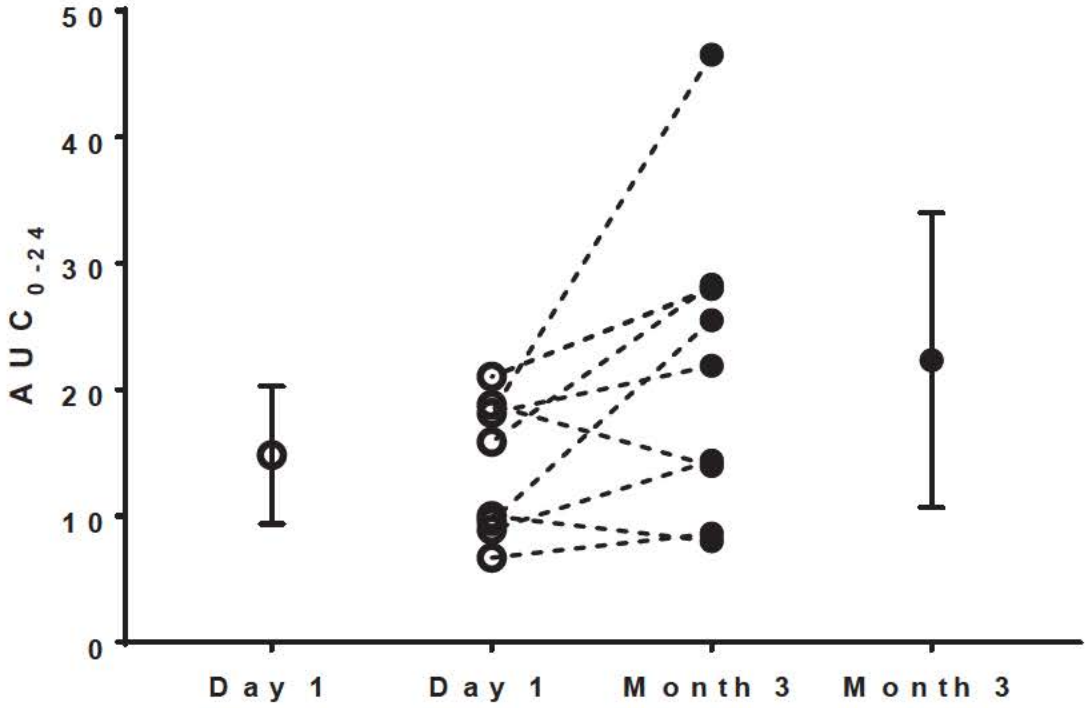
Table 75: Amikacin kinetics and exposure after 590 mg ALIS once-daily for 3 months by NCA[a,b]

	Cmax (mg/L)	AUC_{0-24} (Hr*mg/L)	CL/F (L/Hr)	Vz/F (L)	$T_{1/2\_z}$ (hr)
Mean	**2.8**	**23.5**	**26.8**	**368.6**	**10.1**
CV%	**43.0**	**46.3**	**58.6**	**68.3**	**37.2**
Min	**1.0**	**8.0**	**7.4**	**167.4**	**5.9**
Max	**4.4**	**46.5**	**56.3**	**1035.5**	**19.5**
N	**12**	**12**	**12**	**12**	**12**

[a]Reviewer analysis of individual level data obtained from Applicant's PK dataset (ppkin1.xpt).
[b]Applicant correspondence has confirmed NCA exposure and PK estimates (dataset pk_wide_s212_intensive_subs_18jul2018.csv.

[#]extravascular dose option and linear Up Log Down
[##]1 individual excluded based on λ_z r^2 <0.7

Figure 18: Day 1 Compared to Month 3 AUC0-24 (Source: Reviewer[a])



[a]Reviewer analysis of individual level data obtained from Applicant's PK dataset (ppkin1.xpt).
[##]11 paired inter-occasion individuals. Summarized by mean and SD.

Reviewer Comments

- *Individual Day-1 compared to Month-3 amikacin AUC_{0-24} suggest accumulation (Table 74, Table 75). Variability at Month 3 makes the true magnitude of accumulation difficult to discern especially given the day to day differences in deposited dose/bioavailability typical of orally inhaled drugs.*
- *Using Month 3 NCA estimates (n=25), typical PK parameter and exposure values are broadly in line with the Applicant's population PK model (Table 71). The different methods of calculating volume of distribution should be approximately equivalent for a drug characterized by one-compartment PK.*

Amikacin Exposure in Japanese and White NTM Subjects. Using the final compartmental model, the distributions of AUC_{0-24} and C_{max} were consistent between white (pooled TR02-112 and INS-212) and Japanese subjects (INS-212) as assessed by the Mann-Whitney U test (p>0.4).

Sputum Amikacin Disposition. Substantial variability in amikacin sputum concentrations were observed. Descriptive summary statistics are provided in *Table 76.*

Table 76: Amikacin Sputum Concentrations (INS-212)

All subjects with sputum samples from Study 212, n = 58

Visit	Nominal Sampling Time	N	Mean (%CV)	Median	Min	Max	# of BLQ samples[a]	# of missing samples
Pre-Dose								
MONTH 1b	0-1 HOUR PRE-DOSE	38	20.9 (245)	3.65	0.6	299	12	0
MONTH 3	0-1 HOUR PRE-DOSE	31	17.7 (179)	6.06	0.6	152	10	0
MONTH 6	0-1 HOUR PRE-DOSE	30	69.8 (294)	1.34	0.6	972	11	0
Post-Dose								
DAY 1	8 HOURS POST-DOSE	9	233 (286)	6.96	2.33	2010	0	0
DAY 1	24 HOURS POST-DOSE	15	44.3 (293)	7.14	0.6	512	4	0
MONTH 1b	1-4 HOURS POST-DOSE	36	1720 (183)	426	3.8	14300	0	1
MONTH 3	1-4 HOURS POST-DOSE	30	884 (152)	242	24.8	5640	0	1
MONTH 4	72 HOURS POST-DOSE	20	24 (124)	13.1	0.6	91.2	4	0
MONTH 5	72 HOURS POST-DOSE	23	175 (308)	16.3	0.6	2270	4	0
MONTH 6	1-4 HOURS POST-DOSE	30	1300 (132)	414	5.83	6660	0	0
MONTH 6	72 HOURS POST-DOSE	21	44.6 (133)	17.4	0.6	209	5	0
28 DAY SAFETY FOLLOW-UP		6	0.625 (9.8)	0.6	0.6	0.75	5	0
3 MONTH SAFETY FOLLOW-UP		5	0.636 (8.01)	0.6	0.6	0.708	3	0

Note: Abbreviations are provided in the Abbreviation Listing.
a. Sputum samples assayed as BLQ were assigned a LLOQ value (0.6 µg/g) for the purposes of these summary statistics
b. Note that two sputum samples for (b) (6) were mislabeled; the pre-dose sample (9180 µg/g) was therefore switched with the 1-4 hour sample (24.0 µg/g) for the purposes of these summary statistics

Source: ICPD 00494-1; Clinical information amendment, Appx 1

Applicant Conclusions:

The previous population PK model provided a robust fit to the combined data from Studies TR02-112 and INS-212. Amikacin exposure was similar in subjects enrolled in Study INS-212 to that seen previously in TR02-112. No difference in exposure was observed between Japanese and white subjects. Sputum amikacin concentrations drawn 1 to 4 hr post-dose were much higher than concentrations determined 24 to 72 hours post-dose.

Reviewer's Assessment:

The study adequately explored the pharmacokinetics of inhaled ALIS and were broadly in line with an analysis in which the comprehensive PK subset and observed systemic amikacin exposures were used to evaluate safety and calculate PK parameters via NCA. The review team chose to use the NCA results given the more robust study design and lack of modeling needs for predicting dose adjustments.

15.5 Microbiology Appendices

Table 77: Nontuberculous Mycobacteria present at randomization based on CF status (Study TR02-112 mITT)

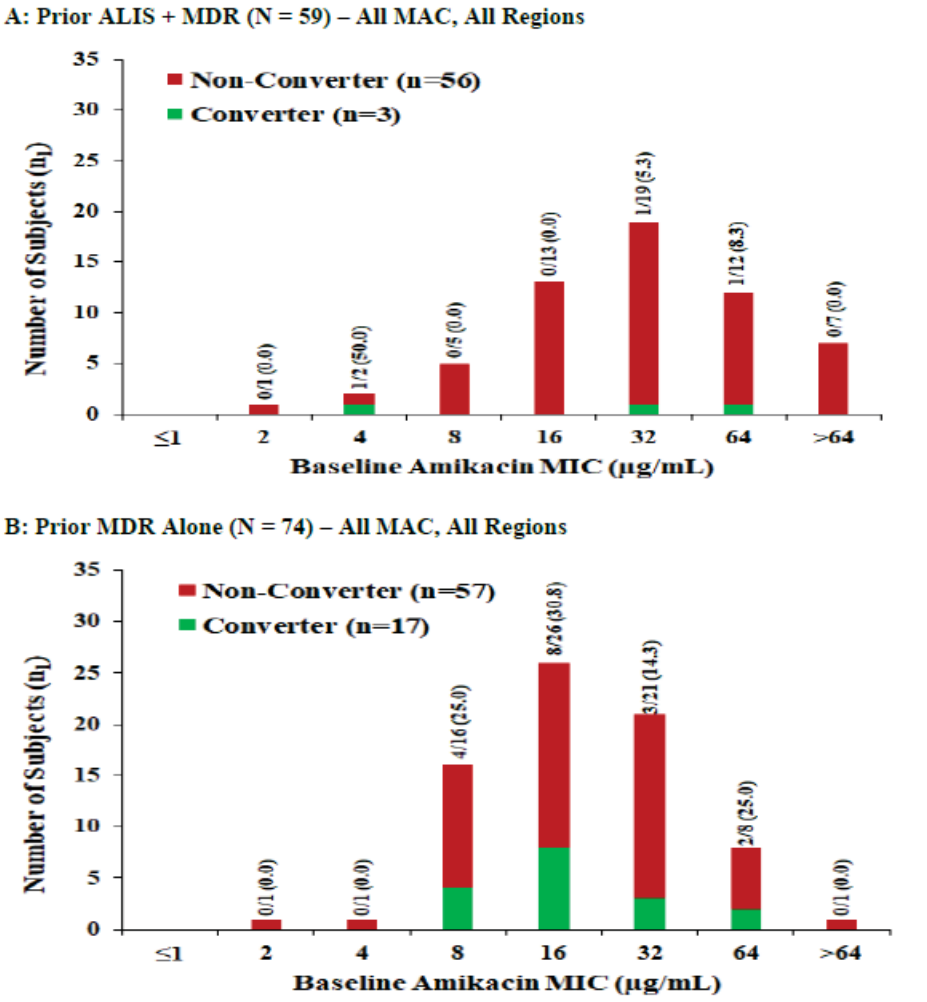
Organism	CF Status	ALIS + MDR (N = 44)	Placebo + MDR (N = 45)	Overall (N = 89)
NTM	Overall	44 (100%)	45 (100%)	89 (100%)
	NCF	36 (81.8%)	37 (82.2%)	73 (82.0%)
	CF	8 (18.2%)	8 (17.8%)	16 (18.0%)
MAC	Overall	29 (65.9%)	28 (62.2%)	57 (64.0%)
	NCF	27 (61.4%)	27 (60.0%)	54 (60.7%)
	CF	2 (4.5%)	1 (2.2%)	3 (3.4%)
M abscessus	Overall	15 (34.1%)	17 (37.8%)	32 (36.0%)
	NCF	10 (22.7%)	10 (22.2%)	20 (22.5%)
	CF	5 (11.4%)	7 (15.6%)	12 (13.5%)

Source: Study TR02-112
Note: For each specified category, the total number of isolates present at randomization (n) are shown alongside the corresponding percentage in parenthesis, % = 100(n/N).
ALIS = amikacin liposome inhalation suspension; CF = cystic fibrosis; MAC = *Mycobacterium avium* Complex; MDR = multidrug regimen; mITT = modified intent-to-treat; N = number of subjects in the indicated treatment group; NCF = non-cystic fibrosis; NTM = nontuberculous mycobacteria(l).

Table 78: Activity of Amikacin Against NTM other than MAC

Organism	Test Method	N	MIC Range	MIC_{50}	MIC_{90}	Reference
RGM	CLSI BMD	40	0.06 to 4	0.25	2	Pang et al, 2015
	CLSI BMD	76	≤ 0.5 to 256	≤ 8	≤ 8	Ramis et al, 2015
M abscessus	CLSI BMD	45	2 to 64	8	32	Jeon et al, 2009
M chelonae	Broth Macrodilution	26	4 to > 64	> 64	> 64	Ho et al, 1997
M fortuitum	CLSI BMD	21	4 to 32	8	32	Goswami et al, 2016
	AD	24	≤ 0.25 to 4	1	2	Udou, 2006
	Broth Macrodilution	31	1 to > 64	8	16	Ho et al, 1997
M kansasii	MGIT 960	49	≤ 1 to 10	4	10	Hombach et al, 2013
	BMD to 7H9	169	1 to 32	4	16	da Silva Telles et al, 2005
	Broth Macrodilution	19	4 to > 64	8	16	Ho et al, 1997
M ulcerans	Agar Proportion	29	0.25 to 2	0.5	2	Ji et al, 2006
M scrofulaceum	Broth Macrodilution	25	2 to 16	4	8	Ho et al, 1997
M marinum	CLSI BMD/AD	46	2 to 4	2	4	Chazel et al, 2016
	Etest	37	0.5 to 8	2	4	Rhomberg and Jones, 2002
	AD	54	1 to 8	2	4	Aubry et al, 2000
M haemophilum	BMD to 7H9	17	2 to 8	4	4	Bernard et al, 1993
M xenopi	Agar Proportion	19	1 to 8	1	2	Dauendorffer et al, 2002

AD = agar dilution; BMD - 7H9 = broth microdilution with Middlebrook 7H9 medium; CLSI BMD = broth microdilution per CLSI M24-A2, 2011; Etest = commercial gradient test strip (agar based test);
MAC = *Mycobacterium avium* Complex; MGIT 960 = commercial fluorescence broth microdilution test system;
MIC = minimum inhibitory concentration (µg/mL); NTM = nontuberculous mycobacteria(l);
RGM = rapidly-growing mycobacteria.



Source: Appendix 11 Table 3.6.1.1
Note: Data labels represent the percentage of subjects who achieved conversion at the indicated amikacin MIC, % = 100 (n of converters at the MIC/n_1 at the MIC).
ALIS = amikacin liposome inhalation suspension; ITT = intent-to-treat (population); MAC = *Mycobacterium av* Complex; MDR = multidrug regimen; MIC = minimum inhibitory concentration (µg/mL); N = number of ALIS + MDR-treated subjects with outcome and baseline amikacin MIC data; n = number of subjects with the indicated outcome; n_1 = number of subjects with the indicated amikacin baseline MIC.

Figure 19: Correlation of Amikacin MIC to Culture Conversion of MAC for ALIS + MDR (Multi-Drug Background Regimen)-Treated Subjects With and Without Prior ALIS Exposure (Study INS-312, ITT), A: Prior ALIS + MDR (N = 59) and B: Prior MDR Alone (N=74)– All MAC

16 Division Director (Clinical)

Concur with review.

17 Office Director (OAP)

Concur with review.

Appears this way on the original

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/s/

DEBORAH WANG
09/28/2018

EDWARD M COX
09/28/2018

Center for Drug Evaluation and Research
Division of Cardiovascular and Renal Products

DCRP Consult NDA 207356

DATE: Date of Document: 3/28/2018
Date of Consult: 7/5/2018
Date Assigned: 7/9/2018
Desired Completion Date: 8/1/2018
Date of Completion: 7/23/2018

FROM: Preston M. Dunnmon, M.D., M.B.A., Medical Officer
Division of Cardiovascular and Renal Products, HFD-110

THROUGH: Karen A. Hicks, M.D., Acting Medical Team Leader
Division of Cardiovascular and Renal Products, HFD-110

Norman Stockbridge, M.D., Ph.D., Division Director
Division of Cardiovascular and Renal Products, HFD-110

TO: Hiwot Hiruy, MD, PhD, Medical Officer
OAP/DAIP, (240) 402-0872

PROCUCT NAME: Arikayce (amikacin liposome inhalation suspension, ALIS)

PRODUCT CLASS: Antibiotic

SPONSOR: Insmed, Inc.

INVESTIGATIONAL INDICATION: for the treatment of nontuberculous mycobacterial (NTM) lung disease caused by *Mycobacterium avium* Complex (MAC) as part of a combination antibacterial regimen in adults

BACKGROUND AND CONSULT QUESTION: "*Please evaluate the results of the Applicant's analysis of the 6-minute walk test in the Phase 3 Study 212. This was a secondary, but clinically relevant endpoint in the study as the primary endpoint was sputum culture conversion by Month 6 of treatment, a surrogate endpoint. Please also be prepared to attend the Advisory Committee meeting scheduled for August 7th on campus to answer any technical questions related to the use of the 6-minute walk test, for example, in other trials or clinical practice."*

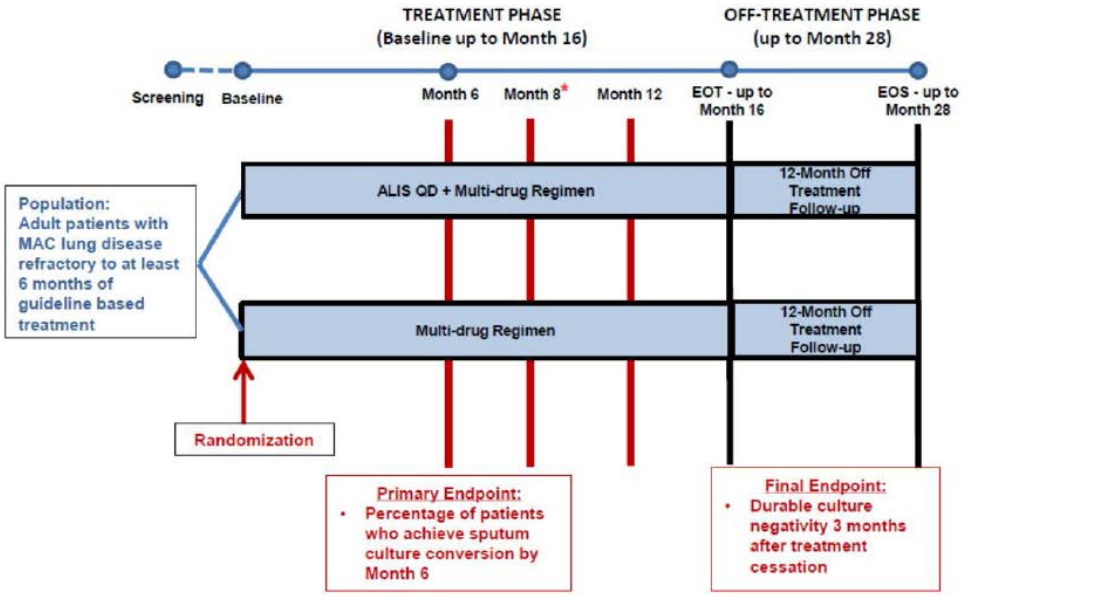
STUDY 212: A Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation (LAI) in Adult Subjects with Nontuberculous Mycobacterial (NTM) Lung Infections Caused by Mycobacterium avium Complex (MAC) That Are Refractory to Treatment

Design

This is an ongoing randomized, open-label, multicenter study of ALIS in adult subjects with NTM lung infections caused by MAC that were refractory to treatment. Enrollment is complete, and this initial report includes data through the cut-off date of 07 July 2017 when the last subject completed the Month 6 visit. A later analysis will be included in an updated final study report at the completion of the study.

The objective of this study was to compare the effectiveness of ALIS (590 mg), administered once daily (QD) when added to a multidrug regimen (MDR), to MDR alone for achieving culture conversion, as defined by 3 consecutive monthly negative sputum cultures by Month 6. The date of conversion was defined as the date of the first of 3 negative monthly cultures. Subjects were randomized 2:1 to ALIS 590 mg administered QD + MDR or MDR alone for a minimum of 8 months. Subjects were stratified by smoking status (current smoker or not) and prior MDR (on treatment or off treatment for at least 3 months) at Screening. At Month 8 (-28 to +7 days), after all sputum culture results were known, up to and including Month 6, subjects were assessed as converters or non-converters. Subjects experiencing "relapse or recurrence" were discontinued from the study at Month 8. Non-converters were discontinued from the study at Month 8. There were 335 subjects in the safety population and 336 subjects in the ITT population. Converters who completed a total of 12 months of treatment (end of treatment [EOT]) returned after the EOT visit for off-treatment follow-up visits at 28 days, and 3, 6, and 12 months. The 12 months off-treatment follow-up visit was the end of study visit. No NTM treatment was administered during the off-treatment phase. The Study Design is provided in Figure 1 below (sponsor):

Figure 1: Study Design



*All converters (at least 3 consecutive monthly negative sputum cultures by Month 6) without relapse or recurrence will remain in study for 12 months, starting from the first negative culture that defines culture conversion.
All non-converters and subjects who experienced a relapse or recurrence after culture conversion by Month 6 will discontinue the study at Month 8 and may be eligible to enter a separate open-label study (INS-312).

Per the protocol, the secondary endpoint of the trial was the change from Baseline (Day 1) to Month 6 in 6-minute walk test (6MWT) distance in the Intent-to-Treat (ITT) population (assuming Missing-Not-at-Random [MNAR]) using a model under the pattern-mixture model framework. This model assumed that subjects on the ALIS + MDR arm with missing data followed the response distribution of an MDR alone arm. It involved 3 steps:

1. The posterior mean and covariance estimates from the SAS MI procedure using the available non-missing data of an MDR alone arm were utilized to multiply impute missing data.
2. The endpoint was then analyzed for each complete data set with imputed data using an analysis of covariance (ANCOVA) with treatment arm and the randomization strata as factors, and the Baseline (Day 1) 6MWD as a covariate.
3. The treatment least squares (LS) mean differences were averaged and the associated standard errors summarized based on within-imputation and between-imputation variances using the SAS MIANALYZE procedure, to yield a final estimate of treatment contrast with associated 95% confidence interval (CI) and P value.

A sensitivity analysis of the change from Baseline (Day 1) to Month 6 in 6MWD was performed using a mixed-effects model for repeated measures (MMRM) with an unstructured covariance matrix implemented with SAS MIXED procedure over Months 4 and 6. This model included treatment, month, the treatment-by-month interaction,

smoking status, and prior MDR (on treatment or off treatment for at least 3 months) as fixed factors, the Baseline (Day 1) 6MWD as a covariate, as well as the Baseline (Day 1) 6MWD-by-month interaction. The Kenward-Rogers correction for denominator degrees of freedom, standard errors, and test statistics was utilized. The between-treatment difference in LS mean change from Baseline (Day 1) to Month 6, the corresponding 95% CI for the true between-treatment difference, and the P value were presented.

Supportive ANCOVA analyses of the change from Baseline (Day 1) to Month 6 in 6MWD with treatment arm and the randomization strata as factors, and the Baseline (Day 1) 6MWD as a covariate were to be performed for both the ITT and the Per Protocol (PP) populations. In the ANCOVA analysis for the ITT population, the last-observation-carried-forward method was used to handle the missing data. In addition, exploratory ANCOVA assessments of change in 6MWD at Month 6 were assessed as follows:

- For converters compared to non-converters overall for the ITT population
- Within the ALIS + MDR arm
- within the MDR alone arm.

Of note, subjects who were unable to perform the 6MWD test at Baseline were excluded from the entire trial.

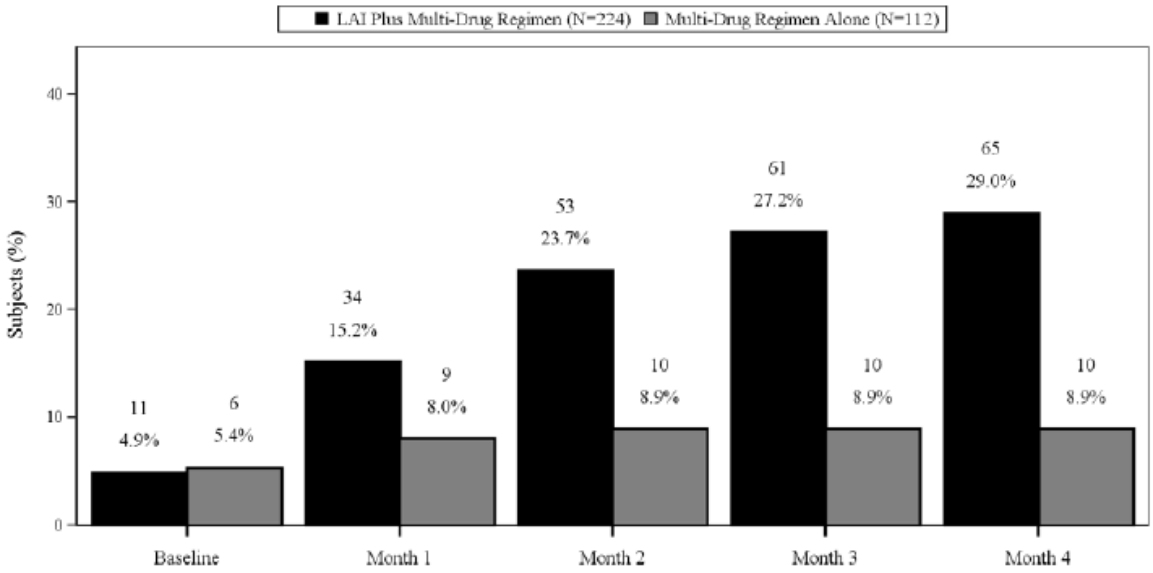
Demographics

The mean age in the total population was 64.7 years (ALIS + MDR: 64.6 years; MDR alone: 64.9 years). Overall, there was a greater percentage of females (69.3%) than males (30.7%) in the study. The demographic characteristics were generally balanced across the ALIS + MDR and MDR alone arms with a higher percentage of female subjects (73.7%) randomized to the ALIS + MDR arm versus the MDR alone arm (60.7%). The highest percentage of subjects was from the United States (42.0%) followed by Japan (14.3%). The majority of subjects were White (69.9%).

Primary Efficacy Outcome

The sponsor reports that significantly more subjects in the ALIS + MDR arm achieved culture conversion (29.0%) compared to the MDR alone arm (8.9%) by Month 6 ($P < 0.0001$). The time course of these conversions by month of treatment is shown in the cummulogram below (sponsor):

Figure 3: Cumulative Proportion of Subjects Achieving Culture Conversion Shown by the First Month of Conversion – ITT Population



Secondary Efficacy Outcome: 6MWD at Month 6

There was no statistically significant difference in the change from Baseline in the 6MWD between subjects in the ALIS + MDR arm compared to the MDR alone arm at Month 6. The difference in the LS mean (ALIS + MDR – MDR alone) (SE) was 3.2 (9.10) meters (95% CI: -21.1, 14.61; P = 0.7223). The tabular results of this analysis are shown below (sponsor):

Table 14: Analysis of Change From Baseline to Month 6 in 6MWT Distance Using Mixed Model Repeated Measures with Pattern-Mixture Modeling – ITT Population

Time Point Statistics	ALIS Plus Multidrug Regimen (N = 224)	Multidrug Regimen Alone (N = 112)
Baseline[a]		
n	220	111
Mean (SD)	425.7 (127.64)	420.4 (126.68)
Median	440.0	438
Min, Max	111.00, 801.00	72.00, 800.00
Month 6		
n	220	111
Mean (SD)	425.2 (141.23)	423.3 (132.73)
Median	440.3	445.0
Min, Max	-45.83, 716.26	3.00, 695.00
Change from Baseline to Month 6[a]		
n	220	111
Mean (SD)	3.5 (72.03)	5.2 (77.19)
Median	2.0	3.0
Min, Max	-183.43, 215.00	-225.00, 196.00
Change from Baseline to Month 6[b]		
LS Mean (SE)	-1.9 (11.23)	1.3 (11.99)
95% CI of LS Mean	-24.0, 20.17	-22.2, 24.88
LS Mean (SE) difference (ALIS Plus Multidrug Regimen – Multidrug Regimen Alone)	-3.2 (9.10)	
95% CI of LS Mean difference (ALIS Plus Multidrug Regimen – Multidrug Regimen Alone)	-21.1, 14.61	
Two-sided *P* value of LS Mean difference (ALIS Plus Multidrug Regimen – Multidrug Regimen Alone)	0.7223	

Source: Table 14.2.2.1

[a] Baseline is defined as the last non-missing value prior to first dose of study drug.

[b] Statistics are obtained from an MMRM model with pattern-mixture modeling of missing values due to dropout, which includes treatment, month, the treatment-by-month interaction, and the combination of smoking status and

The sponsor reports that the various sensitivity analyses of change from Baseline to Month 6 in 6MWT were consistent with the ANCOVA analysis of this outcome.

The sponsor notes that there was a numerical difference in favor of converters in the change from Baseline in the 6MWD at Month 6 as compared to non-converters within the ALIS + MDR arm (LS mean difference [SE]: 31.18 [10.870] meters; 95% CI: 9.71, 52.65; P = 0.0047). However, no difference in favor of converters was observed in subjects in the MDR alone arm with respect to 6MWD (LS mean difference [SE]: 25.18 [25.674] meters; 95% CI: -25.78, 76.14; P = 0.3292).

> *Reviewers Comment: I agree that the lack of significance for this finding in the MDR alone arm is likely a reflection of the low number of converters in this arm versus the ALIS + MDR arm (n=10 versus n=75, respectively). These data suggest that the improvement in 6MWD is the result of pulmonary response to the therapy received, regardless of the assigned therapy. Although more conversions at every time point were noted in the ALIS-treated subjects, the main ANCOVA result comparing the two treatment arms (table 14 above) suggests that the ALIS-treated subjects actually did worse by 3.2 meters compared to MRD alone at Month 6. This result could be an artifact of the statistical imputations and adjustments being performed because we also note that the minimum 6MWD at Month 6 in the ALIS arm (table 14 above) is <u>negative</u> 45.83 meters, suggesting that the worst subject in this group was walking backwards. This non-sense finding was not present in the MMRM and the ANCOVA-LOCF sensitivity analyses, although both of these sensitivity analyses also demonstrated a numerically more negative 6MWD change from baseline at Month six for the ALIS-treated subjects versus those treated with MDR alone (tables 14.2.2.2 and 14.2.2.3, data not shown). An analysis of 6MWD based on the raw, unadjusted data would be interesting.*

<u>Additional Information Regarding Subject Dispositions</u>

Table 14 above suggests no subjects dropped out between baseline and Month 6 and that all of these subjects had follow-up 6MWD testing. However, the following disposition table was provided to the Review Division earlier in the review cycle, suggesting that 44 people had dropped out prematurely from the ALIS arm by Month 6 as opposed to only 4 subjects in the MDR group:

SPONSOR RESPONSE TO REQUEST 1:

The number of patients that completed study visits up to Month 8 by study arm is presented in the below Table. The Table reflects the number of subjects completing each visit. The fluctuation in subject numbers (increasing or decreasing) completing each visit over time is due to missed visits by subjects.

	Study Visit							
Study Arm	Baseline (V2)	M1 (V3)	M2 (V4)	M3 (V5)	M4 (V6)	M5 (V7)	M6 (V8)	M8 (V9)
ALIS+MDR (n=224)	224 (100)	222 (99.1)	201 (89.7)	193 (86.2)	187 (83.5)	183 (81.7)	176 (78.6)	124 (55.4)
MDR Alone (n=112)	112 (100)	109 (97.3)	106 (94.6)	106 (94.6)	107 (95.5)	106 (94.6)	107 (95.5)	77 (68.8)

End of treatment visits are windowed into their appropriate visit month.
At the time of data cut (07JUL2017), 30 ALIS+MDR and 22 MDR Alone subjects completed Month 6 but did not have the opportunity to complete Month 8.

DCRP ASSESSMENT AND RECOMMENDATIONS:

The benefits on 6MWD in this trial appear to be due to successful treatment of the underlying NTM pulmonary infection, regardless of which drug regimen was used to achieve culture conversion to negativity. However, the unaccounted for missing subjects are of concern, particularly if they dropped out prematurely because of adverse events associated with the inhalation of this drug or its liposome carrier. We suggest that Table 14 should be revised to include only subjects that had 6MWD tests at Month 6. This should be done with both their adjusted analysis and an analysis of the unadjusted raw data. The sponsor should identify those subjects who were censored from the month 6 analysis and provide a careful accounting of their dispositions and adverse event profiles. It is possible that Table 14 as currently rendered is a survivor's analysis that is not reflecting important drug toxicity.

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/s/

PRESTON M DUNNMON
07/31/2018

KAREN A HICKS
07/31/2018

NORMAN L STOCKBRIDGE
07/31/2018