AMIKACIN LIPOSOME INHALATION SUSPENSION

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE

ANTIMICROBIAL DRUGS ADVISORY COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviation	Definition
6MWT	6 Minute Walk Test
AIDS	Acquired immune deficiency syndrome
AE	Adverse event
AESI	Adverse event of special interest
AFB	Acid fast bacilli
ALIS	Amikacin liposome inhalation suspension
ANCOVA	Analysis of covariance
ATS	American Thoracic Society
AUC	Area under the concentration-time curve
CF	Cystic fibrosis
CI	Confidence interval
CL_r	renal clearance (L/h)
CL_t/F	Apparent total serum clearance (L/h)
C_{max}	Maximum concentration
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DPPC	Dipalmitoylphosphatidylcholine
EOT	End of treatment
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
HCP	Healthcare professionals
HRQOL	Health-Related Quality of Life
IDSA	Infectious Diseases Society of America
IFU	Instructions for Use
ITT	Intent-to-treat
IV	Intravenous
k0	Zero-order inhalational administration
ka	First-order linear absorption process from lungs
LS	Least squares
MAC	Mycobacterium avium complex
MAUDE	Manufacturer and User Facility Device Experience
MCID	Minimally clinical important difference
MDR	Multidrug regimen
MIC	Minimal inhibitory concentration
MMRM	Mixed model repeated measures

MTB	Mycobacterium tuberculosis
mITT	Modified intent-to-treat
NDA	New drug application
NE	Not estimable
NTM	Nontuberculous mycobacterial
PD	Pharmacodynamic
PFT	Pulmonary function test
PK	Pharmacokinetic
PPK	Population pharmacokinetics
QD	Once daily
SAE	Serious adverse event
SD	Standard deviation
SE	Standard error
SGRQ	St. George's Respiratory Questionnaire
SOC	System organ class
SQS	Semi-quantitative scale
US	United States
V_c/F	Apparent central volume of distribution (L)

1 EXECUTIVE SUMMARY

The Sponsor, Insmed Incorporated (subsequently referred to as Insmed), is seeking regulatory approval for amikacin liposome inhalation suspension (ALIS), a unique liposomal suspension of amikacin formulated for oral inhalation, at a dose of 590 mg once daily (QD) for the treatment of nontuberculous mycobacterial (NTM) lung disease caused by *Mycobacterium avium* complex (MAC) as a part of a combination antibiotic regimen for adult patients. Amikacin is a broad-spectrum aminoglycoside antibiotic with bactericidal activity against both Gram-positive and Gram-negative bacterial species and known activity against NTM lung disease caused by MAC. Amikacin is typically administered parenterally; however, managing toxic side effects can be challenging, and safety for treatment periods longer than 14 days has not been established (Amikacin Prescribing Information, 2012). ALIS is a novel formulation that combines the proven bactericidal activity of amikacin within a liposomal formulation that penetrates biofilm and improves macrophage uptake, allowing for the achievement of high local concentrations of amikacin in the lung while minimizing systemic exposure.

Based on the potential to treat a serious and potentially life-threatening disease and fulfill an unmet medical need, the New Drug Application (NDA) for ALIS was submitted under an Accelerated Approval procedure, which allows for earlier approval of drugs via either a surrogate or intermediate endpoint.

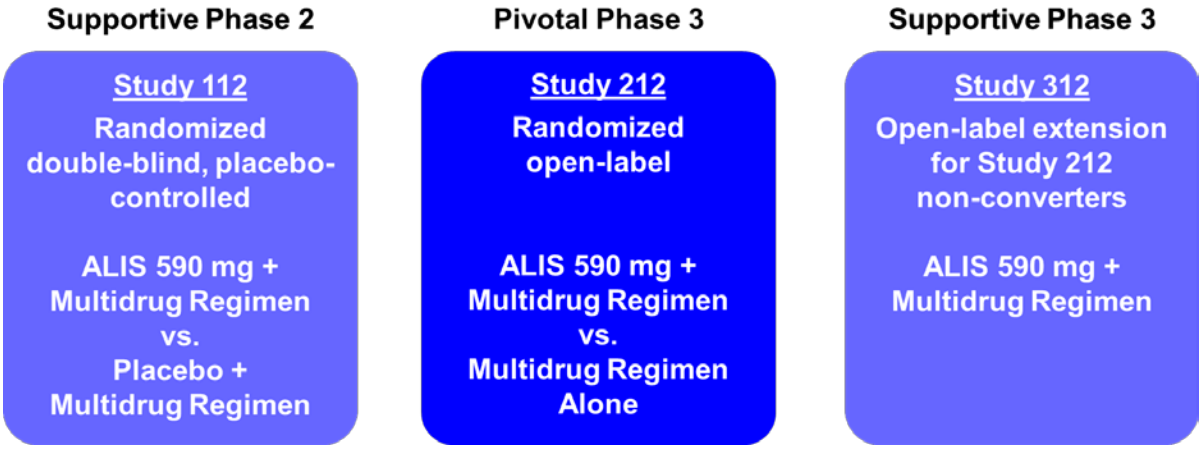
The ALIS NDA submission is supported by 3 key clinical studies conducted in patients with NTM lung disease (Figure 1). The pivotal study, Study INS-212 (subsequently referred to as Study 212), is a Phase 3, randomized, open-label study in adult patients with NTM lung disease caused by MAC who were refractory to treatment with an American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guideline-based Multidrug Regimen. In this superiority design study, patients were randomized to receive either ALIS added to their Multidrug Regimen or their Multidrug Regimen Alone.

The Statistical Analysis Plan (SAP) for the study specified 2 sets of analyses, the initial analysis and the latter analysis. The initial statistical analysis included the primary and several key secondary analyses. The primary endpoint for the study was culture conversion, defined as 3 consecutive monthly negative sputum cultures by Month 6. This analysis has been completed and the study is ongoing in order to collect data for the latter analysis, which addresses outcomes beyond 6 months. Culture conversion by Month 6 is a surrogate endpoint that predicts for persistence of negative sputum samples following the cessation of treatment. This is the primary microbiologic goal of treatment since it is indicative of eradication of the infection and allows patients to discontinue NTM therapy. Thus, in the ALIS clinical development program, culture conversion by Month 6 is a surrogate endpoint that is reasonably likely to predict durable culture conversion 3 months after completion of all NTM antibiotic treatment.

The efficacy and safety of ALIS are supported by 2 additional studies, INS-312 (subsequently referred to as Study 312) and TR02-112 (subsequently referred to as Study 112). Study 312 is an ongoing, Phase 3, open-label study evaluating the long-term safety and efficacy of ALIS in patients who did not achieve culture conversion (ie, non-converters) in Study 212. Study 112 was

a Phase 2 double-blind study comparing ALIS + Multidrug Regimen to an empty liposome placebo added to the Multidrug Regimen. All 3 studies assessed culture conversion as evidence of efficacy when evaluated by Month 6 in Studies 212 and 312 and Day 168 in Study 112.

Figure 1: Overview of Key Clinical Studies in ALIS NTM Development Program



In the ongoing Study 212, patients who achieved sputum culture conversion (ie, converters) continue treatment, with plans to complete 12 months of therapy following the first of 3-consecutive monthly negative sputum cultures that defines culture conversion. The primary endpoint of the pivotal Study 212 serves as the basis for approval under the accelerated approval pathway. The assessment of sputum cultures upon completion of all MAC treatment will provide evidence of sustainability of culture conversion. The durability of culture conversion will be measured after patients have been off all MAC treatment for 3 months. It is this endpoint that will serve as the confirmatory endpoint for full approval under the accelerated approval pathway. Study 212 is fully enrolled; the primary analysis has been completed and the study is ongoing for the purposes of the confirmatory analysis, which will include all randomized patients.

1.1 Background and Unmet Need

Nontuberculous mycobacterial lung disease is a serious, chronic, progressive disease that occurs as a result of the inhalation of NTM organisms from environmental sources. Most patients with NTM lung disease have varying degrees of underlying lung comorbidities such as bronchiectasis and chronic obstructive pulmonary disease (COPD).

The prevalence of NTM lung disease has increased in the United States (US), with a doubling of annual prevalence from 1997 to 2007 (Adjemian et al. 2012). Approximately 50,000 to 95,000 people have confirmed diagnoses of NTM lung infections in the US (American Lung Association 2018). The large majority (≥80%) of definite pulmonary NTM infections in the US are caused by MAC (Prevots et al. 2010).

Pathophysiologically, NTM lung disease caused by MAC has important similarities with infection caused by *M. tuberculosis*, such as the characteristic mycobacterial lung parenchymal immune response to mycobacteria that results in granulomatous inflammation and tissue destruction. The result is a chronic and progressive lung infection with variable rates of disease

progression, from indolent to rapidly progressive, that is invariably associated with significant and sometimes debilitating symptoms, especially cough and fatigue.

The Food and Drug Administration (FDA) held a Patient Focused Drug Development Meeting on 15 October 2015 on the topic of NTM lung disease, seeking to better link patient need with clinical development and the regulatory review process. In the meeting the FDA heard from patients suffering from NTM about the persistent and frequently severe symptoms caused by their disease. In the 'Voice of the Patient' Report from the meeting, the FDA characterized their findings as such: "*The input from the meeting and docket comments underscore the chronic and debilitating effect that NTM has on patients' lives, the challenges they face in finding effective and tolerable therapies to manage their condition, and the diverse experiences of patients with NTM.*" (FDA Patient-Focused Drug Development Workshop 2015).

The degree of morbidity and mortality associated with NTM lung disease is significant. Chronic infection and inflammatory response contributes to progressive, irreversible lung destruction (Olivier et al. 2017) and exacerbates the deterioration of lung function, compounding existing lung conditions measured by pulmonary function tests (Novosad et al. 2017). Park et al. evaluated 40 patients with untreated nodular/bronchiectatic MAC lung disease followed for a mean of 6 years with serial chest computed tomography (CT) scans and found significant radiographic deterioration consistent with progressive MAC lung disease in 39/40 (97%) of patients (Park et al. 2017). NTM lung disease was associated with a 4.3-fold higher incidence of respiratory failure and an increase in mortality rates when compared to the general population (Yeh et al. 2014).

Treatment failure is associated with a substantial decline in lung function in NTM lung disease. In patients who do not respond to an antibiotic treatment, NTM lung disease is associated with rapid decline in lung function. Successful treatment of NTM lung disease likely mitigates the decline in lung function (Park et al. 2016), and may impact mortality. In one prospective, randomized, controlled study, deaths attributable to NTM lung disease were more frequent in patients who remained culture-positive after 12 months of treatment (Jenkins et al. 2008). In a literature review of 13 published studies of 5-year mortality in patients with MAC lung disease, most studies showed an all-cause mortality rate greater than 25%. MAC-related deaths, which were reported in 9 of the 13 studies, as a proportion of all death was as high as 53%. These data indicate that NTM lung disease caused by MAC represents a substantial threat to people with the disease. Five-year all-cause mortality rates range from 5.4% (Hayashi et al. 2012) to 39.7% (Andrejak et al. 2010).

Currently, there are no drugs approved specifically for NTM lung disease in the US. For MAC lung disease, ATS/IDSA guidelines recommend a combination of 3-4 antibiotics, which includes a macrolide, ethambutol, and a rifamycin (rifampin or rifabutin). The microbiological goal of treatment is sustained culture conversion, defined as 12 months of consistently negative sputum cultures while on treatment. This recommendation of treatment is to ensure persistence of negative sputum cultures without microbiologic relapse (Griffith et al. 2007).

Consequently, patients are on this multidrug regimen for a minimum of 12 months and typically 18+ months treatment duration (Johnson and Odell 2014). Other important goals of long-term

antibiotic therapy are to reduce symptoms, reduce lung damage, and slow disease progression, even if long-term sputum conversion is not achieved.

If patients are compliant and well-monitored, they may achieve treatment success (Adjemian et al. 2014). Randomized controlled studies and meta-analyses of antibiotic therapy in patients with MAC lung disease have generally demonstrated culture conversion rates in more than half of patients (Research Committee of the British Thoracic 2001, Kobashi et al. 2007, Jenkins et al. 2008, Fujita et al. 2012, Miwa et al. 2014). However, current multidrug regimens recommended by the ATS/IDSA statement are lengthy and are associated with high rates of adverse events. Completing recommended treatment is often difficult for patients due to side effects and prolonged duration of treatment, further complicating management of NTM lung disease.

There are limited treatment options for patients who do not achieve culture conversion with standard treatment for MAC lung disease. First, there are few effective alternative antibiotics available; therefore, the armamentarium for treating refractory MAC is quite thin. Second, the traditional first choice of alternative therapy has been a parenteral aminoglycoside which requires long-term indwelling venous access. Parenteral, usually intravenous (IV) aminoglycoside administration, typically requires large doses for extended periods to achieve adequate concentrations in the lung (van Ingen et al. 2012), which increases the risk for ototoxicity and nephrotoxicity due to systemic exposure (Rybak et al. 1999, Kovacevic et al. 2016). The utility and effectiveness of parenteral aminoglycosides is limited due to poor penetration into the lung and poor patient tolerance because of frequent and sometimes severe adverse events (AEs). “Salvage” treatment options involve even longer extended therapy and are still associated with poor clinical and microbiological outcome.

Failure to achieve culture conversion with MAC therapy is associated with evidence of disease progression. Koh et al. evaluated 358 patients with nodular/bronchiectatic disease and found that patients with treatment failure and persistently positive sputum AFB results had decline in pulmonary function (forced expiratory volume in 1 second [FEV_1] and forced vital capacity [FVC]) declines than patients who were treated successfully (Koh et al. 2016). Pan et al. evaluated 126 MAC lung disease patients, treated and untreated, and found that microbiologic persistence leads to a significantly increased risk of radiographic progression (Pan et al. 2017). Treatment failure is associated with a substantial decline in lung function in NTM lung disease. Particularly in patients who do not respond to an antibiotic treatment, NTM lung disease may be associated with rapid decline in lung function, which has been overlooked in the past. Successful treatment of NTM may mitigate the decline in lung function (Park et al. 2016).

Patients with macrolide resistant MAC who have persistently positive sputum cultures even with aggressive therapy, including aminoglycosides, have higher mortality rates (Griffith et al. 2006, Ito et al. 2012, Koh et al. 2017).

It is clear that MAC lung disease with microbiologic persistence is an inexorably progressive process. There is an unmet medical need for evidence-based, effective therapeutic options with less systemic toxicities to treat NTM lung disease caused by MAC.

1.2 Regulatory

1.2.1 Indication Statement

The Sponsor's proposed indication is for the treatment of nontuberculous mycobacterial (NTM) lung disease caused by *Mycobacterium avium* complex (MAC) as part of a combination antibiotic treatment. The pivotal trial that supports the application was conducted in a subset of a MAC lung disease population that were unresponsive to previous guideline-based, multidrug therapy. Therefore, the strongest evidence supporting the positive benefit-risk profile of ALIS in the treatment of MAC lung disease is in this population. However, given that this study population represents a very difficult to treat population, the Sponsor believes it is reasonable to extrapolate the demonstrated safety and efficacy to additional subsets of MAC lung disease, including patients who initially present with advanced disease where the physician has already made the decision to treat. While this rationale supports the Sponsor's proposed indication for the use of ALIS for the treatment of NTM lung disease caused by MAC in the broader population, the sponsor recognizes that the final indication statement will be developed in collaboration with the Agency during the NDA review and may more closely reflect the patient population studied in the pivotal trial.

1.2.2 Regulatory History

In recognition of the potential for ALIS to meet a high unmet medical need for the treatment of a serious life-threatening infection, the FDA granted ALIS Fast Track, Qualified Infectious Disease Product, and Breakthrough Therapy designations and assigned it Priority Review. Additionally, ALIS was granted Orphan Drug Designation for the treatment of NTM lung disease in 2013.

Insmed is seeking approval for ALIS at a dose of 590 mg QD administered via oral inhalation for the treatment of NTM lung disease caused by MAC in adults. ALIS is intended to be marketed as a drug-device combination product that is delivered using the Lamira™ Nebulizer System provided as a handset along with the eFlow control unit.

The NDA for ALIS was submitted under an Accelerated Approval procedure (Subpart H). According to FDA guidance, the qualifying criteria for Accelerated Approval include the following:

- Treating a serious condition
- Providing a meaningful advantage over available therapy
- Demonstrating an effect on an endpoint that is reasonably likely to predict clinical benefit

The ALIS NDA fulfils all 3 criteria for Accelerated Approval. First, as detailed in Section 1.1, NTM lung disease caused by MAC is a serious disease, with progressive morbidity and increased mortality risk. The serious nature of NTM lung disease caused by MAC is further supported by the fact that ALIS received Breakthrough Therapy and Qualified Infectious Disease Product designations, both of which are reserved for products intended to treat conditions considered by FDA to be serious or life-threatening.

Second, adding ALIS provides a meaningful advantage over Multidrug Regimen Alone. There is currently no approved product in the US for the treatment of MAC lung disease. In addition, the analysis of the pivotal Phase 3 Study (Study 212) demonstrates that ALIS added to Multidrug Regimen significantly increases the proportion of patients achieving culture conversion by Month 6 compared with Multidrug Regimen Alone.

Third, the achievement of culture conversion by Month 6 is a surrogate endpoint that is reasonably likely to predict the clinical benefit of durable sputum culture negativity 3 months after completion of treatment. The FDA agreed in principal that if Study 212 is successful based on this endpoint, it can be used as the basis for seeking approval under Subpart H. Additional support for the relevance of culture conversion by Month 6 is provided by reports in the literature and preliminary analyses of mortality data from Studies 112 and 212 that suggest that mortality is higher among non-converter patients who remain culture positive compared with those who achieve culture conversion. Patients who achieved culture conversion were also more likely to show improvement in the 6 Minute Walk Test (6MWT), a clinical outcome assessment in Study 212.

1.3 Efficacy

Evidence of efficacy of ALIS for the treatment of adults with NTM lung disease caused by MAC is based on 3 studies: the pivotal, Phase 3 study (Study 212), and the supportive studies (Study 312 and Study 112). Data from these studies establish that ALIS in combination with an antibiotic regimen has superior ability to treat NTM lung disease caused by MAC in adult patients, as demonstrated by culture conversion. Durability of culture conversion will be confirmed in the ongoing Study 212 for full approval of ALIS.

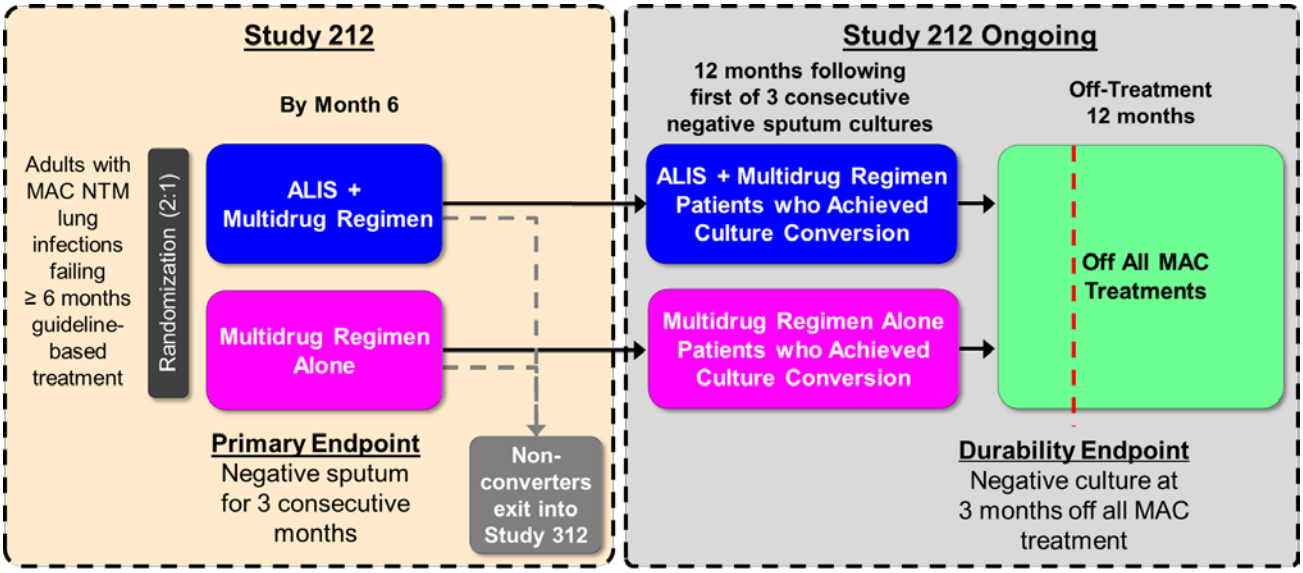
1.3.1 Study 212

Study 212 is an ongoing, randomized, open-label, multicenter study of ALIS in adult patients with treatment refractory NTM lung infections caused by MAC. Patients had to have persistently positive MAC sputum cultures while being treated with a guideline-based Multidrug Regimen for at least 6 months. Patients were randomized 2:1 to receive either ALIS 590 mg QD + Multidrug Regimen or Multidrug Regimen Alone (Figure 2).

The primary endpoint in this study to support Accelerated Approval was the proportion of patients achieving culture conversion, defined as 3 consecutive monthly negative sputum cultures by Month 6 in the ALIS + Multidrug Regimen arm compared to the Multidrug Regimen Alone arm. Patients who achieved culture conversion (converters) by Month 6 continued in the ongoing Study 212 where they went on to complete a treatment course of 12 months, starting from the first of the 3 negative cultures that defined culture conversion. All patients who did not achieve culture conversion (non-converters) exited the study at Month 8 and were offered the opportunity to enroll in Study 312, provided all entry criteria were met for the study (see Section 1.3.2).

A converter who successfully completes treatment will be assessed at 3 months off all treatment for presence of MAC in sputum samples as confirmation of durable efficacy, which will be the confirmatory endpoint to support full approval. The confirmatory endpoint analysis will include all randomized patients, including the non-converters that exited Study 212 and entered Study 312. To achieve the endpoint, patients must exhibit (1) culture conversion by Month 6, (2) sustained culture conversion through 12 months after initial culture conversion while on treatment, and (3) durable sputum culture negativity for 3 months following completion of NTM antibiotic treatment. These patients will continue to be followed through Month 12 off all treatment.

Figure 2: Design of Study 212



Patients in Study 212 were required to have demonstrated persistently positive MAC sputum cultures while on a Multidrug Regimen within the 12 months prior to Screening. The Multidrug Regimen must have consisted of at least 2 antibiotics for at least 6 consecutive months. Confirmation of ongoing MAC lung infection was documented by at least 2 positive sputum cultures consisting of at least 1 positive culture obtained within 6 months prior to Screening and 1 positive culture at Screening. Study 212 only included patients with susceptible amikacin minimal inhibitory concentrations (MICs) of ≤64 µg/mL at Screening.

In consultation with the FDA and clinical experts, a 15% treatment effect in sputum culture conversion was determined to be a meaningful effect in this treatment refractory patient population. Assuming a culture conversion rate by Month 6 of at least 20% in the ALIS + Multidrug Regimen arm and 5% in the Multidrug Regimen Alone arm, randomization of approximately 351 patients (2:1) was predicted to provide ≥90% power at a 2-sided significance level of 0.05. For the primary analysis, all patients who dropped out of the study prior to culture conversion were considered to be treatment failures.

As shown in Table 1, baseline demographics were comparable between treatment arms. Overall, the mean age was 64.7 years, and the majority of patients were female (69.3%) and white

(69.9%), which is consistent with epidemiology of the NTM MAC population in the US. The highest percentage of patients were enrolled from the US (42.0%).

Table 1: Baseline Demographics in Study 212 (ITT Population)

	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Mean age, years (SD)	64.6 (9.59)	64.9 (10.16)
Female	165 (73.7%)	68 (60.7%)
Region		
United States	93 (41.5%)	48 (42.9%)
Rest of the World	83 (37.1%)	44 (39.3%)
Japan	34 (15.2%)	14 (12.5%)
Asia (excluding Japan)	14 (6.3%)	6 (5.4%)
Ethnicity: Hispanic	10 (4.5%)	5 (4.5%)
Race		
White	158 (70.5%)	77 (68.8%)
Asian: Japanese	35 (15.6%)	15 (13.4%)
Asian: Other	23 (10.3%)	10 (8.9%)
Other	3 (1.3%)	6 (5.4%)

Other baseline characteristics were also similar between treatment groups (Table 2). The median duration of NTM lung disease was 3.96 years overall, with a slightly longer duration in the ALIS + Multidrug Regimen arm compared to the Multidrug Regimen Alone arm. Although study entry criteria allowed for patients whose Multidrug Regimen had been stopped for ≤12 months from Screening, most patients were on a Multidrug Regimen (≥2 drugs) at Screening, with a majority on 3 drugs. Most patients had underlying bronchiectasis and were not current smokers, and only a small percentage of patients had received prior nebulized IV amikacin.

Table 2: Baseline Characteristics in Study 212 (ITT Population)

	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Duration of NTM Lung Disease (years; median)	4.45	3.26
Number of Drugs in Regimen at Screening		
0*	2 (0.9%)	3 (2.7%)
2	39 (17.5%)	14 (12.5%)
3	148 (66.1%)	84 (75.0%)
4+	34 (15.2%)	11 (9.8%)
Underlying lung disease		
Bronchiectasis	146 (65.2%)	64 (57.1%)
COPD	29 (12.9%)	19 (17.0%)
COPD & bronchiectasis	22 (9.8%)	18 (16.1%)
Current smoker	26 (11.6%)	10 (8.9%)
Prior nebulized IV amikacin	24 (10.7%)	15 (13.4%)

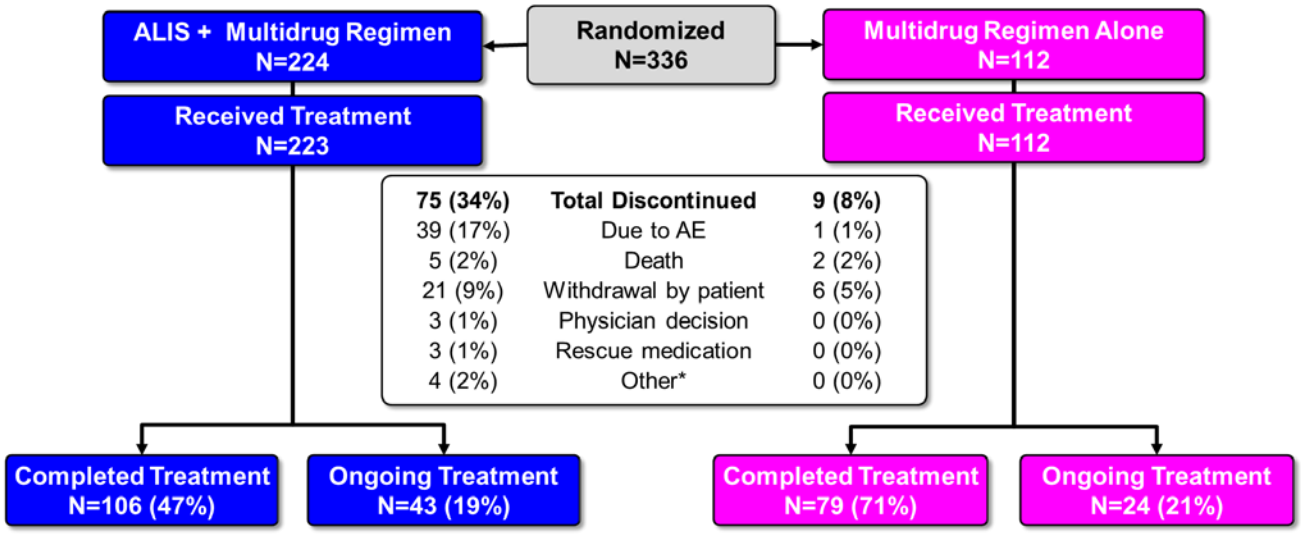
*4 patients reinitiated their multidrug regimen after Day 7, and 1 patient withdrew consent at Baseline.

A total of 336 patients were randomized and 335 received treatment in Study 212 (Figure 3).

Of these patients, 224 were in the ALIS + Multidrug Regimen arm and 112 patients were in the Multidrug Regimen Alone arm. Of the 336 total patients randomized, 185 completed treatment, 84 discontinued treatment, and 67 were still ongoing at the time of the data cutoff. More patients in the ALIS + Multidrug Regimen arm discontinued treatment, most commonly due to an AE or withdrawal by the patient.

At the end of each subject's treatment, the subject was classified as having either "completed treatment" or "discontinued treatment". Note that, at the time of data cut-off for the NDA, some subjects were continuing to receive treatment and were categorized as "ongoing treatment" as shown in Figure 3.

Figure 3: Patient Disposition (End of Treatment) in Study 212 (ITT Population)



*Other includes categories: other, protocol deviation, and non-compliance with study drug

Study 212 met its primary endpoint, supporting Accelerated Approval. The percentage of patients achieving culture conversion by Month 6 was significantly higher in the ALIS + Multidrug Regimen arm compared to the Multidrug Regimen Alone arm (29.0% and 8.9%, respectively; P<0.0001), with an absolute treatment effect size of 20.1% (Figure 4). Figure 5 shows the cumulative proportion of patients achieving culture conversion by the first negative monthly cultures. Patients had to have achieved their first negative culture by Month 4 to meet the primary endpoint of 3 consecutive monthly negative cultures by Month 6. Benefits were observed as early as 1 month following initiation of treatment with ALIS.

Figure 4: Primary Endpoint Initial Analysis in Study 212 – Proportion of Patients Achieving Culture Conversion by Month 6 (ITT Population)

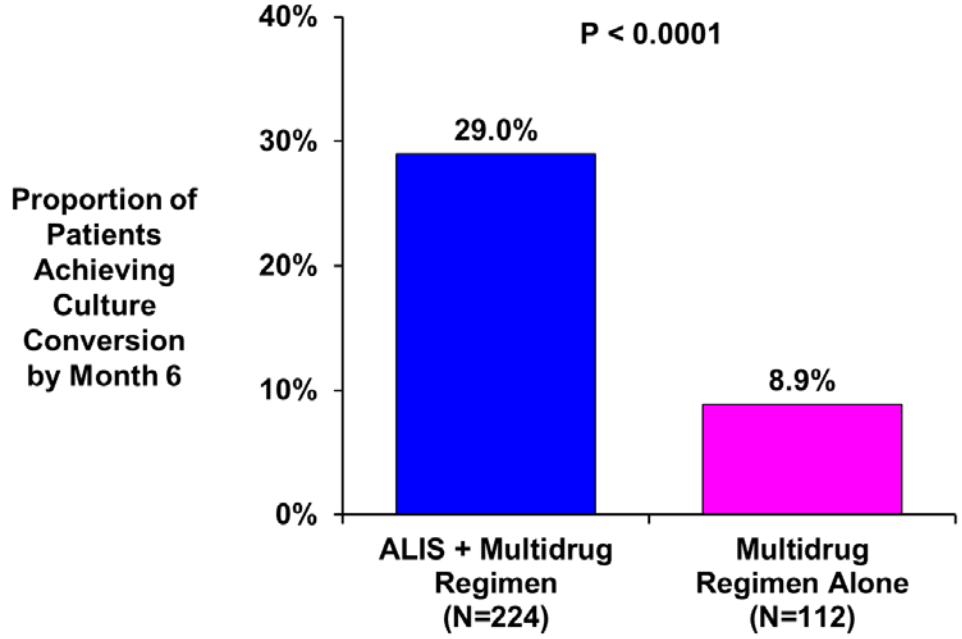
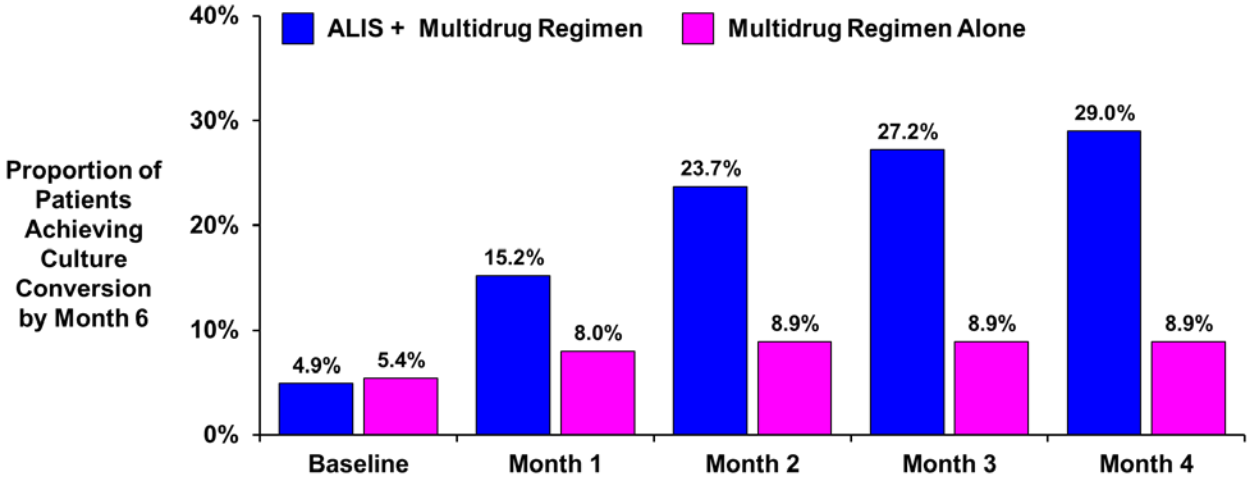


Figure 5: Cumulative Proportion of Patients Achieving Culture Conversion by Month 6 Shown by the First Month of Conversion in Study 212 (ITT Population)



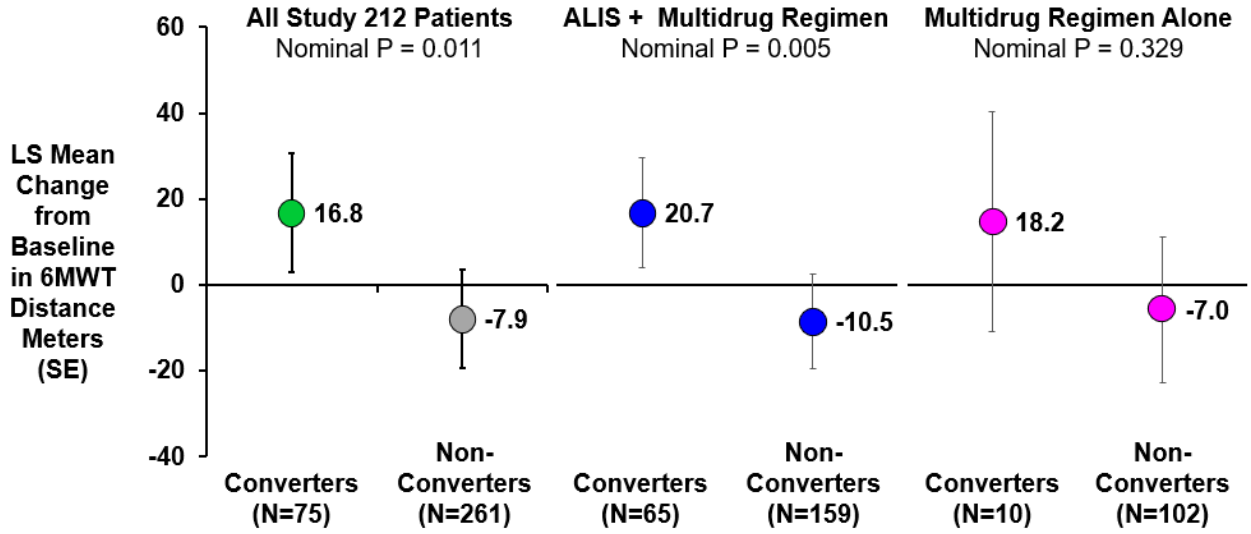
Culture Conversion reported as first month of 3 consecutive monthly negative sputum samples. Patients had to have their first of 3 negative sputum cultures by Month 4 at the latest to meet the endpoint by Month 6.

In a post hoc analysis in patients who achieved culture conversion, sustainability of culture conversion through Month 6 on therapy (defined as consecutive negative sputum cultures [with

no more than 2 consecutive positive broth cultures]) was achieved in 62/65 (95.4%) converters in the ALIS + Multidrug Regimen arm compared to 7/10 (70.0%) converters in the Multidrug Regimen Alone arm.

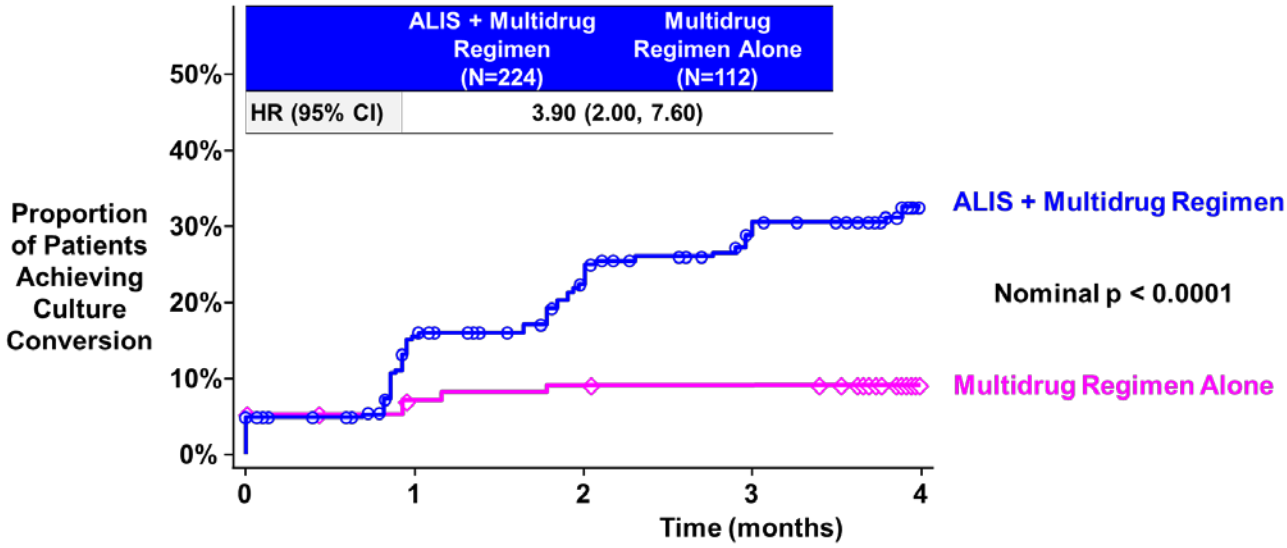
The results for the first secondary endpoint, change from Baseline at Month 6 in the 6MWT distance, did not reach statistical significance. Overall, a least squares (LS) mean difference of -3.2 meters were observed between treatment arms (P=0.7223). It was anticipated that patients who achieved culture conversion may derive a functional benefit, as assessed by the 6MWT distance. Therefore, a prespecified exploratory analysis of change from Baseline at Month 6 in the 6MWT distance was conducted based on conversion status (Figure 6). Overall, converters showed an improvement in the 6MWT distance at Month 6, with a LS mean difference of 24.7 meters between treatment arms (P=0.011). Results were consistent when analyzed by treatment arm. In the ALIS + Multidrug Regimen arm, converters showed an improvement in the 6MWT distance at Month 6, with a LS mean difference of 31.2 (P=0.005). Similarly, in the Multidrug Regimen Alone arm, converters showed an improvement in the 6MWT distance at Month 6 with a LS mean difference of 25.2; however, this difference did not reach statistical significance (P=0.33) due to the small number of converters in this arm (N=10).

Figure 6: Exploratory Endpoint Initial Analysis in Study 212 – Difference in Change from Baseline to Month 6 in 6MWT Distance for Converter Versus Non-converter (Overall, ALIS + Multidrug Regimen, Multidrug Regimen Alone) (ITT Population)



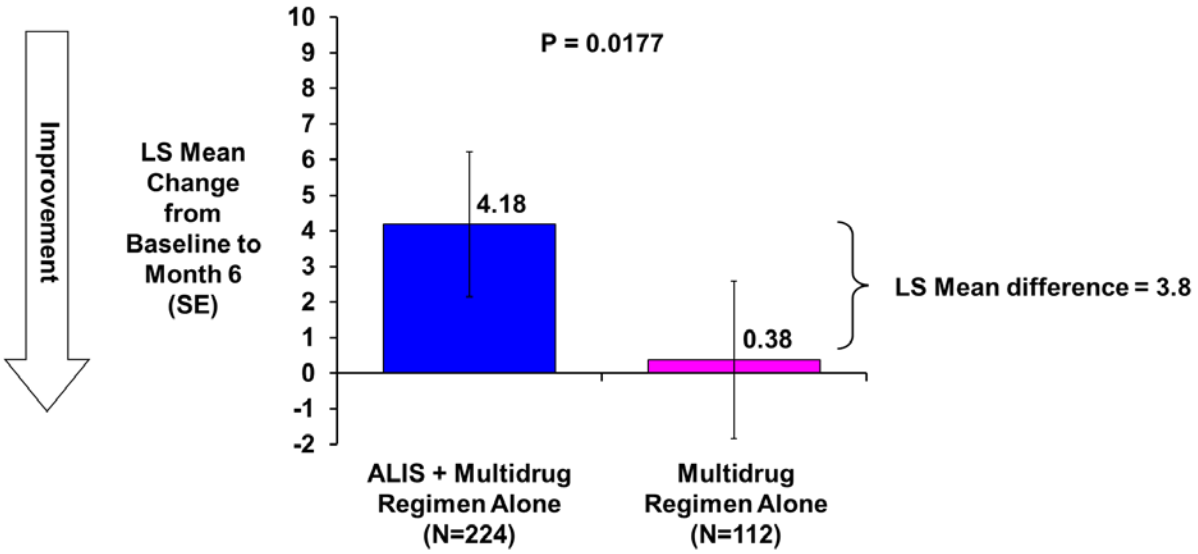
The second secondary endpoint, time to culture conversion, was analyzed using a Cox regression model to estimate the treatment effect (hazard ratio) adjusted for the two binary stratification factors (smoking status and prior multidrug regimen) (Figure 7). Results of this analysis demonstrated a hazard ratio of 3.9, suggesting that patients receiving ALIS + Multidrug Regimen achieved culture conversion were 290% more likely to convert than Multidrug Regimen Alone (nominal P<0.0001). However, the time to culture conversion is not statistically significant given its position in the hierarchical testing.

Figure 7: Secondary Endpoint in Study 212 – Time to Culture Conversion (ITT Population)



There is no validated tool in the St. George's Respiratory Questionnaire (SGRQ), for NTM lung disease, as stated by the FDA. Additionally, no Health-Related Quality of Life (HRQOL) instruments have been validated for use in NTM lung disease. In this study, HRQOL was measured using the SGRQ, which has been validated for use in COPD. SGRQ scores range from 0 to 100, with higher scores indicating more limitations in activities of daily living. A difference of 4 is the minimally clinical important difference (MCID) in COPD. The validity of the instrument and corresponding MCID in NTM lung disease is unknown. Patients in the ALIS + Multidrug Regimen arm had numerically higher increase from Baseline to Month 6 in the SGRQ total score compared to Multidrug Regimen Alone. However, the threshold of 4 was not met, and the endpoint was not statistically significant given its position in the hierarchical testing (Figure 8).

Figure 8: Secondary Endpoint Initial Analysis in Study 212 – Change from Baseline to Month 6 in SGRQ Total Score (ITT Population)



A correlation between acquired amikacin resistance during amikacin therapy, treatment failure, and an amikacin MIC of >64 µg/mL has been reported in the literature (Brown-Elliott et al. 2013). In Study 212, 28/336 (8.3%) patients had MAC isolates with amikacin MIC >64 µg/mL during the study (24/224 [10.3%] in the ALIS + Multidrug Regimen arm and 4/112 [2.7%] in the Multidrug Regimen Alone arm). One patient in each arm had a MAC isolate with amikacin MIC >64 µg/mL at Baseline. In the ALIS + Multidrug Regimen arm, one patient converted after having a MAC isolate with amikacin MIC >64 µg/mL, and one patient converted but subsequently developed a MAC isolate with amikacin MIC >64 µg/mL. In the Multidrug Regimen Alone arm, no patients converted.

Of the 23 patients in the ALIS + Multidrug Regimen arm with post-baseline MAC isolates with amikacin MIC >64 µg/mL, 5/23 (21.7%) subsequently had MAC isolates that reverted back to MIC ≤64µg/mL. Of the 3 patients in the Multidrug Regimen Alone arm with post-baseline MAC isolates with amikacin MIC >64µg/mL, 2/3 (66.7%) subsequently had MAC isolates that reverted back to MIC ≤64µg/mL.

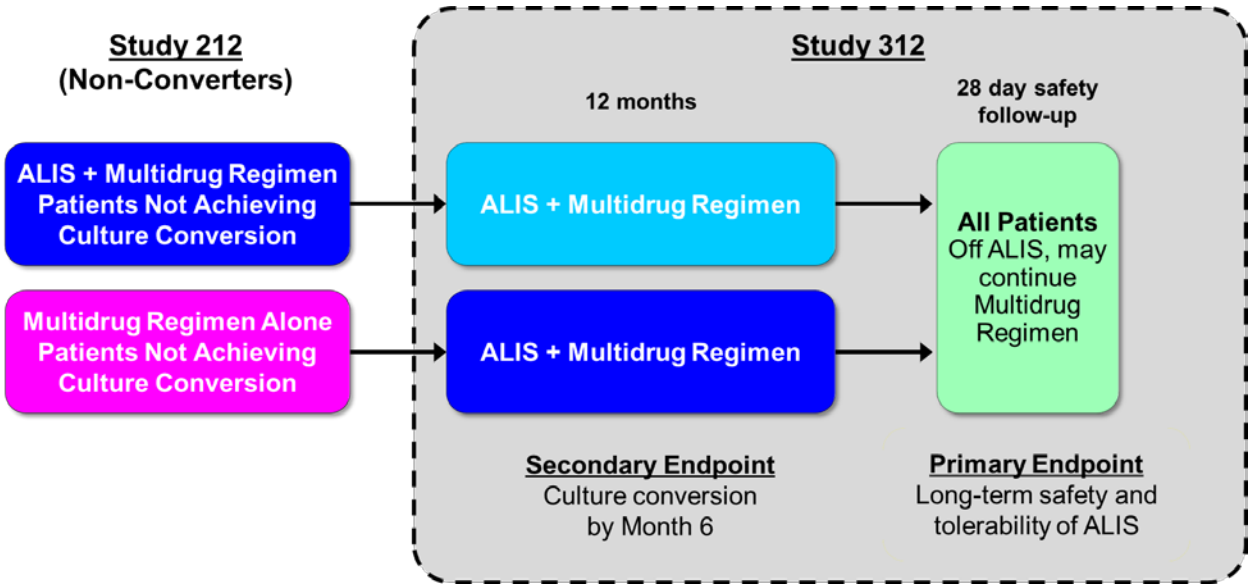
1.3.2 Study 312

Study 312 is an ongoing open-label safety study enrolling patients from Study 212 who failed to achieve culture conversion or experienced a relapse or recurrence, regardless of prior treatment arm (Figure 9). All patients in Study 312 receive ALIS plus their Multidrug Regimen during the 12-month treatment period.

The primary goal of Study 312 is to assess the long-term safety and tolerability of ALIS + Multidrug Regimen for up to 12 months. Additionally, culture conversion by Month 6 will be evaluated, allowing for the assessment of the value of adding or continuing ALIS therapy in patients in the prior ALIS + Multidrug Regimen group from Study 212 and the impact of initiating ALIS treatment for the first time in patients in the prior Multidrug Regimen Alone

group. Although this is a single arm study, the objective nature of the culture conversion endpoint allows for a degree of replication of the culture conversion findings of Study 212, particularly among the prior Multidrug Regimen Alone treatment group.

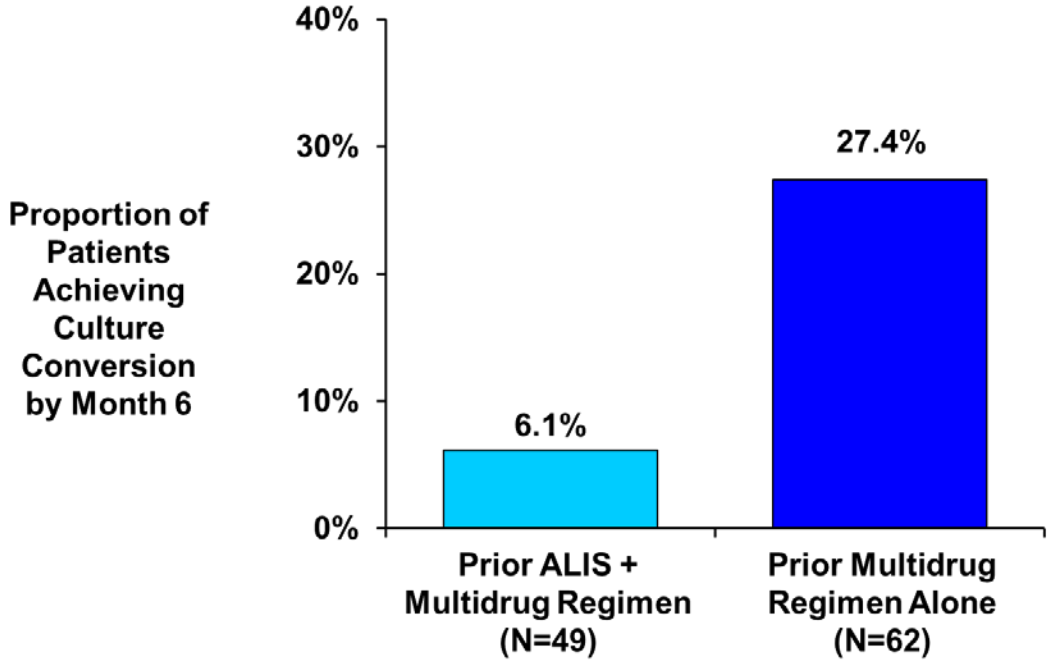
Figure 9: Design of Study 312



Baseline demographics and characteristics in Study 312 were consistent with the original patient population in Study 212. Overall, 74 patients from the prior Multidrug Regimen Alone arm and 59 patients from the prior ALIS + Multidrug Regimen arm enrolled from Study 212. At the time of data cutoff for this ongoing study, a total of 111 patients (62 in the prior Multidrug Regimen Alone arm, and 49 in the prior ALIS + Multidrug Regimen arm) had 3 monthly sputum samples obtained in Study 312 and are therefore classified as “assessable” for culture conversion at this time.

The results from the analysis of culture conversion by Month 6 in patients with available data at the time of the data cutoff showed benefits for patients in both the prior Multidrug Regimen Alone arm and the prior ALIS + Multidrug Regimen arm (Figure 10). Similar to the findings in Study 212, 27.4% of patients in the prior Multidrug Regimen Alone group achieved culture conversion by Month 6. Additionally, 6.1% of patients in the prior ALIS + Multidrug Regimen group achieved culture conversion, suggesting that patients can still achieve culture conversion after Month 6.

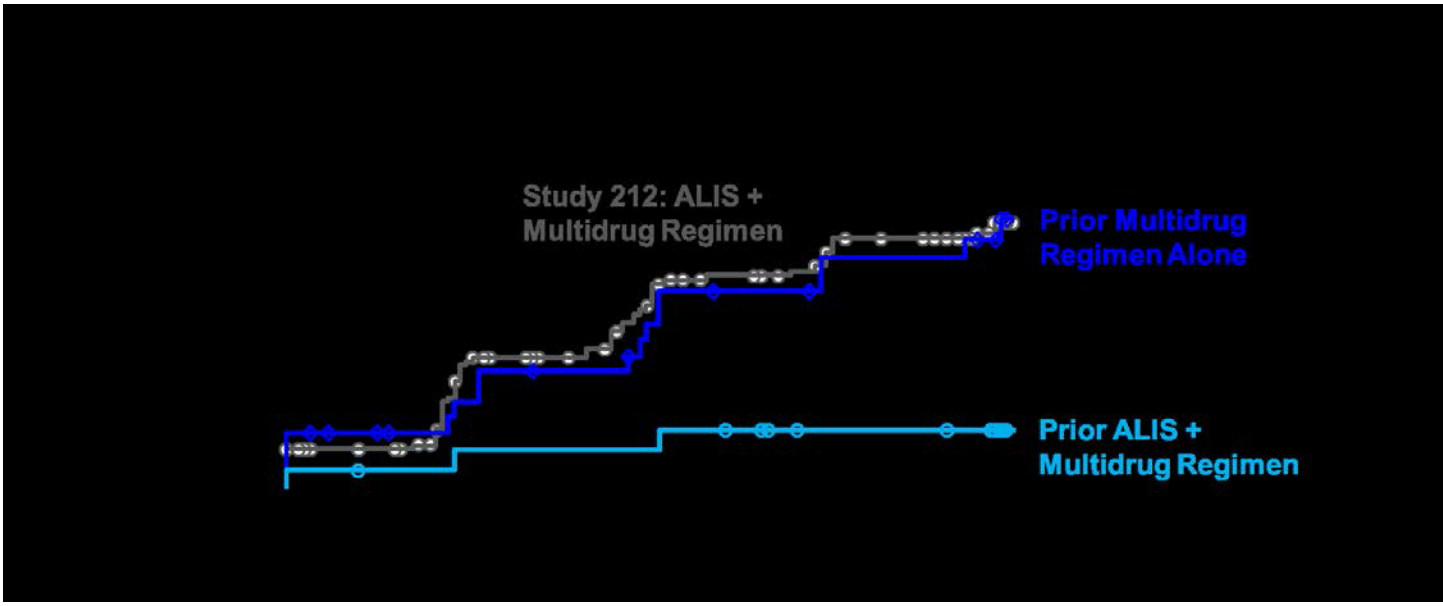
Figure 10: Secondary Endpoint in Study 312 – Proportion of Patients Achieving Culture Conversion by Month 6 (Safety Population)



Note: analysis includes patients with available data at the time of data cutoff

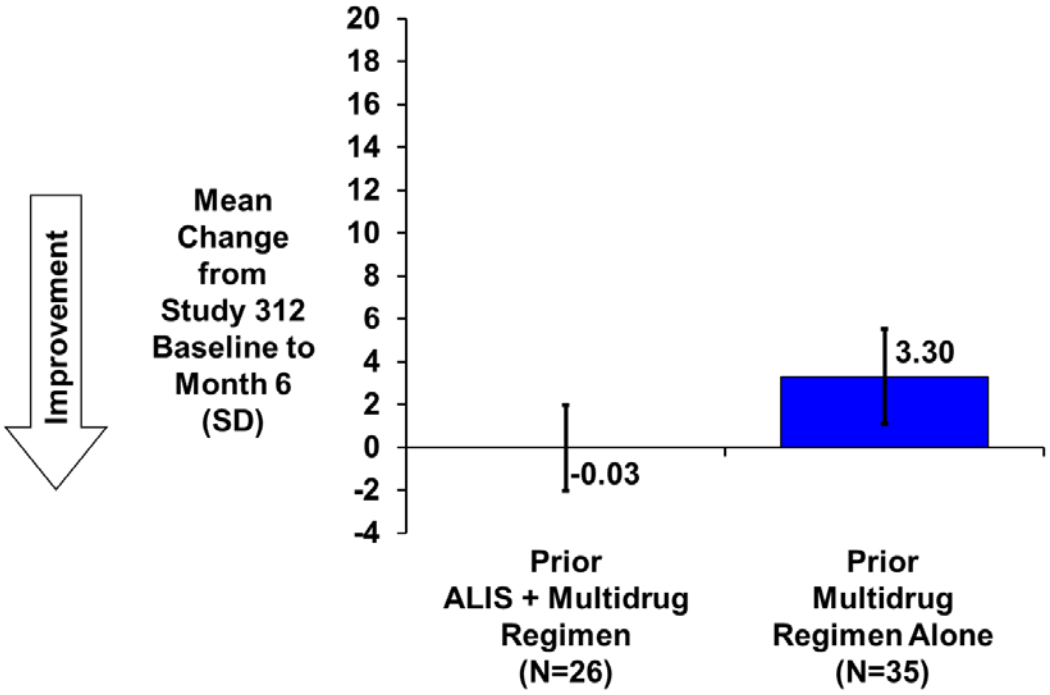
As shown in Figure 11, the mean time to culture conversion in patients in the prior Multidrug Regimen Alone group (2.9 months) was similar to that in the ALIS + Multidrug Regimen arm in Study 212 (2.7 months). Both of these arms represent patients who failed prior treatment and are treated with ALIS for the first time. Patients in the prior ALIS + Multidrug Regimen arm had a mean time to culture conversion of 3.6 months, demonstrating that some of these patients continue to benefit from continued ALIS treatment.

Figure 11: Secondary Endpoint in Study 312 – Time to Culture Conversion (Safety Population)



Finally, the mean change from Baseline at Month 6 in the SGRQ total score (ie, 0-100 points) was 1.88 (3.30 for the prior Multidrug Regimen Alone arm and -0.03 for the prior ALIS + Multidrug Regimen arm), representing a small, non-clinically meaningful change in HRQOL. Importantly, patients in the prior ALIS + Multidrug Regimen arm (with approximately 8 months of prior treatment in Study 212) showed no appreciable change from the Study 312 Baseline at Month 6 in the SGRQ total score (-0.03), suggesting stabilization in the SGRQ total score beyond 6 months of ALIS treatment (Figure 12).

Figure 12: Exploratory Endpoint in Study 312 – Change from Baseline to Month 6 in SGRQ Total Score (Safety Population)



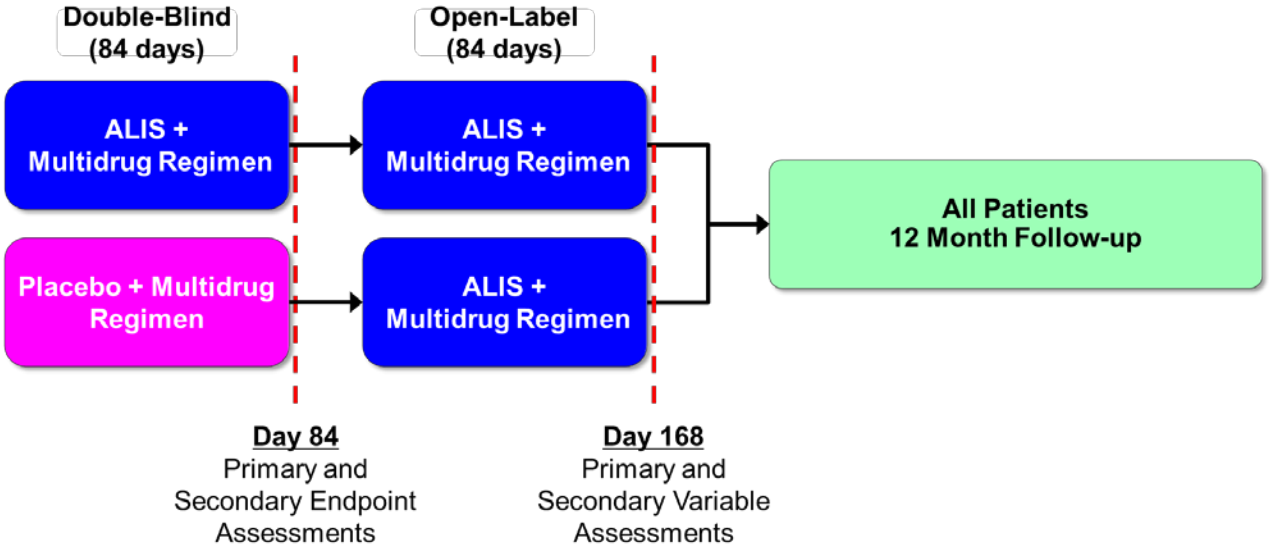
1.3.3 Study 112

Study 112 was a Phase 2 randomized, double-blind, placebo-controlled study of ALIS in patients with treatment refractory NTM lung disease (Figure 13). Study 112 was a proof-of-concept study, providing the earliest evidence of the efficacy of ALIS in NTM lung disease as well as evidence that negative sputum cultures predict for durable culture conversion. The primary objective of Study 112 was to evaluate the safety, efficacy, and tolerability of 84 days of ALIS versus placebo (empty liposomes) added to Multidrug Regimen. Patients were stratified based on the presence or absence of cystic fibrosis (CF) and predominant mycobacterial species in sputum culture at screening (MAC or *M. abscessus*). After 84 days of treatment, all patients entered into an open-label phase, where they received ALIS + Multidrug Regimen for an additional 84 days. Patients were then followed for 12 months off ALIS.

The primary efficacy endpoint in Study 112 was the change from baseline at Day 84 in mycobacterial density as assessed by the semi-quantitative scale (SQS). The SQS is expressed on a 7-step scale, and the maximum improvement for an individual patient was -6 steps and the maximum deterioration was +6 steps (see Table 15 for details). Mycobacterial density, as assessed by the SQS, is a novel endpoint that has not been used in clinical studies for NTM. However, this endpoint was selected because the achievement of negative sputum cultures had not been previously evaluated and was not expected to be common after 84 days of treatment in patients who failed to convert with prior treatment. The proportion of patients with negative sputum cultures, time to negative culture, and change in the 6MWT distance were also evaluated at Day 84. Given the short duration of the double-blind phase, culture conversion, as defined in

Study 212, was not a prespecified endpoint but was analyzed by Day 168 in a post hoc assessment to guide design of the pivotal study.

Figure 13: Design of Study 112



Baseline demographics and characteristics were comparable between treatment arms (Table 3). Overall, the mean age was approximately 58 years, and the majority of patients were female (87.6%) and White (92.1%). While all patients in Study 212 had predominantly MAC lung disease and did not have CF, 64.0% of patients in Study 112 had predominately MAC lung disease and 80.9% of patients did not have underlying CF.

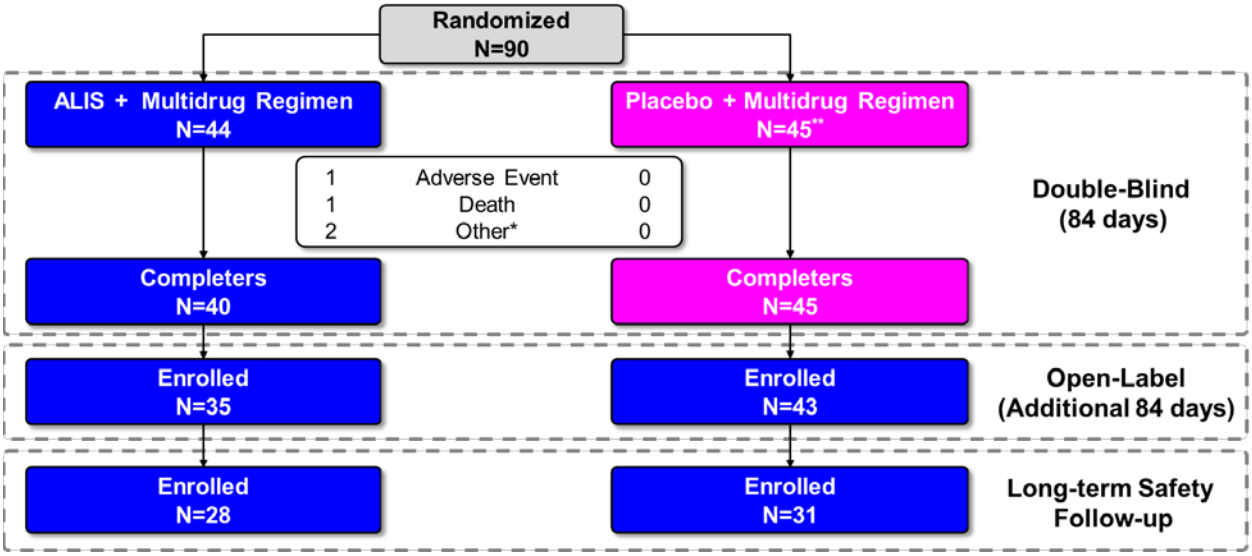
Table 3: Baseline Demographics and Characteristics in Study 112 (mITT Population)

	ALIS + Multidrug Regimen (N=44)	**Placebo + Multidrug Regimen (N=45)**
Mean Age, years (SD)	58.0 (16.61)	59.1 (15.20)
White	42 (95.5%)	40 (88.9%)
Female	38 (86.4%)	40 (88.9%)
Predominant mycobacterial species at screening		
MAC	29 (65.9%)	28 (62.2%)
*M. abscessus**	15 (34.1%)	17 (37.8%)
Comorbid Cystic Fibrosis	8 (18.2%)	9 (20.0%)

*Patients could have NTM lung disease caused by MAC and/or *M. abscessus* in Study 112.

A total of 90 patients were enrolled into the double-blind phase and 89 patients were treated: 44 patients in the ALIS + Multidrug Regimen arm and 45 patients in the placebo + Multidrug Regimen arm (Figure 14). Four patients discontinued the double-blind period, all from the ALIS + Multidrug Regimen arm. A total of 78 patients who completed the double-blind phase were enrolled into the open-label period of an additional 84 days, and 59 enrolled into the long-term 12-month safety follow-up.

Figure 14: Patient Disposition in Study 112 (mITT Population)



*Other includes: withdrawal of consent and lost to follow-up.
**1 patient met exclusion criterion after randomization and did not receive study drug.

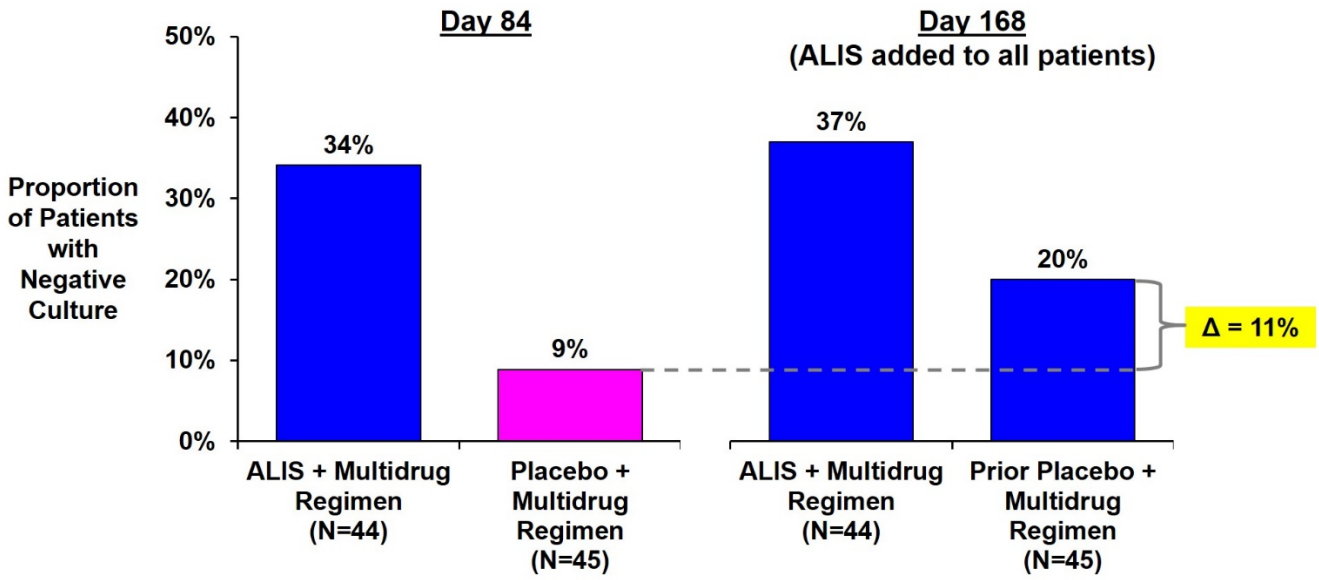
The primary efficacy endpoint of change from Baseline in SQS for mycobacterial showed a trend in favor of the ALIS + Multidrug Regimen arm, but the difference was not statistically significant (P=0.072). However, a numerically greater proportion of ALIS-treated patients had an improvement (decrease) of 1 step or more in bacterial density on the SQS compared with placebo patients (39% vs 24%, respectively), and a numerically smaller proportion of ALIS patients showed a worsening on the SQS compared to placebo (12% vs 24%, respectively).

On the key secondary endpoint, proportion of patients with a negative sputum culture at the end of the double-blind phase (Day 84), a numerically greater proportion of patients in the ALIS + Multidrug Regimen arm achieved a negative NTM culture (34.1%) compared to patients in the placebo + Multidrug Regimen arm (8.9%), with a substantial treatment difference of 25.2% in favor of ALIS (nominal P=0.003).

An exploratory endpoint was analyzed for the proportion of patients with negative sputum culture results at Day 168 (Figure 15). Among patients who received ALIS + Multidrug Regimen during the randomized portion of the study and continued to receive ALIS in the open-label phase, the percentage of patients with a negative sputum culture increased from 34% at Day 84 to 37% at Day 168.

Among patients who received Placebo + Multidrug Regimen during the randomized portion of the study and ALIS + Multidrug Regimen during the open-label phase, the percentage of patients with a negative sputum culture increased from 9% at Day 84 to 20% at Day 168. These results following 84 days of ALIS therapy are similar to those shown during the first 84 days for the ALIS + Multidrug Regimen arm.

Figure 15: Exploratory Endpoint in Study 112 – Proportion of Patients with Negative Sputum Culture at Day 168 (mITT Population)



Additionally, a post hoc analysis was conducted to assess culture conversion as defined in Study 212 (3 consecutive negative sputum cultures) by Day 168. In this analysis, 20 out of 89 patients (22.5%) achieved culture conversion, and 3 additional patients subsequently achieved culture conversion during the 28-day off-treatment period. Of the 23 total converters, 17 completed the 12-month follow-up visit, and 14 (82.4%) demonstrated durability of their negative culture result following 12 months.

An exploratory analysis of 6MWT findings showed a potential improvement in patients treated with ALIS compared to placebo (nominal P=0.0134). Patients in the ALIS + Multidrug Regimen arm had a mean increase from baseline of 23.9 meters compared to a decrease of 25.0 meters in the placebo arm. This finding was not statistically significant due to the failure to achieve statistical significance on the primary endpoint but led to the inclusion of the 6MWT as a secondary endpoint in Study 212.

1.3.4 Efficacy Summary

Overall, results from Studies 212, 312, and 112 demonstrate a consistent benefit of ALIS in combination with Multidrug Regimen for the treatment of patients with NTM lung disease caused by MAC. In the pivotal study, Study 212, a significantly greater percentage of patients treated with ALIS + Multidrug Regimen achieved culture conversion by Month 6 than patients receiving Multidrug Regimen Alone. These findings on culture conversion by Month 6 are reasonably likely to predict the clinical benefit of durable culture negativity, and thus ALIS has been shown to have a meaningful advantage over available therapy.

Results from the pivotal study are supported by the initial analysis from Study 312 showing that patients can still achieve culture conversion after 6 months of treatment with ALIS + Multidrug Regimen. The culture conversion rates by Month 6 in Study 312 for patients in the prior

Multidrug Regimen Alone arm were similar to the culture conversion rates by Month 6 from Study 212, demonstrating consistency of these results.

Study 112 post hoc culture conversion analyses offer similar results to Study 212, providing independent substantiation and consistency. Durability data from Study 112 show that durable culture conversion can be achieved with treatment with ALIS after 12 months, providing evidence that culture conversion by Month 6 is reasonably likely to predict clinical benefit and formed the rationale for using culture conversion by Month 6 as a surrogate endpoint supporting the Subpart H submission.

Finally, the 6MWT data demonstrate that culture conversion is associated with clinical benefit.

1.4 Safety

Overall, the safety data suggest that ALIS has an acceptable safety profile.

The most relevant safety information comes from the pivotal randomized controlled study, Study 212, comparing 223 patients treated with ALIS + Multidrug Regimen to 112 patients treated with Multidrug Regimen Alone. The NTM Pooled Population included all patients from Studies 212, 312, and 112 and includes a total of 388 patients treated with ALIS + Multidrug Regimen. Safety data from Study 212 are based on the NDA data cutoff and include 126 patients treated with ALIS + Multidrug Regimen for at least 6 months and 25 patients treated for at least 1 year. The mean duration of exposure to ALIS from Study 212 was 214 days, representing 105 total patient years of exposure. In the NTM Pooled Population (N=388), mean exposure to ALIS was 199 days with 164 total patient years of exposure.

Overall in the randomized Study 212, a greater proportion of ALIS + Multidrug Regimen treated patients compared to patients receiving Multidrug Regimen Alone experienced at least 1 AE (Table 4). The majority of AEs across both groups were mild to moderate (Grade 1 and Grade 2); Grade 3 or higher AEs were higher in ALIS + Multidrug Regimen patients than patients receiving Multidrug Regimen Alone. Importantly, serious adverse events (SAEs) and AEs leading to death were similar between the treatment arms.

Table 4: Summary of Adverse Events in Study 212

	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
Any AE	219 (98.2%)	102 (91.1%)
AE Grade ≥3	46 (20.6%)	14 (12.5%)
SAEs	45 (20.2%)	20 (17.9%)
AE Leading to Death	6 (2.7%)	5 (4.5%)
AE Leading to ALIS Discontinuation	39 (17.5%)	N/A

The most commonly reported AEs were respiratory (ie, dysphonia, cough, dyspnea, hemoptysis, and oropharyngeal pain), fatigue, or gastrointestinal (ie, diarrhea and nausea). These events were more frequently reported in ALIS + Multidrug Regimen treated patients compared to Multidrug Regimen Alone patients (Table 5). Given that ALIS is an inhalation antibiotic therapy, the respiratory effects might be expected.

Table 5: Common (≥5% in the ALIS + Multidrug Regimen Arm) Adverse Events in Study 212

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
Patients Reporting at Least 1 AE	219 (98.2%)	1504	102 (91.1%)	382
Dysphonia	102 (45.7%)	138	1 (0.9%)	1
Cough	83 (37.2%)	109	17 (15.2%)	20
Dyspnea	48 (21.5%)	62	10 (8.9%)	10
Haemoptysis	39 (17.5%)	54	15 (13.4%)	22
Fatigue	36 (16.1%)	38	8 (7.1%)	8
Diarrhoea	28 (12.6%)	32	5 (4.5%)	5
Nausea	25 (11.2%)	30	4 (3.6%)	4
Oropharyngeal pain	24 (10.8%)	30	2 (1.8%)	2
Headache	22 (9.9%)	25	5 (4.5%)	6
Chronic obstructive pulmonary disease	18 (8.1%)	35	3 (2.7%)	4
Infective exacerbation of bronchiectasis	17 (7.6%)	22	8 (7.1%)	9
Tinnitus	17 (7.6%)	20	1 (0.9%)	1
Pyrexia	16 (7.2%)	21	5 (4.5%)	6
Decreased appetite	15 (6.7%)	17	8 (7.1%)	8
Weight decreased	15 (6.7%)	16	2 (1.8%)	2
Wheezing	15 (6.7%)	16	3 (2.7%)	4
Arthralgia	14 (6.3%)	15	3 (2.7%)	3
Back pain	14 (6.3%)	16	4 (3.6%)	4
Dizziness	14 (6.3%)	15	3 (2.7%)	3
Vomiting	14 (6.3%)	17	3 (2.7%)	4
Chest discomfort	13 (5.8%)	13	3 (2.7%)	4

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
Nasopharyngitis	12 (5.4%)	15	8 (7.1%)	10

Subjects randomized to the MDR Alone arm did not receive a matching comparator, therefore, AEs leading to ALIS discontinuation could only have occurred in the ALIS-treated patients (Table 6). Adverse events leading to ALIS discontinuation were reported in 17.5% of patients in the ALIS + Multidrug Regimen arm. Most AEs leading to discontinuation were respiratory in nature, were non-serious, and resolved once treatment was discontinued.

Table 6: Adverse Events (≥0.5% in Patients in the ALIS + Multidrug Regimen Arm) Leading to ALIS Discontinuation in Study 212 (Safety Population)

Preferred Term	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
≥1 AE Leading to ALIS Discontinuation	39 (17.5%)	N/A
Dyspnoea	7 (3.1%)	N/A
Dysphonia	5 (2.2%)	N/A
Hypoacusis	2 (0.9%)	N/A
Infective exacerbation of bronchiectasis	2 (0.9%)	N/A
Alveolitis allergic	2 (0.9%)	N/A
Chronic obstructive pulmonary disease	2 (0.9%)	N/A
Cough	2 (0.9%)	N/A
Haemoptysis	2 (0.9%)	N/A

As shown in Table 7, AEs leading to discontinuation of Multidrug Regimen were infrequent and similar between treatment arms.

Table 7: Adverse Events (≥0.5% in Patients in either Arm) Leading to Multidrug Regimen Discontinuation in Randomized Study 212

Preferred Term	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
≥1 AE Leading to Multidrug Regimen Discontinuation	9 (4.0%)	3 (2.7%)
Diarrhoea	2 (0.9%)	0
Nausea	2 (0.9%)	0
Lung infiltration	0	1 (0.9%)
Respiratory Failure	0	1 (0.9%)
Fall	0	1 (0.9%)

Serious AEs were reported in a similar proportion of patients in each treatment arm of Study 212. The most common SAEs were respiratory in nature and were reported infrequently (Table 8).

Table 8: Common Serious Adverse Events (≥1% in Patients in the ALIS + Multidrug Regimen Arm) in Study 212 (Safety Population)

Preferred Term	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
Patients with ≥1 SAE	45 (20.2%)	20 (17.9%)
Pneumonia	8 (3.6%)	2 (1.8%)
Chronic obstructive pulmonary disease	7 (3.1%)	1 (0.9%)
Infective exacerbation of bronchiectasis	5 (2.2%)	3 (2.7%)
Haemoptysis	6 (2.7%)	5 (4.5%)
Dyspnoea	3 (1.3%)	0
Pneumothorax	3 (1.3%)	1 (0.9%)

A total of 14 patients experienced SAEs resulting in death. In the NTM Pooled Population (Table 9), one death occurred in a patient receiving ALIS in Study 112, 6 deaths occurred in Study 212 (6 patients receiving ALIS), and 2 deaths occurred in Study 312 (both patients receiving ALIS), ie, total of 9 deaths with ALIS.

Focusing in on the pivotal Study INS-212, a total of 11 patients reported 11 AEs leading to death: 6 (2.7%) patients with 6 reported events in the ALIS + Multidrug Regimen arm and 5 (4.5%) patients with 5 reported events in the Multidrug Regimen Alone arm. Most of the serious AEs resulting in death were respiratory in nature.

All but 1 SAE resulting in death were considered not related to ALIS as determined by the study Investigator, 1 SAE (lung infection) was considered possibly related. Overall, these events are not unexpected in patients suffering from NTM lung infections caused by MAC.

Table 9: Adverse Events Leading to Death in Study 212 and the NTM Pooled Population

	Study 212		NTM Pooled
	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)	ALIS + Multidrug Regimen (N=388)
Any AE leading to death	**6 (2.7%)**	**5 (4.5%)**	**9 (2.3%)**
Respiratory failure	2 (0.9%)	1 (0.9%)	2 (0.5%)
Chronic obstructive pulmonary disease	1 (0.4%)	0	2 (0.5%)
Pneumonia	0	1 (0.9%)	1 (0.3%)
Acute respiratory distress syndrome	0	0	1 (0.3%)
Lower respiratory tract infection	0	0	1 (0.3%)
Lung infection	1 (0.4%)	0	1 (0.3%)
Pulmonary embolism	1 (0.4%)	0	1 (0.3%)
Cachexia	1 (0.4%)	0	1 (0.3%)
Mycobacterium avium complex infection	0	1 (0.9%)	0
Cardiogenic shock	0	1 (0.9%)	0
Interstitial lung disease	0	1 (0.9%)	0

1.4.1 Adverse Events of Special Interest

Since ALIS is an inhaled amikacin product, Insmed investigated 2 categories of AESIs. These events included either respiratory AEs or established toxicities from systemic aminoglycoside use. The NTM Pooled Population, which includes all patients from Studies 212, 312, and 112, was used to assess AESIs.

Four high-level categories of respiratory AEs, including bronchospasm, hemoptysis, COPD exacerbation, and alveolitis allergic, were analyzed for Study 212 and the NTM Pooled Population, to further characterize potential risks from inhaled ALIS treatment. Several preferred terms were included in each main term. Overall rates for these events in Study 212 were higher in ALIS + Multidrug Regimen treated patients compared to Multidrug Regimen Alone. Most events resolved, and few were reported as SAEs. As shown in Table 10, these events were consistent in ALIS-treated patients in Study 212 and the overall NTM Pooled population.

In the patients receiving ALIS in Study 212, 29% experienced bronchospasm, which was primarily due to a greater number of events of dyspnea in the bronchospasm category in patients receiving ALIS + Multidrug Regimen compared to Multidrug Regimen Alone (22% vs 9%, respectively).

The number of patients reporting haemoptysis was 39 (17.5%) in the ALIS + MDR arm and 15 (13.4%) in the MDR alone arm (Table 10). Of the 54 events of haemoptysis which occurred in the ALIS + MDR arm, 31 patients (13.9%) reported mild and 6 (2.7%) moderate events. Of the 22 events in the MDR alone arm, 9 patients (8.0%) reported mild and 5 (4.5%) moderate events. They occurred later in the treatment (>90 days). Nine patients in the ALIS + MDR arm required drug interruption and all resolved. The majority of hemoptysis events lasted <30 days.

Table 10: Respiratory Adverse Events of Special Interest in Study 212 and the NTM Pooled Population

	Study 212		NTM Pooled (Studies 112, 212, 312)
Respiratory AESIs Category	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)	ALIS + Multidrug Regimen (N=388)
Bronchospasm	65 (29.1%)	13 (11.6%)	96 (24.7)
Dyspnoea	48 (21.5%)	10 (8.9%)	67 (17.3%)
Haemoptysis	39 (17.5%)	15 (13.4%)	66 (17.0%)
COPD Exacerbation	18 (8.1%)	4 (3.6%)	25 (6.4%)
Alveolitis Allergic	7 (3.1%)	1 (0.9%)	12 (3.1%)

Adverse events related to the well-known systemic toxicity of aminoglycoside antibiotics, such as nephrotoxicity and neuromuscular AEs, were infrequent and balanced between the treatment groups (Table 11). These findings support the expectation of lessened systemic exposure of amikacin by administering ALIS directly into the lung, compared with parenteral administration.

The AESI of ototoxicity included a number of preferred terms under the system organ classes (SOCs) of Ear and Labyrinth Disorders and Nervous System Disorders. There was an imbalance in the AESI of ototoxicity that was driven by reports of tinnitus in patients treated with ALIS, with only 1 event of tinnitus reported in patients receiving Multidrug Regimen Alone. Seventeen (7.6%) patients experienced a total of 20 events of tinnitus in the ALIS + Multidrug Regimen arm compared to 1 (0.9%) patient in the Multidrug Regimen Alone arm. Events of tinnitus were non-serious and mostly mild in severity (17/20 mild and 3/20 moderate severity). Study drug (ALIS) was not interrupted in 13 of 20 events, interrupted for 6 of 20 events, and 1 was not withdrawn for any event. Of the 20 events, 10 resolved at the time of this report, 1 event was recovering, 1 event recovered with sequelae, 6 events were ongoing, and 2 events had unknown outcomes due to patient withdrawal of consent and patient death.

Table 11: Amikacin-Related Adverse Events of Special Interest

	Study 212		NTM Pooled (Studies 112, 212, 312)
AESI Category	**ALIS + Multidrug Regimen (N=223)**	**Multidrug Regimen Alone (N=112)**	**ALIS + Multidrug Regimen (N=388)**
Nephrotoxicity	3 (1.3%)	3 (2.7%)	16 (4.1%)
Neuromuscular AEs	5 (2.2%)	0	12 (3.1%)
Ototoxicity	38 (17.0%)	11 (9.8%)	59 (15.2%)
Tinnitus	17 (7.6%)	1 (0.9%)	26 (6.7%)
Dizziness	14 (6.3%)	3 (2.7%)	22 (5.7%)

Overall, the addition of ALIS to Multidrug Regimen increased the incidence of AEs but does not appear to add a significant burden to patients, as SAEs were similar between treatment groups. Respiratory events were the most commonly reported AEs, as is expected with an inhaled antibiotic. The majority of all AEs were respiratory in nature, mild to moderate, and most resolved without discontinuation. Finally, systemic toxicities of amikacin-related AEs, with the exception of tinnitus, were infrequent and balanced between arms.

1.5 Conclusions

Overall, ALIS fulfills the regulatory requirements for Accelerated Approval by demonstrating efficacy when added to Multidrug Regimen for the treatment of NTM lung disease caused by MAC. In the pivotal study, Study 212, ALIS + Multidrug Regimen significantly increased the proportion of patients achieving culture conversion by Month 6. The difference between the 29.0% conversion rate in the ALIS + Multidrug Regimen arm and the 8.9% conversion rate in the Multidrug Regimen Alone arm represents an absolute treatment effect size of 20.1%. These efficacy findings are further supported by the results of Studies 312 and 112 that demonstrate a consistency in culture conversion rates by Month 6 or Day 168, respectively, with the addition of ALIS to Multidrug Regimen in patients with NTM lung disease.

The microbiological outcome of culture conversion by Month 6 is considered to be a surrogate endpoint that is reasonably likely to predict the clinical benefit of durable sputum culture negativity at 3 months after treatment cessation. The confirmatory study to support full approval of ALIS is fully enrolled and will assess for durable culture conversion once patients have been off all MAC treatments for 3 months.

In addition to culture conversion by Month 6, ALIS + Multidrug Regimen showed superiority to Multidrug Regimen Alone on several additional endpoints. The mean time to culture conversion occurred approximately 1 month earlier in patients treated with ALIS + Multidrug Regimen, which shortens the duration of overall treatment.

Although the 6MWT did not demonstrate a significant difference between treatment arms in Study 212, a pre-specified exploratory analysis based on conversion status demonstrated a meaningful difference in favor of converters, overall and within each treatment arm. The consistency of benefit observed in converters versus non-converters demonstrated a clear association between culture conversion by Month 6 and improvement in the 6MWT. This result emphasizes the importance of sustained culture conversion and the clinical benefit provided by effective treatments for NTM lung disease caused by MAC.

Based on a thorough and systematic analysis, the safety profile of ALIS has been well characterized and is acceptable relative to Multidrug Regimen in light of the high unmet need and established efficacy. The most common AEs were respiratory in nature, and the respiratory-related AEs were mostly mild to moderate in severity and transient. Adverse events typically occurred soon after initiation of treatment with ALIS and typically decreased in frequency after Month 1, despite continued use. The rate of each of the most common AEs leading to discontinuation of ALIS was relatively low.

The aggregate data support the conclusion that respiratory-related events, mild to moderate in severity, continue to be the most important risks. Other important risks described in this document are ototoxicity which demonstrated a small imbalance. This was driven by higher tinnitus rates in patients treated with ALIS + Multidrug Regimen compared to Multidrug Regimen Alone. There was no imbalance in events indicative of renal toxicity.

Under Subpart H, these findings support a favorable benefit-risk profile for the use of ALIS in the treatment of NTM lung disease caused by MAC as part of a combination antibiotic regimen for adult patients.

2 BACKGROUND ON NONTUBERCULULOUS MYCOBACTERIAL LUNG INFECTIONS

Summary

- NTM lung disease caused by MAC is a life-threatening condition with progressive life-limiting morbidity and increased mortality
- Patients with NTM are heterogeneous, with diverse risk factors, comorbidities, symptomatology, and morbidity
- Patients who do not achieve negative sputum cultures have higher mortality rates
- Currently, in the US, there are no drugs approved specifically for NTM lung infection
- Many patients are unable to attain culture conversion with existing therapy
- For patients not able to achieve culture conversion, current options are lengthy, and treatment with combination antibiotic therapy can be indefinite

2.1 Overview of Nontuberculous Mycobacterial Lung Infections

NTM lung disease caused by MAC is a chronic, debilitating and sometimes life-threatening condition associated with cough, sputum production, shortness of breath, fatigue, and weight loss (Kim et al. 2008). MAC most commonly complicates debilitating comorbid lung diseases such as bronchiectasis and COPD. In patients without a prior diagnosis of underlying lung comorbidities, MAC has been implicated in progressive lung disease (Prince et al. 1989).

2.1.1 Epidemiology

The *Mycobacterium* genus represents more than 180 naturally-occurring *Mycobacterium* species that are ubiquitous in the environment and can be found in multiple environmental niches, most commonly in water and soil (Griffith et al. 2007). *Mycobacterium avium* and *Mycobacterium intracellulare* primarily comprise MAC and are the predominant infective species in NTM pulmonary disease in the US, Japan, European countries, and elsewhere.

Pulmonary disease due to NTM was traditionally reported as primarily upper lobe fibrocavitary disease occurring in male smokers with emphysema (Contreras et al. 1988). More recently, it has become clear that the spectrum of disease is much wider with somewhat unsuspected susceptible populations, specifically older white women without apparent predisposing conditions to pulmonary disease such as cigarette smoking. In this population with MAC pulmonary disease, the radiographic presentation is predominantly nodular and fibronodular densities in the mid and lower lung fields (Prince et al. 1989).

The prevalence of human disease attributable to MAC organisms appears to have increased over the past 2 decades (Khan et al. 2007, Marras et al. 2007, Khan et al. 2008). The annual prevalence of NTM caused by MAC increased from approximately 20 to 47 cases per 100,000 or 8.2% per year in US Medicare beneficiaries (Adjemian et al. 2012). The American Lung Association estimates that there are about 50,000 to 95,000 people living with NTM lung

infections in the US, with a higher frequency in older adult women than men (American Lung Association 2018). The prevalence of NTM now greatly surpasses that of *M. tuberculosis* complex in the US.

At least 80% of definite pulmonary NTM infections in the US are caused by MAC (Prevots et al. 2010).

2.1.2 Morbidity and Mortality in Nontuberculous Mycobacterial Lung Infections

Nontuberculous mycobacterial pulmonary infection is characterized by a range of signs and symptoms that are variable and nonspecific and often worsen over time. These signs and symptoms can include chronic cough, sputum production, and fatigue. Fatigue and loss of energy were reported as the "most common symptoms" by 80% of meeting attendees in an informal poll while 40% reported chronic cough and coughing up blood and phlegm (FDA Patient-Focused Drug Development Workshop 2015). Less commonly, and usually with advanced NTM lung infection, malaise, dyspnea, fever, hemoptysis, and weight loss can also occur. Co-existing lung diseases such as bronchiectasis, COPD, previous mycobacterial diseases, CF, and pneumoconiosis often complicate the evaluation (Wilson et al. 1997).

The following is an excerpt from FDA Patient-Focused Drug Development Workshop in 2015:

On October 15, 2015, FDA held a public meeting on non-tuberculous mycobacterial (NTM) lung infections to gain insight into the experiences of NTM patients, caregivers, and other patient representatives on the most significant symptoms of their disease, its impact on daily life, and on currently available therapies."

Several key themes emerged from this meeting:

- *NTM infections cause chronic and devastating lung disease with debilitating symptoms, including fatigue, chronic cough, and shortness of breath. Participants described the significant detrimental impact of these and other symptoms on daily life. Participants emphasized how these symptoms worsen over time and are exacerbated by common environmental factors that irritate the lungs.*
- *NTM affects all aspects of patients' lives. Participants described the drastic change from their active and independent lives before diagnosis. Many participants noted that the significant decline in health caused them or their loved ones to limit or completely stop participating in activities that they once enjoyed or were able to do, which led to social isolation. Participants also shared that their constant coughing was often misunderstood by others as being contagious, which left them or their loved ones feeling stigmatized and embarrassed.*
- *Participants described using a combination of antibiotic drugs, steroids, and pain medicines in addition to non-drug therapies, all of which had varying degrees of success with managing symptoms. Participants emphasized the downsides of treatment and the life-threatening side effects they experienced. Participants shared that they valued the benefits of non-drug therapies, especially exercise and pulmonary rehabilitation.*

- *Nearly all participants expressed the need for treatment that was effective in slowing down disease progression in addition to stopping lung deterioration. Participants focused on the need for treatments that have minimal life-threatening side effects, decreased the number of exacerbations they experienced, and on drugs with shorter, less complicated dosing schedules.*

MAC lung disease has been reported to carry a 5-year, all-cause mortality risk ranging from 5.4% (Hayashi et al. 2012) to 39.7% (Andrejak et al. 2010). In a review of 13 studies, 10 reported 5-year mortality rates exceeding 25% in patients with MAC lung disease. In retrospective studies from the literature, the failure to achieve negative sputum cultures in patients with MAC lung disease has been associated with higher mortality rates (Griffith et al. 2006, Ito et al. 2012). In one study of patients with definite MAC lung disease, the 5-year mortality rate was 33.3% in untreated patients compared to 22.2% in treated patients (Ito et al. 2012). In the treated group, sputum conversion was 53.7% compared to 0.0% in the untreated group. Although this study reflects an indirect measure of treatment success, these data suggest that treatment followed by sputum conversion decreases mortality risk.

2.2 Current Treatment Options

There are currently no drugs approved in the US specifically for NTM lung infection. Treatment guidelines are largely based on the results of case series and observational studies from single centers with limited number of patients and supported by expert opinion (Griffith et al. 2007). In the past, the treatment of NTM infection, including MAC lung disease, was adopted from treatment approaches used in managing TB therapy with less than optimal clinical outcomes. That scenario changed during the Acquired Immune Deficiency Syndrome (AIDS) epidemic of the 80's and early 90's with the emergence of disseminated MAC disease as the bacterial pathogen most associated with mortality. It was during that era that macrolides were discovered to be effective treatment and prophylaxis agents for disseminated MAC disease. It could be said that the macrolide era for treating NTM infections was born at that time and has persisted since. Unfortunately, there has been no major advance in the treatment of NTM disease, including MAC disease since that time.

The current treatment of MAC lung disease is with a macrolide-based multidrug regimen similar to the regimen used for treating disseminated MAC infection in AIDS patients (Table 12). Multiple studies have confirmed the efficacy of this regimen for treating MAC lung disease. The recommendation for most patients with nodular/bronchiectatic MAC lung disease is a 3-drug antibiotic regimen that includes a macrolide, ethambutol, and a rifamycin (rifampin or rifabutin). The goal of treatment is 12 months sputum culture negativity, which means a minimum of 12 months therapy but more often requires 18+ months treatment duration and typically exceeds 18 months (Johnson and Odell 2014).

Parenteral amikacin or streptomycin are recommended as initial therapy for patients with fibrocavitary disease or severe nodular/bronchiectatic disease. However, the optimal treatment has yet to be established, and parenteral aminoglycoside use is limited by poor penetration into

lung tissue and the potential for ototoxicity, loss of motor function, and impaired kidney function with high or prolonged systemic exposure (Rybak et al. 1999, Kovacevic et al. 2016).

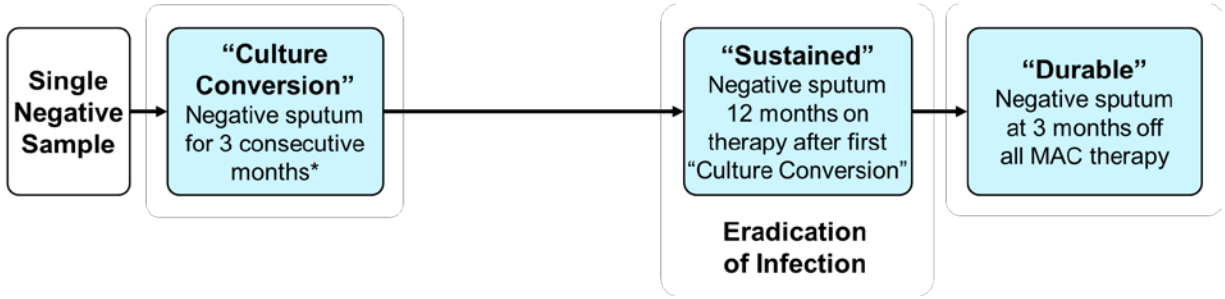
Table 12: Current Treatment Options for NTM Lung Disease

	Initial Therapy		Advanced (severe) or Previously Treated Disease
	Nodular/ Bronchiectatic Disease*	Cavitary Disease	
Macrolide	Clarithromycin 1000 mg TIW or azithromycin 500-600 mg TIW	Clarithromycin 500†-1000 mg/d or azithromycin 250-300 mg/d	Clarithromycin 500†-1000 mg/d or azithromycin 250-300 mg/d
Ethambutol	25 mg/kg TIW	15 mg/kg/d	15 mg/kg/d
Rifamycin	Rifampin 600 mg TIW	Rifampin 450†-600 mg/d	Rifabutin 150†-300 mg/d or rifampin 450†-600 mg/d
IV aminoglycoside	None	Streptomycin or amikacin or none	Streptomycin or amikacin

* Not recommended for severe or previously treated disease.
† Lower dose for weight <50 kg.
Griffith et al. (2007)

The microbiological goal of treatment is 12 months of negative sputum cultures while on treatment (Griffith et al. 2007). Negative sputum samples predict for persistence of negative samples, which predict for durable negative samples and sustained culture conversion. Culture conversion, defined as negative sputum samples for 3 consecutive months, is expected to predict for negative sputum samples following an additional 12 months of treatment (Figure 16). This enduring efficacy allows patients to stop all NTM therapy and predicts for durable culture conversion, which is confirmed once patients are off all therapy for 3 months. Thus, culture conversion is expected to predict for durable culture conversion once NTM therapy is completed.

Figure 16: Timeline for Sustained Efficacy



*Multiple negative sputum samples on 3 consecutive months

Culture conversion has been reported to occur in the majority of patients with nodular bronchiectatic disease who complete a full course of guideline-based treatment (Wallace et al. 2014). While most patients retain negative cultures once off therapy, recurrence can occur as

either reinfection (new species or subspecies) or relapse (original infection), highlighting the difficulty in maintaining negative cultures and the need for effective first-line treatment.

In patients who fail treatment and/or have more severe underlying conditions such as fibrocavitary disease, culture conversion is more difficult to achieve, even with extended treatment. As previously discussed, alternative therapeutic options are limited (Lam et al. 2006, Griffith and Aksamit 2012, Kwak et al. 2017).

For patients not able to achieve culture conversion, current options are lengthy, and treatment with combination antibiotic therapy can be indefinite.

2.3 Patient Medical Need

There are currently no approved pharmacological treatments for NTM lung disease. There is a clear unmet medical need for evidence-based, effective, and less toxic therapeutic options for the treatment of NTM lung disease caused by MAC.

3 PRODUCT DESCRIPTION

Summary

- ALIS is an inhaled liposomal formulation of amikacin, an antibiotic that is known to have potent activity against MAC
- The liposome formulation penetrates biofilms
- ALIS accumulates within macrophages to a greater degree than inhaled free amikacin
- Nebulized delivery of ALIS allows for the achievement of high local concentrations of amikacin in the lung while minimizing systemic exposure
- The results of an *in vitro* experiment demonstrated that residual amikacin in sputum samples had no impact on MAC culture results. Negative sputum culture results for MAC isolates during treatment with ALIS reliably reflect the true microbiological status of the patient

3.1 ALIS Description

ALIS is a unique liposomal formulation of amikacin intended for administration by oral inhalation using an eFlow Nebulizer System provided as the Lamira nebulizer handset along with the eFlow control unit. The Lamira Nebulizer System is a high efficiency electronic nebulizer that uses a vibrating perforated membrane to generate inhalable aerosol. The formulation and route of administration of ALIS allow for the achievement of high local concentrations of amikacin in the lungs, while minimizing systemic exposure.

Amikacin is an aminoglycoside antibiotic with a broad-spectrum of activity against numerous clinically important Gram-positive and Gram-negative bacterial species. Multiple studies from the literature provide reliable reference data on the activity of amikacin against MAC as well as the activity of amikacin against other NTM species (Brown-Elliot et al. 2013, Van Ingen et al. 2014, Schön et al. 2017, Eglund et al. 2015).

3.2 Lamira Nebulizer System

The Lamira Nebulizer System for ALIS is based on PARI's eFlow technology electronic nebulizer, which has been marketed for over a decade in the US for use with various products administered by oral inhalation. The PARI eFlow device has been previously cleared via 510(k).

PARI and Insmed collaborated to develop the product-specific nebulizer. Compared to the original eFlow device, the Lamira Nebulizer System features an increased medication reservoir fill volume and the laser-drilled holes in the aerosol head membranes have a slightly different geometry compared to the originator device. These changes optimize the delivery of the ALIS liposome suspension product and have minimally altered the physical appearance of the device compared to the other longtime PARI eFlow devices.

Lamira Nebulizer System

Lamira Control Unit and Handset

3.3 Mechanism of Action

Amikacin, the active pharmaceutical ingredient in ALIS, is a well-known antimicrobial drug substance that has been used in medical practice for decades. Amikacin is a 4,6-disubstituted 2-deoxystreptamine aminoglycoside of the same subclass as gentamicin, kanamycin, and tobramycin. For all aminoglycosides, the primary mechanism of action is inhibition of protein synthesis with a bactericidal mode of action. The mechanism of action of amikacin relies on the disruption and inhibition of protein synthesis in target bacteria by binding to the ribosome at the A site of the 16S rRNA.

ALIS liposomes are composed of 2 lipids, di-saturated phospholipid, dipalmitoylphosphatidylcholine (DPPC) and cholesterol, which are the major constituents of the endogenous pulmonary surfactant. The liposomes have a neutral charge and shield the cationic amikacin, allowing for the penetration of biofilms. *In vivo* studies established that ALIS accumulates within macrophages to a greater degree than inhaled free amikacin. ALIS has no apparent impact on macrophage function *in vivo*. The ability to penetrate biofilms and increase intracellular accumulation of ALIS is highly relevant in the context of treating MAC infections, due to the capability of MAC to survive and replicate within pulmonary macrophages (Honda et al. 2015, Daley 2017).

3.4 *In Vitro* and *In Vivo* Studies

Based on its *in vitro* spectrum, amikacin administered by injection is indicated for the treatment of serious infections caused by *Staphylococcus spp., Pseudomonas spp., E. coli, Proteus spp., Klebsiella spp., Enterobacter spp., Serratia spp.,* and *Acinetobacter spp.,* and the *in vitro* activity of amikacin against these species has been well established.

The *in vitro* activity of amikacin against *Mycobacterium spp*. has also been well-characterized, and IV amikacin has been used as a second-line agent for the treatment of *Mycobacterium tuberculosis* (MTB) and as a recommended agent for the treatment of NTM lung disease caused by MAC in patients with concurrent fibrocavitary or severe nodular/bronchiectatic lung disease. The *in vitro* activity against MAC has been demonstrated in several studies, including 2 studies involving large volumes of isolates tested by standard methods where amikacin had an MIC_{50} of 16 µg/mL and an MIC_{90} of 32 µg/mL. Similar activity against MAC was observed in other *in vitro* studies. The *in vitro* activity against other non-MAC mycobacteria including other NTM and MTB has also been demonstrated in multiple studies.

In addition, the efficacy of ALIS in a murine lung disease model was evaluated *in vivo* (Rose et al. 2014). Based on data using a murine MAC pulmonary infection model, ALIS administered via a nebulizer effectively kills MAC in the lung.

3.4.1 Biodistribution

The biodistribution of nebulized, inhaled ALIS has been investigated in rats, healthy human volunteers, and NTM patients. In rats, ALIS demonstrated equal dose-dependent deposition across all lung lobes and regions. Furthermore, sectioned lungs imaged with fluorescent microscopy exhibited diffuse ALIS extracellular colocalization and accumulation inside of macrophages (Malinin et al. 2016). In a human biodistribution study in four NTM patients, inhaled radiolabeled ALIS resulted in 43% deposited into the lungs (Olivier et al. 2016). Scintigraphy studies demonstrated that the inhalation of ALIS results in distribution to both central and peripheral compartments of the lung, and prolonged retention.

3.4.2 Intracellular Accumulation of Amikacin in Macrophages

The intracellular accumulation and activity of amikacin in macrophages after treatment with ALIS is highly relevant in the context of treating MAC infections due to the capability of MAC to survive and replicate in pulmonary macrophages (Honda et al. 2015, Daley 2017). When

administered as ALIS by inhalation using a nebulizer, liposomal amikacin demonstrates dose-dependent accumulation and elimination in the lungs of rats and accumulation within lung macrophages. ALIS has no apparent impact on macrophage function *in vitro* (Malinin et al. 2016). ALIS is bactericidal against MAC internalized within macrophages and has increased activity against intracellular MAC due to the higher amount of amikacin that accumulates in macrophages relative to free amikacin.

3.4.3 Effect of Residual Amikacin in Sputum Samples

Sputum culture results were critical efficacy endpoints in the ALIS clinical efficacy studies. As the inhalation of ALIS delivers amikacin directly to the lung, and amikacin may still be detected in sputum 72 hours post-dose, the effects of residual amikacin on MAC culture results were evaluated. This experiment demonstrated that ALIS did not bias sputum mycobacterial culture results and supports the assumption that negative sputum culture results for MAC isolates during treatment with ALIS reliably reflect the true microbiological status of the patient (Eagle et al. 2018).

An *in vitro* experiment was designed to replicate the potential effects of residual amikacin in sputum samples on the growth of MAC. Banked sputum samples were prepared with varying concentrations of amikacin (0 [control], 4, 16, 64, and 128 µg/mL amikacin). Sixteen unique *M. avium* or *M. intracellulare* isolates with known amikacin MIC values were obtained from banked clinical isolates. The first cohort of sputum samples was prepared with cultured isolates at a density equivalent to a 0.75 McFarland and differing amikacin concentrations of 0, 4, 16, 64, and 128 µg/mL. Each sputum sample was then cultured after 24 hours and 72 hours incubation (160 total combinations). The second cohort of sputum samples was prepared with cultured isolates at a density equivalent to a 1.0 McFarland and amikacin concentrations of 4, 16, 64, and 128 µg/mL (128 total combinations). Incubation periods of 24 and 72 hours represented transportation times to the microbiology laboratory for processing. From the 288 unique sputum samples prepared, MAC growth inhibition was not seen with increasing concentrations of amikacin across all MIC values after either 24 or 72 hours incubation.

4 REGULATORY AND DEVELOPMENT HISTORY

Summary

- The FDA granted ALIS with Qualified Infectious Disease Product and Breakthrough Therapy designations for the treatment of NTM infections, supporting the unmet need for patients with NTM caused by MAC
- The NDA for ALIS for NTM lung disease was submitted under an Accelerated Approval procedure (Subpart H) with culture conversion by Month 6 as a surrogate endpoint that is reasonably likely to predict the confirmatory endpoint of durable culture negativity at 3 months off therapy for full approval
- The ALIS clinical development program comprises 1 adequate and well-controlled pivotal Phase 3 study, Study 212, and 2 supportive clinical studies, Studies 312 and 112

4.1 Regulatory History

Amikacin has been approved by the FDA for IV use since 1976. ALIS is a combination product of amikacin formulated for oral inhalation using the Lamira Nebulizer System that is provided as a handset along with the eFlow control unit. The current NDA for ALIS is a 505(b)(2) application based on the existing literature. A 505(b)(2) NDA contains full safety and effectiveness reports but allows for some of the information required for NDA approval, such as safety and efficacy information on the active ingredient, to come from studies not conducted by or for the applicant.

The NDA for ALIS for NTM lung disease was submitted under an Accelerated Approval procedure (Subpart H). Under Subpart H, the FDA may grant marketing approval for a new drug product on the basis of adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate or intermediate endpoint that is reasonably likely to predict clinical benefit or on the basis of an effect on a clinical endpoint other than survival or irreversible morbidity. Approval under this section is subject to the requirement that the applicant study the drug further in postmarketing studies to verify and describe its clinical benefit. In addition to demonstrating an effect on a surrogate endpoint, products for Accelerated Approval must treat a serious condition and provide a meaningful advantage over available therapy.

ALIS was granted Orphan Drug Designation for the treatment of NTM lung disease on 25 March 2013. On 28 June 2013, in recognition of the potential for ALIS to meet a high unmet medical need for the treatment of a serious life-threatening infection, the FDA granted ALIS with both Fast Track and Qualified Infectious Disease Product designations for the treatment of NTM infections. The FDA also granted ALIS with Breakthrough Therapy Designation on 16 June 2014 based on sputum culture negativity in adult patients with NTM lung disease who failed to convert with prolonged Multidrug Regimen as demonstrated in the Phase 2 Study 112.

4.2 Clinical Development Program

Evidence for the efficacy of ALIS for the treatment of adults with NTM lung disease caused by MAC is based upon 1 adequate and well-controlled pivotal Phase 3 study, Study 212, and 2 supportive clinical studies, Studies 312 and 112.

Study 212 is an ongoing, Phase 3, randomized, open-label, multicenter study of ALIS 590 mg QD + Multidrug Regimen vs Multidrug Regimen Alone in adult patients with NTM lung disease caused by MAC who were unable to convert with prolonged Multidrug Regimen therapy. The primary objective of this study is to evaluate the efficacy of ALIS 590 mg QD, when added to Multidrug Regimen, for achieving culture conversion (defined as 3 consecutive monthly negative sputum cultures) by Month 6 compared to Multidrug Regimen Alone. Culture conversion is considered to be a surrogate endpoint reasonably likely to predict durable sputum culture negativity 3 months after completion of treatment. Patients who achieved culture conversion (converters) by Month 6 continued their treatment in Study 212 for 12 months from the first of the 3 consecutive negative sputum cultures that defined culture conversion (total maximum treatment up to 16 months). An evaluation of the durability of culture conversion at 3 months off all NTM therapy will provide confirmatory data for full approval. Patients then returned for follow-up visits for 12 months off all MAC treatment. Patients who did not achieve culture conversion (non-converters) exited Study 212 at Month 8/End of Treatment (EOT) and were offered the option to enter the separate open-label Study 312.

The confirmatory endpoint analysis assessed at 3 months off-treatment will assess Study 212 responders who demonstrate sequential attainment of (1) culture conversion by Month 6, (2) sustained culture conversion through 12 months after initial culture conversion, and (3) durable sputum culture negativity for 3 months following completion of treatment.

Study 312 is an ongoing, Phase 3, single-arm, open-label study. To enroll, patients must have completed the Month 6 and EOT visits in Study 212 and have not achieved the protocol definition of culture conversion by Month 6 or have experienced a protocol defined relapse or recurrence by Month 6. The primary objective of this study is to evaluate the long-term safety and tolerability of ALIS 590 mg QD added to the patient's Multidrug Regimen administered for up to 12 months in patients who failed to convert in Study 212 regardless of which treatment arm they had previously been in; secondary objectives include culture conversion and time to culture conversion.

Study 112 was a Phase 2 randomized, double-blind, placebo-controlled study to evaluate efficacy, safety, and tolerability of 84 days of ALIS 590 mg QD when added to Multidrug Regimen in patients with NTM lung disease who were unable to convert with prolonged Multidrug Regimen. In this study, culture conversion was the subject of a post hoc analysis. At the completion of the double-blind phase, patients had the option to continue in the open-label phase of the study and receive add-on ALIS 590 mg QD for an additional 84 days. All patients were required to complete a 28-day safety follow-up visit after the EOT visit. Patients completing this study had the option to enroll in a long-term safety follow-up and returned to the study site at 12 months after the last dose of study drug (either after completing the randomized

double-blind phase or the open label phase). Study 112 was completed at the time of this submission.

Table 13: Overview of Clinical Studies in the ALIS Development Program for NTM

Study	Status	Treatment	Primary Endpoint	Secondary Endpoints	Exploratory Endpoints
212	Ongoing	ALIS 590 mg QD	Culture conversion by Month 6	• Time to culture conversion • Change in 6MWT distance from Baseline in at Month 6 • Change in SGRQ from Baseline at Month 6	• Change in 6MWT from Baseline to Month 6 for converters vs non-converters for all patients • Change in 6MWT from Baseline to Month 6 for converters vs non-converters in ALIS + MDR arm • Change in 6MWT from Baseline to Month 6 for converters vs non-converters in MDR alone arm
312	Ongoing	ALIS 590 mg QD	Safety and tolerability of ALIS	• Culture conversion by Month 6 • Time to culture conversion • Change in 6MWT from Baseline at Month 6	• Change in SGRQ from Baseline at Month 6
112	Completed	ALIS 590 mg QD	Change in SQS from Baseline to Day 84	• Proportion of patients with negative NTM culture at Day 84	• Change in 6MWT from Baseline to Day 84 • Culture conversion by Day 168 (post hoc) • Change in SGRQ from Baseline to Day 84

ALIS = amikacin liposome inhalation suspension; MDR = Multidrug Regimen; 6MWT = six-minute walk test; SQS = semi-quantitative scale; NTM = nontuberculous mycobacterium

5 CLINICAL PHARMACOLOGY

Summary

- Clinical pharmacology studies demonstrated that serum exposure of amikacin was low throughout the program
- Median $t_{1/2}$ was approximately 5.5 hours in NTM patients
- None of the covariates evaluated for their relationship with the interindividual variability in amikacin PK (age, body weight, height, sex, ideal body weight, body surface area, and creatinine clearance) were found to be significant
- The risk of nephrotoxicity and/or ototoxicity secondary to inhaled administration of ALIS at a daily dose of 590 mg in NTM lung disease is minimal

Clinical pharmacology studies demonstrate that inhalational delivery of ALIS results in significantly higher amikacin concentrations in the lung than in serum (as reflected by differences in sputum concentrations compared to serum concentrations). Therefore, as serum concentrations after ALIS administration do not consistently reflect the drug concentration at the site of infection in the lung, conventional pharmacokinetic (PK)/pharmacodynamic (PD) surrogates correlating serum concentrations with clinical and microbiological response of the pathogen causing lung disease could not be used as a basis for dose selection. In contrast, IV administration of amikacin has been shown to achieve low concentrations of amikacin in sputum (Canis et al. 1997). This is an important distinction, as the desired PK profile of ALIS provides maximal amikacin in the lung, the site of infection, while minimizing systemic exposure and thus reducing the potential for aminoglycoside-associated toxicities.

5.1 Dose Rationale

The dose selected for the Phase 3 study (Study 212) was based on the efficacy signal and safety profile seen in the Phase 2 study (Study 112), the safety profile seen in the CF program, as well as the PK profile based on all studies in the ALIS clinical development program. Given these observations, and in light of the difficulties in conducting extensive dose-ranging evaluations in the setting of a very rare disease, further dose exploration was not conducted for the NTM indication.

In Study 112, more patients who received ALIS achieved a negative sputum culture at the end of the double-blind phase (Day 84) and culture conversion (defined post hoc as 3 consecutive monthly negative sputum cultures) by the 28-day off-ALIS follow-up visit, compared to patients who received placebo. During the double-blind phase, a greater percentage of patients who received ALIS experienced AEs, most of which were respiratory in nature. Patients who received ALIS also had a higher incidence of SAEs, with bronchiectasis (2 patients who received ALIS versus 1 patient who received placebo) and pneumonia (1 patient who received ALIS and 2 patients who received placebo) as the most frequently reported. During the double-blind phase,

discontinuations due to AEs only occurred in patients who received ALIS. Based on the efficacy signal and safety profile seen in this population of patients with refractory NTM lung disease, the same dosing regimen was taken into the pivotal Phase 3 study (Study 212).

Patients in Study 212 were randomized to receive either ALIS 590 mg QD + Multidrug Regimen or Multidrug Regimen Alone. More patients in the ALIS group achieved culture conversion by Month 6 compared to patients who received Multidrug Regimen Alone. Patients in the ALIS group experienced more AEs, most of which were respiratory in nature, than patients in the Multidrug Regimen Alone group. The rate of SAEs was similar between the 2 groups. Most SAEs were respiratory in nature and mild to moderate in severity. The rate of SAEs in the Study 312 was similar between patients who received ALIS + Multidrug Regimen and those who received Multidrug Regimen Alone in the preceding Study 212.

The safety and tolerability of the 590-mg dose in the CF program were also considered during dose selection in the NTM program. ALIS 590 mg demonstrated a favorable safety and tolerability profile in patients with CF during on-treatment cycles. The overall incidence of AEs decreased over successive treatment cycles (up to 12 cycles), including AEs that were respiratory in nature.

PK parameters are important for evaluating the potential for antibiotic efficacy, and for aminoglycoside antibiotics such as amikacin, which exhibit concentration-dependent killing, include the peak concentration (C_{max}) and the area under the concentration-time curve (AUC) at the site of activity (Moore et al. 1987, Frimodt-Moller 2002, Owens and Shorr 2009). The primary measure of antibiotic bactericidal or bacteriostatic activity is the MIC. The MIC is the lowest concentration of an antibiotic that completely inhibits the growth of a microorganism *in vitro*.

ALIS delivers amikacin directly to the lung via nebulization, maximizing PK/PD by delivering drug directly to the site of infection. The PK of amikacin after administration of ALIS was established during the ALIS CF program, based on both sputum and serum PK parameters. This was repeated in the Phase 2 study (Study 112). Based on the PK data, serum exposures of amikacin after ALIS administration are low and not predictive of amikacin concentration at the site of infection in the lung. In Study 112 and Study 212, serum C_{max} and AUC_{0-24} were similar at Day 1 and steady state and indicated little or no accumulation of amikacin in serum, and minimal systemic exposure for a QD nebulized dose of 590 mg of ALIS. Therefore, serum exposures are not relevant for determining the efficacy of ALIS. ALIS delivers amikacin directly to the lung via nebulization with substantially higher median post-dose sputum concentrations than the highest observed serum concentration. Thus, even though sputum concentrations of amikacin are not a direct measure of the concentration of amikacin in lung tissue nor intracellularly (macrophage uptake), sputum PK is the most valuable PK indicator to predict microbiological outcomes in lung disease.

Antibiotic dose selection is also guided by safety and tolerability, which was also taken into consideration for Study 212. In contrast to the PK/PD for efficacy discussed above, serum concentrations of amikacin after ALIS administration are relevant when evaluating the potential

for systemic toxicity of candidate doses in NTM lung disease. Several studies have evaluated risk factors for the development of nephrotoxicity with systemic administration of aminoglycosides (Bertino et al. 1993, Murry et al. 1999, Rybak et al. 1999). In addition to risk factors such as patient age and concomitant medications, systemic aminoglycoside exposure (trough concentrations and/or AUC) has been consistently identified as a modifiable risk factor for aminoglycoside nephrotoxicity. Although the PK/PD parameter that is most predictive of toxicity has not been fully elucidated, it is clear that lower aminoglycoside exposure and more infrequent dosing are associated with lower risk of nephrotoxicity. Data from Rybak and colleagues suggest that, even with twice daily (BID) dosing of systemic aminoglycosides, the risk of nephrotoxicity is extremely low at daily AUC values below 50 mg•h/L. The risk of nephrotoxicity when aminoglycosides are administered QD is significantly lower (Rybak et al. 1999). In the ALIS development program, the mean daily amikacin AUC at steady state after administration of ALIS at a dose of 590 mg QD in NTM patients is approximately 21 mg•h/L, and the maximum observed value was 55.4 mg•h/L. Furthermore, the mean steady state C_{max} in NTM patients receiving ALIS 590 mg QD was approximately 2.4 mg/L, which would be considered an acceptable trough amikacin concentration (5 to10 mg/L) in aminoglycoside monitoring algorithms (Kovacevic et al. 2016). Overall, these data suggest that the risk of nephrotoxicity secondary to inhaled administration of ALIS at a daily dose of 590 mg in NTM lung disease is minimal.

For ototoxicity, the publication by Modongo et al. provides a particularly relevant example given that it evaluated the risk of ototoxicity in patients being treated with amikacin for multidrug-resistant tuberculosis. Patients received systemic amikacin treatment for up to 866 days (approximately 28 months). The authors found that cumulative AUC for amikacin was highly predictive of the occurrence of ototoxicity in these patients. Regression analyses indicated that a cumulative AUC of 87,232 days•mg•h/L was associated with a 10% risk of ototoxicity. For reference, if the patient with the highest amikacin serum AUC_{0-24} at steady state (55.6 mg•h/L) were to receive ALIS continuously for 16 months (480 days), their cumulative AUC would be 26,688 days•mg•h/L. That cumulative AUC would be associated with a probability of 0.13% risk of ototoxicity (Modongo et al. 2015). While the true risk of ototoxicity in a given patient is governed by complex factors such as age and prior damage due to ototoxic drugs, these data suggest that the additive risk due to ALIS administration in NTM patients is relatively low.

Based on the efficacy signal and safety profile seen in the NTM and CF programs, as well as the PK profile based on all studies in the ALIS development program, the ALIS dose of 590 mg QD was selected. These safety and efficacy data are consistent with standard PK/PD principles in antibiotic development that aim to maximize antibiotic concentrations at the site of infection while minimizing systemic exposure.

5.2 Pharmacokinetics

Table 14 summarizes the PK findings in studies with ALIS.

Age, sex, and race did not appear to have significant effects on ALIS PK. Specific studies of ALIS in patients with renal and hepatic impairment were not conducted. Based on the low

systemic bioavailability, it is not necessary to adjust ALIS doses on the basis of renal function. Since amikacin is not metabolized, the PK of amikacin is not expected to be affected by hepatic impairment.

No specific drug-drug interaction studies have been conducted with ALIS. Based on the low systemic bioavailability of amikacin following ALIS administration and the fact that amikacin is not metabolized, PK-related drug-drug or drug-food interactions are not expected in patients receiving ALIS.

Table 14: Summary of Pharmacokinetic Results

Pharmacokinetics	Observations	Evidence
Absorption	Serum exposure of amikacin was low throughout the program	Approximately 7% of the loaded dose reached systemic circulation within the first 24 hours
	Liposomes were retained within the lung	Deposition of ^{99m}Tc-ALIS in areas of cavitation and air trapping was not apparent
Distribution	ALIS significantly increased amikacin uptake into human macrophages relative to free amikacin over a range of concentrations.	Biphasic retention with more than half of the deposited dose retained 24 hours post-dose followed by a slower phase up to 48 hours and delivered to both central and peripheral compartments of the lung
Metabolism	Amikacin sulfate is not metabolized	
Elimination	Median half-life for the 53 NTM PK subjects was approximately 5.5 hours	Median half-lives of NTM patients was 5.3 hours in Study 112 and 5.5 hours in Study 212
Dose-Proportionality	Sputum amikacin concentrations and exposures generally increased in a dose-proportional manner	
	Serum amikacin exposure (C_{max} and AUC) did not consistently increase with ALIS dose	Likely due to the variability in absorption and the low systemic concentrations
PPK Intrinsic	None of the covariates evaluated for their relationship with the interindividual variability in amikacin PK (age, body weight, height, sex, ideal body weight, body surface area, and creatinine clearance) were found to be significant.	The only covariate effect was the relationship between body weight and renal clearance.
PPK Extrinsic	Amikacin exposure was similar in Japanese and White subjects (regardless of study).	Median AUC_{24} and C_{max} were similar for the 2 PK populations with more variability in Japanese subjects which may reflect the higher number of Japanese subjects in the PK population

5.3 Population Pharmacokinetics

The population pharmacokinetic (PPK) model originally developed for the CF population (a three-compartment model [absorptive lung compartment, central serum compartment, and urine compartment] with a zero-order inhalational administration (k0), first-order linear absorption process from lungs (ka), and a first order elimination process (apparent total clearance [CL_t/F], apparent central volume of distribution [V_c/F], and renal clearance [CL_r]) provided a robust fit to the combined data from Studies 112 and 212 and allowed for the estimation of serum amikacin exposure in patients with NTM. Amikacin exposure was similar in patients enrolled in Study 212 to Study 112 and confirmed the previous observation of low systemic bioavailability of ALIS.

As detailed in Table 14, none of the covariates evaluated for their relationship with the interindividual variability in amikacin PK (age, body weight, height, sex, ideal body weight, body surface area, and creatinine clearance) were found to be significant. Amikacin exposure was similar in Japanese and white patients regardless of study.

6 CLINICAL EFFICACY

Summary

- ALIS in combination with Multidrug Regimen demonstrated superior ability to achieve culture conversion in adult patients with NTM lung disease caused by MAC; the study findings support that culture conversion is predictive of durable culture negativity and may be associated with functional benefit
- In Study 212, treatment with ALIS + Multidrug Regimen led to a significantly greater percentage of patients achieving culture conversion by Month 6 than patients receiving Multidrug Regimen Alone (29.0% vs 8.9%, respectively; P<0.0001)
- Results across studies demonstrated a consistent effect of ALIS + Multidrug Regimen on culture conversion
- The interim analyses of Study 312 showed that 27.4% of patients who received Multidrug Regimen Alone in Study 212 but failed to culture convert were able to achieve culture conversion by Month 6 when ALIS was added to Multidrug Regimen, showing similar efficacy to Study 212
- Study 312 also showed that some patients who received ALIS + Multidrug Regimen in Study 212 and did not culture convert were able to convert following additional ALIS + Multidrug Regimen treatment
- Results from Study 212 are supported by post hoc analysis in Study 112 showing 22.5% of patients achieved culture conversion by Day 168
- In Study 212, the 6MWT did not show a meaningful change from baseline to Month 6 between the treatment arms. However, improvements were seen in patients who achieved culture conversion compared to patients who did not achieve culture conversion in a pre-specified exploratory analysis. These findings suggest that culture conversion by Month 6 is likely associated with a concurrent meaningful clinical benefit

6.1 Efficacy Overview

Evidence for the efficacy of ALIS in the treatment of adults with NTM lung disease caused by MAC is based on the ongoing pivotal study, Study 212, and 2 supportive studies – the completed, randomized, controlled Phase 2 study, Study 112, and the ongoing open-label supportive Phase 3 study, Study 312, in patients who failed to convert in Study 212.

6.1.1 Endpoint Definitions

6.1.1.1 Culture Conversion

Culture conversion, defined as 3 consecutive monthly negative sputum cultures by Month 6, was the primary endpoint in Study 212 and was based on sputum samples that were processed and cultured in central laboratories. Sputum collection across all studies consisted of multiple

samples collected 1 day apart for visits requiring sputum samples. All sputum samples were required to be negative for the patient to be declared culture negative at that visit.

Patients met the endpoint of culture conversion if they had 3 consecutive monthly negative sputum cultures. For Study 212, a patient must achieve the first of 3-consecutive monthly negative sputum cultures (that defines culture conversion) by Month 4 at the latest in order to meet the primary endpoint by Month 6.

All 3 studies evaluated culture conversion either as a pre-specified analysis (ie, Studies 212 and 312) or post hoc (ie, Study 112).

6.1.1.2 Semi-Quantitative Scale for Mycobacterial Culture

Study 112 used a novel endpoint to help quantify the change in mycobacterial burden since at the time of study design it was believed that most patients with NTM lung disease were unable to convert with prolonged Multidrug Regimen were not expected to achieve negative culture within 84 days. The endpoint used the SQS mycobacterial culture reporting method, a 7-step scale based on results of solid and liquid media, as described in Table 15.

At each time point, sputum cultures were scored based on evidence of growth in broth medium and estimated number of colonies observed on solid medium as shown in Table 15. The change in the SQS was based on an ordinal scale. The mean was calculated from the Step change for each patient (Step number at Day 84 subtracted from the Step number at Baseline). The maximum improvement for an individual patient was -6 steps and the maximum deterioration was +6 steps. The analysis of the primary endpoint included a pre-specified imputation for death such that the change in Step from Baseline to Day 84 was imputed as +7 steps for any patient who died during the study, regardless of cause.

The study population of patients with NTM lung infection who did not convert and were treated for 84 days. Quantitative reductions may represent meaningful clinical improvement and may correlate with subsequent negative sputum cultures for NTM. Published data in NTM disease is sparse, but any change in this scale was considered to be potentially clinically meaningful by the experts treating these patients.

Table 15: Semi-Quantitative Mycobacterial Culture Reporting Method

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 (manual count on agar)	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+

6.1.1.3 6 Minute Walk Test (6MWT)

The standardized protocol based on the ATS guidelines was used for the 6MWT. In Study 212, the 6MWT was conducted by a site member who was blinded to the patient's open-label treatment assignment.

6.1.1.4 St. George's Respiratory Questionnaire (SGRQ)

The SGRQ is a self-administered questionnaire that has been validated in patients with airways disease, specifically in patients with COPD and bronchiectasis (Jones 1991, Jones et al. 1991, Wilson et al. 1997). The SGRQ assesses HRQOL in patients with chronic pulmonary disease by evaluating 3 health domains: Symptoms (distress caused by respiratory symptoms); activity (effects of disturbances on mobility and physical activity); and impacts (the effect of disease on factors such as employment, personal control of one's health, and need for medication).

A composite total score is derived as the sum of domain scores for symptoms, activity, and impact, with 0 being the best possible score and 100 being the worst possible score. A difference of 4 is the MCID in disease states in which the SGRQ has been validated, such as for COPD. A minimal important difference has not been established in NTM lung disease.

6.2 Study 212

6.2.1 Study Design

Study 212 is an ongoing, randomized, open-label, multicenter study of ALIS in adult patients with NTM lung disease caused by MAC who were unable to convert with prolonged Multidrug Regimen therapy. The primary objective of this study is to evaluate the efficacy of ALIS 590 mg QD when added to Multidrug Regimen in achieving culture conversion (defined as 3 consecutive monthly negative sputum cultures) by Month 6 compared to Multidrug Regimen Alone. Culture conversion by Month 6 is a surrogate endpoint for the confirmatory endpoint to establish treatment success, durability of culture conversion, defined as sustained negative cultures for 12 months from the first culture conversion and for 3 months after treatment cessation. The confirmatory endpoint will be evaluated in the final analysis of this currently ongoing study.

Patients were randomized (2:1) to receive either ALIS 590 mg QD + Multidrug Regimen or Multidrug Regimen Alone without a matching comparator (Figure 17). Patients were stratified by smoking status (current smoker or not) and Multidrug Regimen use at screening (on treatment or off treatment for 3-12 months prior to screening). Throughout the duration of the study, patients were to continue on the same Multidrug Regimen of ≥2 antibiotics based on the 2007 ATS/IDSA Guidelines. The Multidrug Regimen was not to change during the treatment period except for safety concerns or if rescue medication was required, in which case the patient was discontinued from the study.

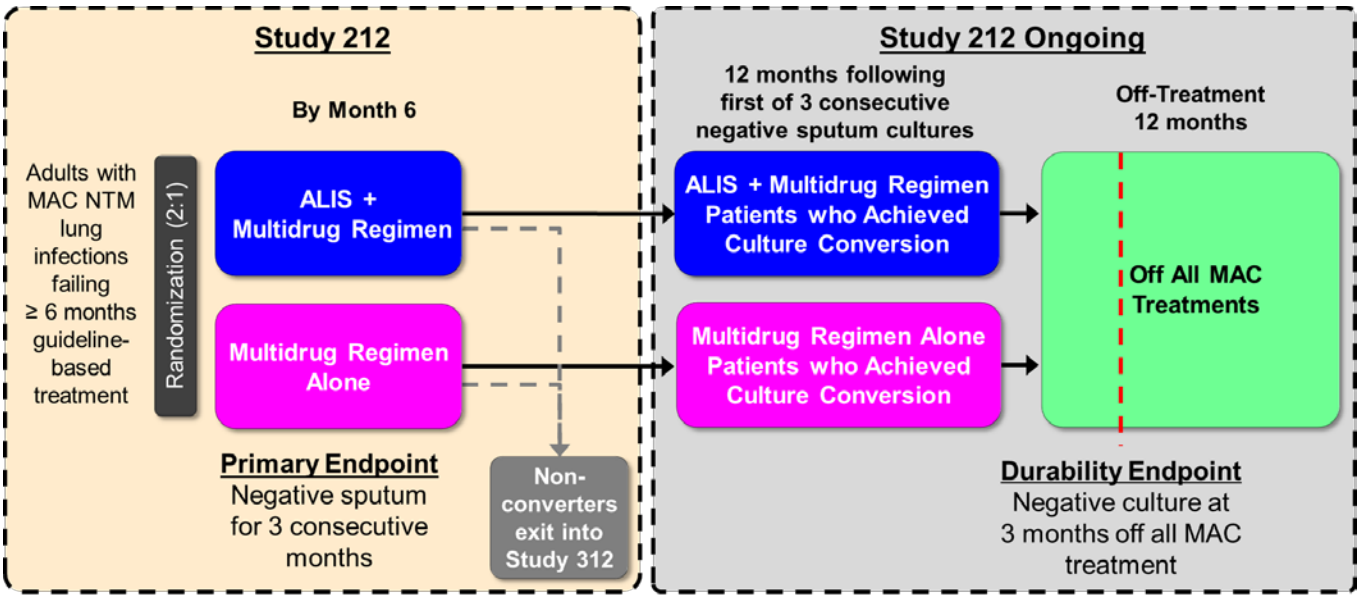
Sputum culture results remained blinded until the Month 8 visit. At the Month 8 visit, all sputum culture results were provided to the study sites, and patients were assessed for culture conversion based on the sputum culture results at visits from Baseline to Month 6. The initial analysis, upon which the current application is based, occurred when the last patient completed the Month 8

visit. Patients who achieved culture conversion (converters) by Month 6 continued treatment in Study 212 for 12 months from the first of the 3 consecutive negative sputum cultures that defined culture conversion (total maximum treatment up to 16 months). These patients returned after the EOT visit for follow-up visits at 28 days and 3, 6, and 12 months off all MAC treatment. The 12 months off all MAC treatment follow-up visit was the End of Study (EOS) visit.

Patients who did not achieve culture conversion (non-converters) exited Study 212 at Month 8/EOT. Patients who experienced a relapse or recurrence after achieving culture conversion also exited Study 212 at their Month 8/EOT visit. Patients who left Study 212 were offered the option to enter the separate open-label study, Study 312.

A full schedule of events in Study 212 can be found in Appendix 10.1.

Figure 17: Design of Study 212



ALIS = amikacin liposome inhalation suspension; MAC = Mycobacterium avium complex; NTM = nontuberculous mycobacterium

6.2.1.1 Inclusion and Exclusion Criteria

A full list of inclusion and exclusion criteria can be found in Appendix 10.2.1. Key inclusion criteria include:

1. Male or female, 18 years of age or older (20 years of age or older in Japan).
2. Positive for MAC on culture while being treated with Multidrug Regimen (at least 2 antibiotics) for a minimum duration of 6 consecutive months.
3. MAC lung infection documented by at least 2 positive cultures 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).

Key exclusion criteria include:

1. Patients with CF.
2. Patients whose MAC NTM infection was resistant to amikacin (as identified by MIC susceptibility >64 µg/mL).

6.2.1.2 Statistical Analyses

In consultation with the FDA and clinical experts, a 15% treatment effect in culture conversion was determined to be a meaningful effect in this treatment refractory patient population. Assuming a culture conversion rate by Month 6 of at least 20% in the ALIS + Multidrug Regimen arm and 5% in the Multidrug Regimen Alone arm, randomization of approximately 351 patients (2:1) was predicted to provide ≥90% power at a 2-sided significance level of 0.05.

The primary efficacy analysis of culture conversion by Month 6 was performed using the Cochran-Mantel-Haenszel test stratified by smoking status and Multidrug Regimen at screening (on treatment or off treatment for at least 3 months) at the 2-sided significance level of 0.05. Any missing sputum samples prior to confirmed conversion were considered culture positive in the derivation of each patient's conversion status. Patients for whom culture conversion could not be evaluated due to missing sputum culture results were considered non-converters.

Secondary objectives were assessed in a hierarchical order as presented below:

- 6MWT: The change from Baseline (Day 1) to Month 6 in 6MWT distance was analyzed for the intent-to-treat (ITT) population (assuming missing-not-at-random) using a model under the pattern-mixture model framework. This model assumed that patients in the ALIS + Multidrug Regimen arm with missing data followed the response distribution of a patient in the Multidrug Regimen Alone arm. Missing data in the 6MWT for this analysis were imputed using the multiple imputation method
- Time to Culture Conversion: Time to culture conversion was defined as the first of 3 consecutive negative sputum cultures and was analyzed using the Cox regression model to estimate hazards ratio. The Cox regression model included effects for smoking status, current Multidrug Regimen status at Baseline (on treatment or off treatment for at least 3 months), and treatment. Kaplan-Meier estimates for the distribution of time to culture conversion were constructed for each treatment arm
- SGRQ: The change from Baseline (Day 1) to Month 6 in SGRQ total score was analyzed for the ITT population using a mixed model repeated measures (MMRM) model including effects for smoking status, current Multidrug Regimen status at Baseline (on treatment or off treatment for at least 3 months), treatment, month, treatment-by-month interaction as fixed effects, and baseline score and baseline score-by-month interaction as a covariate

6.2.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics were comparable between treatment arms (Table 16). The mean age in the total population was 64.7 years (ALIS + Multidrug Regimen 64.6 years, Multidrug Regimen Alone 64.9 years). Overall, there was a greater percentage of females (69.3%) than males (30.7%) in the study, and the majority of patients were White (69.9%).

Table 16: Baseline Demographics in Study 212 (ITT Population)

	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Age (years)		
n	224	112
Mean (SD)	64.6 (9.59)	64.9 (10.16)
Median	65.0	66.0
Min, Max	40, 87	32, 85
Region		
United States of America	93 (41.5%)	48 (42.9%)
Asia (excluding Japan)	14 (6.3%)	6 (5.4%)
Japan	34 (15.2%)	14 (12.5%)
Rest of the World	83 (37.1%)	44 (39.3%)
Sex		
Male	59 (26.3%)	44 (39.3%)
Female	165 (73.7%)	68 (60.7%)
Ethnicity		
Hispanic or Latino	10 (4.5%)	5 (4.5%)
Not Hispanic or Latino	211 (94.2%)	102 (91.1%)
Not recorded	0	4 (3.6%)
Unknown	3 (1.3%)	1 (0.9%)
Race[a]		
American Indian or Alaska Native	0	1 (0.9%)
Asian: Japanese	35 (15.6%)	15 (13.4%)
Asian: Other	23 (10.3%)	10 (8.9%)
Black or African American	3 (1.3%)	3 (2.7%)
Native Hawaiian or other Pacific Islander	1 (0.4%)	0
White	158 (70.5%)	77 (68.8%)
Multi-racial	1 (0.4%)	0
Not recorded	3 (1.3%)	6 (5.4%)

a. Patients who mark more than one race are classified as multiracial and counted only once.
ALIS = amikacin liposome inhalation suspension (590 mg); ITT = intent-to-treat; IUD = intrauterine device; SD = standard deviation.

A summary of other baseline characteristics (antibiotics in the Multidrug Regimen at enrollment, smoking status, underlying lung disease, and prior nebulized IV amikacin solution use) for patients in the ITT population is presented in Table 17. Baseline characteristics were balanced across the 2 treatment arms. Most patients were on Multidrug Regimen treatment at Screening, were not current smokers, had underlying bronchiectasis, and had not received prior IV amikacin solution by inhalation via nebulizer.

A summary of the duration of NTM lung disease is presented by treatment arm in Table 18. The median duration of NTM lung disease was 3.96 years overall; a slightly longer median duration of NTM lung disease was observed in the ALIS + Multidrug Regimen arm versus the Multidrug Regimen Alone arm (4.45 years versus 3.26 years, respectively).

Table 19 shows a summary of the different combinations of Multidrug Regimen patients were currently taking at Baseline, and Table 18 shows the duration of treatment with Multidrug Regimen prior to Baseline in Study 212.

Table 17: Baseline Characteristics in Study 212 (ITT Population)

	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Duration of NTM Lung Disease (years; median)	4.45	3.26
Number of Drugs in Regimen at Screening		
0*	2 (0.9%)	3 (2.7%)
2	39 (17.5%)	14 (12.5%)
3	148 (66.4%)	84 (75.0%)
4+	34 (15.2%)	11 (9.8%)
Underlying lung disease		
Bronchiectasis	146 (65.2%)	64 (57.1%)
COPD	29 (12.9%)	19 (17.0%)
COPD & bronchiectasis	22 (9.8%)	18 (16.1%)
Current smoker	26 (11.6%)	10 (8.9%)
Prior nebulized IV amikacin	24 (10.7%)	15 (13.4%)

*Four patients reinitiated their multidrug regimen after Day 7, and 1 patient withdrew consent at Baseline.
ALIS = amikacin liposome inhalation suspension (590 mg); COPD = chronic obstructive pulmonary disease; ITT = intent-to-treat; IV = intravenous.

Table 18: Summary of Duration of NTM Lung Disease (Years) (Safety Population)

	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
n	221[a]	112
Mean	6.18	4.54
Standard Deviation	5.525	3.858
Median	4.45	3.26
Minimum	0.0	0.0
Maximum	32.5	20.3

a. Two patients in the ALIS + Multidrug Regimen arm reported MAC as "Mycobacterial infection."
ALIS = amikacin liposome inhalation suspension (590 mg); MAC = *Mycobacterium avium* complex.

Table 19: Multidrug Regimen Combinations at Baseline in Study 212 (Safety Population)

Drug combination	ALIS + Multidrug Regimen Total (N=223)	Multidrug Regimen Alone Total (N=112)
Ethambutol/Macrolide/Rifamycin/Other	30 (13.5%)	8 (7.1%)
Ethambutol/Macrolide/Rifamycin	123 (55.2%)	61 (54.5%)
Ethambutol/Macrolide/Other	6 (2.7%)	6 (5.4%)
Ethambutol/Macrolide	13 (5.8%)	3 (2.7%)
Ethambutol/Rifamycin /Other	8 (3.6%)	6 (5.4%)
Ethambutol/Rifamycin	3 (1.3%)	1 (0.9%)
Ethambutol/Other	1 (0.4%)	0
Macrolide/Rifamycin /Other	13 (5.8%)	12 (10.7%)
Macrolide/Rifamycin	13 (5.8%)	5 (4.5%)
Macrolide/Other	9 (4.0%)	6 (5.4%)
Rifamycin/Other	1 (0.4%)	1 (0.9%)
Other	1 (0.4%)	0

Table 20: Duration of Treatment with Multidrug Regimen Prior to Baseline in Study 212 (ITT Population)

Months	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Mean	51	37
Min, Max	4, 265	3, 225
	N (%)	**N (%)**
6-12	20 (8.9)	11 (9.8)
> 12-24	62 (27.7)	33 (29.5)
> 24	137 (61.2)	65 (58.0)

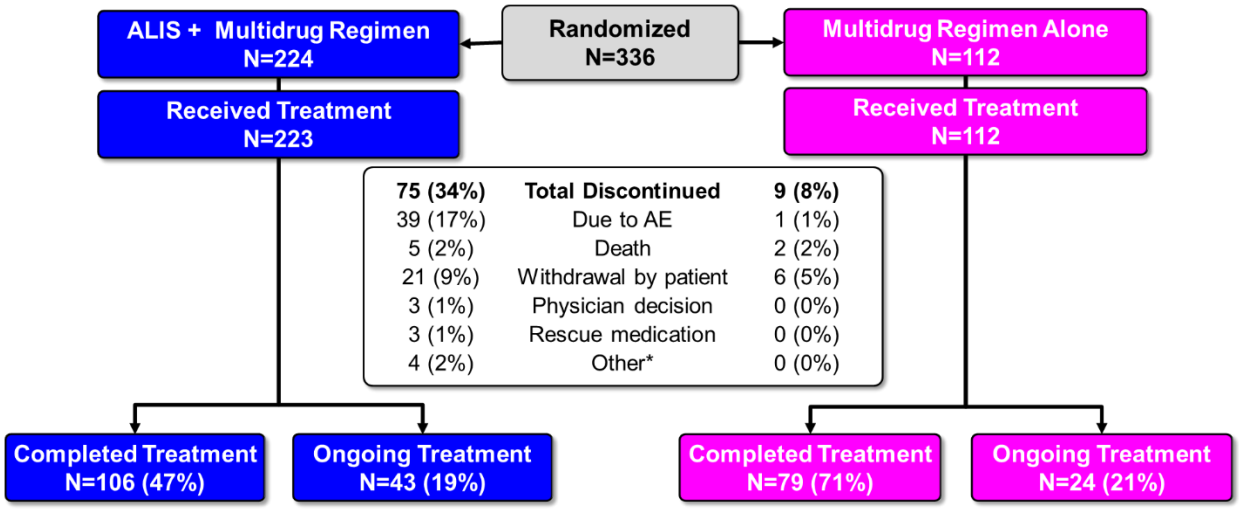
6.2.3 Patient Disposition

Patient disposition is shown in Figure 18. At the time of this initial analysis, enrollment for the study was completed. Of the 492 patients screened, a total of 336 patients were randomized (ITT population): 224 patients in the ALIS + Multidrug Regimen arm and 112 patients in the Multidrug Regimen Alone arm. Of the 336 total patients randomized, 185 completed treatment as defined in the protocol (47.3% ALIS + Multidrug Regimen arm; 70.5% Multidrug Regimen Alone arm), 84 discontinued prior to completing treatment as defined in the protocol (33.5% ALIS + Multidrug Regimen arm; 8.0% Multidrug Regimen Alone arm), and the remainder of the patients were ongoing on treatment.

The most common reasons for discontinuation prior to completing treatment as defined in the protocol in the ALIS + Multidrug Regimen arm were AE (17.4%) and withdrawal by patient (9.4%). For the Multidrug Regimen Alone arm, the most common reason for early

discontinuation of treatment was withdrawal by patient (5.4%). Only 1 patient in the Multidrug Regimen Alone arm discontinued treatment due to an AE, which may reflect the nature of the treatment arm in this study, with open label use of a Multidrug Regimen to which the patients had previously been exposed for at least 6 months.

Figure 18: Patient Disposition (End of Treatment) in Study 212 (ITT Population)



*Other includes: other, protocol deviation and non-compliance with study drug

6.2.4 Primary Endpoint – Proportion of Patients Achieving Culture Conversion by Month 6

The study met its pre-specified primary endpoint of culture conversion by Month 6, defined as 3 consecutive monthly negative sputum cultures by Month 6. At each month, multiple samples were obtained, and all samples had to be negative to be considered negative for that month. The proportion of patients achieving culture conversion by Month 6 was significantly higher in the ALIS + Multidrug Regimen arm compared to the Multidrug Regimen Alone arm (29.0% and 8.9%, respectively, P<0.0001), with an absolute treatment effect size of 20.1% (Table 21).

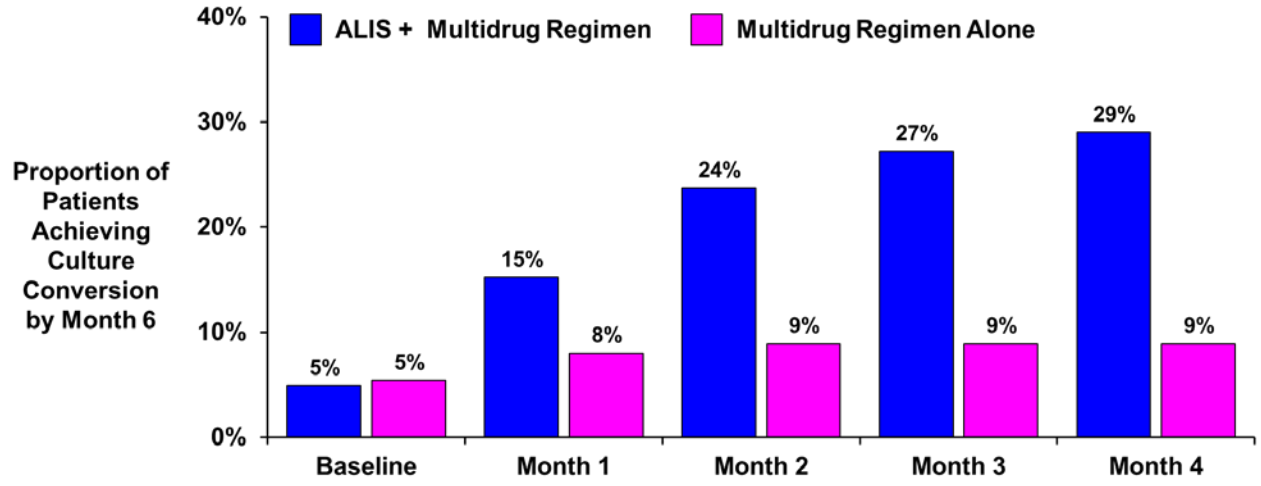
Table 21: Analysis of Culture Conversion by Month 6 in Study 212 (ITT Population)

Statistic	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Number of Patients Assessed	224	112
Converters	65 (29.0%)	10 (8.9%)
Non-Converters	159 (71.0%)	102 (91.1%)
Adjusted Odds Ratio (95% CI)	4.220 (2.078, 8.570)	
P-Value	<0.0001	

Note: Converters are defined as patients who have 3 consecutive MAC-negative sputum cultures within the first 6 months (inclusive) of study.
Note: Adjusted odds ratio (ALIS + Multidrug Regimen/Multidrug Regimen Alone) and P-value are calculated using Cochran-Mantel-Haenszel test, with stratification factors of the combination of smoking status and prior Multidrug Regimen as fixed factors. An adjusted odds ratio >1 indicates a better treatment response for the patients who take ALIS + Multidrug Regimen compared with those who take Multidrug Regimen alone.
ALIS = amikacin liposome inhalation suspension (590 mg); ITT = intent-to-treat; CI = confidence interval;
MAC = *Mycobacterium avium complex*.

Figure 19 shows the cumulative proportion of patients achieving culture conversion displayed by first month of conversion. Patients must achieve the first of 3 consecutive negative sputum cultures (that defines conversion) by Month 4 in order to meet the primary endpoint by Month 6.

Figure 19: Cumulative Proportion of Patients Achieving Culture Conversion by Month 6 Shown by the First Month of Conversion in Study 212 (ITT Population)



Culture Conversion reported as first month of 3 consecutive monthly negative sputum samples. Patients had to have their first of 3 negative sputum cultures by Month 4 at the latest to meet the endpoint by Month 6.
ITT = intent-to-treat.

6.2.5 Secondary Endpoints

6.2.5.1 Change from Baseline in 6 Minute Walk Test (6MWT) Distance at Month 6

The change from Baseline to Month 6 in the 6MWT distance in the ITT population using MMRM with pattern-mixture modeling is summarized in Table 22. There was no statistically significant difference in the change from Baseline in the 6MWT distance between patients in the

ALIS + Multidrug Regimen arm compared to the Multidrug Regimen Alone arm at Month 6. The difference in the LS mean (ALIS + Multidrug Regimen – Multidrug Regimen Alone) (SE) was -3.2 (9.10) meters (95% confidence interval [CI]: -21.1, 14.61; P=0.7223). Based on the predefined hierarchical testing procedure (Section 6.2.1.2), statistical testing of secondary endpoints therefore ended.

It is important to note that in a pre-specified exploratory analysis (Section 6.2.6), improvements in the 6MWT were seen in patients who achieved culture conversion compared to patients who did not achieve culture conversion. This suggests that culture conversion is associated with improvement in patient functional capacity.

Table 22: Analysis of Change from Baseline to Month 6 in 6MWT Distance Using Mixed Model Repeated Measures in Study 212

Time Point Statistics	ALIS + 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	438.0
Min, Max	111, 801	72, 800
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, 695
Change from Baseline to Month 6[b]		
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, 196
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 + Multidrug Regimen – Multi Drug Regimen Alone)	**-3.2 (9.10)**	
95% CI of LS Mean difference (ALIS + Multidrug Regimen – Multidrug Regimen Alone)	-21.1, 14.61	
Two-sided P-value of LS Mean difference (ALIS + Multidrug Regimen – Multidrug Regimen Alone)	**0.7223**	

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 which includes treatment, month, the treatment-by-month interaction, and the combination of smoking status and prior multidrug regimen as fixed factors, the baseline 6MWT distance as a covariate and baseline 6MWT distance-by-month interaction. MMRM includes postbaseline data through Month 6.
Note: For baseline, n is the number of patients with a baseline score and at least one post-baseline score. For Month 4 and Month 6, n is the number of patients with a baseline score and a postbaseline score at the summarized visit.
6MWT = 6-minute walk test; ALIS = amikacin liposome inhalation suspension (590 mg); CI = confidence interval; ITT = intent-to-treat; LS = least squares; Max = maximum; Min = minimum; MMRM = mixed model repeated measures; SD = standard deviation; SE = standard error.

6.2.5.2 Time from Baseline to Culture Conversion

Time to culture conversion in the ALIS + Multidrug Regimen arm compared to Multidrug Regimen Alone arm is shown in Table 23. There was a numerical difference in favor of the ALIS + Multidrug Regimen arm in the meantime from Baseline to culture conversion compared to Multidrug Regimen Alone arm (2.7 months versus 3.5 months, respectively [nominal P<0.0001]). However, the time to culture conversion is not statistically significant given its position in the hierarchical testing.

Table 23: Time from Baseline to Culture Conversion in Study 212

Statistic	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Number of Patients Assessed	224	112
Number of Converters	65 (29.0%)	10 (8.9%)
Number of Patients Censored	159 (71.0%)	102 (91.1%)
Overall Time to Culture Conversion in Months:		
Mean (SD)	2.724 (1.4614)	3.502 (1.2660)
Median	3.734	3.914
Min, Max	0.00, 4.77	0.00, 8.13
Kaplan-Meier Estimate		
1st quartile (95% CI)	2.07 [1.78, 3.78]	NE
Median (95% CI)	NE [4.14, NE]	NE
3rd quartile (95% CI)	NE	NE
Cox Proportional Hazards Model[a]		
Hazard Ratio (95% CI)	3.90 [2.00, 7.60]	
P-value	<0.0001 (nominal)	

a Results of hazard ratio, its CI and P-value are obtained from Cox regression model with treatment, stratification factors as covariates that include smoking status and prior multidrug regimen use. A hazard ration > 1 indicates that patients receiving ALIS + Multidrug Regimen are more likely to have culture conversion compared to those receiving Multidrug Regimen Alone after adjusting for baseline smoking status and prior multidrug regimen use.
ALIS = amikacin liposome inhalation suspension (590 mg); CI = confidence interval; ITT = intent-to-treat; Max = maximum; Min = minimum; NE = not estimable; SD = standard deviation.

6.2.5.3 Change from Baseline in St. George's Respiratory Questionnaire (SGRQ) at Month 6

There was a numerical difference in favor of the Multidrug Regimen Alone arm in the change from Baseline in total SGRQ score at Month 6 compared with the ALIS + Multidrug Regimen arm (Table 24); however, the difference is not statistically significant given its position in the hierarchical evaluation of endpoints. Further, the MCID of 4 was not met (least squares mean [SE] difference: 3.800 [1.5919]; 95% CI: [0.666, 6.935]; P=0.0177) suggesting that the addition of ALIS to Multidrug Regimen is unlikely to add significantly to a patient's treatment burden.

Table 24: Change from Baseline in St. George's Respiratory Questionnaire (SGRQ) in Study 212 (ITT Population)

Time Point Statistics	ALIS + Multidrug Regimen (N=224)	Multidrug Regimen Alone (N=112)
Baseline[a]		
n	189	105
Mean (SD)	37.929 (21.4294)	38.506 (21.4943)
Median	36.670	38.100
Min, Max	0.76, 85.75	1.05, 89.13
Month 6		
n	161	103
Mean (SD)	38.838 (22.6287)	37.050 (23.8588)
Median	36.900	35.180
Min, Max	2.12, 87.28	0.00, 85.10
Change from Baseline to Month 6[a]		
n	161	103
Mean (SD)	2.059 (13.5370)	-1.331 (11.6768)
Median	1.800	-1.090
Min, Max	-45.33, 47.59	-28.19, 37.25
Change from Baseline to Month 6[b]		
LS Mean (SE)	4.182 (2.0401)	0.382 (2.2138)
95% CI of LS Mean	0.167, 8.197	-3.975, 4.739
LS Mean (SE) difference (ALIS + Multidrug Regimen – Multi Drug Regimen Alone)	**3.800 (1.5919)**	
95% CI of LS Mean difference (ALIS + Multidrug Regimen – Multidrug Regimen Alone)	0.666, 6.935	
Two-sided P-value of LS Mean difference (ALIS + Multidrug Regimen – Multidrug Regimen Alone)	**0.0177**	

Note: CI = Confidence Interval, LS = Least Squares, MMRM = Mixed Model Repeated Measures, SE = Standard Error.
Note: For baseline, n is the number of patients with a baseline score and at least one post-baseline score. For Month 6, n is the number of patients with a baseline score and a post-baseline score at the summarized visit.
a Baseline is defined as the last non-missing value prior to first dose of study drug.
b Statistics are obtained from the MMRM analysis of change from baseline to Months 6 which includes treatment, month, the treatment-by-month interaction, and the combination of smoking status and prior multidrug regimen as fixed factors, the baseline score as a covariate and the baseline score-by-month interaction.

6.2.6 Exploratory Endpoints

6.2.6.1 Change from Baseline in 6 Minute Walk Test (6MWT) at Month 6 for Converters vs Non-converters for All Patients

The analysis of change from Baseline to Month 6 in the 6MWT distance using analysis of covariance (ANCOVA) for converters versus non-converters in the ITT population is presented in Table 25. There was a numeric difference in favor of converters in the change from Baseline to Month 6 in the 6MWT distance compared to non-converters in the ITT population (LS mean difference [SE] 24.71 [9.623] meters; 95% CI: 5.76, 43.66; P=0.0108).

Table 25: Change from Baseline to Month 6 in 6MWT Distance by Converter Status for All Patients in Study 212 (ITT Population)

Time Point Statistic	Converter (N=75)	Non-Converter (N=261)
Baseline[a]		
n	73	203
Mean (SD)	457.9 (120.64)	427.7 (120.53)
Median	471.0	447.0
Min, Max	111, 800	72, 720
Month 6		
n	70	191
Mean (SD)	469.3 (114.24)	424.9 (131.52)
Median	491.5	445.0
Min, Max	60, 648	3, 708
Change from Baseline to Month 6		
n	70	191
Mean (SD)	16.9 (58.62)	-7.4 (72.32)
Median	4.0	-5.0
Min, Max	-102, 170	-376, 255
Change from Baseline to Month 6[b]		
LS Mean (SE)	16.83 (13.717)	-7.88 (11.464)
95% CI of LS Mean	-10.18, 43.84	-30.46, 14.69
LS Mean (SE) difference (Converter - Non-Converter)	**24.71 (9.623)**	
95% CI of LS Mean difference (Converter - Non-Converter)	5.76, 43.66	
Two-sided P-value of LS Mean difference (Converter - Non-Converter)	**0.0108**	

a Baseline is defined as the last non-missing value prior to first dose of study drug.
b Statistics are obtained from the ANCOVA model of change from baseline to each visit in 6MWT which includes converter status, the combination of smoking status and prior multidrug regimen as fixed factors, and the baseline 6MWT distance as a covariate.
Note: For baseline, n is the number of patients with a baseline score and at least one post-baseline score. For Month 4 and Month 6, n is the number of patients with a baseline score and a post-baseline score at the summarized visit.
6MWT = 6-minute walking test; ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; SE = standard error.

6.2.6.2 Change from Baseline in 6 Minute Walk Test (6MWT) at Month 6 for Converters vs Non- converters in the ALIS + Multidrug Regimen Arm

The analysis of change from Baseline to Month 6 in the 6MWT distance using ANCOVA for converters versus non-converters within the ALIS + Multidrug Regimen arm is presented in Table 26. There was a numerical difference in favor of converters in the change from Baseline in the 6MWT distance at Month 6 compared with non-converters within the ALIS + Multidrug Regimen arm (P=0.0047).

Table 26: Change from Baseline to Month 6 in 6MWT Distance by Converter Status for ALIS + Multidrug Regimen in Study 212 (ITT Population)

Time Point Statistic	Converter (N=65)	Non-Converter (N=159)
Baseline[a]		
n	63	109
Mean (SD)	460.5 (108.33)	430.5 (124.44)
Median	471.0	447.0
Min, Max	111, 682	120, 720
Month 6		
n	61	98
Mean (SD)	475.3 (109.33)	422.5 (132.01)
Median	493.0	441.5
Min, Max	60, 648	53, 708
Change from Baseline to Month 6		
n	61	98
Mean (SD)	15.3 (60.73)	-14.6 (69.08)
Median	4.0	-11.5
Min, Max	-102, 170	-376, 186
Change from Baseline to Month 6[b]		
LS Mean (SE)	20.66 (15.629)	-10.52 (13.652)
95% CI of LS Mean	-10.22, 51.53	-37.49, 16.45
LS Mean (SE) difference (Converter - Non-Converter)	**31.18 (10.870)**	
95% CI of LS Mean difference (Converter - Non-Converter)	9.71, 52.65	
Two-sided P-value of LS Mean difference (Converter - Non-Converter)	**0.0047**	

a Baseline is defined as the last non-missing value prior to first dose of study drug.
b Statistics are obtained from the ANCOVA model of change from baseline to each visit in 6MWT which includes converter status, the combination of smoking status and prior multidrug regimen as fixed factors, and the baseline 6MWT distance as a covariate.
Note: For baseline, n is the number of patients with a baseline score and at least one postbaseline score. For Month 4 and Month 6, n is the number of patients with a baseline score and a postbaseline score at the summarized visit.
6MWT = 6-minute walk test; ALIS = amikacin liposome inhalation suspension (590 mg); ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; SE = standard error.

6.2.6.3 Change from Baseline in 6 Minute Walk Test (6MWT) at Month 6 for Converters vs Non-converters in the Multidrug Regimen Alone Arm

The analysis of change from Baseline to Month 6 in the 6MWT distance using ANCOVA for converters versus non-converters within the Multidrug Regimen Alone arm is presented in Table 27. Although the point estimate of the LS Mean difference in the change from Baseline to Month 6 in the 6MWT distance between converters and non-converters in the Multidrug Regimen Alone arm (25.18 [25.674] meters [95% CI: -25.78, 76.14]) was similar to that seen in the overall population (24.71 meters), the p-value associated with this finding was 0.3292. This may be due to the small sample size (n=10 converters).

Table 27: Analysis of Covariance of Change from Baseline in the 6MWT Distance by Converter Status for Multidrug Regimen Alone in Study 212 (ITT Population)

Time Point Statistic	Converter (N=10)	Non-Converter (N=102)
Baseline[a]		
n	10	94
Mean (SD)	441.0 (187.70)	424.5 (116.41)
Median	470.0	444.5
Min, Max	195, 800	72, 645
Month 6		
n	9	93
Mean (SD)	428.8 (144.19)	427.4 (131.67)
Median	430.0	450.0
Min, Max	243, 581	3, 695
Change from Baseline to Month 6		
n	9	93
Mean (SD)	27.7 (42.72)	0.2 (75.21)
Median	2.0	0.0
Min, Max	-9, 111	-220, 255
Change from Baseline to Month 6[b]		
LS Mean (SE)	18.15 (31.443)	-7.03 (20.923)
95% CI of LS Mean	-44.27, 80.56	-48.57, 34.50
LS Mean (SE) difference (Converter - Non-Converter)	**25.18 (25.674)**	
95% CI of LS Mean difference (Converter - Non-Converter)	-25.78, 76.14	
Two-sided P-value of LS Mean difference (Converter - Non-Converter)	**0.3292**	

a Baseline is defined as the last non-missing value prior to first dose of study drug.
b Statistics are obtained from the ANCOVA model of change from baseline to each visit in 6MWT, which includes converter status, the combination of smoking status and prior multidrug regimen as fixed factors, and the baseline 6MWT distance as a covariate.
Note: For baseline, n is the number of patients with a baseline score and at least one post-baseline score. For Month 4 and Month 6, n is the number of patients with a baseline score and a post-baseline score at the summarized visit.
6MWT = 6-minute walk test; ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; SE = standard error.

6.2.7 Amikacin Resistance

The development of amikacin resistance has been linked to mutations in the rrs gene of the rrn operon that results in changes to the 16S rRNA binding site of aminoglycosides. A correlation between acquired amikacin resistance during amikacin-therapy, treatment failure, and an amikacin MIC of >64 µg/mL has been reported in the literature (Brown-Elliott et al. 2013).

In Study 212, MAC isolates with an amikacin MIC of >64 µg/mL were seen in 28/336 (8.3%) patients overall (24/224 [10.3%] in the ALIS + Multidrug Regimen arm and 4/112 [2.7%] in the Multidrug Regimen Alone arm).

Of the 24 patients randomized to the ALIS + Multidrug Regimen arm:

- 1 patient had an isolate with an amikacin MIC >64 µg/mL at Baseline

- 23 patients had isolates with an amikacin MIC >64 µg/mL post-baseline
  - Culture Conversion:
    - 1 patient achieved culture conversion at Month 2 after having a MAC isolate with amikacin MIC >64 µg/mL
    - 1 patient achieved culture conversion at Month 1, but subsequent developed an isolate with amikacin MIC >64 µg/mL at Month 5
  - Persistence of MIC >64 µg/mL:
    - 18/23 (78.3%) patients had persistent MAC isolates with MIC >64 µg/mL
    - 5/23 (21.7%) patients subsequently had MAC isolates that reverted back to MIC ≤64µg/mL

Of the 4 patients randomized to the Multidrug Regimen Alone arm:

- 1 patient had an isolate with an amikacin MIC >64 µg/mL at Baseline
- 3 patients had isolates with an amikacin MIC >64 µg/mL at Baseline
  - Culture Conversion:
    - No patients achieved culture conversion
  - Persistence of MIC >64 µg/mL
    - 1/3 (33.3%) patients had persistent MAC isolates with MIC >64 µg/mL
    - 2/3 (66.7%) patients subsequently had MAC isolates that reverted back to MIC ≤64µg/mL

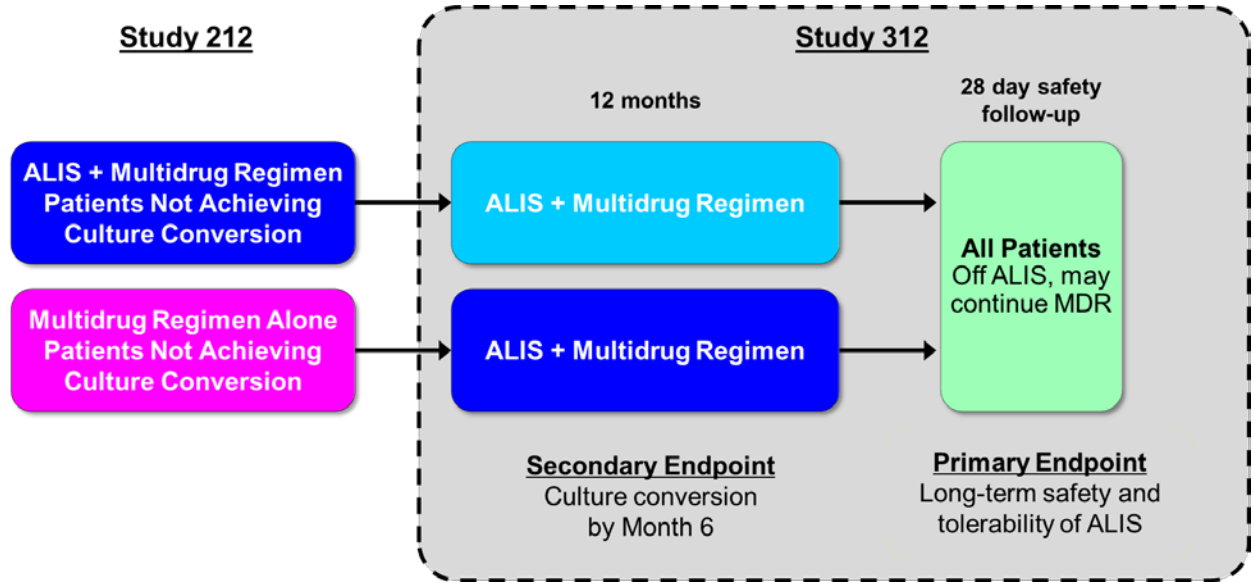
6.3 Study 312

6.3.1 Overview

Study 312 is an open-label study in patients with NTM lung infections who did not respond to treatment in Study 212. Patients participating in Study 212 who had not achieved the protocol definition of culture conversion (3 consecutive monthly negative sputum cultures) or who had experienced a relapse or recurrence (agar positive or more than 2 consecutive broth positive results after culture conversion had occurred) by Month 6, as determined by their sputum culture results from Day 1 through Month 6 and confirmed at their scheduled Month 8 visit, were eligible to participate in Study 312 (Figure 20). Study 312 therefore provides long-term data in patients taking ALIS for up to 20 months.

The primary objective of Study 312 is to evaluate the long-term safety and tolerability of ALIS 590 mg QD added to Multidrug Regimen for up to 12 months in patients who failed to convert with prolonged Multidrug Regimen and failed to convert in Study 212. Secondary endpoints included culture conversion by Month 6 and time to culture conversion.

Figure 20: Design of Study 312



MDR=Multidrug Regimen

6.3.2 Demographic and Baseline Characteristics

Since this study only enrolled patients from Study 212, the demographic and disease characteristics for patients in Study 312 are consistent with baseline characteristics in Study 212 (Table 28). Overall, the mean age of patients was 64.7 years. The majority of patients were female (63.9%), White (61.7%), and Non-Hispanic or Latino (88.7%). The highest percentage of patients were from the US (39.1%).

Table 28: Summary of Patient Demographics and Baseline Characteristics in Study 312 (Safety Population)

	Prior ALIS + Multidrug Regimen (N=59)	Prior Multidrug Regimen Alone (N=74)
Age (years)		
n	59	74
Mean (SD)	65.1 (8.64)	64.4 (10.35)
Median	65.0	65.5
Min, Max	46, 85	33, 83
Region		
United States	19 (32.2%)	33 (44.6%)
Asia (excluding Japan)	3 (5.1%)	3 (4.1%)
Japan	17 (28.8%)	11 (14.9%)
Rest of the World	20 (33.9%)	27 (36.5%)
Sex		
Male	18 (30.5%)	30 (40.5%)
Female	41 (69.5%)	44 (59.5%)
Ethnicity		
Hispanic or Latino	6 (10.2%)	4 (5.4%)
Not Hispanic or Latino	52 (88.1%)	66 (89.2%)
Not recorded	0	4 (5.4%)
Unknown	1 (1.7%)	0
Race[a]		
American Indian or Alaska Native	0	0
Asian: Japanese	17 (28.8%)	11 (14.9%)
Asian: Other	6 (10.2%)	6 (8.1%)
Black or African American	1 (1.7%)	3 (4.1%)
Native Hawaiian or Other Pacific Islander	0	0
White	33 (55.9%)	49 (66.2%)
Multiracial	1 (1.7%)	0

a Patients who marked more than 1 race were classified as multiracial and counted only once.
Note: Unless otherwise mentioned, for percentages calculations, the number of Safety patients is used as the denominator.
ALIS = amikacin liposome inhalation suspension (590 mg); Max = maximum; Min = minimum; SD = standard deviation.

6.3.3 Patient Disposition

At the time of data cutoff for the interim report, a total of 133 patients were enrolled in Study 312 (74 from the prior Study 212 Multidrug Regimen Alone arm; 59 in the prior ALIS + Multidrug Regimen arm) with enrollment still ongoing. Overall, 24 (18%) patients completed treatment as defined in the protocol (20.3% prior Multidrug Regimen Alone arm; 15.3% prior ALIS + Multidrug Regimen arm), 28 (21.1%) patients discontinued treatment prior to completion (20.3% prior Multidrug Regimen Alone arm; 22% prior ALIS + Multidrug Regimen arm), and the remainder of patients (n=81) were continuing treatment in the study.

6.3.4 Secondary Endpoints

6.3.4.1 Proportion of Patients Achieving Culture Conversion by Month 6

Patients entering Study 312 from the prior Study 212 Multidrug Regimen Alone arm represent a population of patients with MAC lung disease who failed prior treatment and received ALIS for the first time in Study 312. Therefore, this population is analogous to the population of patients in Study 212 who were randomized to receive ALIS in Study 212. As shown in Table 29, 23% of these patients attained culture conversion by 6 months. These findings are similar to the 29% of patients achieving culture conversion in the ALIS + Multidrug treatment arm in Study 212, providing further evidence of culture conversion rates with ALIS added to Multidrug Regimen. Additionally, the interim results of Study 312 demonstrate the potential benefits from continuing ALIS in patients that do not achieve culture conversion by Month 4. The fact that 5.1% of patients in the prior ALIS + Multidrug Regimen group converted in Study 312 suggests that patients may convert with continued treatment.

Table 29: Analysis of Culture Conversion by Month 6 in Study 312 (Safety Population)

Statistic	Prior ALIS + Multidrug Regimen (N=59)	Prior Multidrug Regimen Alone (N=74)
Number of Patients Assessed	49	62
Converter	3 (5.1%)	17 (23.0%)
Non-Converter	46 (78.0%)	45 (60.8%)
Unassessable	10 (16.9%)	12 (16.2%)

Note: Unassessable patients have less than 3 monthly sputum samples (ie, have not yet reached Month 2) at time of analysis.
Note: Patients with 3 monthly sputum samples (ie, reached Month 2 or beyond) are assessed for converter status; non-converters may still convert after the data cutoff date.
Note: Converters are defined as patients who have 3 consecutive MAC-negative sputum cultures within the first 6 months (inclusive) of study.
ALIS = amikacin liposome inhalation suspension (590 mg); MAC = *Mycobacterium avium* complex.

6.3.4.2 Time from Baseline to Culture Conversion

For the prior Multidrug Regimen Alone arm, the mean time to culture conversion was 2.9 months (Table 30). Patients in the prior ALIS + Multidrug Regimen arm (with approximately 8 months of prior treatment in Study 212) had a mean time to culture conversion of 3.6 months.

Table 30: Summary of Time to Culture Conversion in Study 312 (Safety Population)

Statistic	Prior ALIS + Multidrug Regimen (N=59)	Prior Multidrug Regimen Alone (N=74)
Number of Patients Assessed	59	74
Number of Converters	3 (5.1%)	17 (23.0%)
Number of Patients Censored	56 (94.9%)	57 (77.0%)
Overall Time to Culture Conversion in Months:		
Mean (SD)	3.5343.560 (1.00800.9236)	2.8942.908 (1.46394392)
Median	3.914	3.882
Min, Max	0.00, 4.34	0.00, 4.61

ALIS = amikacin liposome inhalation suspension (590 mg); Max = maximum; Min = minimum; SD = standard deviation.

6.3.4.3 Change from Baseline in St. George's Respiratory Questionnaire at Month 6

Patients in the prior Multidrug Regimen Alone arm showed an increase in mean (SD) total SGRQ score (3.96 [12.13]); however, the magnitude of this increase was relatively small and did not meet the MCID of 4. Patients in the prior ALIS + Multidrug Regimen arm showed relatively no change in total SGRQ score (-0.028 [7.5]), suggesting there may be stabilization in SGRQ score beyond 6 months of treatment with ALIS.

6.4 Study 112

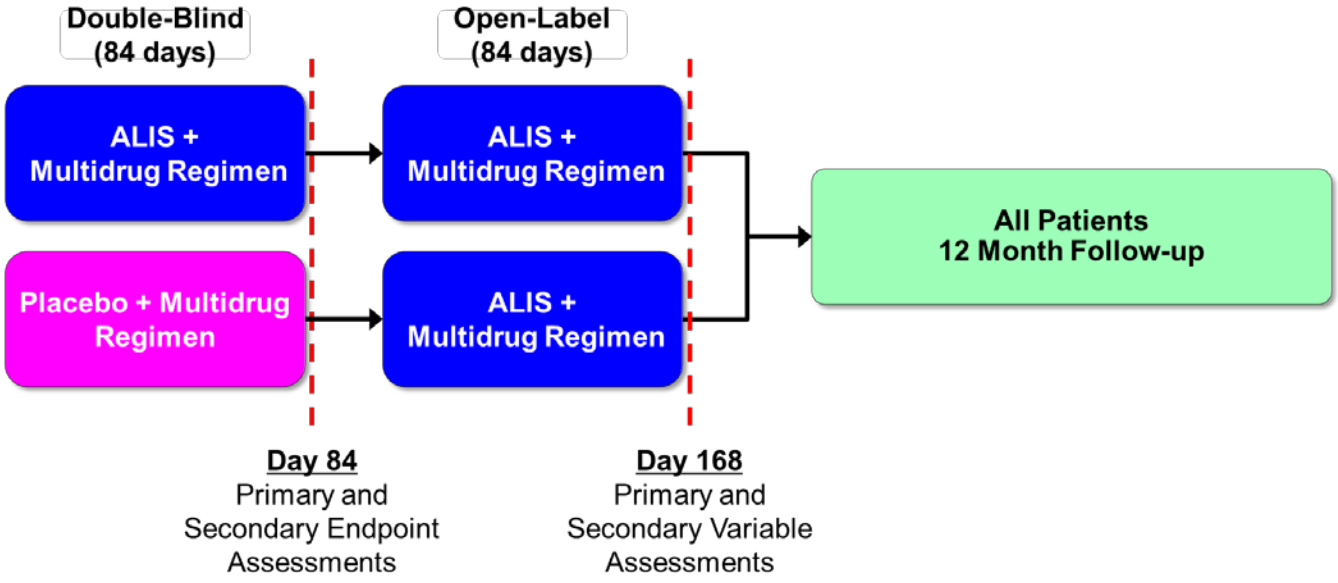
6.4.1 Study Design

Study 112 was a Phase 2, randomized, double-blind, placebo-controlled study of ALIS for inhalation in patients with treatment-resistant NTM lung disease. The primary objective of this study was to evaluate the efficacy of ALIS 590 mg QD when added to Multidrug Regimen compared to placebo added to Multidrug Regimen. The primary efficacy endpoint in this study was the change from Baseline to Day 84 in mycobacterial burden, assessed using an SQS.

Patients were randomized (1:1) in the double-blind phase to receive either ALIS 590 mg QD or placebo QD (empty liposome) added to Multidrug Regimen for 84 days (Figure 21). Patients were stratified based on the presence or absence of CF and predominant mycobacterial species in sputum culture at screening (MAC or *M. abscessus*). At the completion of the double-blind phase, patients had the option to continue in the open-label phase of the study and receive add-on ALIS 590 mg QD for an additional 84 days.

All patients were required to complete a 28-day safety follow-up visit after the EOT visit. Patients completing this study had the option to enroll in a long-term safety follow-up and returned to the study site at 12 months after the last dose of study drug (either after completing the randomized double-blind phase or the open-label phase).

Figure 21: Design of Study 112



6.4.1.1 Inclusion and Exclusion Criteria

A full list of inclusion and exclusion criteria is in Appendix 10.2.

Key inclusion criteria included the following:

1. Male or female patients, ages 18 years to 85 years.
2. 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.
3. 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.
4. Positive sputum culture obtained during the screening period with either MAC or *M. abscessus* or mixed infection with 1 dominant species.
5. Adherence to a standard of care Multidrug Regimen for at least 6 months prior to screening with persistently positive mycobacterial cultures.

6.4.1.2 Statistical Analyses

For the double-blind phase of the study, all efficacy analyses were performed using the modified intent-to-treat (mITT) population, which was defined as all randomized patients who received at least 1 dose of study drug.

The primary efficacy analysis of change from baseline on the SQS for mycobacterial culture in the ALIS arm compared to placebo at Day 84 was conducted in the mITT population using a stratified Wilcoxon rank sum test to compare the treatment arms at a 2-sided significance level of 0.05, adjusting for the randomization strata (presence/absence of CF and MAC versus *M. abscessus*).

Deaths were scored as +7 steps. Note that the worst possible outcome using the 7-point SQS would be +6 (ie, a change from Step 1 to Step 7). Therefore, the imputation of +7 for missing values due to death was more severe than could be applied for any patient with Baseline and Day 84 samples.

Secondary endpoints were analysed using a stratified Cochran-Mantel-Haenszel test of treatment arm adjusting for the randomization strata. Time to negative sputum sample during the 84-day double-blind phase was summarized using Kaplan-Meier estimation using a stratified log-rank test of treatment arm adjusting for the randomization strata.

6.4.2 Demographic and Baseline Characteristics

Patient baseline demographics and disease characteristics were comparable between treatment groups for the mITT population (Table 31). Most patients were White (92.1%), the majority were female (87.6), and the mean age was 58.5 years. There were no noteworthy differences between treatment groups in lung function parameters at baseline. The proportions of patients with negative sputum cultures at baseline were similar for the ALIS and placebo groups (3/44 [6.8%] and 3/45 [6.7%], respectively).

Approximately 19% of patients had CF, and 64% had predominately MAC lung infection and 36% had predominately *M. abscessus* lung infection (Table 32).

Table 31: Demographics and Baseline Characteristics in Study 112 (mITT Population)

	ALIS 590 mg QD (N=44)	Placebo (N=45)	Total (N=89)
Race/Ethnicity, n (%)			
White (not of Hispanic origin)	42 (95.5)	40 (88.9)	82 (92.1)
Hispanic	0	2 (4.4)	2 (2.2)
African	0	1 (2.2)	1 (1.1)
Asian	2 (4.5)	2 (4.4)	4 (4.5)
Sex, n (%)			
Male	6 (13.6)	5 (11.1)	11 (12.4)
Female	38 (86.4)	40 (88.9)	78 (87.6)
Baseline age (Mean [SD] years)	58.0 (16.61)	59.1 (15.20)	58.5 (15.83)
Baseline height (Mean [SD] cm)	165.4 (7.63)	164.2 (9.12)	164.8 (8.39)
Baseline weight (Mean [SD] kg)	59.71 (10.132)	60.15 (15.373)	59.93 (12.976)
Baseline FEV_1 (Mean [SD] L)	1.730 (0.6910)	1.637 (0.5168)	1.683 (0.6075)
Baseline FEV_1 % predicted (Mean [SD]	63.56 (21.339)	62.56 (17.168)	63.06 (19.239)
Baseline FEV_1 % predicted by stratum, n (%)			
25%-50%	14 (31.8)	11 (24.4)	25 (28.1)
>50%-75%	18 (40.9)	25 (55.6)	43 (48.3)
>75%	12 (27.3)	9 (20.0)	21 (23.6)
Baseline mycobacterial load			
Culture negative (confirmed with no growth in liquid medium)	3 (6.8)	3 (6.7)	6 (6.7)
Growth in liquid medium only (liquid positive)	3 (6.8)	3 (6.7)	6 (6.7)
1-49 colonies (manual count on agar) (agar positive)	17 (38.6)	10 (22.2)	27 (30.3)
1+ (50-100 colonies)	2 (4.5)	4 (8.9)	6 (6.7)
2+ (>100-200 colonies)	2 (4.5)	2 (4.4)	4 (4.5)
3+ (>200-500 colonies)	3 (6.8)	4 (8.9)	7 (7.9)
4+ (>500 colonies)	14 (31.8)	19 (42.2)	33 (37.1)

BMI = body mass index; FEV_1 = forced expiratory volume in 1 second; FEV_1 % predicted = forced expiratory volume in 1 second % predicted; mITT = modified intent-to-treat; QD = once daily

Table 32: Stratification at Screening for Randomization in Study 312 (mITT Population)

	ALIS 590 mg QD (N=44)	Placebo (N=45)	Total (N=89)
Predominantly MAC lung disease	29 (65.9)	28 (62.2)	57 (64.0)
Predominantly *M. abscessus* lung disease	15 (34.1)	17 (37.8)	32 (36.0)
Presence of CF	8 (18.2)	9 (20.0)	17 (19.1)
Absence of CF	36 (81.8)	36 (80.0)	72 (80.9)
Predominantly MAC lung disease and presence of CF	2 (4.5)	1 (2.2)	3 (3.4)
Predominantly MAC lung disease and absence of CF	27 (61.4)	27 (60.0)	54 (60.7)
Predominantly *M. abscessus* lung disease and presence of CF	6 (13.6)	8 (17.8)	14 (15.7)
Predominantly *M abscessus* lung disease and absence of CF	9 (20.5)	9 (20.0)	18 (20.2)

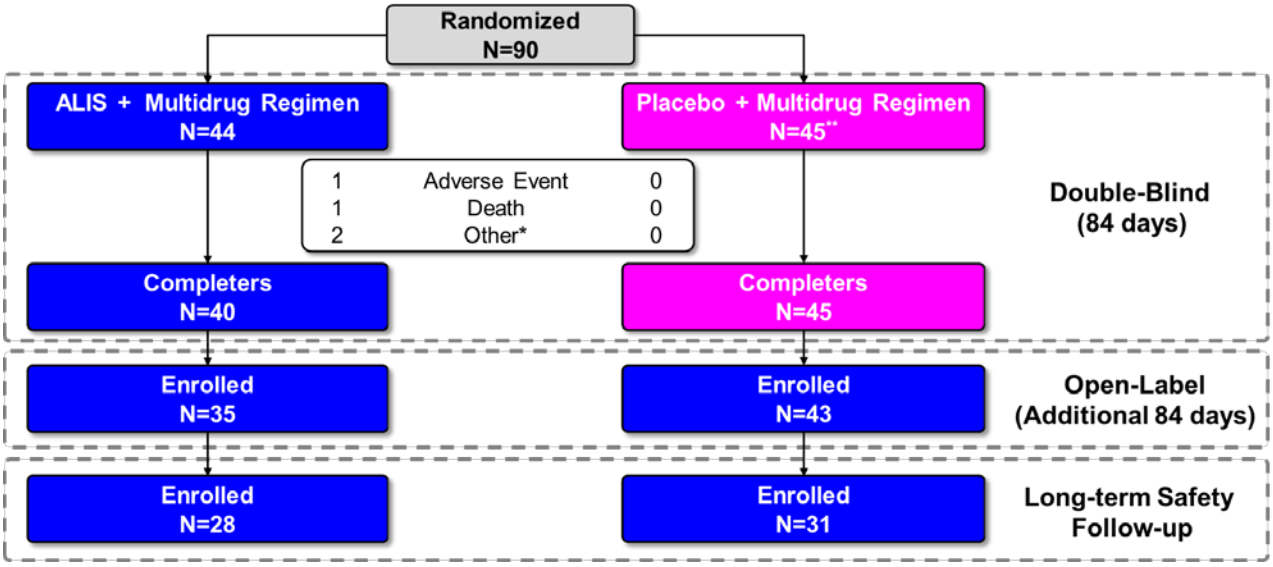
6.4.3 Patient Disposition

Ninety patients were randomized into the double-blind phase (Figure 22). One patient randomized to placebo met exclusion criteria after randomization and did not receive study drug; therefore, 89 patients were included in the mITT/safety population for the double-blind phase.

Approximately 10% of the patients in the mITT/safety population did not complete dosing per protocol; these patients were in the ALIS group (9/44 patients, 20.5%). Most patients completed all study visits, with 4/44 (9.1%) patients in the ALIS group and 0/45 (0%) patients in the placebo group discontinuing the study early.

Of the 85 patients who completed dosing in the double-blind phase, 78 patients enrolled in the open-label phase of the study.

Figure 22: Patient Disposition in the Double-blind Phase in Study 112 (mITT Population)



*Other includes categories: withdrawal of consent and lost to follow-up.
**1 patient met exclusion criterion after randomization and did not receive study drug

6.4.4 Primary Endpoint – Mycobacterial Density on the Full Semi-Quantitative Scale (SQS)

The analysis of the primary efficacy endpoint of the change from Baseline at Day 84 on the SQS for mycobacterial culture demonstrated a trend in favor of the ALIS group compared to placebo, but this difference was not statistically significant (P=0.072).

6.4.5 Secondary Endpoints

6.4.5.1 Proportion of Patients with Negative Sputum Culture at Day 84

As shown in Table 33, a numerically greater proportion of patients in the ALIS group achieved a negative sputum culture (14/41; 34.1%) compared to patients in the placebo group (4/45; 8.9%) at Day 84 (nominal P=0.003). A numerical difference between treatments in patients achieving negative sputum culture status was also seen at Day 56 (P=0.038).

Table 33: Proportion of Patients with Negative Sputum Culture at Day 84 in Study 112 (mITT Population)

	Number (%) of Patients			
	ALIS 590 mg QD (N=44)	**Placebo (N=45)**	**Overall (N=89)**	**P-value[a]**
Baseline	5/44 (11.4)	5/45 (11.1)	10/89 (11.2)	
Day 28	10/42 (23.8)	5/45 (11.1)	15/87 (17.2)	0.100
Day 56	11/42 (26.2)	4/45 (8.9)	15/87 (17.2)	0.038
Day 84	14/41 (34.1)	4/45 (8.9)	18/86 (20.9)	0.003

mITT = modified intent-to-treat; NTM = nontuberculous mycobacterium; QD = once daily.
[a] *P*-value for stratified Cochran-Mantel-Haenszel test of treatment arm adjusting for the randomization strata.
Note: Missing values were excluded under the assumption of missing at random, for which missing baseline or post-baseline values were excluded but all non-missing data were included (ie, exclusion was not at patient level, but rather at time point level).

6.4.5.2 Time from Baseline to Negative Sputum Culture

A numerical difference was observed in time to a negative NTM culture during the 84-day double-blind treatment phase for the mITT population in favor of the ALIS group compared with the placebo group (Table 34). The median time to a negative NTM culture was not able to be estimated for either treatment group.

Table 34: Time from Baseline to Negative NTM Culture in Study 112 (mITT Population)

	ALIS 590 mg QD (N=44)	**Placebo (N=45)**	**Total (N=89)**
Censoring at date of last contact or at initiation of open-label ALIS			
Number of patients with the event	11	2	13
Number censored	33	43	76
Kaplan-Meier median	NA	NA	NA
Cox proportional hazards model[a]			
p-value for treatment effect			0.0244
Hazard ratio (ALIS: placebo)			5.68
95% CI for hazard ratio			(1.25, 25.79)
p-value for stratified log rank test of treatment arm adjusting for randomization strata			0.0129

CI = confidence interval; mITT = modified intent-to-treat; NA = not applicable; NTM = nontuberculous mycobacterium; QD = once daily.
Note: Patients who had negative NTM cultures at baseline were censored.
[a] The Cox proportional hazards model has effects for treatment arm and for the randomization strata, with a test of the treatment effect at a 2-sided significance level of 0.05.

6.4.6 Exploratory Endpoints

6.4.6.1 Change from Baseline to 6 Minute Walk Test (6MWT) at Day 84

At Day 84, patients in the ALIS group had a mean increase from baseline of 23.9 meters on the 6MWT distance, while patients treated with placebo had a mean decrease of 25.0 meters (Table 35). The difference between the treatment groups was statistically significant in favor of the ALIS arm using the Wilcoxon rank sum analysis (P=0.0134) and using ANCOVA (P=0.0090). Positive results were surprising given the short duration of the study and prompted inclusion of the 6MWT distance as a key secondary endpoint in Study 212.

Table 35: Change from Baseline in 6MWT Distance at Day 84 in Study 112 (mITT Population)

	ALIS 590 mg QD (N=44)	Placebo (N=45)	Total (N=89)
Baseline (meters)			
Mean (SD)	441.636 (133.6408)	441.778 (111.5671)	441.708 (122.2726)
Median	435.000	457.000	456.000
Min, max	53.00, 660.00	120.00, 751.00	53.00, 751.00
Change at Day 84 (meters)			
Mean (SD)	23.895 (66.7046)	-25.032 (100.2323)	-2.632 (89.4425)
Median	12.500	-6.000	5.000
Min, max	-99.00, 264.00	-387.00, 220.00	-387.00, 264.00
p-value for stratified Wilcoxon rank sum test of treatment arm adjusting for the randomization strata			0.0134
p-value from treatment effect in ANCOVA model			0.0090

mITT = modified intent-to-treat; SD = standard deviation
Note: Missing values are excluded under the assumption of missing at random, for which missing baseline or post-baseline values are excluded but all non-missing data are included (ie, exclusion is not at patient-level, but rather at timepoint level). Baseline is defined as the measurement prior and closest to the administration of the first dose of study drug (ALIS or placebo). The ANCOVA model includes effects for treatment, baseline walk distance, and the randomization strata.

6.4.6.2 Culture Conversion at Day 168 and Durability at 12-month follow-up

Culture conversion by the end of the open-label phase in the main study (Day 168) was assessed as a post hoc analysis, using the definition of 3 consecutive negative monthly samples. Overall, 20 out of 89 patients (22.5%) achieved culture conversion. In addition to the 20 patients who converted by Day 168, 3 patients met the definition of culture conversion at the Day 28 off-ALIS treatment follow-up (all of which were randomized to the placebo arm during the double-blind phase, and their first negative culture occurred during the open-label phase at Day 140). Of the 23 total converters, 17 completed the 12-month follow-up visit, and 14 (82.4%) demonstrated durability of their negative culture result following 12 months.

6.4.6.3 Change from Baseline in St. George's Respiratory Questionnaire (SGRQ) at Day 84

Change in quality of life from baseline to Day 84 and to Day 168 was assessed using the SGRQ in non-CF patients only. There was no statistically significant difference between treatment groups based on the change from baseline in SGRQ total quality of life score from baseline through Day 84 of the double-blind phase or through the open-label phase (P=0.6080 and P=0.5894, respectively).

6.5 Summary of Efficacy Findings

Study 212 was designed to assess the efficacy of ALIS + Multidrug Regimen compared with Multidrug Regimen Alone on the surrogate endpoint of culture conversion by Month 6, with a subsequent analysis of ALIS + Multidrug Regimen compared with Multidrug Regimen Alone on durable culture conversion to support full approval. Results from the initial analysis of Study 212 show that treatment of ALIS + Multidrug Regimen led to a significantly higher percentage of patients achieving culture conversion by Month 6 than patients receiving Multidrug Regimen Alone ($P<0.0001$). The culture conversion rates in the ALIS + Multidrug Regimen arm and the Multidrug Regimen Alone arm were 29.0% and 8.9%, respectively. The findings in Study 212 are supported by the initial analysis from the single-arm open-label Study 312 evaluating culture conversion by Month 6, as well as the post hoc analysis of Study 112 evaluating culture conversion by Day 168. Thus, ALIS has been shown to have an effect on a surrogate endpoint reasonably likely to predict clinical benefit and has also been shown to have a meaningful advantage over available therapy (ie, Multidrug Regimen). These results from clinical investigations together with supportive evidence of efficacy provided by nonclinical *in vitro* and *in vivo* studies meet the statutory substantial evidence standard for effectiveness.

The initial analysis of Study 312 demonstrates that culture conversion rates are consistent with the primary outcome of Study 212, particularly in patients initiating add-on ALIS. Further, the fact that there were patients in the prior ALIS + Multidrug Regimen group of Study 212 who converted in Study 312 suggests that patients who do not achieve culture conversion by Month 4 may go on to convert with continued treatment. Across the 3 NTM clinical studies, ALIS administered with Multidrug Regimen resulted in consistent MAC culture conversion rates.

The study design elements in this NTM clinical program provide the foundation to support the microbiological endpoint of culture conversion by Month 6 as a surrogate reasonably likely to predict clinical benefit (durability of negativity 3 months post-treatment).

All 3 NTM studies showed that culture conversion occurred within the first few months of treatment. In the subset of patients who converted in the pivotal Phase 3 study (Study 212), patients who received ALIS + Multidrug Regimen achieved culture conversion approximately 1 month earlier on average than patients who received Multidrug Regimen Alone (mean 2.7 months versus 3.5 months, respectively). Time to culture conversion in patients in the prior Multidrug Regimen Alone group, initiating ALIS in Study 312, was similar to the mean time to culture conversion in the ALIS + Multidrug Regimen arm in Study 212 (mean 2.9 months).

The post hoc preliminary results for durability of negative cultures in Study 112 provide support for culture conversion by Month 6 as a surrogate for the confirmatory endpoint at 3 months off treatment. These early indicators are encouraging for the persistence of effect of ALIS in the treatment of NTM lung disease.

In Study 212, the 6MWT did not show a meaningful difference between treatment arms in change from baseline to Month 6. It is important to note that in a pre-specified exploratory analysis, improvements in the 6MWT were seen in patients who achieved culture conversion compared with patients who did not achieve culture conversion. The relationship between culture

conversion and improved 6MWT is an important factor in understanding the relationship between clinical benefit, as characterized by 6MWT, and sustained culture conversion. The consistent improvement in 6MWT at Month 6 in converters compared with non-converters provides evidence that culture conversion by Month 6, in addition to be a predictor of future clinical benefit (durable culture conversion), is also associated with contemporaneous functional benefit.

7 CLINICAL SAFETY

Summary

- The safety profile of ALIS when added to Multidrug Regimen is acceptable relative to Multidrug Regimen Alone given the significant risk of the disease, the inadequacy of current treatment approaches, and early indications of positive clinical outcomes from the use of ALIS, including a potential mortality benefit
- ALIS demonstrated a consistent safety profile across the populations studied in the clinical development program, minimizing the known systemic toxicities of IV amikacin
- AEs related to known amikacin toxicities such as nephrotoxicity and peripheral neuropathy were balanced between groups. An imbalance in ototoxicity was driven by higher tinnitus rates in patients treated with ALIS + Multidrug Regimen compared to Multidrug Regimen Alone
- There is a greater incidence of AEs when ALIS was added to combination antibiotic therapy, most events were mild to moderate, and most resolved without discontinuation
- The most common AEs for ALIS were respiratory in nature
- The rate of AEs leading to discontinuation of ALIS was relatively low, indicating that AEs were mostly tolerated and manageable
- Serious AEs were reported in a similar proportion of patients in each treatment arm (ALIS + Multidrug Regimen arm 20.2%, Multidrug Regimen Alone arm 17.9%) and were mostly respiratory in nature
- AEs leading to death occurred in both treatment arms (2.7% and 4.5% in the ALIS + Multidrug Regimen and Multidrug Regimen Alone arms, respectively) and were attributable to the underlying NTM infection

7.1 Treatment Exposure

A summary of the number of patients in the ALIS NTM clinical development program is presented in Table 36 below. A total of 428 patients were treated in the NTM studies, 388 of whom were treated with ALIS 590 mg QD + Multidrug Regimen, and 157 of whom were treated with Multidrug Regimen Alone during the controlled stages of the studies. The comparator group, Multidrug Regimen Alone, may have utilized an inactive comparator (ie, empty liposomes in Study 112) or no blinded comparator (Study 212). Of the 157 patients treated with Multidrug Regimen Alone, 117 entered an open-label phase or extension study and were among the 388 who received ALIS: 74 in Study 312 after having been treated with Multidrug Regimen Alone in Study 212, and 43 in the open-label extension of Study 112 after having been treated with Multidrug Regimen + empty liposomes in the double-blind phase of Study 112.

Table 36: Enumeration of Patients in the ALIS NTM Development Program

Study	ALIS			Comparator		Total
	Treatment	Dose (mg)	N	Treatment	N	N
Study 212	ALIS + Multidrug Regimen	590	223[a]	Multidrug Regimen Alone	112	335
Study 312	ALIS + Multidrug Regimen / ALIS + Multidrug Regimen	590	59	-		59
	Multidrug Regimen Alone/ ALIS + Multidrug Regimen	590	74	-		74
Study 112 (Blinded)	ALIS + Multidrug Regimen	590	44	Multidrug Regimen + empty liposomes	45	89
Study 112 (Open-Label)	ALIS + Multidrug Regimen / ALIS + Multidrug Regimen Multidrug Regimen Alone// ALIS + Multidrug Regimen Scintigraphy	590	35 43 4	-		82
Unique Patients NTM[b]	All ALIS	All	388	Comparator	157	428

[a] 224 patients were randomized. One patient immediately discontinued upon randomization without dosing and was excluded from the safety population.
b Study 112 patients with both NTM and CF are counted in both Unique NTM and Unique CF patient counts.
ALIS = amikacin liposome inhalation suspension; NTM = nontuberculous mycobacteria

The duration of exposure in the NTM Pooled Population, which includes patients from Studies 212, 312, and 112, is presented below in Table 37 . The durations of exposure to ALIS differed among patients due to study design, randomization, and includes participation in open-label extension studies. In the NTM Pooled Population, the median duration of exposure to ALIS was 169.0 days. The median durations of exposure to ALIS in Studies 212, 312, and 112 were 235.5, 327.5, and 85.0 days, respectively. The patient years of exposure to ALIS in Studies 212, 312, and 112 were 105.3, 33.5, and 25.3 patient years, respectively.

Table 37: Duration of Exposure in the NTM Pooled Population

Parameter/ Category/Statistic	Study 112		Study 212		Study 312	Study 212 + Study 312[d]	Total	
	ALIS + Multidrug Regimen (N=91) n (%)	Multidrug Regimen + Empty Liposomes (N=45) n (%)	ALIS + Multidrug Regimen (N=223) n (%)	Multidrug Regimen Alone (N=112) n (%)	ALIS + Multidrug Regimen (N=133) n (%)	ALIS + Multidrug Regimen (N=297) n (%)	ALIS + Multidrug Regimen (N=388) n (%)	Multidrug Regimen Alone (N=157) n (%)
Duration of Exposure Group (Days)								
Any Exposure	91 (100%)	45 (100%)	223 (100%)	112 (100%)	133 (100%)	297 (100%)	388 (100%)	157 (100%)
<14	2 (2.2%)	0	7 (3.1%)	2 (1.8%)	4 (3.0%)	10 (3.4%)	12 (3.1%)	2 (1.3%)
14 to 28	8 (8.8%)	0	8 (3.6%)	1 (0.9%)	2 (1.5%)	10 (3.4%)	18 (4.6%)	1 (0.6%)
29 to 84	34 (37.4%)	33 (73.3%)	23 (10.3%)	2 (1.8%)	6 (4.5%)	26 (8.8%)	60 (15.5%)	35 (22.3%)
85 to 168	35 (38.5%)	12 (26.7%)	16 (7.2%)	1 (0.9%)	7 (5.3%)	19 (6.4%)	54 (13.9%)	13 (8.3%)
169 to 366	12 (13.2%)	0	101 (45.3%)	80 (71.4%)	32 (24.1%)	106 (35.7%)	118 (30.4%)	80 (51.0%)
367+	0	0	25 (11.2%)	2 (1.8%)	1 (0.8%)	39 (13.1%)	39 (10.1%)	2 (1.3%)
Ongoing	0	0	43 (19.3%)	24 (21.4%)	81 (60.9%)	87 (29.3%)	87 (22.4%)	24 (15.3%)
Summary (Days)[a]								
n	91	45	180	88	52	210	301	133
Mean (SD)	101.4 (52.65)	82.8 (4.95)	213.6 (123.03)	231.7 (58.43)	235.1 (137.48)	241.3 (155.34)	199.0 (147.61)	181.3 (85.20)
Median	85.0	84.0	235.5	241.0	327.5	236.0	169.0	238.0
Min, Max	2, 177	57, 93	1, 485	1, 388	5, 367	1, 615	1, 615	1, 388
Total Exposure[b] (Patient-Years)	25.3	10.2	105.3	55.8	33.5	138.7	164.0	66.0
Patient-Years Study[c]	25.3	10.2	105.3	55.8	33.5	138.7	164.0	66.0

a Does not include ongoing patients.
b Patient-Years of Exposure is time patients were taking study treatment, removing off treatment cycles.
c Patients-Years on Study Treatment is time from first to last dose of study treatment.
c Patients received ALIS + Multidrug Regimen in Study 212 and continued on to receive ALIS + Multidrug Regimen in Study 312.
ALIS = amikacin liposome inhalations suspension; Max = maximum; Min = minimum; NTM = nontuberculous mycobacteria; SD = standard deviation
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7.2 Safety Presentation

The primary safety population comes from the pivotal, randomized study, Study 212; as some patients remain on treatment, the data presented here are through the NDA submission cutoff date. Safety results from Study 312 and the NTM Pooled Population are also presented.

7.3 Overall Summary of Adverse Events in Studies 212 and 312

Pivotal Study 212

As shown in Table 38, most patients in both arms reported at least one AE (ALIS + Multidrug Regimen: 98.2%, Multidrug Regimen Alone: 91.1%). Most AEs were Grade 1 (mild) or Grade 2 (moderate) in severity; Grade 3 and Grade 4 AEs were reported in a higher percentage of patients in the ALIS + Multidrug Regimen arm vs the Multidrug Regimen Alone arm (Grade 3: 12.6% and 8.0%, respectively; Grade 4: 5.4% and 0%, respectively). However, Grade 5 AEs were reported in a lower percentage of patients in the ALIS + Multidrug Regimen arm versus the Multidrug Regimen Alone arm (2.7% and 4.5%, respectively).

Serious AEs were reported in a similar percentage of patients in each treatment arm (20.2% ALIS + Multidrug Regimen and 17.9% Multidrug Regimen Alone). Adverse events leading to ALIS discontinuation were reported in 17.5% of patients in the ALIS + Multidrug Regimen arm. Note that because of the nature of the comparator arm (open label randomization with no matching placebo), study drug discontinuation was only applicable to the ALIS + Multidrug Regimen arm.

Table 38: : Overall Summary of Adverse Events in Study 212 (Safety Population)

	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
Patients reporting ≥1 AE	219 (98.2%)	102 (91.1%)
Grade 1 (mild)	72 (32.3%)	49 (34.8%)
Grade 2 (moderate)	101 (45.3%)	39 (34.8%)
Grade 3 (severe)	28 (12.6%)	9 (8.0%)
Grade 4 (life threatening)	12 (5.4%)	0
Grade 5 (death)	6 (2.7%)	5 (4.5%)
Patients reporting ≥1 SAE	45 (20.2%)	20 (17.9%)
Patients reporting ≥1 AE leading to study drug discontinuation	39 (17.5%)	N/A

Note: AE = Adverse Event, AE = Adverse Event.
Note: AEs that occurred on or after Study Day 1 and within 28 days after the last dose are considered AEs.
Note: the number (N) in the safety population is used as the denominator for calculating the percentages.

Study 312 (Open-label Study in Non-Converters)

An overview of AEs in Study 312 is presented in Table 39. At least 1 AE was reported in most patients (overall: 84.2%; prior ALIS + Multidrug Regimen arm: 72.9%; prior Multidrug Regimen Alone arm: 93.2%). The highest percentage of AEs were Grade 1 or Grade 2.

Serious AEs were reported in a similar number of patients in the prior ALIS + Multidrug Regimen and prior Multidrug Regimen Alone arms (16.9% vs 20.3%, respectively). Adverse events leading to ALIS discontinuation were low in patients previously treated with ALIS + Multidrug Regimen (1.7%), while patients in the prior Multidrug Regimen Alone arm showed a rate similar to Study 212 (12.2%).

Table 39: Overall Summary of Adverse Events in Study 312 (Safety Population)

	Prior ALIS + Multidrug Regimen (N=59)	Prior Multidrug Regimen Alone (N=74)
Patients reporting ≥1 AE	43 (72.9%)	69 (93.2%)
Grade 1 (mild)	19 (32.2%)	24 (32.4%)
Grade 2 (moderate)	13 (22.0%)	28 (37.8%)
Grade 3 (severe)	8 (13.6%)	15 (20.3%)
Grade 4 (life threatening)	1 (1.7%)	2 (2.7%)
Grade 5 (death)	2 (3.4%)	0
Patients reporting ≥1 SAE	10 (16.9%)	15 (20.3%)
Patients reporting ≥1 AE leading to ALIS discontinuation	1 (1.7%)	9 (12.2%)

Note: AE = Adverse Event.
Note: AEs that occurred on or after Study Day 1 and within 28 days after the last dose are considered AEs.
Note: the number (N) in the safety population is used as the denominator for calculating the percentages.

7.3.1 Common Adverse Events in Studies 212 and 312

7.3.1.1 Pivotal Study 212

Respiratory AEs were the most commonly reported AEs in both treatment groups in Study 212 (87.4% in ALIS + Multidrug Regimen and 50.0% in Multidrug Regimen Alone;(Table 40)). The most commonly reported respiratory AEs in ALIS-treated patients were dysphonia (45.7%), cough (37.2%), dyspnea (21.5%), hemoptysis (17.5%), and oropharyngeal pain (10.8%). Given that ALIS is an inhalation antibiotic therapy, these respiratory events were not unexpected.

Table 40: Most Common (≥5% ALIS-treated Patients and More Frequent than Placebo) Adverse Events by System Organ Class and Preferred Term in Study 212

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
Patients Reporting at Least 1 AE	219 (98.2%)	1504	102 (91.1%)	382
Respiratory, thoracic and mediastinal disorders	195 (87.4%)	576	56 (50.0%)	98
Dysphonia	102 (45.7%)	138	1 (0.9%)	1
Cough	83 (37.2%)	109	17 (15.2%)	20
Dyspnoea	48 (21.5%)	62	10 (8.9%)	10
Haemoptysis	39 (17.5%)	54	15 (13.4%)	22
Oropharyngeal pain	24 (10.8%)	30	2 (1.8%)	2
Chronic obstructive pulmonary disease	18 (8.1%)	35	3 (2.7%)	4
Wheezing	15 (6.7%)	16	3 (2.7%)	4
Infections and infestations	99 (44.4%)	164	48 (42.9%)	70
Infective exacerbation of bronchiectasis	17 (7.6%)	22	8 (7.1%)	9
Gastrointestinal disorders	87 (39.0%)	143	24 (21.4%)	31
Diarrhoea	28 (12.6%)	32	5 (4.5%)	5
Nausea	25 (11.2%)	30	4 (3.6%)	4
Vomiting	14 (6.3%)	17	3 (2.7%)	4
General disorders and administration site conditions	76 (34.1%)	114	17 (15.2%)	29
Fatigue	36 (16.1%)	38	8 (7.1%)	8
Pyrexia	16 (7.2%)	21	5 (4.5%)	6
Chest discomfort	13 (5.8%)	13	3 (2.7%)	4
Musculoskeletal and connective tissue disorders	50 (22.4%)	88	17 (15.2%)	19
Back pain	14 (6.3%)	16	4 (3.6%)	4
Arthralgia	14 (6.3%)	15	3 (2.7%)	3
Nervous system disorders	51 (22.9%)	78	10 (8.9%)	12
Headache	22 (9.9%)	25	5 (4.5%)	6
Dizziness	14 (6.3%)	15	3 (2.7%)	3
Investigations	40 (17.9%)	64	14 (12.5%)	18
Weight decreased	15 (6.7%)	16	2 (1.8%)	2
Ear and labyrinth disorders	31 (13.9%)	42	10 (8.9%)	12
Tinnitus	17 (7.6%)	20	1 (0.9%)	1

Note: ALIS = amikacin liposome inhalation suspension + Multidrug Regimen; Placebo consists of Multidrug Regimen Alone

Details including severity and interruption for the common respiratory AEs (occurring in ≥10% of patients) are provided below in Table 41.

In the ALIS + Multidrug Regimen arm in Study 212, dysphonia was the most commonly reported AE (45.7% or 102/223 ALIS patients). A majority of AEs of dysphonia were Grade 1 or Grade 2, with only a few patients overall (1.8% or 4/223) reporting dysphonia of Grade ≥3 in

severity. No patients reported SAEs of dysphonia. In 39.5% (or 88/223) of patients, an AE of dysphonia occurred within 30 days after initiating ALIS. Overall, 36.3% (81/223) of patients had an event that resolved. Overall, 14.8% (33/223) of patients had AEs of dysphonia that led to ALIS interruption; the event resolved in 12.6% (28/223) of these patients following ALIS interruption. Overall, few (2.2%) patients had an AEs of dysphonia that led to discontinuation of ALIS; all patients had resolution of the event following ALIS withdrawal. Twenty-three percent (23.3%) (52/223) of patients had an event of dysphonia that lasted for ≤30 days and 18.4% (41 out of 223) had an event that lasted for >90 days. In 8.1% of patients with AEs of dysphonia, concomitant treatment was required for an event.

In the ALIS + Multidrug Regimen arm in Study 212, cough was the second most frequently reported AE (37.2% or 83/223). Most patients with AE of cough had Grade 1 or 2 events, with only a few patients overall (0.4%) reporting cough of Grade ≥ 3 in severity. No patients reported SAEs of cough. In 27.8% (62/223) of patients, , an event of cough occurred within 30 days after initiating ALIS. Twenty-four percent (24.2%) (54/223) of patients had an event of cough that resolved, and 14.3% (32/223) had an event that did not resolve. Overall, 8.1% (18/233) of patients had an AE of cough that led to ALIS interruption, and seven percent (7.2%) (16/223) had resolution of the event following ALIS interruption. Overall, only 0.9% of patients had an AE of cough which led to discontinuation of ALIS, and all events resolved following ALIS withdrawal. Twenty-four percent (24.2%) (54/223) of patients had an event of cough that lasted for a duration of >90 days. Seventeen percent (17%) of patients had an event of cough that required concomitant treatment.

In the ALIS + Multidrug Regimen arm in Study 212, 21.5% of patients (48/223) reported dyspnea. Most patients with AEs of dyspnea had a Grade 1 or Grade 2 events, with only a few (2.7%) patients overall reporting dyspnea of Grade ≥3 in severity. Few (1.3%) patients overall reported SAEs of dyspnea. Fifteen percent (14.8%) of patients (33/223) had an AE of cough occurring within 30 days after initiating ALIS. Seventeen percent (16.6%) of patients (37/223) had an AE of dyspnea that resolved. Overall, 8.5% of patients had an AE of dyspnea that led to ALIS interruption, and seven percent (7.2%) (16/223) had resolution of event following ALIS interruption. Overall, few (3.1%) patients experienced AEs of dyspnea that led to discontinuation of ALIS, and most (2.2%) had resolution of the event following ALIS withdrawal. Ten percent10.3% of patients had AEs of dyspnea that lasted for ≤30 days, and 8.5% had an event that lasted >90 days. Eleven percent (11.2%) had AEs of cough required concomitant treatment.

In the ALIS + Multidrug Regimen arm in Study 212, 17.5% of patients (39/223) reported AEs of hemoptysis. Most AEs of hemoptysis were Grade 1 or Grade 2 events, with only a few (2.7%) patients overall reporting hemoptysis of Grade ≥3 in severity. Three percent (2.7%) of the patients reported an SAE of hemoptysis. Eight percent (8.1%) of the patients (18/223) with AEs of hemoptysis had an event occurring >90 days after initiating ALIS. Seventeen percent (17%) of patients (38/223) had hemoptysis that resolved. Overall, 4.0% of patients had a hemoptysis event that led to ALIS interruption, with resolution all events resolved following ALIS interruption. Overall, few (0.9%) patients had an AEs of hemoptysis that led to discontinuation of ALIS, and all resolved following ALIS withdrawal. In 13.5% of patients (30/223), hemoptysis lasted for

≤30 days. Approximately 5.4% of patients had AEs of hemoptysis requiring concomitant treatment.

In the ALIS + Multidrug Regimen arm in Study 212, 10.8% of patients (24/223) reported AEs of oropharyngeal pain. All AEs of oropharyngeal pain were Grade 1 or Grade 2. No patients overall reported a SAE of oropharyngeal pain. Eight (7.6%) percent of patients (17/223) had AEs of oropharyngeal pain occurring within 30 days after initiating ALIS. Nine percent (9.4%) of patients (21/223) had AEs of oropharyngeal pain that resolved. Overall, 2.2% of patients had AEs of oropharyngeal pain that led to ALIS interruption, and all had resolution of the event following ALIS interruption. No patients had AEs of oropharyngeal pain that led to discontinuation of ALIS. In approximately 5% of patients (12/223) the AEs of oropharyngeal pain lasted for ≤30 days. Approximately 8 (3.6%) patients had AEs of oropharyngeal pain requiring concomitant treatment.

Table 41: Study 212 Details of the Most Common (≥10% ALIS-treated Patients) Respiratory Adverse Events

		Dysphonia (N=223)		Cough (N=223)		Dyspnea (N=223)		Hemoptysis (N=223)		Oropharyngeal Pain (N=223)	
		ALIS + MDR	MDR Alone	ALIS + MDR	MDR Alone	ALIS + MDR	MDR Alone	ALIS + MDR	MDR Alone	ALIS + MDR	MDR Alone
Patients with ≥1 AE		102 (45.7)	1 (0.9)	83 (37.2)	17 (15.2)	48 (21.5)	10 (8.9)	39 (17.5)	15 (13.4)	24 (10.8)	2 (1.8)
Grade ≥3		4 (1.8)	0	1 (0.4)	0	6 (2.7)	0	6 (2.7)	3 (2.7)	0	0
Grade 5 (fatal)		0	0	0	0	0	0	0	0	0	0
Serious		0	0	0	0	3 (1.3)	0	6 (2.7)	5 (4.5)	0	0
Interruption of ALIS		33 (14.8)	0	18 (8.1)	0	19 (8.5)	0	9 (4.0)	0	5 (2.2)	0
Interruption/resolution*		28 (12.6)	0	16 (7.2)	0	16 (7.2)	0	9 (4.0)	0	5 (2.2)	0
Discontinuation of ALIS		5 (2.2)	0	2 (0.9)	0	7 (3.1)	0	2 (0.9)	0	0	0
Discontinuation/resolution*		5 (2.2)	0	2 (0.9)	0	5 (2.2)	0	2 (0.9)	0	0	0
Onset*	≤30 days	88 (39.5)	1 (0.9)	62 (27.8)	4 (3.6)	33 (14.8)	3 (2.7)	8 (3.6)	3 (2.7)	17 (7.6)	0
	31 – 90 days	20 (9.0)	0	16 (7.2)	1 (0.9)	9 (4.0)	1 (0.9)	15 (6.7)	2 (1.8)	5 (2.2)	0
	> 90 days	14 (6.3)	0	19 (8.5)	13 (11.6)	13 (5.8)	6 (5.4)	18 (8.1)	13 (11.6)	5 (2.2)	2 (1.8)
Duration*	≤30 days	52 (23.3)	0	22 (9.9)	8 (7.1)	23 (10.3)	5 (4.5)	30 (13.5)	13 (11.6)	12 (5.4)	2 (1.8)
	31 – 90 days	18 (8.1)	1 (0.9)	18 (8.1)	4 (3.6)	11 (4.9)	2 (1.8)	7 (3.1)	2 (1.8)	6 (2.7)	0
	> 90 days	41 (18.4)	0	54 (24.2)	7 (6.3)	19 (8.5)	3 (2.7)	4 (1.8)	0	7 (3.1)	0
Required Treatment		18 (8.1)	1 (0.9)	38 (17.0)	7 (6.3)	25 (11.2)	2 (1.8)	12 (5.4)	8 (7.1)	8 (3.6)	0

*Subject count is unique within each category
Denominator is the number of patients with interruption/discontinuation for that AE
MDR=Multidrug Regimen

7.3.1.2 Study 312 (Open-label Study in Non-Converters)

As with Study 212, respiratory AEs were the mostly commonly reported AEs when ALIS was added to guideline-based therapy in patients previously in the prior Multidrug Regimen Alone arm in Study 312 (Table 42). These respiratory events were less frequent for patients who continued ALIS from the Prior ALIS + Multidrug Regimen arm.

Table 42: Most Common (≥5%) Adverse Events by System Organ Class and Preferred Term in Study 312

System Organ Class/ Preferred Term	Prior ALIS + Multidrug Regimen (N=59)		Prior Multidrug Regimen Alone (N=74)	
	Patients	Events	Patients	Events
Patients Reporting at Least 1 AE	43 (72.9%)	167	69 (93.2%)	359
Respiratory, thoracic and mediastinal disorders	18 (30.5%)	34	60 (81.1%)	146
Dysphonia	2 (3.4%)	3	33 (44.6%)	44
Cough	3 (5.1%)	4	26 (35.1%)	30
Haemoptysis	5 (8.5%)	5	8 (10.8%)	9
Dyspnoea	3 (5.1%)	4	10 (13.5%)	11
Chronic obstructive pulmonary disease	3 (5.1%)	3	4 (5.4%)	6
Productive cough	1 (1.7%)	2	6 (8.1%)	6
Sputum increased	1 (1.7%)	2	5 (6.8%)	5
Oropharyngeal pain	1 (1.7%)	1	4 (5.4%)	5
Infective exacerbation of bronchiectasis	3 (5.1%)	3	6 (8.1%)	7
Nasopharyngitis	5 (8.5%)	5	2 (2.7%)	2
Mycobacterium avium complex infection	3 (5.1%)	3	1 (1.4%)	1
General disorders and administration site conditions	6 (10.2%)	9	22 (29.7%)	25
Fatigue	1 (1.7%)	1	9 (12.2%)	9
Chest discomfort	0	0	4 (5.4%)	4
Nervous system disorders	6 (10.2%)	6	15 (20.3%)	18
Dizziness	1 (1.7%)	1	5 (6.8%)	5
Headache	1 (1.7%)	1	4 (5.4%)	4
Investigations	5 (8.5%)	8	11 (14.9%)	14
Weight decreased	1 (1.7%)	1	4 (5.4%)	4
Musculoskeletal and connective tissue disorders	9 (15.3%)	11	6 (8.1%)	10
Musculoskeletal chest pain	3 (5.1%)	3	2 (2.7%)	2
Renal and urinary disorders	5 (8.5%)	9	7 (9.5%)	11
Haematuria	4 (6.8%)	4	4 (5.4%)	5
Ear and labyrinth disorders	3 (5.1%)	4	8 (10.8%)	10
Tinnitus	1 (1.7%)	1	5 (6.8%)	6

Note: The number (N) in the Safety population is used as the denominator for calculating the percentages.
Note: At each level of summation (overall, System Organ Class, Preferred Term), patients reporting more than 1 AE are counted at most once. AEs are coded by using MedDRA version 17.1.
AE = adverse event; ALIS = amikacin liposome inhalation suspension (590 mg).

7.4 Adverse Events ≥Grade 3 in Pivotal Study 212

In Study 212, Grade 3 and Grade 4 AEs were reported in a higher percentage of patients in the ALIS + Multidrug Regimen arm (Grade 3 [12.6%]; Grade 4 [5.4%]) than in the Multidrug Regimen Alone arm (Grade 3 [8.0%]; Grade 4 [0%]). Grade 5 (fatal) AEs, however, were reported in a lower percentage of patients in the ALIS + Multidrug Regimen arm than in the Multidrug Regimen Alone arm (2.7% versus 4.5%, respectively).

In the ALIS + Multidrug Regimen arm, 46 subjects experienced an AE Grade ≥3:

- 15.2 % (34/223) of patients had an AE Grade ≥3 that resolved
- 7.2 % (16/223) patients had an AE Grade ≥3 that was ongoing at the time of data cutoff/last follow-up
- Overall, 2.7 % (6/223 patients) of the patients had an AE Grade ≥3 that led to death (discussed further in Section 7.8)
- Overall, 9.0 % (20/223) patients had an AE Grade ≥3 that led to ALIS interruption, with most 7.6 % (17/223) patients having resolution of the event following interruption
- Overall, 5.4% (12/223) of patients had an AE Grade ≥3 that led to ALIS withdrawal, with most 4.0% (9/223) having resolution following withdrawal

7.5 Adverse Events Leading to Interruption of ALIS in Pivotal Study 212

Adverse events leading to study drug (ALIS) interruption in >2% of patients are presented in Table 43. Per protocol, if a patient experienced an AE, after discussion between the site and the medical monitor, interruption of ALIS was permitted until symptoms subsided. The most common AEs leading to interruption of ALIS were in respiratory in nature (32.3%): Dysphonia (14.8%), dyspnea (8.5%), and cough (8.1%). Adverse events leading to interruption of ALIS occurred mostly early in treatment. Most AE requiring interruption of ALIS resolved. In Study 212, 103 patients experienced a total of 272 AEs leading to interruption of ALIS, and 243 of these events (89.3%) resolved. The remaining 29 events were still ongoing at time of the data cut-off. Relative to the incidence rate of AEs (incidence rate in ALIS + Multidrug Regimen arm: Dysphonia 45.7%, dyspnea 21.5%, cough 37.2%), the respective incidence of AE leading to interruption ALIS was low.

Table 43: Adverse Events Leading to Interruption of ALIS (>2% of Patients)

Adverse Event	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)
≥1 Adverse Event Leading to ALIS Interruption	103 (46.2%)	N/A
Dysphonia	33 (14.8%)	N/A
Dyspnea	19 (8.5%)	N/A
Cough	18 (8.1%)	N/A
Hemoptysis	9 (4.0%)	N/A
Fatigue	8 (3.6%)	N/A
COPD	7 (3.1%)	N/A
Headache	6 (2.7%)	N/A
Tinnitus	6 (2.7%)	N/A
Oropharyngeal pain	5 (2.2%)	N/A
Infective Exacerbation of Bronchiectasis	5 (2.2%)	N/A

7.6 Adverse Events Leading to Discontinuation of ALIS in Pivotal Study 212

Assessment of AEs leading to discontinuation of ALIS is relevant only for the ALIS + Multidrug Regimen arm since the protocol focused on the discontinuation of ALIS from Multidrug Regimen.

In Study 212, a total of 39 patients (17.5%) reported AEs that led to discontinuation of ALIS in the ALIS + Multidrug Regimen arm (Table 44). Most AEs leading to discontinuation of ALIS were non-serious (27/39). Of the 12 patients with SAEs leading to discontinuation of ALIS, 9 had resolution of the event following discontinuation of ALIS. One patient had an SAE of infective exacerbation of bronchiectasis that was ongoing at the time of the date cutoff, and 2 patients had SAEs leading to death (respiratory failure and exacerbation of COPD) that were not considered related to ALIS (See Section 7.8). Adverse events leading to discontinuation of ALIS were most commonly reported in the Respiratory, Thoracic and Mediastinal SOC (11.2%), followed by Infections and Infestations SOC (2.2%). The most common AEs leading to discontinuation of ALIS were dyspnea in 7 patients (3.1%), dysphonia in 5 patients (2.2%), and allergic alveolitis, COPD, cough, hemoptysis and infective exacerbation of bronchiectasis, each in 2 patients (0.9%). All other AEs leading to discontinuation of ALIS were reported in 1 patient each.

Relative to the incidence rate of dysphonia and dyspnea (45.7% and 21.5%, respectively, in the ALIS + Multidrug Regimen arm), the respective incidence of these AE leading to discontinuation of ALIS was low. Adverse events leading to discontinuation mainly occurred early in treatment. Most patients (30/39) had resolution of AEs following discontinuation of ALIS. The remaining 9 patients had AEs still ongoing at time of their last follow-up.

Table 44: Adverse Events Leading to Discontinuation of ALIS by System Organ Class and Preferred Term in >1 Patient in Study 212 (Safety Population)

	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)
System Organ Class/ Preferred Term	**Patients**	**Events**	
Patients reporting at least one AE leading to Discontinuation of ALIS	39 (17.5%)	39	N/A
Respiratory, thoracic and mediastinal disorders	25 (11.2%)	25	N/A
Dyspnoea	7 (3.1%)	7	N/A
Dysphonia	5 (2.2%)	5	N/A
Alveolitis allergic	2 (0.9%)	2	N/A
Chronic obstructive pulmonary disease	2 (0.9%)	2	N/A
Cough	2 (0.9%)	2	N/A
Haemoptysis	2 (0.9%)	2	N/A
Infections and infestations	5 (2.2%)	5	N/A
Infective exacerbation of bronchiectasis	2 (0.9%)	2	N/A
Ear and labyrinth disorders	4 (1.8%)	4	N/A
Hypoacusis	2 (0.9%)	2	N/A
General disorders and administration site conditions	2 (0.9%)	2	N/A

Note: AE = Adverse Event
Note: the number (N) in the safety population is used as the denominator for calculating the percentages.
Note: Only AEs are summarized in this table. AEs that occurred on or after Study Day 1 and within 28 days after the last dose are considered AEs.
Note: At each level of summation (overall, system organ class, preferred term), patients reporting more than one AE are counted at most once. AEs are coded by using MedDRA version 17.1.

For comparison, 12 patients (3.6%) experienced AEs that led to discontinuation of Multidrug Regimen (9 patients [4.0%] in the ALIS + Multidrug Regimen arm and 3 patients [2.7%] in the Multidrug Regimen Alone arm; Table 45). Additionally, 4 patients (1.8%) reported AEs that led to discontinuation of both ALIS and Multidrug Regimen in the ALIS + Multidrug Regimen arm.

Table 45: Adverse Events Leading to Discontinuation of Multidrug Regimen by System Organ Class and Preferred Term in >1 Patient in Study 212

	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
System Organ Class/ Preferred Term	**Patients**	**Events**	**Patients**	**Events**
Patients reporting ≥1 AE leading to Discontinuation of Multidrug Regimen	9 (4.0%)	16	3 (2.7%)	3
Gastrointestinal disorders	3 (1.3%)	5	0	0
Diarrhoea	2 (0.9%)	2	0	0
Nausea	2 (0.9%)	2	0	0
Vomiting	1 (0.4%)	1	0	0
Respiratory, thoracic and mediastinal disorders	2 (0.9%)	2	2 (1.8%)	2
Lung infiltration	0	0	1 (0.9%)	1
Respiratory failure	0	0	1 (0.9%)	1
Dysphonia	1 (0.4%)	1	0	0
Pulmonary cavitation	1 (0.4%)	1	0	0
Ear and labyrinth disorders	2 (0.9%)	2	0	0
Deafness	1 (0.4%)	1	0	0
Deafness neurosensory	1 (0.4%)	1	0	0
Investigations	2 (0.9%)	2	0	0
Hepatic enzyme increased	1 (0.4%)	1	0	0
Weight decreased	1 (0.4%)	1	0	0
Eye disorders	1 (0.4%)	1	0	0
Visual acuity reduced	1 (0.4%)	1	0	0
Injury, poisoning and procedural complications	0	0	1 (0.9%)	1
Fall	0	0	1 (0.9%)	1
Metabolism and nutrition disorders	1 (0.4%)	1	0	0
Decreased appetite	1 (0.4%)	1	0	0
Musculoskeletal and connective tissue disorders	1 (0.4%)	1	0	0
Arthralgia	1 (0.4%)	1	0	0
Skin and subcutaneous tissue disorders	1 (0.4%)	1	0	0
Pruritus	1 (0.4%)	1	0	0
Vascular disorders	1 (0.4%)	1	0	0
Hot flush	1 (0.4%)	1	0	0

Note: AE = Adverse Event.
Note: the number (N) in the safety population is used as the denominator for calculating the percentages.
Note: Only AEs are summarized in this table. AEs that occurred on or after Study Day 1 and within 28 days after the last dose are considered AEs.
Note: At each level of summation (overall, system organ class, preferred term), patients reporting more than one AE are counted at most once. AEs are coded by using MedDRA version 17.1.

7.7 Serious Adverse Events in Pivotal Study 212

A summary of the SAEs that occurred in >1 patient in either the ALIS + Multidrug Regimen arm or the Multidrug Regimen Alone arm in Study 212 is provided in Table 46. Serious AEs were reported in a similar proportion of patients in each treatment arm (ALIS + Multidrug Regimen arm 20.2%, Multidrug Regimen Alone arm 17.9%). Serious AEs of COPD (3.1% vs 0.9%) were reported with higher frequency in the ALIS + Multidrug Regimen arm, while SAEs of

hemoptysis (2.7% vs 4.5%) were reported with higher frequency in the Multidrug Regimen Alone arm.

Table 46: Serious Adverse Events (>1 Patient) by System Organ Class and Preferred Term in Study 212

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
Patients Reporting ≥1 SAE	45 (20.2%)	90	20 (17.9%)	38
Respiratory, thoracic and mediastinal disorders	26 (11.7%)	36	11 (9.8%)	19
Haemoptysis	6 (2.7%)	8	5 (4.5%)	7
Chronic obstructive pulmonary disease	7 (3.1%)	12	1 (0.9%)	1
Pneumothorax	3 (1.3%)	3	1 (0.9%)	1
Dyspnoea	3 (1.3%)	3	0	0
Respiratory failure	2 (0.9%)	2	1 (0.9%)	1
Pneumonitis	2 (0.9%)	2	0	0
Pulmonary cavitation	0	0	2 (1.8%)	3
Infections and infestations	20 (9.0%)	31	6 (5.4%)	10
Pneumonia	8 (3.6%)	9	2 (1.8%)	2
Infective exacerbation of bronchiectasis	5 (2.2%)	8	3 (2.7%)	3
Infective exacerbation of chronic obstructive airways disease	2 (0.9%)	2	1 (0.9%)	1
Mycobacterium avium complex infection	1 (0.4%)	1	2 (1.8%)	2
Lung infection	2 (0.9%)	4	0	0
Cardiac disorders	1 (0.4%)	1	5 (4.5%)	5
Acute myocardial infarction	0	0	2 (1.8%)	2
Psychiatric disorders	2 (0.9%)	3	0	0
Anxiety	2 (0.9%)	2	0	0

Note: The number (N) in the safety population is used as the denominator for calculating the percentages.
Note: Only AEs are summarized in this table. AEs that occurred on or after the date of first visit and within 28 days after the last dose are considered AEs.
Note: At each level of summation (overall, system organ class, preferred term), patients reporting more than 1 AE are counted at most once. AEs are coded using MedDRA version 17.1.
AE = adverse event; ALIS = amikacin liposome inhalation suspension (590 mg); incl = including, SAE = serious adverse event.

In the ALIS + Multidrug Regimen arm, 45 subjects out of 223 had a serious adverse event

- 16.1% (36/223) patients with an SAE had an event that resolved
- 6.7% (15/223) patients had an SAE that was ongoing at the time of data cutoff/last follow-up
- Overall, 2.7%% (6/223) of the patients had an SAE that led to death (discussed further in Section 7.8)
- Overall 8.5% (19/223) patients had an SAE that led to ALIS interruption, with most 7.2% (16/223) having resolution of the event following interruption
- Overall, 5% (12.223) patients had an SAE that led to ALIS withdrawal, with most 4.0% (9/223) having resolution following withdrawal

7.8 Adverse Events Leading to Death

NTM Pooled Population

A total of 14 patients experienced SAEs resulting in death in the NTM Pooled Population (Table 47). One death occurred in a patient receiving ALIS in Study 112, 11 deaths occurred in Study 212 (6 patients receiving ALIS, 5 patients receiving Multidrug Regimen Alone), and 2 deaths occurred in Study 312 (both patients receiving ALIS). Most SAEs resulting in death were respiratory in nature. All but one SAE resulting in death were considered not related to ALIS as determined by the study investigator; one SAE was considered possibly related. Of the SAEs resulting in death in the NTM Pooled Population, 13 of the 14 occurred in patients who had persistently positive sputum cultures for NTM and did not achieve culture conversion.

In the NTM Pooled Population, patients were exposed to ALIS for 164.0 patient years compared to patients exposed to Multidrug Regimen Alone for 66.0 patient years. There were 5.5 deaths per 100 patient years in the ALIS group compared to 7.6 deaths per 100 patient years in the Multidrug Regimen Alone group.

Pivotal Study 212

A total of 11 patients reported 11 AEs leading to death: 6 (2.7%) patients with 6 reported events in the ALIS + Multidrug Regimen arm and 5 (4.5%) patients with 5 reported events in the Multidrug Regimen Alone arm. The most common AEs leading to death were reported in the Respiratory, Thoracic, and Mediastinal Disorders SOC and included respiratory failure in 3 total patients (ALIS + Multidrug Regimen arm: 2, Multidrug Regimen Alone arm: 1); COPD and pulmonary embolism, each in 1 patient in the ALIS + Multidrug Regimen arm; and interstitial lung disease in 1 patient in the Multidrug Regimen Alone arm. Adverse Events leading to death were also reported in the Infections and Infestations SOC (lung infection in 1 patient in the ALIS + Multidrug Regimen arm, and MAC infection and pneumonia, each in 1 patient in the Multidrug Regimen alone arm), Cardiac Disorders SOC (cardiogenic shock in 1 patient in the MDR Alone arm), and Metabolism and Nutrition Disorders SOC (cachexia in 1 patient in the ALIS + Multidrug Regimen arm).

Brief narratives for the patients treated with ALIS + Multidrug Regimen and Multidrug Regimen with AEs leading to death are provided in Appendix 10.6.1.

Table 47: Serious Adverse Events Resulting in Death in the NTM Pooled Population

System Organ Class/ Preferred Term	Study 112		Study 212		Study 312	Total NTM	
	ALIS + Multidrug Regimen (N=91) n (%)	Multidrug Regimen + Empty Liposomes (N=45) n (%)	ALIS + Multidrug Regimen (N=223) n (%)	Multidrug Regimen Alone (N=112) n (%)	ALIS + Multidrug Regimen (N=133) n (%)	ALIS + Multidrug Regimen (N=388) n (%)	Multidrug Regimen Alone (N=157) n (%)
At Least One	1 (1.1%)	0	6 (2.7%)	5 (4.5%)	2 (1.5%)	9 (2.3%)	5 (3.2%)
Cardiac disorders	0	0	0	1 (0.9%)	0	0	1 (0.6%)
Cardiogenic shock	0	0	0	1 (0.9%)	0	0	1 (0.6%)
Infections and infestations	1 (1.1%)	0	1 (0.4%)	2 (1.8%)	1 (0.8%)	3 (0.8%)	2 (1.3%)
Lower respiratory tract infection	0	0	0	0	1 (0.8%)	1 (0.3%)	0
Lung infection	0	0	1 (0.4%)	0	0	1 (0.3%)	0
Mycobacterium avium complex infection	0	0	0	1 (0.9%)	0	0	1 (0.6%)
Pneumonia	1 (1.1%)	0	0	1 (0.9%)	0	1 (0.3%)	1 (0.6%)
Metabolism and nutrition disorders	0	0	1 (0.4%)	0	0	1 (0.3%)	0
Cachexia	0	0	1 (0.4%)	0	0	1 (0.3%)	0
Respiratory, thoracic and mediastinal disorders	1 (1.1%)	0	4 (1.8%)	2 (1.8%)	1 (0.8%)	6 (1.5%)	2 (1.3%)
Acute respiratory distress syndrome	1 (1.1%)	0	0	0	0	1 (0.3%)	0
Chronic obstructive pulmonary disease	0	0	1 (0.4%)	0	1 (0.8%)	2 (0.5%)	0
Interstitial lung disease	0	0	0	1 (0.9%)	0	0	1 (0.6%)
Pulmonary embolism	0	0	1 (0.4%)	0	0	1 (0.3%)	0
Respiratory failure	0	0	2 (0.9%)	1 (0.9%)	0	2 (0.5%)	1 (0.6%)

Note: Data presented through the cutoff date (07 July 2017) for ongoing Studies 212 and312.
ALIS = amikacin liposome inhalation suspension; NTM = nontuberculous mycobacteria

7.9 Adverse Events of Special Interest

Important potential risks categories identified as AESIs that may be associated with ALIS include alveolitis allergic, bronchospasm, infective exacerbation of underlying disease, hemoptysis, exacerbation of COPD, and dysphonia. Additionally, neuromuscular disorders, nephrotoxicity, and ototoxicity are AESI categories known to be related to use of parenteral amikacin that were therefore examined in the ALIS safety assessment.

Results for AESI assessments are shown for the NTM Pooled Population, defined as all patients who participated in the NTM studies (Studies 212, 312, and 112).

7.9.1 Respiratory Adverse Events

7.9.1.1 Bronchospasm

To investigate the relationship between ALIS and reported pulmonary events, the following grouped terms indicative of bronchospasm-related events were analyzed: asthma, bronchospasm, bronchial hyperreactivity, dyspnea, dyspnea exertional, throat tightness, and wheezing.

As shown in Table 48, AEs in the bronchospasm category were reported in a similar percentage of patients treated with ALIS + Multidrug Regimen in Study 212 and the NTM Pooled Population (29.1% and 24.7%, respectively).

Table 48: Adverse Events of Special Interest: Bronchospasm

	Study 212		NTM Pooled
Respiratory AESI Category	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)	ALIS + Multidrug Regimen (N=388)
Bronchospasm	65 (29.1%)	13 (11.6%)	96 (24.7%)
Bronchospasm	6 (2.7%)	0	10 (2.6%)
Bronchial hyperreactivity	1 (0.4%)	0	2 (0.5%)
Dyspnea	48 (21.5%)	10 (8.9%)	67 (17.3%)
Wheezing	15 (6.7%)	3 (2.7%)	23 (5.9%)
Asthma	3 (1.3%)	0	3 (0.8%)
Dyspnea exertional	1 (0.4%)	0	2 (0.5%)
Throat tightness	1 (0.4%)	0	1 (0.3%)

Note: Only terms that appeared in Study 212 ALIS + Multidrug Regimen group are shown in the table

In Study 212, AEs in the bronchospasm category were reported in a higher percentage of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (ALIS + Multidrug Regimen: 29.1%; Multidrug Regimen Alone: 11.6%) (Table 49). Serious AEs of bronchospasm were low (ALIS + Multidrug Regimen: 1.3%; Multidrug Regimen: 0%). Although 29% of patients receiving ALIS were considered to have an AESI of “bronchospasm” as defined by these broader terms, this was driven mainly by dyspnea, which was reported in 21.5% of patients receiving ALIS. Preferred terms of “bronchospasm” and “wheezing” were reported in 2.7% and 6.7%, respectively, of patients receiving ALIS in Study 212.

Most patients with AESIs in the bronchospasm category had Grade 1 or Grade 2 events. Overall, only a few patients had AESIs in the bronchospasm category of Grade ≥3 (2.7% or6/223) or a serious event (1.3% or 3/223). No patients had a Grade 5 (fatal) event. A majority (20.2% or 45/223) of patients had AEs in the bronchospasm category with onset within 30 days after initiating ALIS. Twenty-two percent (21.5% or 48/223) of patients had AEs in bronchospasm category that resolved. Overall, 10.3% of patients experienced AEs in the bronchospasm category that led to ALIS interruption, with most (9.0%) having resolution following ALIS interruption. Few (4%) patients had an AE in the bronchospasm category that led to discontinuation of ALIS, with three (2.7%) percent of patients having resolution following ALIS withdrawal. Thirteen percent (13.0% or 29/223) of patients had AEs in bronchospasm category that lasted for ≤30 days and 13% (or 29/223) had an event that lasted >90 days. Seventeen (16.6%) of patients had AEs in the bronchospasm category that required concomitant treatment.

Among Study 212 patients receiving ALIS, 65 patients reported 96 AEs in the bronchospasm category. Eleven of the 96 AEs (12%) were bronchospasm or bronchial hyperreactivity, reported in 7 patients (table 46). These adverse events were mild in 6 patients and moderate in 1 patient. For 6 (2.7%) patients the events occurred within 30 days of starting treatment. ALIS dosing was interrupted in 2 (0.9%) patients, and 1 patient (0.4%) withdrew from the study due to bronchospasm. All resolved following interruption and withdrawal.

Comorbidities were very common among the patient population in Study 212, with multiple past medical history conditions noted among the 65 patients.

Table 49: Adverse Events Relating to Bronchospasm (AESI) and Bronchospasm or Bronchial Hyperactivity in Study 212

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
Bronchospasm Incidence Rate	65 (29.1%)		13 (11.6%)	
Number of AEs		96		15
Toxicity Grade				
Grade 1	48 (21.5%)		9 (8.0%)	
Grade 2	18 (8.1%)		4 (3.6%)	
Grade 3	4 (1.8%)		0	
Grade 4	2 (0.9%)		0	
Grade 5	0		0	
Toxicity Grade ≥ 3	6 (2.7%)		0	
Serious AE	3 (1.3%)		0	
AE Onset				
Onset ≤ 30 days	45 (20.2%)		4 (3.6%)	
Onset 31 to 90 days	14 (6.3%)		2 (1.8%)	
Onset > 90 days	18 (8.1%)		8 (7.1%)	
AE Resolution				
Not Resolved	21 (9.4%)		8 (7.1%)	
Resolved	48 (21.5%)		6 (5.4%)	
ALIS Interruption	23 (10.3%)		0	
ALIS Interruption and Resolution	20 (9.0%)		0	
ALIS Withdrawal	9 (4.0%)		0	
ALIS Withdrawal and Resolution	6 (2.7%)		0	
AE Duration				
< 30 days	29 (13.0%)		7 (6.3%)	
30 to 90 days	17 (17.6%)		2 (1.8%0	
> 90 days	29 (13.0%)		5 (4.5%)	
Bronchospasm or Bronchial Hyperreactivity Incidence Rate	7 (3.1%)		0	
Number of AEs		11	0	
Toxicity Grade				
Grade 1	6 (2.7%)		0	
Grade 2	1 (0.4%)		0	
Grade 3	0		0	
Grade 4	0		0	
Grade 5	0		0	
Toxicity Grade ≥ 3	0		0	
Serious AE	0		0	
AE Onset				
Onset ≤ 30 days	6 (2.7%)		0	
Onset 31 to 90 days	2 (0.9%)		0	
Onset > 90 days	1 (0.4%)		0	

System Organ Class/ Preferred Term	ALIS + Multidrug Regimen (N=223)		Multidrug Regimen Alone (N=112)	
	Patients	Events	Patients	Events
AE Resolution				
Not Resolved	1 (0.4%)		0	
Resolved	6 (2.7%)		0	
ALIS Interruption	2 (0.9%)		0	
ALIS Interruption and Resolution	2 (0.9%)		0	
ALIS Withdrawal	1 (0.4%)		0	
ALIS Withdrawal and Resolution	1 (0.4%)		0	
AE Duration				
< 30 days	5 (2.2%)		0	
30 to 90 days	0		0	
> 90 days	2 (0.9%)		0	

7.9.1.2 Hemoptysis

Adverse events in the hemoptysis category were reported in a similar percent of patients treated with ALIS + Multidrug Regimen in Study 212 and the NTM Pooled Population (17.5% and 17.0%, respectively) (Table 50).

In Study 212, AEs in the hemoptysis category were reported in a higher percent of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (ALIS + Multidrug Regimen: 17.5%; Multidrug Regimen Alone: 13.4%). Most hemoptysis events were mild to moderate in nature, with only a few subjects overall with events of Grade ≥ 3 severity in either arm (2.7% in ALIS + Multidrug Regimen arm and 2.7% in Multidrug Alone arm). However, SAEs of hemoptysis were lower with the ALIS + Multidrug Regimen group (ALIS + Multidrug Regimen: 2.7% (6/223); Multidrug Regimen Alone: 4.5% (5/112).. When they occurred, they occurred later in the treatment (>90 days), with almost all events resolving. Some patients required drug interruption, and the events subsequently resolved. The majority of hemoptysis events lasted <30 days.

Table 50: Adverse Events of Hemoptysis

Respiratory AESIs Category	Study 212		NTM Pooled
	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen (N=112)	ALIS + Multidrug Regimen (N=388)
Hemoptysis	39 (17.5%)	15 (13.4%)	66 (17.0%)

7.9.1.3 Exacerbation of Chronic Obstructive Pulmonary Disease (COPD)

Adverse events of the exacerbation of COPD category included “infective exacerbation of chronic obstructive airways disease” for exacerbation due to infection and “chronic obstructive airways disease” for exacerbation of non-infective etiology.

Adverse events in the exacerbation of COPD category were reported in a similar percent of patients in treated with ALIS + Multidrug Regimen in Study 212 and the NTM Pooled Population (8.1% and 6.4%, respectively) (Table 51).

Table 51: Adverse Events of Special Interest: Exacerbation of COPD

	Study 212		NTM Pooled
Respiratory AESI Category	**ALIS + Multidrug Regimen (N=223)**	**Multidrug Regimen Alone (N=112)**	**ALIS + Multidrug Regimen (N=388)**
Exacerbation of COPD	18 (8.1%)	4 (3.6%)	25 (6.4%)
Infective exacerbation of COPD	2 (0.9%)	1 (0.9%)	2 (0.5%)
COPD	18 (8.1%)	3 (2.7%)	25 (6.4%)

Note: Only terms that appeared in Study 212 ALIS + Multidrug Regimen group are shown in the table

In Study 212, AEs in the exacerbation of COPD category were reported in a higher percent of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (ALIS + Multidrug Regimen 8.1%; Multidrug Regimen Alone 3.6%). Serious AEs of exacerbation of COPD also higher in the ALIS + Multidrug Regimen arm (ALIS + Multidrug Regimen: 3.1%; Multidrug Regimen Alone: 1.8%).

In the ALIS + Multidrug Regimen arm, 18 patients experienced 37 AEs of COPD exacerbation. Dosing with study drug (ALIS) was interrupted for 7 (3.1%) patients, all resolved subsequently, ALIS was withdrawn in 2 (0.4%) patients; the event resolved in 1 patient and was ongoing in the other patient at time of data cut-off.

7.9.1.4 Allergic Alveolitis

Adverse events in the allergic alveolitis category were reported in a similar percent of patients in treated with ALIS + Multidrug Regimen in Study 212 and the NTM Pooled Population (3.1% and 3.1%, respectively) (Table 52).

Table 52: Adverse Events of Special Interest: Allergic Alveolitis

	Study 212		NTM Pooled
Respiratory AESI Category	**ALIS + Multidrug Regimen (N=223)**	**Multidrug Regimen Alone (N=112)**	**ALIS + Multidrug Regimen (N=388)**
Allergic Alveolitis	7 (3.1%)	1 (0.9%)	12 (3.1%)
Pneumonitis	4 (1.8%)	0	6 (1.5%)
Allergic alveolitis	2 (0.9%)	0	3 (1.0%)
Interstitial lung disease	1 (0.4%)	1 (0.9%)	2 (0.5%)
Respiratory disorder	0	0	1 (0.3%)

The AESI for allergic alveolitis included allergic alveolitis, pneumonitis, and interstitial lung disease. In study 212, AEs in the allergic alveolitis category were reported in a higher percent of patients in the ALIS + Multidrug Regimen group (3.1% [7/223]) than the Multidrug Regimen Alone group (0.9% [1/112]).

Four patients in the ALIS + Multidrug Regimen arm and the 1 patient in the Multidrug Regimen Alone arm had a SAE. ALIS was withdrawn with an AE in the allergic alveolitis category in 3/7 patients and resolved in those 3 patients. For all other events, patients receiving study drug (ALIS) continued treatment without dose interruption. All events in patients in the ALIS + Multidrug Regimen arm resolved except for 1 fatal event of worsening interstitial lung disease considered secondary to scleroderma. The outcome of the event of worsening interstitial lung disease in the patient in the Multidrug Regimen Alone arm was also fatal.

7.9.2 Adverse Events Known to be Related to Use of Parenteral Amikacin

7.9.2.1 Nephrotoxicity

Adverse events in the nephrotoxicity category were reported in a lower percent of patients in treated with ALIS + Multidrug Regimen in Study 212 than the NTM Pooled Population (1.3% and 4.1%, respectively) (Table 53). The higher incidence rate in the NTM Pooled Population was mainly driven by the higher rate of hematuria reported in the Study 312.

Table 53: Adverse Events of Special Interest: Nephrotoxicity

	Study 212		NTM Pooled
Respiratory AESIs Category	**ALIS + Multidrug Regimen (N=223)**	**Multidrug Regimen (N=112)**	**ALIS + Multidrug Regimen (N=388)**
Nephrotoxicity	3 (1.3%)	3 (2.7%)	16 (4.1%)
Blood creatinine increased	1 (0.4%)	0%	3 (0.8%)
Glomerular filtration rate decreased	1 (0.4%)	1 (0.9%)	2 (0.5%)
Haematuria	1 (0.4%)	2 (1.8%)	9 (2.3%)
Leukocyturia	1 (0.4%)	1 (0.9%)	2 (0.5%)
Proteinuria	1 (0.4%)	2 (1.8%)	3 (0.8%)

Note: Only terms that appeared in Study 212 ALIS + Multidrug Regimen group are shown in the table.

In Study 212, AEs in the nephrotoxicity category were reported in a smaller percent of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (1.3%; Multidrug Regimen Alone 2.7%).

In Study 212, there were no SAEs of nephrotoxicity in either treatment arm.

In Study 212, no patients reported nephrotoxicity events within the first month of ALIS.

7.9.2.2 Neuromuscular Disorder

Adverse events in the neuromuscular category were reported in a similar percent of patients in treated with ALIS + Multidrug Regimen in Study 212 as the NTM Pooled Population (2.2% and 3.1%, respectively) (Table 54).

Table 54: Adverse Events of Special Interest: Neuromuscular Disorder

Respiratory AESIs Category	Study 212		NTM Pooled
	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen (N=112)	ALIS + Multidrug Regimen (N=388)
Neuromuscular	5 (2.2%)	0%	12 (3.1%)
Muscular weakness	1 (0.4%)	0%	1 (0.3%)
Balance disorder	3 (1.3%)	0%	5 (1.3%)
Neuropathy peripheral	1 (0.4%)	0%	6 (1.5%)

In Study 212, AEs in the neuromuscular category were reported in a higher percent of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (2.2%; Multidrug Regimen Alone 0%). However, overall the incidence of AEs in the neuromuscular category were low, and all events were mild and non-serious. One AE of balance disorder led to interruption ALIS and subsequently resolved. All but 1 AE resolved; 1 AE of muscle weakness was ongoing at the time of last follow-up for the patient.

In Study 212, 0.9% of events in the neuromuscular category occurred within the first month of ALIS treatment.

7.9.2.3 Ototoxicity

Adverse events in the ototoxicity category was reported in a similar percent of patients in treated with ALIS + Multidrug Regimen in Study 212 than the NTM Pooled Population (17.0% and 15.2%, respectively) (Table 55).

Table 55: Adverse Events of Special Interest: Ototoxicity

	Study 212		NTM Pooled
Respiratory AESI Category	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)	ALIS + Multidrug Regimen (N=388)
Ototoxicity	38 (17.0%)	11 (9.8%)	59 (15.2%)
Tinnitus	17 (7.6%)	1 (0.9%)	26 (6.7%)
Deafness	2 (0.9%)	0	3 (0.8%)
Deafness neurosensory	3 (1.3%)	1 (0.9%)	4 (1.0%)
Hypoacusis	5 (2.2%)	6 (5.4%)	5 (1.3%)
Vertigo	2 (0.9%)	0	5 (1.3%)
Balance Disorder	3 (1.3%)	0	5 (1.3%)
Dizziness	14 (6.3%)	3 (2.7%)	22 (5.7%)
Presyncope	1 (0.4%)	0	2 (0.5%)

Note: Only terms that appeared in Study 212 ALIS + Multidrug Regimen group are shown in the table

In Study 212, the AESI for ototoxicity included hypoacusis, deafness neurosensory, deafness, tinnitus, and vertigo. Adverse events in the ototoxicity category were reported in a higher percent of patients in the ALIS + Multidrug Regimen group than the Multidrug Regimen Alone group (ALIS + Multidrug Regimen 17.0%; Multidrug Regimen Alone 9.8%).

There were no SAEs in the ototoxicity category for either regimen.

A total of 17 (7.6%) patients experienced 20 events of tinnitus in the ALIS + Multidrug Regimen arm compared to 1 (0.9%) patient in the Multidrug Regimen Alone arm. Events of tinnitus were non-serious and mostly mild in severity (17/20 mild and 3/20 moderate severity). Study drug (ALIS) was not interrupted in 13 of 20 events, interrupted for 6 of 20 events, and was not withdrawn for any event. Of the 20 events, 10 resolved at the time of this report, 1 event was recovering, 1 event recovered with sequelae, 6 events were ongoing, and 2 events had unknown outcomes due to patient withdrawal of consent and patient death. Of the 17 patients who experienced tinnitus, 3 patients also experienced hearing loss: 1 patient had a past medical history of tinnitus and experienced worsening of tinnitus at Study Day 7 followed by bilateral neurosensory hearing loss at Day 43 – study drug (ALIS) was not interrupted for either event with the event of tinnitus resolving and the event of bilateral neurosensory hearing loss ongoing at the time of this report; 1 patient experienced tinnitus at Study Day 72 to Day 76, and subsequently experienced hypoacusis on Day 357 – study drug (ALIS) was not interrupted for either event with hypoacusis ongoing at the time of this report; 1 patient had a past medical history of mild hearing loss and experienced tinnitus and hypoacusis at Study Day 90 – study drug (ALIS) was not interrupted with both events ongoing at the time of this report. The 1 patient in the Multidrug Regimen Alone arm had tinnitus of mild severity, which was ongoing at the time of this report.

7.10 Audiology Evaluations

NTM Pooled Population

The audiology assessment schedule in studies with the NTM Population was as follows:

- **Study 112:** Screening, Day 84, Day 168, 28 days off treatment if prior assessment was abnormal
- **Study 212:** Screening, Month 3, Month 6, Month 12, EOT
- **Study 312:** Screening, Month 6, Month 12

Based on this schedule, the 3, 6, and 8 months post-baseline timepoints provide the most information, with a meaningful number of patients with audiology data available at these timepoint. The 12 months and 14 months post-baseline timepoints, although important in assessing the longer-term audiology data, had a small number of patients (12 months post-baseline: ALIS + Multidrug Regimen 7.7% [30/388]; Multidrug Regimen Alone 1.9% [3/157]; 14 months post-baseline 5.4% [21/388]; Multidrug Regimen Alone 0% [0/157]) due to:

- Studies 212 and 312 were still ongoing and not all patients had reached this timepoint at the time of the data cut-off
- In Study 212, only converters continued in the study after Month 8
- As the ratio of converters was small in the Multidrug Regimen Alone arm of Study 212, the number of patients at the 12 months and 14 months post-baseline timepoint was very small

There was no trend in change from Baseline in mean decibels noted over time at 3, 6, or 8 months post-baseline at any hertz in the NTM Pooled Population or in Studies 212, 312, or 112.

7.11 Clinical Laboratory Evaluations

NTM Pooled Population

In the NTM Pooled Population, there were no notable differences between patients in the ALIS + Multidrug Regimen and Multidrug Regimen Alone groups in the Baseline values or shifts from Baseline of chemistry or hematology parameters.

Potentially clinically meaningful chemistry laboratory events of patients in the NTM Controlled Population were assessed, and no clinically meaningful changes were observed in the NTM Pooled Population.

7.12 Pulmonary Function Tests

The actual and change from baseline in the percent-predicted pulmonary function tests (PFTs) (FEV1, FVC, and FEF25-75) were summarized. Only the percent-predicted values were summarized because the actual values were correlated with sex, age, height, and weight. Therefore, actual values were highly variable making it difficult to interpret the summary statistics, especially in small sample sizes.

PFTs were categorized as potentially clinically meaningful events. The number and percentage of patients satisfying each of the potentially clinically meaningful criteria are presented.

The PFT assessment schedule for each study included in the PFT analysis is detailed below. NTM studies PFTs assessment schedule:

- Study 112: Screening, Baseline (Day 1), Day 28, Day 56, Day 85, Day 112, Day 140, Day 168, 28-day safety follow-up
- Study 212: Baseline (Day 1), Month 6, 6-month safety follow-up

Pulmonary Function Tests in Study 212

Overall, 88.5% of patients had baseline spirometry (90.1% and 84.8% in the ALIS + Multidrug Regimen and Multidrug Regimen Alone arms, respectively); change from Baseline to Month 6 was evaluated in 67.2% of patients (62.8% and 75% in the ALIS + Multidrug Regimen and Multidrug Regimen alone arms, respectively). There were no clinically relevant changes in pulmonary function tests (Table 56).

Table 56: Pulmonary Function Tests in Study 212 (Safety Population)

Test	Time Point	ALIS + Multidrug Regimen (N=223)	Multidrug Regimen Alone (N=112)	Total (N=335)
Forced Expiratory Volume in 1 sec (L)	Baseline[1]			
	n	201	95	296
	Mean (SD)	1.689 (0.6475)	1.711 (0.7224)	1.696 (0.6713)
	Median	1.610	1.600	1.600
	Min, Max	0.59, 3.76	0.40, 4.81	0.40, 4.81
	Month 6			
	n	151	98	249
	Mean (SD)	1.748 (0.6632)	1.705 (0.7242)	1.731 (0.6868)
	Median	1.650	1.565	1.620
	Min, Max	0.30, 3.78	0.51, 4.78	0.30, 4.78
	Change from Baseline to Month 6			
	n	140	84	224
	Mean (SD)	-0.055 (0.1673)	-0.059 (0.2099)	-0.056 (0.1840)
	Median	-0.040	-0.040	-0.040
	Min, Max	-0.79, 0.43	-1.15, 0.73	-1.15, 0.73
Forced Vital Capacity (L)	Baseline[1]			
	n	201	95	296
	Mean (SD)	2.548 (0.8471)	2.631 (0.9758)	2.575 (0.8896)
	Median	2.480	2.500	2.480
	Min, Max	0.92, 5.43	0.92, 5.88	0.92, 5.88
	Month 6			
	n	151	98	249
	Mean (SD)	2.593 (0.8604)	2.668 (1.0476)	2.622 (0.9372)
	Median	2.440	2.485	2.470
	Min, Max	0.69, 5.37	0.62, 6.02	0.62, 6.02
	Change from Baseline to Month 6			
	n	140	84	224
	Mean (SD)	-0.085 (0.2410)	-0.065 (0.2323)	-0.077 (0.2374)
	Median	-0.060	-0.100	-0.080
	Min, Max	-1.00, 0.90	-0.79, 0.57	-1.00, 0.90
Forced Expiratory Flow (L)	Baseline[1]			
	n	196	95	291

	Mean (SD)	1.256 (0.8961)	1.251 (0.8497)	1.254 (0.8798)
	Median	1.010	1.100	1.040
	Min, Max	0.20, 4.60	0.19, 5.01	0.19, 5.01
	Month 6			
	n	149	98	247
	Mean (SD)	1.328 (0.8898)	1.173 (0.7623)	1.266 (0.8433)
	Median	1.070	0.985	1.070
	Min, Max	0.19, 4.37	0.20, 4.41	0.19, 4.41
	Change from Baseline to Month 6			
	n	135	84	219
	Mean (SD)	0.004 (0.5216)	-0.063 (0.3878)	-0.022 (0.4749)
	Median	-0.030	-0.005	-0.020
	Min, Max	-3.88, 2.82	-1.65, 0.96	-3.88, 2.82

[1] Baseline is defined as the last non-missing value prior to first dose of study drug.

Summary of Potentially Clinically Meaningful Pulmonary Function Test Results in the NTM Controlled Population:

- Percent-predicted FEV1 (decrease of >20%):
  In the NTM Controlled Population, a lower percentage of patients in the ALIS group (12.4%, 33/267) had a percent-predicted FEV1 decrease of >20% in at least 1 post baseline visit as compared to the percentage of patients in the “placebo” group (20.4%, 32/157)
- Percent-predicted FEV1 (increase of >20%):
  In the NTM Controlled Population, a lower percentage of patients in the ALIS group (3.7%, 10/267) had a percent-predicted FEV1 increase of >20% in at least 1 post baseline visit as compared to the percentage of patients in the “placebo” group (7.0%, 11/157)
- Percent-predicted FEV1 (≤49%):
  In the NTM Controlled Population, a lower percentage of patients in the ALIS group (22.8%, 61/267) had a percent-predicted FEV1 ≤49% in at least 1 post baseline visit as compared to the percentage of patients in the “placebo” group (35.7%, 56/157)
- Percent-predicted FVC (decrease of >20%):
  In the NTM Controlled Population, a similar percentage of patients in the ALIS group (12.7%, 34/267) and in the “placebo” group (18.5%, 29/157) had a percent-predicted FVC decrease of >20% in at least 1 post baseline visit
- Percent-predicted FVC (increase of >20%):
  In the NTM Controlled Population, a lower percentage of patients in the ALIS group (4.1%, 11/267) had a percent-predicted FVC increase of >20% in at least 1 post baseline visit as compared to the percentage of patients in the “placebo” group (8.9%, 14/157)
- Percent-predicted FVC (≤49%):

In the NTM Controlled Population, a lower percentage of patients in the ALIS group (14.2%, 38/267) had a percent-predicted FVC ≤49% in at least 1 post baseline visit as compared to the percentage of patients in the "placebo" group (22.9%, 36/157)

7.13 Safety Conclusions

Based on a thorough and systematic safety analysis, ALIS has a consistent safety profile across the populations studied in the NTM clinical development program. A higher percentage of AEs occurred in the ALIS group than in the Multidrug Regimen Alone group, with the majority of AEs of Grade 1 or Grade 2 in severity. More Grade 3 and Grade 4 AEs were observed in patients receiving ALIS compared to Multidrug Regimen Alone; however, a lower percentage of patients receiving ALIS experienced a Grade 5 AE compared with patients receiving Multidrug Regimen Alone.

The majority of AEs were respiratory in nature, related to the inhaled route of administration and most occurred within the first month of ALIS administration. The most common respiratory AEs were almost exclusively mild to moderate in nature. The incidence frequency of time of common AEs showed the highest frequency after initiation of ALIS with frequent decline in the incidence frequency after the first month, indicating an assumed improved tolerability over time.

Adverse events of allergic alveolitis occurred at a low frequency and were mostly serious AEs, with a higher incidence in the ALIS group compared to the Multidrug Regimen Alone group. All events in patients in the ALIS + Multidrug Regimen arm resolved except for 1 fatal event of worsening interstitial lung disease considered secondary to scleroderma. The outcome of the event of worsening interstitial lung disease in the patient in the Multidrug Regimen Alone arm was also fatal.

For AESIs of bronchospasm (29%; 65/223), the PTs with the highest frequency were dyspnea and wheezing and occurred more frequently in the ALIS group compared to the Multidrug Regimen Alone group.

Bronchospasm and bronchial hyperactivity within the AESI of bronchospams were reported by 3% (7/223) of all patients, and approximately 0.9% (2/223) of those patients interrupted ALIS as an outcome of the event and it resolved. Bronchodilators were used to treat bronchospasm in 2.7% (6/223) of the patients.

Pre-existing respiratory conditions that might predispose to events of bronchospasm and dyspnea, and which confound the interpretation of the relationship with ALIS of reported respiratory events, were, as expected, very common in the NTM population. Taken together, the pattern of bronchospasm- and dyspnea-related AEs is suggestive of a mix of respiratory symptoms triggered by inhalation of ALIS and symptoms due to co-existing pulmonary or cardiovascular pathology. The events that occurred soon after starting ALIS, and that led to dose interruption or withdrawal with subsequent resolution, likely represent ALIS-induced respiratory symptoms. In most cases, including all of those reported as bronchospasm or bronchial hyperreactivity, the events were of low severity (Grade 1 or 2) and in most (65%) treatment with ALIS was not interrupted.

The rates of infective exacerbation of underlying bronchiectasis and underlying COPD were similar in the ALIS group and the Multidrug Regimen Alone group. A higher frequency of other respiratory AESI occurred in the ALIS group compared to the Multidrug Regimen Alone group.

The proposed product information and patient leaflet will warn that bronchospasm and dyspnea have been reported with ALIS and that ALIS may exacerbate underlying COPD. The product information will also contain information on the management of symptoms including advise that pre-treatment with short-acting selective beta-2 agonists should be considered for patients with known hyperreactive airway disease, chronic obstructive pulmonary disease, asthma, or bronchospasm.

Adverse events related to the well-known systemic toxicity of aminoglycoside antibiotics such as nephrotoxicity and peripheral neuropathy were infrequent and were balanced between patients in the ALIS and Multidrug Regimen Alone groups. For AESIs of neuromuscular disorders, the overall frequency was low, with a rate much lower than reported for parenteral amikacin.

The higher incidence in ototoxicity events in patients treated with ALIS was driven by tinnitus and dizziness. The majority of ototoxicity events were mild, began in the first month of treatment, and resolved within the same month. Prior hearing events in the patients' medical histories could not be excluded as potential confounding factors.

Interruptions of ALIS were permitted for the management of AEs, particularly local respiratory events, with reintroduction of ALIS after symptom resolution. The vast majority of the patients that interrupted ALIS had their AE resolve. The overall rate of AEs leading to discontinuation of ALIS was relatively low compared to their incidence, suggesting that discontinuation is not a major contributor to the decline in incidence of AEs over time.

Serious AEs were reported in a similar percentage of patients in the ALIS group and the Multidrug Regimen Alone groups. Importantly, the incidence of serious AEs did not increase in the ALIS group after additional exposure to ALIS with continued treatment.

Of the deaths that occurred in patients with NTM lung disease, most occurred in patients who had not achieved culture conversion. As expected in severe lung disease, most events were respiratory. Many patients also had past comorbidities that might have influenced outcome (eg COPD and active smoker at Baseline).

Overall, the safety assessment showed that ALIS inhalation minimizes the known systemic toxicities of IV amikacin apart from tinnitus. While the AEs increased when added to combination antibiotic therapy, most events were mild to moderate, mainly reported in the first month of treatment, and most resolved without discontinuation. More severe side-effects of ALIS include allergic alveolitis and bronchospasm, and are generally manageable and of limited duration. Serious events including death occurred no more frequently with ALIS than in the control group.

Overall, the safety profile of ALIS has been well characterized and is acceptable relative to Multidrug Regimen, especially in light of the high unmet need and established efficacy.

8 BENEFIT RISK ANALYSIS

Nontuberculous mycobacterial lung disease caused by MAC has the potential to pose a serious threat to public health as supported by the designation of ALIS as a Breakthrough Therapy. The infection of vulnerable patients gives rise to NTM lung disease for which there is no currently approved therapy. Current recommended standard of care treatment consists of a combination of drugs for prolonged periods. The goal of treatment is eradication as defined as sustained culture conversion (ie, sputum culture negativity). However, these therapies are poorly tolerated, and they do not achieve the goals of eradication in a significant number of patients. There are consequences to unsuccessful treatment, including a higher mortality risk. The addition of ALIS to guideline-based therapy in patients demonstrated to be refractory to that therapy achieves culture conversion in a substantial proportion of patients. Conversion by Month 6 is reasonably likely to predict the clinical benefit of culture negativity at 3 months after treatment cessation.

Clinical outcomes of the 6MWT and overall mortality were superior in patients who achieved culture conversion compared with NTM patients who did not achieve culture conversion regardless of treatment group. The relationship between culture conversion and improved 6MWT is an important factor in understanding the relationship between clinical benefit, as characterized by 6MWT, and sustained culture conversion. The consistently better improvement in 6MWT at Month 6 in converters compared with non-converters provides evidence that culture conversion by Month 6 is associated with contemporaneous clinical benefit.

The safety of ALIS has been well characterized. While the administration of ALIS has been associated with primarily respiratory-related AEs, they were mostly local and were mild to moderate in severity. The rate of AEs leading to discontinuation of ALIS was relatively low compared to the overall incidence. Dose interruptions are often sufficient to manage local respiratory AEs. The safety profile is acceptable given the significant risk of the disease, the inadequacy of current treatment approaches, and early indications of positive clinical outcomes from the use of ALIS, including a potential mortality benefit. The aggregate data support the conclusion that respiratory-related events, mild to moderate in severity, continue to be the most important risks. Other important risks described in this document are ototoxicity which demonstrated a small imbalance. This was driven by higher tinnitus rates in patients treated with ALIS + Multidrug Regimen compared to Multidrug Regimen Alone. There was no imbalance in events indicative of renal toxicity.

Under Subpart H, these findings support a positive benefit-risk profile for the use of ALIS in the treatment of NTM lung disease caused by MAC as part of a combination antibiotic regimen for adult patients.

Overall, ALIS in combination with an antibiotic regimen significantly increases the likelihood of sustained culture conversion in adult patients. These findings will be confirmed in the ongoing Study 212, which is fully enrolled and has had the primary analysis completed. ALIS offers the opportunity of a durable cure which may lead to improved morbidity and mortality outcomes.

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10 APPENDICES

10.1 Schedule of Events in Study 212

Table 57: Schedule of Events in Study 212

	Screening	TREATMENT PHASE												OFF-TREATMENT PHASE			
		Baseline	Month 1[a]	Month 2[a]	Month 3	Month 4	Month 5[a]	Month 6	Month 8	Month 10	Month 12	Month 14	EOT up to Month 16	28 Day Safety Follow-Up	3 Month Safety Follow-Up	6 Month Safety Follow-Up	EOS (12 Month Safety Follow-Up)
	(V1)	(V2)	(V3)	(V4)	(V5)	(V6)	(V7)	(V8)	(V9)	(V10)	(V11)	(V12)	(V13)	(V14)	(V15)	(V16)	(V17)
Visit Window	Approximately -98 to -70 (10-14 weeks)[b]	Day 1	(±3)	(±3)	(±3)	(±3)	(±3)	(±3)	(-28; +7)	(±5)	(±5)	(±5)	(±5)	(±5)	(±7)	(±7)	(±7)
Informed consent	X	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pregnancy test[c]	X	X	X	X	X	X	X	X	X	–	X	–	X	–	–	–	-
ECG	X	–	–	–	–	–	–	–	–	–	–	–	X	–	–	–	–
SGRQ	–	X	–	–	X	–	–	X	X	–	X	–	X	–	X	–	-
EQ-5D-3L	–	X	–	–	X	–	–	X	X	–	X	–	X	–	X	–	-
Medical history	X		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Physical examination	X	X			X	X		X	X	–	X	–	X	–	X	–	X
Vital signs and pulse oximetry	X	X[d]			X[d]	X[d]		X[d]	X[d]	–	X[d]	–	X	–	X	–	X
6 minute walk test[e]	–	X	–	–	–	X	–	X	X	–	–	–	X	–	X	–	-
Pulmonary Function Tests[f] (FEV_1[L], FEF(25-75%), FVC)	–	X	–	–	–	–	–	X	–	–	–	–	–	–	–	X	–

Audiology test	X[g]	–	–	X	–	–	X	–	–	X	–	X	–	–	–	–	
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Health Care Resource Utilization	–	X	X	X	X	X	X	X	X	–	X	–	X	–	–	–	–
AE assessment	–	X[h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sputum collection for microbiology	X	X	X	X	X	X	X	X	X	–	X	–	X	X	X	X	X
Chemistry	X	X	X	–	X	–	–	X	X	–	X	–	X	–	–	–	–
Hematology	X	X	X	–	X	–	–	X	X	–	X	–	X	–	–	–	–
Urinalysis	X	X	X	–	X	–	–	X	X	–	X	–	X	–	–	–	–
Serum for Biomarkers (CRP and IL-6)	–	X	–	–	–	–	–	X	–	–	–	–	X	–	–	–	–
Send sputum collection containers home	X	X	X	X	X	X	X	X	X	–	X	–	X	X	X	X	–
Administer study drug at site	–	X	X	X	X	X	X	X	X	–	X	–		–	–	–	–
Dispense study drug[i]	–	X	X	X	X	X	X	X	X	X	X	X	–	–	–	–	–
Collection of study drug vials	–	–	X	X	X	X	X	X	X	X	X	X	X	–	–	–	–
Start consent process for INS-312								X[j]									

Note: At visits where study drug is administered, all patient-reported outcomes, 6MWT, Physical Exam should be performed before study drug administration**.**

Note: Culture conversion is defined to occur when the first of 3 consecutive monthly sputum cultures are MAC negative. All NTM treatment will stop when a subject has completed 12 months of treatment starting from their first of 3 negative cultures when they were defined as a 'converter'.

Abbreviations: AE, adverse event; CRP, C-reactive protein; CT, computed tomography; ECG, electrocardiogram; EOS, End of Study; EOT, End of Treatment; ED-5D-3L, EuroQol 5D; $FEF_{(25-75\%)}$, forced expiratory flow at 25-75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; PK, pharmacokinetic; SGRQ, St. George's Respiratory Questionnaire.

[a] Home Healthcare visits may be available for sites with IRB/EC approval to conduct Home Healthcare visits, and for qualifying subjects who may have difficulty attending clinic visits.

[b] The Screening window allows time for sputum culture results, minimum inhibitory concentration (MIC) determination, and scheduling of Screening assessments. Any delay in obtaining microbiological results and MICs must be reported to the Sponsor immediately and documented within the

study source documents. In the event that sputum culture results are not known by 8 weeks (Day -14), the Baseline (Day 1) visit should be scheduled as soon as possible in order to maintain the Screening window, and an extension of the Screening window to 14 weeks is allowed, if necessary. If sputum culture results are known prior to 8 weeks (Day -14), the Screening window of 10 weeks must be maintained.

[c] Serum pregnancy testing will be performed on women of child bearing potential at Screening. A urine pregnancy will test will be performed on women of child bearing potential at all other visits. Women not of child bearing potential are defined as postmenopausal (i.e., amenorrheic for at least 1 year), or surgically or naturally sterile.

[d] Vital signs and pulse oximetry will be assessed both before and after dosing on visits where study drug is administered.

[e] The 6MWT must be conducted by a site member who is blinded to the subject's open-label treatment assignment.

[f] The PFTs will be performed at sites with access to spirometers and trained personnel to perform spirometry tests.

[g] The Baseline audiology examination must be performed during Screening period or on Day 1 before study drug administration.

[h] At Baseline (Day 1), AEs will be assessed only after study drug administration or first dose of multi-drug regimen. Any AE that has occurred prior to the first dose must be included in the subject's medical history, including any AEs that occur within the Screening period.

[i] Study drug will be dispensed to all subjects up to and including the Month 6 visit. At Month 8, subjects who are non-converters or experienced a relapse or recurrence after achieving culture conversion will exit the study at Month 8/EOT visit. Subjects who remain in the study after Month 8 will receive study drug for 12 months beginning from the first of 3 negative cultures that defines culture conversion.

[j] If Study INS-312 has been IRB/EC approved, start the ICF consent process at Month 6, in order to provide sufficient time for the subject to make an informed decision.

10.2 Inclusion and Exclusion Criteria

10.2.1 Study 212 Inclusion and Exclusion Criteria

To be eligible for enrollment, a prospective patient must have met all of the following criteria:

1. Was male or female, 18 years of age or older (20 years of age or older in Japan).
2. Was positive for MAC on culture as defined in inclusion criterion No. 4 while being treated with a Multidrug Regimen (at least 2 antibiotics) for a minimum duration of 6 consecutive months that was either ongoing or had been stopped no more than 12 months before Screening (exceptions to treatment with a Multidrug Regimen for 6 consecutive months included treatment with doses or frequencies below those recommended by guidelines and/or short interruptions of therapy, both occurring due to safety/tolerability issues).
3. Was diagnosed with MAC NTM lung infection with evidence of underlying lung disease such as nodular BR and/or fibrocavitary disease by chest radiography or chest CT. High-resolution CT scan was preferred, if available.
4. Had a 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.
6. Was willing to adhere to treatment with a Multidrug Regimen during the course of the study.
7. Was able to produce approximately 3 mL of sputum or was willing to undergo an induction that produced approximately 3 mL of sputum for mycobacteriology.
8. Females of childbearing potential agreed to practice an acceptable method of birth control (eg, true abstinence [refraining from heterosexual intercourse during the entire study], hormonal or barrier methods, partner sterilization, or intrauterine device [IUD]) while participating in the study. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of the study, and withdrawal were not acceptable methods of contraception.
9. Provided written informed consent before performing any study-related procedure.
10. Was willing to have serum specimens stored.
11. Was able to comply with study drug use, study visits, and study procedures as determined by the Investigator.

A prospective patient with any of the following conditions was excluded from this study:

1. Patients with CF.
2. Patients whose MAC NTM infection was resistant to amikacin (as identified by MIC susceptibility > 64 µg/mL).
3. Patients who were not able to perform the 6MWT.

4. Positive pregnancy test or lactation at Screening. All women of childbearing potential were tested. Women not of childbearing potential were defined as postmenopausal (ie, amenorrhoeic for at least 1 year) or surgically or naturally sterile.
5. Active pulmonary malignancy (primary or metastatic) or any malignancy requiring chemotherapy or radiation therapy within 1 year before Screening or anticipated during the study period.
6. Active allergic bronchopulmonary mycosis or any other condition requiring chronic systemic corticosteroids at a dose greater than the equivalent of 10 mg/day of prednisone within 3 months before Screening.
7. Active pulmonary tuberculosis requiring treatment at Screening.
8. History of lung transplantation.
9. Initiation of chronic therapy (eg, high-dose ibuprofen, inhaled anti-inflammatory agents including steroids, low-dose maintenance steroids, recombinant human deoxyribonuclease [rhDNase-]) within 28 days before Baseline (Day 1).
10. Administration of any investigational drug within 8 weeks before Screening.
11. Prior exposure to ALIS (including clinical study).
12. Known hypersensitivity to aminoglycosides.
13. Use of inhaled or systemic aminoglycosides with activity against MAC (eg, amikacin, kanamycin, or streptomycin) within 28 days before Baseline (Day 1).
14. Acquired and primary immunodeficiency syndromes (eg, HIV-positive patients regardless of CD4 counts).
15. Significant (as determined by the Investigator) hearing loss, vestibular dysfunction, neuromuscular weakness, or a diagnosis of myasthenia gravis, where the potential risk of aminoglycoside toxicity outweighs the potential benefit.
16. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) $\geq 3 \times$ the upper limit of normal (ULN) or total bilirubin $\geq 2 \times$ ULN at Screening.
17. Absolute neutrophil count (ANC) $\leq 500/\mu L$ at Screening.
18. Serum creatinine $> 2 \times$ ULN at Screening.
19. Current alcohol, medication, or illicit drug abuse.
20. Any condition that, in the opinion of the Investigator, interfered with ability to safely complete the study or adhere to study requirements.
21. Persons who have been committed to an institution by virtue of an order issued either by the judicial or the administrative authorities.
22. In the opinion of the Investigator, patients who were not expected to survive the duration of the study.
23. Disseminated MAC infection.

10.3 Prior and Concomitant Therapy

Throughout the duration of the study, subjects in both arms of the study continued the same multidrug (at least 2 antibiotics) antimycobacterial regimen. The regimen was based on the 2007 ATS/IDSA guidelines or respective local guidelines, and they were not to change during the treatment phase except for safety concerns or if "rescue" antimycobacterial therapy was required. Any subject who required "rescue" medication was discontinued from the study. Subjects who achieved culture conversion and completed treatment stopped all NTM treatment at the EOT visit. No NTM treatment was given during the safety follow-up period.

Where allowed, and if approved by IRB/EC, the Sponsor provided reimbursement for the multidrug antimycobacterial regimen for as long as the subject participated in the study and was adherent to all protocol requirements. These drugs may have included, but were not limited to azithromycin, clarithromycin, clofazimine, ethambutol, ethionamide, rifabutin, and rifampicin.

All medications used during the study were entered into the concomitant medications section of the electronic case report form (eCRF).

Although systemic exposure to amikacin is low after ALIS administration, precaution was taken if subjects required the following systemic medications that may have possible interactions with amikacin: potent diuretics (such as ethacrynic acid and furosemide), beta lactam antibiotics (such as penicillins and cephalosporins), bisphosphonates, platinum compounds, and thiamine.

Bronchodilator therapy was allowed. Subjects who developed bronchospasm were permitted to be pretreated with a bronchodilator before study drug administration.

10.4 Discontinuation of Study Drug

Study drug administration may be discontinued for any patient who meets 1 or more of the criteria listed below:

1. The Investigator may discontinue study drug in the interest of patient safety. The Investigator must identify specific AEs (laboratory abnormality, intercurrent illness, other medical condition or situation) that result in premature discontinuation of study drug in the eCRF.
2. A patient who withdraws consent to receive study drug will be discontinued. All attempts must be made to conduct an EOS visit.
3. Patients who meet the following criteria established by Hy's law will be discontinued: alanine aminotransferase or aspartate aminotransferase $\geq$ 3 ULN AND total bilirubin > 2 ULN.
4. Patients who need "rescue" medication.
5. Patients who become pregnant will be discontinued and followed until the pregnancy is concluded.

6. Administration of study drug will be discontinued to all patients seen by an Investigator who terminates participation in the study prematurely.
7. Administration of study drug will be discontinued to all patients if the study is terminated.

The Investigator must contact the medical monitor immediately before discontinuing a patient from study drug if possible, or no later than 24 hours after the event. When a patient is discontinued from study drug prematurely, the Investigator will clearly document the reason in the medical record and complete the appropriate eCRF page describing the reason for discontinuation. If the patient is not able to continue to the end of the study for safety reasons, procedures required for an early discontinuation visit must be performed.

In the event that a patient is withdrawn from the study treatment because of an AE, the patient should be followed and treated by the Investigator until the abnormal parameter or symptom is resolved or stabilized. It is up to the Investigator to determine and document that the AE is either resolved or that it has reached a stable state, after which no further follow-up is necessary.

If a patient fails to attend scheduled assessments in the study, the Investigator must determine the reasons and the circumstances as completely and accurately as possible. "Lost to follow-up" should be marked only in an exceptional case when all documented attempts to reach the patient by the Investigator or other staff members were unsuccessful.

10.5 Adverse Events ≥Grade 3

Table 58: Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Maximum Grade in Study 212 (Safety Analysis Set)

	ALIS + Multidrug Regimen (N=223)			Multidrug Regimen Alone (N=112)		
System Organ Class/ Preferred Term	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5
Patients reporting at least one TEAE	28 (12.6%)	12 (5.4%)	6 (2.7%)	9 (8.0%)	0	5 (4.5%)
Respiratory, thoracic and mediastinal disorders	20 (9.0%)	9 (4.0%)	4 (1.8%)	3 (2.7%)	1 (0.9%)	2 (1.8%)
Acute respiratory failure	0	1 (0.4%)	0	0	0	0
Alveolitis allergic	2 (0.9%)	0	0	0	0	0
Aspiration	0	0	0	1 (0.9%)	0	0
Chronic obstructive pulmonary disease	2 (0.9%)	3 (1.3%)	1 (0.4%)	0	0	0
Cough	1 (0.4%)	0	0	0	0	0
Dysphonia	4 (1.8%)	0	0	0	0	0
Dyspnoea	4 (1.8%)	2 (0.9%)	0	0	0	0

	ALIS + Multidrug Regimen (N=223)			Multidrug Regimen Alone (N=112)		
System Organ Class/ Preferred Term	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5
Interstitial lung disease	0	1 (0.4%)	0	0	0	1 (0.9%)
Lung infiltration	0	0	0	1 (0.9%)	0	0
Pneumonitis	1 (0.4%)	0	0	0	0	0
Pneumothorax	1 (0.4%)	2 (0.9%)	0	1 (0.9%)	0	0
Pulmonary embolism	0	0	1 (0.4%)	0	0	0
Rales	1 (0.4%)	0	0	0	0	0
Respiratory acidosis	0	0	0	0	1 (0.9%)	0
Respiratory failure	1 (0.4%)	0	2 (0.9%)	0	0	1 (0.9%)
Infections and infestations	9 (4.0%)	5 (2.2%)	1 (0.4%)	5 (4.5%)	0	2 (1.8%)
Aspergillus infection	1 (0.4%)	0	0	0	0	0
Clostridium difficile infection	0	0	0	1 (0.9%)	0	0
Infectious pleural effusion	0	1 (0.4%)	0	0	0	0
Infective exacerbation of bronchiectasis	2 (0.9%)	1 (0.4%)	0	2 (1.8%)	0	0
Infective exacerbation of chronic obstructive airways disease	1 (0.4%)	0	0	0	0	0
Laryngitis	1 (0.4%)	0	0	0	0	0
Lung infection	0	0	1 (0.4%)	0	0	0
Lung infection pseudomonal	1 (0.4%)	0	0	0	0	0
Mycetoma mycotic	0	0	0	1 (0.9%)	0	0
Mycobacterium abscessus infection	1 (0.4%)	0	0	0	0	0
Mycobacterium avium complex infection	1 (0.4%)	0	0	0	0	1 (0.9%)
Pneumonia	2 (0.9%)	3 (1.3%)	0	1 (0.9%)	0	1 (0.9%)
Pneumonia pseudomonal	0	1 (0.4%)	0	0	0	0
Respiratory tract infection	1 (0.4%)	0	0	0	0	0
Scedosporium infection	1 (0.4%)	0	0	0	0	0
Tooth abscess	0	0	0	1 (0.9%)	0	0
Upper respiratory tract infection	0	0	0	1 (0.9%)	0	0
Gastrointestinal disorders	2 (0.9%)	0	0	0	0	0
Gastrointestinal toxicity	1 (0.4%)	0	0	0	0	0
Pneumatosis intestinalis	1 (0.4%)	0	0	0	0	0
General disorders and administration site conditions	2 (0.9%)	0	0	0	0	0
Exercise tolerance decreased	1 (0.4%)	0	0	0	0	0
Pain	1 (0.4%)	0	0	0	0	0
Musculoskeletal and connective tissue disorders	1 (0.4%)	0	0	0	0	0
Back pain	1 (0.4%)	0	0	0	0	0
Nervous system disorders	3 (1.3%)	0	0	0	0	0
Aphonia	3 (1.3%)	0	0	0	0	0
Skin and subcutaneous tissue disorders	1 (0.4%)	0	0	1 (0.9%)	0	0
Dermatitis	1 (0.4%)	0	0	0	0	0
Night sweats	0	0	0	1 (0.9%)	0	0
Metabolism and nutrition disorders	1 (0.4%)	0	1 (0.4%)	0	0	0
Cachexia	0	0	1 (0.4%)	0	0	0
Decreased appetite	1 (0.4%)	0	0	0	0	0
Eye disorders	2 (0.9%)	0	0	0	0	0
Cataract	1 (0.4%)	0	0	0	0	0
Optic neuropathy	1 (0.4%)	0	0	0	0	0
Psychiatric disorders	2 (0.9%)	0	0	0	0	0
Anxiety	1 (0.4%)	0	0	0	0	0
Insomnia	1 (0.4%)	0	0	0	0	0
Libido decreased	0	0	0	0	0	0

	ALIS + Multidrug Regimen (N=223)			Multidrug Regimen Alone (N=112)		
System Organ Class/ Preferred Term	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5
Major depression	1 (0.4%)	0	0	0	0	0
Injury, poisoning and procedural complications	0	0	0	1 (0.9%)	0	0
Fall	0	0	0	1 (0.9%)	0	0
Cardiac disorders	0	0	0	1 (0.9%)	1 (0.9%)	1 (0.9%)
Acute myocardial infarction	0	0	0	1 (0.9%)	0	0
Cardiac failure	0	0	0	0	1 (0.9%)	0
Cardiogenic shock	0	0	0	0	0	1 (0.9%)
Blood and lymphatic system disorders	0	1 (0.4%)	0	0	0	0
Thrombocytopenia	0	1 (0.4%)	0	0	0	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (0.4%)	0	0	1 (0.9%)	0	0
Colon adenoma	1 (0.4%)	0	0	0	0	0
Prostate cancer	0	0	0	1 (0.9%)	0	0
Hepatobiliary disorders	0	1 (0.4%)	0	0	0	0
Hepatic function abnormal	0	1 (0.4%)	0	0	0	0
Immune system disorders	1 (0.4%)	0	0	0	0	0
Drug hypersensitivity	1 (0.4%)	0	0	0	0	0

AE = Adverse Event, LAI = Liposomal Amikacin for Inhalation (590 mg), TEAE = Treatment-Emergent Adverse Event.
Note: the number (N) in the safety population is used as the denominator for calculating the percentages.
Note: Only TEAEs are summarized in this table. AEs that occurred on or after Study Day 1 and within 28 days after the last dose are considered TEAEs.
Note: At each level of summation (overall, system organ class, preferred term), patients reporting more than one TEAE are counted at most once at highest severity. AEs are coded by using MedDRA version 17.1.

10.6 Patient Narratives

10.6.1 Deaths for Studies TR02-112, INS-212, and INS-312

10.6.1.1 Study TR02-112

Subject No. (b) (6) ALIS + MDR, ACUTE RESPIRATORY DISTRESS SYNDROME, a 64-year-old female with underlying bronchiectasis enrolled into the TR02-112 study and was randomized to receive ALIS + MDR. The subject's other relevant medical history included hemoptysis requiring bronchial arterial embolization, pneumonia, losing weight. She did not achieve negative sputum cultures. On Day 81 of the study, the subject was hospitalized with bacterial pneumonia and declined suddenly with acute respiratory distress syndrome (ARDS). She was transferred to the intensive care unit. A chest CT scan showed pulmonary edema and multifocal pneumonia and diffuse ground glass opacities. She became febrile with increased oxygen requirements (15 L via Oxymizer) and was treated with IV antibiotics. The following day, she continued to decline and required intubation and vasopressor agents. Influenza test was negative and a repeat chest x-ray showed progressive ARDS. Corticosteroids were initiated on Day 87 however her condition remained tenuous and on Day 90 the family asked for withdrawal of active care if there was no improvement. The subject died the following day of hypoxic respiratory failure due to ARDS.

10.6.1.2 Study INS-212

Subject No. [redacted], ALIS + Multidrug Regimen, RESPIRATORY FAILURE: A 61-year-old male with underlying bronchiectasis and COPD (stage 4) was diagnosed with MAC lung infection 4 years prior to enrolling in Study 212. The patient's other relevant medical history included smoking (43 pack years), right upper lobectomy and coronary artery disease with right heart enlargement. His BMI at baseline was 14.6. During the screening period, the patient was hospitalized for the treatment of exacerbation of COPD with oxygen saturation at 90%. Subsequently, he enrolled in Study 212 and was randomized to receive ALIS added to MDR. He did not achieve sputum culture conversion. On Day 56 of the study, the patient was hospitalized with shortness of breath and weight loss. He was treated for the possibility of pneumonia with IV antibiotics and continued treatment with ALIS+MDR. On Day 59, he was discharged showing clinical improvement. Antibiotics were stopped as pneumonia was not confirmed. On Day 70, he continued to deteriorate and was withdrawn from the study. Home hospice was organized, and he died 11 days later.

Subject No. [redacted] ALIS + Multidrug Regimen, PULMONARY EMBOLISM: A 67-year-old female with underlying pulmonary sarcoidosis and end-stage lung fibrosis for 5 years prior to enrolling in Study 212. She was randomized to receive ALIS added to MDR. She did not achieve sputum culture conversion. On Day 45 of the study, the patient was hospitalized for the treatment of pseudomonal pneumonia and pulmonary embolism while continuing treatment with ALIS + Multidrug Regimen. On Day 61, the patient died secondary to pulmonary embolism and superimposed pseudomonal pneumonia on a background of end-stage fibrosis secondary to sarcoidosis.

Subject No. [redacted], ALIS + Multidrug Regimen, RESPIRATORY FAILURE: A 79-year-old male with underlying COPD was diagnosed with MAC lung infection 3 years prior to enrolling in Study 212. The patient's other relevant medical history included smoking (60+ pack years), asbestos pleural plaques and laryngeal squamous cell carcinoma. He did not achieve sputum culture conversion. At Baseline, he had an oxygen saturation of 93% with severe airflow obstruction on spirometry (FEV_1 35% predicted). On Day 52 of the study, the patient was hospitalized with exacerbation of COPD, respiratory acidosis (arterial pH 7.16) and oxygen saturation 92%. It was noted that he had experienced significant weight loss and decreased food intake over the prior 4-6 months. Upon admission the patient was treated with Bilevel Positive Airway Pressure, however his condition worsened (arterial pH 7.12 and oxygen saturation 87%) and he was admitted for respiratory failure to the intensive care unit, however, his family requested palliative care only and the patient died the following morning from type 2 respiratory failure.

Subject No. [redacted] ALIS + Multidrug Regimen, CACHEXIA: A 61-year-old male with underlying COPD/emphysema and a history of lung lobectomy was diagnosed with MAC lung infection 17 years prior to enrolling in Study 212. The patient's other relevant medical history included smoking (40 pack years) and cachexia. His BMI at baseline was 18.0. He was randomized to receive ALIS added to Multidrug Regimen. He did not achieve sputum culture

conversion. On Day 14 of the study, the patient had further weight loss and was unable to walk without assistance and was hospitalized due to worsening of pulmonary cachexia. He underwent percutaneous endoscopic gastrostomy tube insertion for high-calorie nutrition and continued treatment with ALIS + Multidrug Regimen. On Day 23, the patient developed respiratory failure secondary to severe cachexia and died.

Subject (b) (6) ALIS + Multidrug Regimen, COPD: A 60-year-old male with underlying COPD and bronchiectasis was diagnosed with MAC lung infection 2 years prior to enrolling in Study 212. The patient's other relevant medical history included smoking (40 pack years). He was randomized to receive ALIS added to Multidrug Regimen. He did not achieve sputum culture conversion. On Day 14 of the study, the patient was hospitalized for 8 days with exacerbation of COPD. On Day 56, he was re-hospitalized for exacerbation of COPD and discharged 3 weeks later with partial clinical improvement. He was re-hospitalized 12 days later with progression of COPD for a further 15 days. He died 70 days later due to a combination of exacerbation and progression of underlying COPD.

Subject No. (b) (6), ALIS + Multidrug Regimen, LUNG INFECTION: A 70-year-old female with underlying bronchiectasis was diagnosed with MAC lung infection 6 years prior to enrolling in Study 212. The patient's other relevant medical history included tuberculosis 24 years ago. Her BMI at baseline was 18.1. She was randomized to receive ALIS added to MDR. She did not achieve sputum culture conversion. On Day 119 of the study, the patient was reported to have a chronic pulmonary infection, with dyspnea, cough and fatigue requiring supplemental oxygen. She was hospitalized for the treatment of pulmonary infection on Day 170; treatment with ALIS was interrupted. The patient died in the hospital 3 weeks later due to pulmonary infection.

Subject No. (b) (6) (MDR alone) (INTERSTITIAL LUNG DISEASE), a 71-yearold male with underlying bronchiectasis, COPD/emphysema, interstitial lung disease, cavitary lung disease and asthma was diagnosed with MAC lung infection 2 years prior to enrolling in Study INS-212. The subject's other relevant medical history included smoking (50 pack years), cardiomyopathy with implantable cardioverter/defibrillator. During the screening period, the subject was hospitalized for the treatment of pneumonia. Subsequently, he enrolled in Study INS-212 and was randomized to receive MDR alone. The subject achieved sputum culture conversion at Month 1 and maintained negative sputum cultures until the EOT visit (Month 13) while on MDR alone. During the 28-day safety follow up phase, he experienced 2 events of hemoptysis, the first one massive requiring an embolization procedure. He continued to decline with worsening interstitial lung disease and was placed into hospice care. The subject died 42 days after the EOT visit.

Subject No. (b) (6) (MDR alone) (MAC INFECTION), a 75-year-old female with underlying bronchiectasis and COPD was diagnosed with MAC lung infection 4 years prior to enrolling in Study INS-212. The subject's other relevant medical history included mycetoma and liver sarcoidosis with hepatocellular carcinoma 25 and 43 years ago. Her BMI at baseline was 17.5. She was randomized to receive MDR alone. She did not achieve sputum culture

conversion. On Day 29 of the study, the subject experienced MAC lung infection exacerbation and treatment with inhaled amikacin (IV solution) daily was initiated as a rescue medication. The subject discontinued the study per protocol. She was hospitalized on Day 32 due to inability to swallow after approximately one week of home hospice care and died 3 days later due to exacerbation of MAC lung infection.

Subject No. (b) (6) (MDR alone) (CARDIOGENIC SHOCK), a 71-year-old male with underlying COPD was diagnosed with MAC lung infection 3 years prior to enrolling in Study INS-212. The subject's other relevant medical history included tobacco use (50 pack years). He was randomized to receive MDR alone. He did not achieve sputum culture conversion. On Day 10 of the study, the subject underwent shoulder replacement surgery and experienced worsening shortness of breath post-surgery. He was discharged from the hospital 2 days later with persistent shortness of breath, chest pain and sweatiness. The following day he was re-hospitalized with respiratory distress and chest pain (oxygen saturation 92% on 4L oxygen). His pro-b-type natriuretic peptide and troponin levels were elevated and he had a significant obstructive coronary artery disease in his left main and left anterior descending coronary artery on cardiac catheterization. The subject died during the procedure from cardiogenic shock due to systolic dysfunction and malignant refractory arrhythmias.

Subject No. (b) (6) (MDR alone) (PNEUMONIA), a 64-year-old female with underlying bronchiectasis and COPD was diagnosed with MAC lung infection for an unknown period prior to enrolling in Study INS-212. The subject's other relevant medical history included mycetoma and malnutrition. Her BMI at baseline was 14.6. She was randomized to receive MDR alone. She did not achieve sputum culture conversion. On Day 31 of the study, the subject was hospitalized with recurrence of mycetoma. One week later despite bilevel positive airway pressure she respiratory failure (arterial pH 7.1) requiring mechanical ventilation. She recovered and was discharged on Day 53. On Day 162, she was hospitalized for 3 days with exacerbation of COPD. On Day 195, she was hospitalized for one day with exacerbation of bronchiectasis. On Day 219, she was hospitalized for clinical deterioration, pneumonia was diagnosed 7 days later and the subject died of pneumonia 3 days after diagnosis.

Subject No. (b) (6) (MDR alone) (RESPIRATORY FAILURE), a 74-year-old male with underlying bronchiectasis was diagnosed with MAC lung infection 3 years prior to enrolling in Study INS-212. The subject's other relevant medical history included right ventricular hypertrophy and enlargement. He was randomized to receive MDR alone. He did not achieve sputum culture conversion. On Day 141 of the study, the subject was hospitalized with cardiac failure, oxygen saturation 86% and severe acidosis (pH 7.09) for which he was mechanically ventilated in the intensive care unit for 6 days. He then transferred to the ward on noninvasive ventilatory support. One week later, he was re-intubated and considered to be in respiratory failure. He required prolonged chest drain insertion for pneumothorax and bronchial fistula. The subject died 20 days later due to respiratory failure.

10.6.1.3 Study INS-312

Subject No. (b) (6) ALIS + Multidrug Regimen, COPD: A 63-year-old female with underlying emphysema and bronchiectasis was diagnosed with MAC lung infection 3 years prior to enrolling in Study 212 and then Study 312. She was randomized to receive ALIS + Multidrug Regimen in Study 212, did not achieve sputum culture conversion in Study 212 and entered Study 312. She did not achieve sputum culture conversion in Study 312. Her overall health status declined progressively during Study 212 with pneumonia, acute respiratory failure, aspergillosis and >10% weight loss. On Day 76 in Study 312, the patient was hospitalized for 3 days the treatment of bronchiectasis exacerbation, while continuing treatment with ALIS + Multidrug Regimen. On Day 121, the patient was visited by homecare and she was mobilizing and reported overall improvement however 3 days later the patient died due to COPD.

Subject No. (b) (6), ALIS + Multidrug Regimen, LOWER RESPIRATORY TRACT INFECTION: A 71-year-old female with underlying COPD and bronchiectasis was diagnosed with MAC lung infection 3 years prior to enrolling in Study 212 and then Study 312. The patient's other relevant medical history included smoking (50 pack years). She did not achieve sputum culture conversion in Study 212 and entered Study 312. She did not achieve sputum culture conversion in Study 312. At the Study 312 baseline visit her BMI dropped to 14.6 and oxygen saturation to 93%. The patient experienced 12 serious adverse events during Study 212, including 2 events of COPD exacerbation and 1 event of lower respiratory infection in the screening period and a further 6 events of COPD during the study, all of which resolved after a duration of 1-12 days without study drug interruption. On Day 64 of the Study 312, the patient was hospitalized with a chest infection, continued to clinically deteriorate and was withdrawn from the study and began palliative care on Day 90. The patient died 22 days later due to COPD and bronchiectasis.