



Liposomal Amikacin for Inhalation (LAI)

NCT02344004

INS-212

A Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation (LAI) in Adult Patients with Nontuberculous Mycobacterial (NTM) Lung Infections Caused by *Mycobacterium avium* complex (MAC) That are Refractory to Treatment

Phase 3

Protocol Amendment #3

Version Date: 22 February 2016

EudraCT Number
2014-005010-31

Insmmed Incorporated
10 FINDERNE AVENUE, BUILDING 10
BRIDGEWATER, NJ 08807-3365

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SIGNATURE PAGE FOR INSMED INCORPORATED

(Hereinafter called Insmed)

Drug name

Liposomal Amikacin for Inhalation (LAI)

Protocol Title

A Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation (LAI) in Adult Patients with Nontuberculous Mycobacterial (NTM) Lung Infections Caused by *Mycobacterium avium* complex (MAC) That are Refractory to Treatment

Protocol Number

INS-212

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2014-005010-31

Name of Approver

[REDACTED]

Title of Approver

[REDACTED]

Signature of Approver _____

Date: 22 February 2016

[REDACTED]

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SIGNATURE PAGE FOR THE INVESTIGATOR

Protocol Number
INS-212

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Institutional Review Board/Independent Ethics Committee (IRB/EC) and will fulfill all responsibilities for submitting pertinent information to the IRB/EC responsible for this study.

I agree that the Sponsor, Insmmed Incorporated, or its representatives shall have access to any source documents from which case report form information may have been generated.

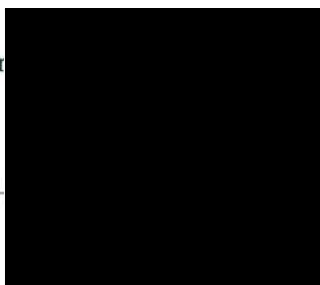
I further agree not to originate or use the name of Insmmed Incorporated, or study drug code in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol, without the prior written consent of Insmmed Incorporated.

Name of Approver

Type or print name of signer

Signature of Approver

Date: mm/dd/yyyy



05/25/2016

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IMPORTANT CONTACTS**Insmmed Incorporated**

10 Finderne Avenue, Building 10
 Bridgewater, NJ 08807-3365

Insmmed Global Clinical And Drug Safety			
Name:	Title	Telephone	e-Mail Address

Clinical Research Organization

Vendor Name	SynteractHCR
Address	5759 Fleet Street Suite 100 Carlsbad, CA 92008
Telephone Number	
Fax Number	
E-mail Address	www.synteracthcr.com

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SUMMARY OF CHANGES

Section	Change	Rationale
Various	Typographical errors	Corrected any misspellings, removed extra punctuation marks and spaces and revised phrases to ensure consistency throughout the document
Important Contacts	Updated Insmmed personnel.	Decision was made to change the department included in the contact information and to add email addresses.
Table 3-1 Schedule of Events	<p>Changed from Table 1 to Table 3-1.</p> <p>Changed visit windows for Months 10, 12, 14, EOT, and 28-day follow-up (Visits 10, 11, 12, 13, and 14) from ± 3 days to ± 5 days.</p> <p>Changed the location of the row for the CT Scan Substudy and added a row for the Japan CT Scan Substudy.</p> <p>Added row for starting the consent process for Study INS-312 with the activity added to Month 6 (Visit 8).</p>	<p>Changed to have consistent table numbering format throughout.</p> <p>Change required for practical reasons related to scheduling visits.</p> <p>Rows reorganized for clarity. Row for Japan CT Scan Substudy added because Japan requested to have a CT Scan Substudy substantially different from the one for the rest of the world.</p> <p>Subjects will be provided with information about Study INS-312 at Month 6 so that they have time to make an informed decision before Month 8 when some subjects will become eligible for Study INS 312.</p>
Table 3-1 Schedule of Events and Sections 6.3, 7.2.2.1, and 7.2.3.1	Physical examination and vital signs removed from Months 1, 2, and 5 (Visits 3, 4, and 7).	Removed physical examination and vital signs at Months 1, 2, and 5 to simplify study procedures.
Table 3-1 Schedule of Events	Deleted “in a subset of subjects” from the row for the Population PK sampling.	The phrase was redundant as “Sub-Set” is already at the top of the row.
Table 3-1 Schedule of Events	“Comprehensive” was added as a descriptor for the PK sampling in Japanese subjects. Sample type and Japanese subjects were removed.	The name of the Japanese PK substudy was changed to include the word “Comprehensive”. The sample type and the inclusion of only Japanese subjects in this substudy are both described in the footnote.

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Table 3-1 Schedule of Events	Remove dosing at EOT visit.	There is no dosing at EOT visit.
Table 3-1 Schedule of Events	Table footnotes were modified (see rationale). Footnote lettering was updated.	<p>Some footnotes were deleted and others modified/added. Wording and typographical errors were edited/fixed for clarity. The abbreviations list was modified to include all abbreviations used in the table and its footnotes. Redundant visit window footnotes were deleted. Redundant footnote on patient-reported outcomes being assessed before study drug administration was deleted.</p> <p>The pregnancy test footnote was modified to reflect a serum pregnancy test only at screening with all other pregnancy tests done using urine. A footnote for the treatment blinding requirement for the site personnel conducting the 6MWT was added. The audiology and chest CT scan footnotes were edited for clarity. The chest CT scan footnote was modified to reflect the desire for a high resolution scan if possible. The AE footnote was edited for clarity. The footnote on discontinuation procedures was deleted. The 2 footnotes on population PK were combined and edited for clarity and inclusion of geographic region. The Japanese PK substudy footnote was changed to the Comprehensive PK substudy footnote to reflect the change in the name of the substudy and details were replaced with a reference to the substudy protocol. The study drug dispensing footnote was edited for clarity. The 28-day safety follow-up footnote was deleted because it was confusing and not necessary.</p> <p>New footnotes were added to provide details on the length of the Screening period and scheduling the Day 1 visit in case of delays in obtaining Screening sputum results; to provide for home health visits for Months 1, 2, and 5 for qualifying patients who may have difficulty attending clinic visits; to start the Study INS-312 consent process at Month 6; and to specify that PFTs will be performed at sites with appropriate equipment and trained personnel.</p>
Synopsis and Section 2.1	Changed 3 consecutive negative cultures to 3 consecutive monthly negative cultures.	Clarified the frequency of sputum cultures
Synopsis and Sections 2.2 and 2.3	<p>In secondary objective 4, changed 2 consecutive broth positive cultures to 2 consecutive monthly broth positive cultures.</p> <p>In exploratory objective 8, added EOT as a timepoint.</p>	<p>Clarified the frequency of sputum cultures</p> <p>Changed for consistency. The secondary objective of evaluation of EQ-5D-3L change from baseline applies to both Month 6 and EOT.</p>
Synopsis and Sections 2.5, 6.3, and 7.4.1	Changed 72 hours off LAI to 2 days interruption of LAI.	Sputum sample for PK will be collected at the clinic visit which is preceded by 2 days of a scheduled/planned interruption in LAI dosing for subjects randomized to LAI plus multi-drug per Section 7.1.1. The original intent of the protocol was for subjects to interrupt dose for 2

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		days as described. 72 hours assumes the subject takes the dose in the morning, have 2 days of interruption, and have their clinic visit in the morning of the next day (accounting for 72 hours). Since 72 hours was being misunderstood as 3 days, the protocol was revised to reflect 2 days of interruption as per original intention and per protocol. There is no change from 3 to 2 days.
Synopsis and Sections 2.5.1 and 7.4.3.	Changed Japanese to Comprehensive. In Section 2.5.1, listed the 3 specific substudy objectives.	The name of the substudy was changed from Japanese PK Substudy to Comprehensive PK Substudy based on a request from Japan. Section 2.5.1 was modified to match the synopsis and the objectives listed in the substudy.
Synopsis and Section 1	Background and rationale were updated/edited.	Edits were made for clarity. In Section 1.3, but not synopsis, information about treatment-related lung tumors observed at the end of 2 years of dosing in 2 of 120 rats was added.
Synopsis and Section 3.1	Study description and design were extensively edited.	Incorporated changes from Japanese specific amendment and clarified exceptions to the study entry requirement for 6 consecutive months of multi-drug treatment regimen. Exceptions included treatment doses/frequencies below those recommended by guidelines and/or drug interruptions due to safety/tolerability issues. This was done to represent clinical practice, as medications are tailored based on patient needs. Clarified that a multi-drug regimen consists of at least 2 antibiotics. Removed RNA sequencing and replace with MIC determination to clarify that study eligibility will be based on MIC determination instead of RNA sequencing. Clarified definitions of converters and non-converters. Clarified process for determining converter/non-converter status and subsequent study progress. Clarified that all NTM treatment ends at the EOT visit and that no NTM treatment will be given during the safety follow-up or off-treatment period. Clarified that subjects eligible for a separate open-label study will include non-converters and converters who experienced a relapse/recurrence by Month 6. Removed detailed information on follow-up procedure for patients who prematurely discontinue from the study (not needed here in the synopsis). Added the option for home health visits for Months 1, 2, and 5 for qualifying patients who may have difficulty attending clinic visits. More details are in Section 3.1 than the synopsis. Added instructions regarding the scheduling of Day 1 in case of delays in obtaining Screening sputum culture results.
Synopsis	PK assessments and their time points were edited and placed clearly in specific categories based on activity, region, and sample type. Removed details about timing relative to dosing for the Population PK blood	Activities were placed in one of the following 3 categories to clarify which samples would be taken from subjects in what locations for what purpose: <ul style="list-style-type: none"> • Population PK Serum and Sputum (US and Japan only) • Sputum PK Substudy (Global) • Comprehensive PK Serum and Sputum Substudy (Japan only)

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	draws. Added baseline as a timepoint and changed 72 hours after LAI dosing to 2 days interruption of LAI dosing for Months 4, 5, and 6 for Sputum PK Substudy. Changed Japanese to Comprehensive for the serum and sputum PK substudy in Japan only. Added “in a subset” of Japanese subjects to the activity for the Comprehensive PK Serum and Sputum substudy (Japan only).	Removed unnecessary details from the synopsis. Added necessary details to the synopsis. Changed the name of the Japan only substudy to reflect the change in the substudy’s name.
Synopsis and Section 4.1	Limited subject age to 20 or older in Japan in criterion #1. Revised wording about prior multi-drug regimens in criterion #2. Made the high-resolution aspect of the diagnostic CT scan a preference rather than mandatory in criterion #3. Revised text for the requirement of MAC positive cultures in criterion #4. Revised text for the contraception methods in criterion #8.	Japan requested a lower limit of 20 years old rather than 18 years old. Incorporated changes from Japanese specific amendment and clarified exceptions to the study entry requirement for 6 consecutive months of multi-drug treatment regimen. Clarified that high resolution CT scan is not required for inclusion criteria 3. High resolution CT scan is preferred, if available. Regular CT scan can be used. Clarified that at least 2 positive cultures are needed to be eligible to participate in the study. One positive culture should be obtained within 6 months prior to Screening, and one positive culture at Screening. Edited for clarity.
Synopsis and Section 4.2	Revised MIC susceptibility to >64 µg/mL in criterion #2. Changed gentamicin to kanamycin in criterion #13. Changed criterion #22 from disallowing continuous oxygen use to disallowing subjects unlikely to survive long	Added units for MIC. Clarified from amendment 2 that MICs with >64 µg/mL will be excluded. Subjects with MIC = 64 µg/mL can be included. Corrected the antibiotic that was listed as an example. Oxygen use (continuous or otherwise) is allowed. The concern was including subjects too sick to survive the duration of the study. Modified exclusion criterion to address the concern directly.

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	enough to finish the study.	
Synopsis and Section 4.3	Reason 2 was edited to clarify that not all AEs automatically lead to discontinuation. Reason 4 was edited to provide more detail. Added Reason 8: Any subject who has to have rescue medication will be discontinued.	Clarified that Investigator and/or subject discretion should be applied when deciding if an AE should result in subject withdrawal from the study. Added definition of subject noncompliance and clarified that Investigator discretion should be applied in deciding if the subject should be withdrawn from the study. Added for consistency throughout the protocol.
Section 4.3	Reference to end-of-study visit replaced with end-of-treatment visit and added a reference to Section 5.12.1.	Discontinued subjects are supposed to have an EOT visit, not an EOS visit. Section 5.12.1 is a new section with scenarios for follow-up procedures based on when (during the study) the subject discontinues study drug or study participation.
Synopsis and Sections 3.2 and 3.3	Added “monthly” and deleted “collected monthly without relapse or recurrence” from the description of sputum cultures in the primary endpoint. Month 6 was added as timepoint for exploratory endpoint 8.	Modified to include the frequency of sputum cultures and match the text in the primary objective. Clarified the definition of culture conversion. Added Month 6 as a timepoint to match the corresponding exploratory objective.
Synopsis and Sections 9.4 and 9.6.2	Analysis specified as the final analysis for the primary endpoint and other key endpoints based on Month 6 data. Primary and sensitivity analyses methods for comparing the treatment groups for the change from baseline to Month 6 in 6MWT distance were switched (flipped).	Analyses specified per agreement with FDA. Analyses methods for primary and sensitivity analyses were switched per FDA recommendation.
Synopsis and Section 3.6.3	Recruitment time for the study changed from 12 months to 18-24 months.	Recruitment period modified to reflect latest feasibility assessment.
Study Schematic	Modified figure.	Modified to reflect clarifications in the Synopsis/Section 3.1 Study Design

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Figure (in synopsis and Figure 3-1)		
Section 3.6.3	Added reference to Section 5.12.1 for procedures for study discontinuation.	Section 5.12.1 is a new section with scenarios for follow-up procedures based on when (during the study) the subject discontinues study drug or study participation.
Section 4.4 and Section 6.2	Added instructions regarding the scheduling of Day 1 in case of delays in obtaining Screening sputum culture results.	Added for consistency with the rest of the protocol.
Section 4.6	Added “and Converter” to the section heading. Added definition of converter, relapse/recurrence, and non-converter. For the definition of completer, switched the order of presentation to converters before non-converters.	Added definitions of converter, relapse/recurrence, and non-converter for clarity. Switched the order of converters and non-converters in the definition of completer to parallel the order of the definitions of converter and non-converter.
Section 5.3	Updated LAI administration time from 13 minutes to 14 minutes.	LAI administration is projected to last approximately 14 minutes.
Section 5.3 and Section 5.4 and Section 5.8.4	Added dose interruption information.	Clarified that LAI dose interruptions are allowed if due to respiratory adverse events that may affect continuation in the study. Re-introduction of LAI is recommended.
Section 5.8.5	Added text to the description of dispensing of study drug.	Clarified that the amount of LAI dispensed to subjects in that treatment group would include extra supply in case of delays in visit scheduling.
Section 5.8.6	Added text regarding documentation of destruction of drug supplies.	Clarified that documentation should be provided to the Sponsor.
Section 5.9	Defined multi-drug regimen as including at least 2 antibiotics. Added text regarding the cessation of all NTM lung infection treatment after the EOT visit.	Clarified what is a multi-drug regimen and that it can be based on local guidelines. Clarified that all NTM treatment ends at the EOT visit and that no NTM treatment will be given during the safety follow-up period. Specified that the Sponsor will pay for it where allowed. Provided examples of drugs that could be part of it.

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	Added text specifying payee for multi-drug regimen and drugs that could be in that regimen.	
Section 5.11	Removed paragraph restricting certain types of oxygen therapy. Added a Table with examples of precautionary medications that may interact with LAI.	Subjects are allowed to use oxygen therapy. Provided concrete examples of potential drug-drug interactions. Per agreement with PMDA.
Section 5.12	Added “and/or Study Participation” to the section heading. Modified existing text and added text specifying that discontinuation of study drug does not automatically mean discontinuation from study participation (except in Japan). Added Section 5.12.1 Procedures for Discontinuation	This section is about discontinuation of study drug and/or study participation not just discontinuation of study drug. It is possible to discontinue study drug but continue study participation, depending on the circumstances. Text was edited for clarity and to incorporate the requirement that discontinuing study drug also means discontinuing study participation only in Japan. Added scenarios for follow-up procedures based on when (during the study) the subject discontinues study drug or study participation.
Section 6 (multiple places in this section)	Added cross references to Appendix 5 for CT scans. Added activities in the Japan-specific CT Scan Substudy. Changed Japanese to Comprehensive. Changed cross reference for sputum collection containers from Section 7.2.7 to 7.1.1. Changed 72 hours to 2 days interruption of LAI for the sputum-only PK substudy.	Request from Japan to have a CT Scan Substudy substantially different from the one for the rest of the world. Request from Japan to change the title of the Japan-specific PK substudy. Corrected a previous error. Sputum sample for PK will be collected at the clinic visit which is preceded by 2 days of a scheduled/planned interruption in LAI dosing for subjects randomized to LAI plus multi-drug per Section 7.1.1. The original intent of the protocol was for subjects to interrupt dose for 2 days as described. 72 hours assumes the subject takes the dose in the morning, have 2 days of interruption, and have their clinic visit in the morning of the next day (accounting for 72 hours). Since 72 hours was being misunderstood as 3 days, the protocol was revised to reflect 2 days of interruption as per original intention and per protocol. There is no change from 3 to 2 days.

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	<p>Added “and sputum” and “in Japanese subjects only” for the bullet about the Japanese (now Comprehensive) PK Substudy.</p> <p>Visit windows for Months 10, 12, 14, EOT, and 28-day safety follow-up were changed from ± 3 days to ± 5 days.</p>	<p>Sampling includes sputum as well as blood (serum). Clarified to the rest of the world that the Comprehensive PK Substudy applies only in Japan.</p> <p>Change required for practical reasons related to scheduling visits.</p>
Section 6.3	<p>Added sentence on home healthcare visits for qualified subjects for Months 1, 2, and 5.</p> <p>Removed physical examinations and vital signs at Months 1, 2, and 5</p> <p>Added “and sputum” and specified only at Month 6 for biomarker samples.</p> <p>Added “by the central microbiology laboratory” in regards to culture conversion assessment.</p> <p>Deleted definitions of converter, relapse, and other details about assessing conversion, remaining in this study, or going to the separate open-label study.</p>	<p>Added the option for home health visits for Months 1, 2, and 5 for IRB/EC approved sites approved to conduct Home Healthcare visits, and for qualifying patients who may have difficulty attending clinic visits.</p> <p>Physical examinations and vital signs no longer done at Months 1, 2, and 5.</p> <p>Biomarkers are being assessed in sputum as well as serum.</p> <p>Added to specify who makes the culture conversion assessment and to discourage sites from using local laboratories for this.</p> <p>This information is provided in Section 3.1. Unnecessarily repeated information was deleted.</p>
Section 6.7	<p>Removed administer study drug at the study site. Removed references to before or after study drug administration.</p>	<p>Study drug is not administered at the EOT visit.</p>
Section 6.8	<p>Added “for subjects who complete 12 months of treatment” to the section heading.</p> <p>Added dispense sputum collection containers to the 28-day, 3-month, and</p>	<p>Subjects who do not finish 12 months of treatment starting from the first of 3 negative cultures that defines culture conversion will not participate in the off-treatment phase.</p> <p>Subjects are still collecting 3 sputum samples (2 at home on the 2 consecutive days before the clinic visit) per timepoint.</p>

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	6-month off treatment visits.	
Section 6.9	Modified text regarding timing of the visit.	Clarified timing of the EOS visit as occurring 12 months after the EOT visit. Clarified the EOS visit is for all subjects who complete 12 months of treatment from their first of 3 negative cultures that classified them as a converter.
Section 7.1.1	Instructed sites not to perform local mycobacterium sputum cultures	Clarification why sites cannot perform their own sputum cultures for mycobacteria. Performing local mycobacterium sputum cultures would introduce bias to patient treatment plans.
Section 7.2.4	Include provision for PFTs before and after LAI dosing for subjects randomized to LAI plus multi-drug.	Included provision for airway hypersensitivity testing for patients prior to LAI administration if necessary.
Sections 7.2.5.1 and 7.2.5.2	Specified that the baseline ECG will be done at Screening.	Revised for consistency within protocol.
Section 7.2.7.2	Added “and hematology”	Central laboratory will be used to perform hematology assessments as well as clinical chemistry assessments.
Section 7.3.3	Added “or Different” to section heading.	Clarified that strain or species of MAC will be analyzed in subjects who achieved culture conversion but subsequently have a positive culture to determine causative agent of recurrence or relapse of NTM disease.
Section 7.3.3.1	Included more details on the methodology of MAC identification.	
Section 7.3.3.2	Modified text.	
Section 7.3.4	Included wording that high resolution CT scan is preferred, if available. Added cross reference to Appendix 5.	Clarification that high resolution CT scan is preferred, if available. Request from Japan to have a CT Scan Substudy substantially different from the one for the rest of the world.
Section 7.4.1	Modified text for the population PK subset. Added Baseline timepoint for the sputum-only PK substudy.	Clarify that the population PK subset only applies to US and Japan sites. Other edits were also made for clarity. Sputum sample for PK assessment will be collected at Baseline for this substudy.
Section 7.5	Added “except Month 10, 14, and off-treatment visits.”	Healthcare resource utilization is not being assessed at these visits.
Section 8.1	Added clinically relevant laboratory abnormalities to the list of examples of AEs.	Added for clarity.

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Section 8.2	Identified the IB as the reference safety information for determining expectedness of an AE.	Modified previous text to be more relevant.
Section 8.3	Added paragraph on how to handle expected events of pulmonary exacerbations.	Provided instructions on how to define and report pulmonary exacerbations.
Section 8.4	Specified that corrections and additions to an SAE report should be reported to the Sponsor/delegate within 24 hours. Deleted text that appeared to request the site to notify health authorities of deaths and life-threatening events. Deleted Section 8.4.3.	Corrected previous text that specified 48 hours. The Sponsor or designee will notify the health authorities of such events as required. No such “other reportable events” have been identified. Pulmonary exacerbation text was modified and moved to Section 8.3.
Section 8.6	Changed SAEs to SUSARs.	Corrected a previous error.
Section 9.5.2	Deleted “did not miss more than 20% of doses to Month 6.”	Modified text to clarify the definition of per protocol analysis based on allowance of dose interruptions due to respiratory events per Investigator discretion and medical monitor discussion.
Section 9.6.1.	Added text to the sentence on subjects with missing sputum cultures being identified as non-converters.	Text was modified because, depending on which culture timepoints are missing and the results of the cultures surrounding those missing timepoints, it may be possible to classify a subject with limited missing sputum cultures as a converter.
Section 9.6.2	Added statement that LOCF will be used in a supportive ANCOVA for change from Baseline to Month 6 in 6MWT distance for the ITT population.	Added for clarity.
Section 9.6.3	Specified that a prior CT scan will be used in the analysis if an EOT CT scan is not performed.	Added for clarity.
Section 9.7	Removed text about how to follow up subjects who	Text is redundant with content in Section 5.12 and does not belong in the Safety Analysis section.

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	prematurely discontinue the study.	
Sections 12.4 and 12.5	Modified text regarding sample storage.	Specified which samples for what purpose will be stored in what location for how long.
Appendix 1	Language was softened to show the information provided is a suggestion and not mandatory.	Original text was too prescriptive and did not allow sites to follow their standard procedures for sputum induction.
Appendix 3 Table 14-2	Changed “Japanese” to “Comprehensive” in the name of the substudy. Revised wording and schedule of events.	Name changed per request from Japan. Clarified procedures for including Japanese subjects in Japan in the substudy. Provided more details on sampling times for both sputum and blood (serum).
Appendix 4	Added preference for high resolution chest CT scans, if available.	Modified text to be in alignment with the preference for high resolution CT scans everywhere else in the protocol.
Appendix 5	Added Appendix 5	Request from Japan to have a CT Scan Substudy substantially different from the one for the rest of the world.

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STUDY SYNOPSIS

Study Title	A Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation (LAI) in Adult Patients with Nontuberculous Mycobacterial (NTM) Lung Infections caused by <i>Mycobacterium avium</i> complex (MAC) that are refractory to treatment
Name of Test Drug/ Investigational Product	Liposomal Amikacin for Inhalation (LAI)
Active Pharmaceutical Ingredient	Amikacin sulfate
Objectives	<p>Primary Objective</p> <ol style="list-style-type: none"> 1. To evaluate the efficacy of LAI (590 mg) administered once daily (QD), when added to a multi-drug regimen, for achieving culture conversion (3 consecutive monthly negative sputum cultures) by Month 6 compared to a multi-drug regimen alone <p>Secondary Objectives</p> <ol style="list-style-type: none"> 1. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the six-minute walk test (6MWT) at Month 6 compared to a multi-drug regimen alone 2. To evaluate the efficacy of LAI (590 mg) QD when added to a multi-drug regimen on the durability of treatment success 3 months after the end of total treatment course (negative sputum culture after 3 months off treatment) 3. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen for time to culture conversion compared to a multi-drug regimen alone 4. To evaluate the efficacy of LAI (590 mg) administered QD, when added to a multi-drug regimen for achieving sustainability (consecutive negative sputum cultures [with no more than 2 consecutive monthly broth positive cultures] for 12 months on treatment) compared to a multi-drug regimen alone 5. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the 6MWT at End of Therapy (EOT) compared to a multi-drug regimen alone 6. To evaluate patient-reported symptoms of NTM and change from Baseline (Day 1) in quality of life scores on the St. George's Respiratory Questionnaire (SGRQ) at Month 6 <p>Exploratory Objectives</p>

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	<ol style="list-style-type: none"> 1. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters within the LAI (590 mg) administered QD added to a multi-drug regimen arm 2. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters for all subjects 3. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters within the multi-drug regimen alone arm 4. To evaluate the change from Baseline to Month 6 on Body Mass Index (BMI) for those randomized to LAI (590 mg) administered QD added to a multi-drug regimen compared to a multi-drug regimen alone 5. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the 6MWT at Month 8 and 3 months off-treatment compared to a multi-drug regimen alone 6. To evaluate the proportion of subjects achieving culture conversion with durability after 12 months off treatment (EOS) in the LAI arm compared to a multi-drug regimen alone 7. To compare patient-reported symptoms of NTM and change from Baseline (Day 1) in quality of life scores on the SGRQ – Part 2 (Activities of Daily Living) at Month 6 8. To compare change from Baseline (Day 1) in the EQ-5D-3L patient reported health outcomes at Month 6 and EOT 9. To evaluate the number of subjects in each treatment arm who develop a new strain of MAC during the study at EOS 10. To compare all-cause mortality between treatment arms 12 months after treatment (EOS) <p>Safety Objective</p> <ol style="list-style-type: none"> 1. To evaluate the safety and tolerability of LAI (590 mg) QD added to a multi-drug regimen arm <p>Pharmacokinetic Objectives</p> <ol style="list-style-type: none"> 1. To evaluate the pharmacokinetics (PK) of LAI via a population PK approach 2. To evaluate the concentration of amikacin in sputum after 2 days interruption of LAI dosing in a subset of subjects 3. To evaluate the concentration of amikacin in sputum after 28 days off-LAI in a subset of subjects 4. To evaluate the concentration of amikacin in sputum after 3 months off-LAI in a subset of subjects <p>Comprehensive Pharmacokinetic Sub-Study Objectives</p>
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	<ol style="list-style-type: none"> 1. To characterize systemic amikacin exposure, including approximate systemic bioavailability in Japanese subjects, using a population PK model to describe the disposition of amikacin in the serum 2. To compare population PK modeling between Japanese subjects to historical population PK modeling for LAI 3. To evaluate the amikacin concentration in sputum <p>CT Scan Sub-Study Objective</p> <ol style="list-style-type: none"> 1. To compare assessments of chest CT scans, read by a trained medical professional, from Baseline (Day 1) to EOT in a subset of subjects within each treatment arm
Study Description	<p>Study Rationale</p> <p>Treatment guidelines for patients with MAC lung infection in the published American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) consensus document were based primarily on small uncontrolled or non-comparative studies in subjects with predominantly severe or refractory MAC disease [Griffith et al, 2007].</p> <p>Overall, there remains a lack of sufficiently powered, prospective clinical trials aimed at the treatment of pulmonary MAC infection. Many of the drugs used in the prolonged, recommended multi-drug regimens are expensive and poorly tolerated [Ballarino, et al 2009]. It is clear from these single-site studies that there is an unmet need for more effective, less-toxic therapeutic options.</p> <p>Study TR02-112 was a randomized double-blind placebo controlled study that dosed 89 subjects with LAI or placebo added to a background multi-drug regimen. At Day 84, 25.0% of subjects with add-on LAI had a negative sputum culture compared to 6.7% of subjects with add-on placebo. This is an important result in this population as subjects were refractory to therapy for a minimum of 6 months to more than 2 years prior to enrollment.</p> <p>Study INS-212 will evaluate whether the signal identified in TR02-112 is further confirmed with a longer duration of LAI treatment in subjects with NTM MAC lung infections who are refractory to a stable multi-drug regimen for at least 6 months. INS-212 is a randomized, open-label study of efficacy, safety and tolerability of once daily dosing of 590 mg LAI plus a multi-drug regimen compared to a multi-drug regimen alone in subjects with NTM lung infections that are refractory to therapy. Eligible subjects are those diagnosed with MAC and with persistently positive cultures while being treated with a multi-drug treatment regimen (at least 2 antibiotics) for a minimum duration of 6 consecutive months that is either ongoing or completed no more than 12 months before Screening (exceptions to multi-drug treatment regimen for 6 consecutive months include treatment with doses or frequencies below those recommended by guidelines and/or short interruptions of therapy, both occurring due to safety/tolerability issues).</p> <p>One to three sputum samples collected at Screening will be used to determine eligibility of the subject. At Baseline, subjects will start or continue on a multi-drug anti-mycobacterial regimen (at least 2 antibiotics) and will be randomized to LAI (590 mg) QD plus a multi-drug regimen or to a multi-drug regimen alone for a</p>

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	<p>minimum of 8 months. Subjects who demonstrate culture conversion by Month 6 will complete a total treatment course of 12 months starting from the first of 3 negative cultures that defines culture conversion.</p> <p>Randomization will be stratified according to subject smoking status (current smoker or not) and current multi-drug regimen status (on treatment or off treatment for at least 3 months) at Screening.</p> <p>Design</p> <p>The Screening window allows time for sputum culture results, susceptibility by 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. Once Screening assessments are complete and sputum culture results are known, eligible subjects will be randomized 2:1 to LAI administered QD plus a multi-drug regimen or a multi-drug regimen alone. The primary efficacy endpoint is the proportion of subjects achieving culture conversion (3 consecutive monthly negative sputum cultures) by Month 6 in the LAI plus a multi-drug regimen arm compared to a multi-drug regimen alone.</p> <p>Converters are defined as subjects who have 3 consecutive monthly MAC-negative sputum cultures at any time within the first 6 months of the study. After culture conversion, relapse or recurrence is defined as having MAC-positive sputum cultures in liquid broth media (agar negative) for 3 or more consecutive months, or having 1 MAC-positive sputum culture on solid media (agar positive). Non-converters are defined as subjects who do not have 3 consecutive monthly MAC-negative sputum cultures at any time within the first 6 months of the study.</p> <p>Sputum culture results will only be available to the site after the Month 6 sputum result is known, in time for the Month 8 visit. At Month 8 (-28 to +7 days), after all sputum culture results are known, up to and including Month 6, subjects will be assessed as converters or non-converters.</p> <p>All converters will remain in Study INS-212. All converters who, after culture conversion, subsequently have MAC-positive sputum cultures in liquid broth media (agar negative) for 1 or 2 consecutive months only, will also remain in Study INS-212. These subjects will continue on their randomized treatment regimen until they complete a total of 12 months of treatment (EOT), starting from the first of 3 negative cultures that defines culture conversion. These subjects will return after the EOT visit for 28 day, 3, 6 and 12 months off-treatment follow-up visits. The 12 months off-treatment follow-up visit will be the end of study (EOS) visit. No NTM treatment will be administered during the off-treatment phase.</p> <p>At Month 8, all non-converters will be discontinued from INS-212. All subjects who experienced a relapse or recurrence after culture conversion by Month 6 will also be discontinued from INS-212 at their Month 8 visit. These subjects may be eligible to</p>
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	<p>enter a separate open-label study of LAI (Study INS-312), provided all entry criteria have been met for that study.</p> <p>Expectorated sputum (spontaneous or induced e.g., with nebulized hypertonic saline solution as needed) will be collected at Day 1 and every month through Month 6, at Month 8, Month 12, EOT, and at 28 days, 3, 6, and 12/EOS months off-treatment.</p> <p>All subjects will come in monthly for routine visits through Month 6, at Month 8, 10, 12, 14, EOT, and at 28 days, 3, 6 and 12/EOS months off-treatment. Home Healthcare visits may be available at Months 1, 2, and 5 for sites with IRB/EC approval to conduct Home Healthcare visits, and for qualifying subjects who may have difficulty attending clinic visits. Unscheduled visits will occur as needed should subject's symptoms worsen between visits.</p>
Pharmacokinetics	<p>Population PK Serum and Sputum (US and Japan only)</p> <ol style="list-style-type: none"> 1. Sputum and serum samples will be collected at Months 1, 3 and 6 in a subset of subjects on LAI. <p>Sputum PK Sub-Study (Global)</p> <ol style="list-style-type: none"> 1. Sputum samples only will be collected at Baseline, Months 4, 5, 6 (after 2 days of interruption of LAI dosing) and at the 28-day follow-up visits in a separate subset of subjects on LAI. 2. Sputum samples only will be collected at the 3-month follow-up visit in a separate subset of subjects on LAI identified at EOT. <p>Comprehensive PK Serum and Sputum Sub-Study (Japan only)</p> <ol style="list-style-type: none"> 1. Comprehensive PK sampling of blood and sputum will be collected at Baseline, Months 1, 3, and 6 in a sub-set of Japanese subjects.
Study Duration	<p>This study has an anticipated recruitment period of 18-24 months, with each subject receiving treatment in the study for up to 16 months (excluding Screening and observational follow-up). Subjects will remain in the study for up to 31 months, including approximately 2.5 months for Screening, up to 16 months in the randomized open-label treatment phase, and 12 months in the off-treatment phase.</p>
Study Population	<p>The study is designed to enroll enough subjects to ensure at least 261 evaluable subjects reach Month 6. Accounting for discontinuation, it is anticipated that up to approximately 351 randomized subjects may be required. Eligible subjects are those diagnosed with NTM lung infections caused by MAC who meet the entry criteria below. Subjects will be randomized 2:1 to treatment with LAI plus a multi-drug regimen or a multi-drug regimen alone. This study is planned to be conducted at approximately 150 sites in North America, Europe, and the Asia-Pacific regions.</p>
Inclusion Criteria	<p>To be eligible for enrollment, a patient must meet all of the following criteria:</p> <ol style="list-style-type: none"> 1. be male or female, 18 years or older (20 years or older in Japan) 2. be positive for MAC on culture as defined in inclusion criterion No. 4, while being treated with a multi-drug treatment regimen (at least 2 antibiotics) for a minimum duration of 6 consecutive months that is either ongoing or was

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	<p>stopped no more than 12 months before Screening (exceptions to multi-drug treatment regimen for 6 consecutive months include treatment with doses or frequencies below those recommended by guidelines and/or short interruptions, both occurring due to safety/tolerability issues)</p> <ol style="list-style-type: none"> 3. be diagnosed with MAC NTM lung infection with evidence of underlying lung disease such as nodular bronchiectasis and/or fibrocavitary disease by chest radiography (CXR) or chest computed tomography. High resolution CT (HRCT) scan is preferred, if available. 4. have 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 one positive culture obtained within 6 months prior to Screening and one positive culture at Screening (cultures to be at least 1 month apart). Cultures may be obtained from sputum or bronchoscopy. 5. have a MAC-positive sputum at Screening 6. be willing to adhere to multi-drug treatment regimen during the course of the study 7. be able to produce approximately 3 mL of sputum or be willing to undergo an induction that produces approximately 3 mL of sputum for mycobacteriology 8. female of child bearing potential agrees to practice an acceptable method of birth control (e.g., true abstinence [refraining from heterosexual intercourse during the entire study], hormonal or barrier methods, partner sterilization, or intrauterine device [IUD]) while participating in the trial. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of the study, and withdrawal are not acceptable methods of contraception. 9. the patient will provide written informed consent before performing any study related procedure 10. be willing to have serum specimens stored 11. be able to comply with study drug use, study visits, and study procedures as determined by the Investigator
Exclusion Criteria	<p>A patient with any of the following conditions must be excluded from this study:</p> <ol style="list-style-type: none"> 1. patients with cystic fibrosis 2. patients whose MAC NTM infection is resistant to amikacin (as identified by MIC susceptibility >64 µg/mL) 3. patients who are not able to perform the 6MWT 4. positive pregnancy test or lactation at Screening. All women of child bearing potential will be tested. Women not of child bearing potential are defined as postmenopausal (i.e., amenorrheic for at least 1 year), or surgically or naturally sterile.

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	<ol style="list-style-type: none"> 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 (e.g., high-dose ibuprofen, inhaled anti-inflammatory agents including steroids, low dose maintenance steroids, recombinant human deoxyribonuclease [rhDNase]) within 28 days before Day 1. 10. administration of any investigational drug within 8 weeks before Screening 11. prior exposure to LAI (including clinical study). 12. known hypersensitivity to aminoglycosides 13. use of inhaled or systemic aminoglycosides with activity against MAC (e.g., amikacin, kanamycin, or streptomycin) within 28 days before Day 1 14. acquired and primary immunodeficiency syndromes (e.g. 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 or alanine aminotransferase ≥ 3 times the upper limit of normal (ULN) or total bilirubin ≥ 2 times the upper limit of normal (ULN) at Screening 17. absolute neutrophil count $\leq 500/\mu\text{L}$ 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, interferes 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 are not expected to survive the duration of the study 23. patients with disseminated MAC infection
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Study Withdrawal Criteria	<p>A subject may be withdrawn from the study for any of the following reasons:</p> <ol style="list-style-type: none"> 1. the event of a subject's death 2. the subject experiences an AE and the Investigator or the subject determines that withdrawal from the study is appropriate 3. a major protocol deviation that interferes with the integrity of the study data for this subject 4. the subject is noncompliant (does not comply with dosing instructions provided by the Investigator or does not comply with the study visit schedule) and the Investigator determines that withdrawal of the subject is appropriate 5. the subject withdraws consent to participate in the study 6. the site is unable to contact the subject after all reasonable efforts have been exhausted (Lost to Follow-Up) 7. any condition that, in the judgment of the Investigator, would compromise the ability of the subject to comply with the study protocol or complete the study (physician decision). 8. any subject who has to have "rescue" medication will be discontinued
Study Drug Administration	<p>LAI will be supplied by Insmmed in clear glass 10 mL vials via nebulization for a delivered dose of 590 mg. The study drug will be administered via inhalation using the PARI eFlow[®] nebulizer (eFlow[®]), a small machine that delivers medication in the form of a mist inhaled into the lungs, which is approved by the European Medicines Agency for use in European Union (elsewhere it is an investigational medical device that is not yet commercially approved).</p> <p>Study drug will be administered QD. Subjects who develop bronchospasm may be pre-treated with a bronchodilator before study drug administration.</p>
Methodology and Study Procedures	See Table 3-1 Schedule of Events
Study Endpoints	<p>PRIMARY EFFICACY ENDPOINT</p> <p>The primary endpoint is the proportion of subjects achieving culture conversion (3 consecutive monthly negative sputum cultures) by Month 6 in the LAI arm compared to multi-drug regimen alone.</p> <p>SECONDARY EFFICACY ENDPOINTS</p> <ol style="list-style-type: none"> 1. Change in 6MWT distance at Month 6 in the LAI arm compared to a multi-drug regimen alone 2. Proportion of subjects achieving culture conversion with durability after 3 months off treatment in the LAI arm compared to a multi-drug regimen alone

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	<ol style="list-style-type: none"> 3. Time to culture conversion in the LAI arm compared to a multi-drug regimen alone by Month 6 4. Proportion of subjects achieving culture conversion with sustainability at the EOT in the LAI arm compared to a multi-drug regimen alone 5. Change in 6MWT distance at EOT in the LAI arm compared to a multi-drug regimen alone 6. Change from Baseline (Day 1) at Month 6 in the SGRQ. <p>EXPLORATORY ENDPOINTS</p> <ol style="list-style-type: none"> 1. Change in 6MWT distance at Month 6 for converters versus non-converters in LAI arm 2. Change in 6MWT distance at Month 6 for converters versus non-converters for all subjects 3. Change in 6MWT distance at Month 6 for converters versus non-converters in the multi-drug regimen alone arm 4. Change in BMI at Month 6 in LAI arm compared to a multi-drug regimen alone 5. Change in 6MWT distance at Month 8 and 3 months off-treatment in the LAI arm compared to a multi-drug regimen alone 6. Proportion of subjects achieving culture conversion with durability after 12 months off treatment (EOS) in the LAI arm compared to a multi-drug regimen alone 7. Change from Baseline (Day 1) at Month 6 in the SGRQ – Part 2 (Activities of Daily Livings) 8. Change from Baseline (Day 1) at Month 6 and EOT in the EQ-5D-3L 9. Proportion of subjects who develop a new strain of MAC in the LAI arm compared to multi-drug regimen alone 10. Radiological changes in chest CT Scan at EOT in the LAI arm compared to multi-drug regimen alone, in a sub-set of subjects 11. Mortality at EOS.
Efficacy Variables	Organisms identified in sputum specimens cultured in agar and broth, 6MWT results, SGRQ scores
Safety Variables	<p>The frequency of AEs, AEs leading to withdrawal from study and treatment-emergent serious AEs, laboratory test results, vital signs and pulmonary function measurements will be summarized.</p> <p>AEs of special interest include bronchospasm, hemoptysis, ototoxicity, and allergic alveolitis.</p>

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Statistical Methods	<p>Sample Size Determination</p> <p>The sample size was determined for the primary comparison between the treatment arms LAI plus a multi-drug regimen and a multi-drug regimen alone with respect to the proportion of subjects achieving culture conversion by Month 6 using nQuery Advisor® 7.0. Assuming a culture conversion rate by Month 6 of no less than 20% for the LAI plus a multi-drug regimen treatment arm, the rate by Month 6 of 5% for the multi drug regimen alone treatment arm, and a 2:1 randomization ratio, a sample size of 261 subjects (174 for LAI plus a multi-drug regimen and 87 for the multi-drug regimen) will provide at least 90% power for the continuity corrected Chi square test at the 2 sided significance level of 0.05.</p> <p>The sample size was also evaluated for the secondary endpoint of change from Baseline (Day 1) to Month 6 in 6MWT distance. Assuming a common standard deviation of 100 and a 2:1 randomization ratio to either LAI plus a multi-drug regimen or a multi-drug regimen alone, a sample size of 192 subjects (128 for LAI plus a multi-drug regimen and 64 for the multi-drug regimen) will provide at least 90% power to detect a between-treatment difference of 50 meters in mean change from Baseline (Day 1) to Month 6 in 6MWT distance using a two arm t-test at the 2-sided significance level of 0.05.</p> <p>Due to lack of durability data, it is difficult to estimate the power with a total sample size of 261 subjects for the comparison between treatment arms with respect to the proportion of subjects achieving culture conversion with durability after 3 months off-treatment. Assuming the proportion of subjects achieving culture conversion with durability is 16% and 4% for LAI plus a multi-drug regimen and a multi-drug regimen respectively, and a 2:1 randomization ratio, a sample size of 261 subjects (174 for LAI plus a multi-drug regimen and 87 for the multi-drug regimen) will provide at least 83% power for Fisher's exact test at the 2-sided significance level of 0.05.</p> <p>Statistical Methodology</p> <p>The final analyses of the primary efficacy endpoint, the change from Baseline to Month 6 in 6MWT distance, and time to culture conversion by Month 6 will be performed when the last subject completes Month 6 and data for all subjects who have completed Month 6 are available. These final analyses will be performed prior to the completion of the study. Interim analysis of some safety data will be included.</p> <p>The primary efficacy endpoint is the proportion of subjects achieving culture conversion by Month 6. The primary efficacy analysis will be performed for the intent-to-treat population using the Cochran-Mantel-Haenszel test stratified by smoking status and prior multi-drug regimen at the 2-sided significance level of 0.05. The null hypothesis assumes that the culture conversion by Month 6 is independent of treatment, and the alternative hypothesis assumes that the culture conversion by Month 6 is associated with treatment.</p> <p>A supportive analysis of the primary efficacy endpoint will be performed for the per-protocol population similarly using the Cochran-Mantel-Haenszel test stratified by smoking status and prior multi-drug regimen.</p> <p>The change from Baseline (Day 1) to Month 6 in 6MWT distance will be analyzed for the ITT population (assuming Missing-Not-at-Random [MNAR]) using a model</p>
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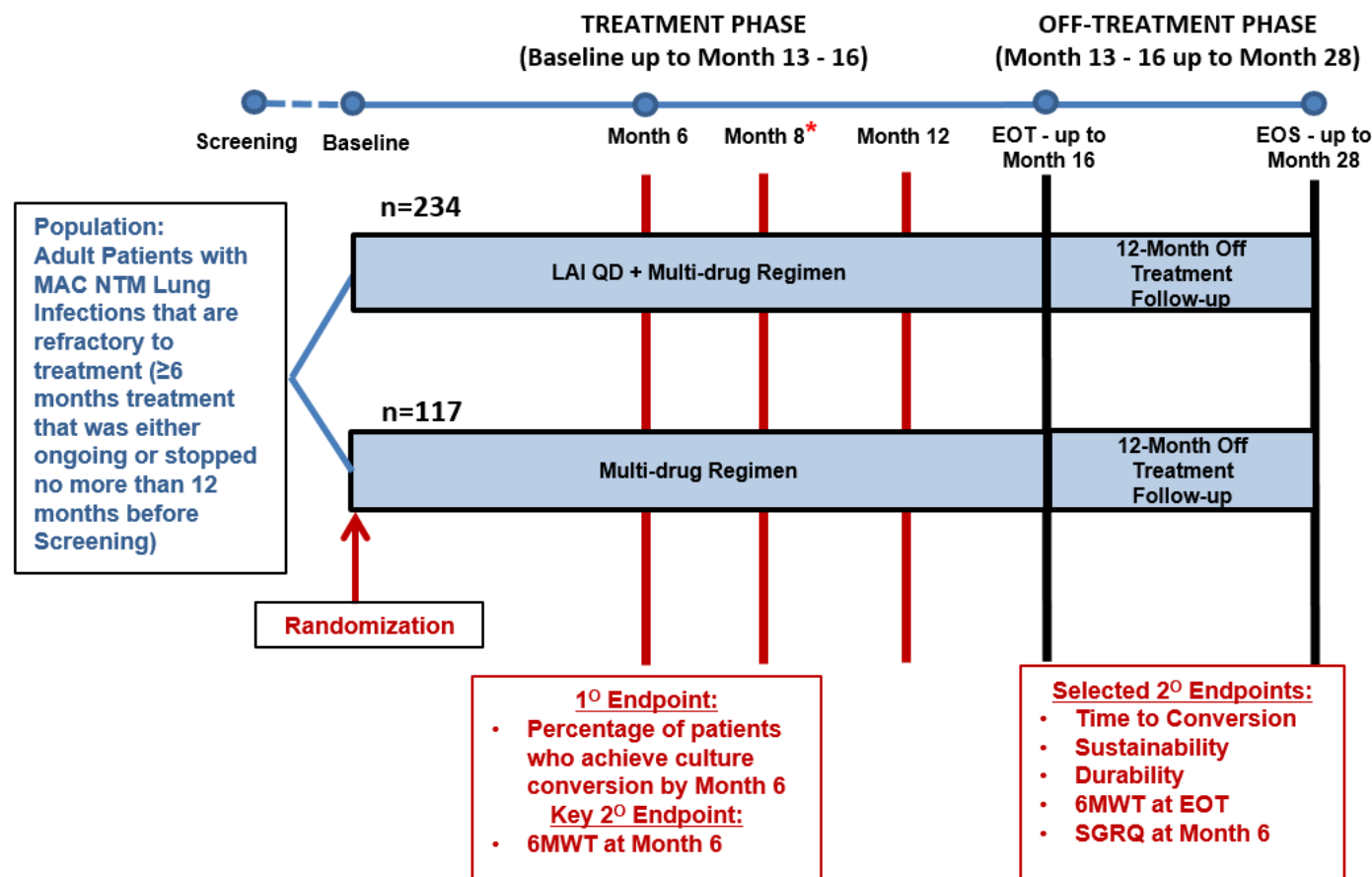
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	<p>under the pattern-mixture model framework. This model assumes that subjects on the LAI plus a multi-drug regimen arm with missing data follow the response distribution of a multi-drug regimen alone arm. It involves 3 steps:</p> <ol style="list-style-type: none"> 1. The posterior mean and covariance estimates from the SAS MI procedure using the available non-missing data of a multi-drug regimen alone arm will be utilized to multiply impute missing data. 2. The endpoint will then be analysed for each complete data set with imputed data using an analysis of covariance (ANCOVA) with treatment arm and the randomization strata as factors, and the Baseline 6MWT distance as a covariate. 3. The treatment LSMEAN differences will be averaged and the associated standard errors will be summarized based on within-imputation and between-imputation variances using the SAS MIANALYZE procedure, to yield a final estimate of treatment contrast with associated 95% confidence interval (CI) and p-value. <p>A sensitivity analysis using a mixed-effects model for repeated measures (MMRM) and supportive analyses will be performed.</p> <p>The changes from Baseline to Month 8 (exploratory endpoint), EOT, and 3 months off-treatment (exploratory endpoint) in 6MWT distance will be analyzed for the ITT population using the similar mixed-effects model for repeated measures (MMRM) with an unstructured covariance matrix implemented with SAS MIXED procedure over all post-randomization visits.</p> <p>Kaplan Meier estimates for the distribution of time to culture conversion will be constructed for treatment arms. The treatment comparison will be made using the stratified log rank test for the ITT population. The estimated median time to culture conversion for each treatment arm will be presented. The time to culture conversion will also be analyzed using Cox regression model to estimate hazards ratio.</p> <p>The proportion of subjects achieving culture conversion with sustainability by the EOT and the proportion of subjects achieving culture conversion with durability after 3 months off-treatment will be analyzed for both the ITT and PP populations similarly using the Cochran Mantel Haenszel test stratified by smoking status and prior multi-drug regimen.</p> <p>The change from Baseline (Day 1) to Month 6 in SGRQ total score and the SGRQ – Part 2 subdomains (exploratory endpoint) will be analyzed for the ITT population similarly using the same MMRM method as the analysis of the change from Baseline to EOT in 6MWT distance.</p>
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*All converters (at least 3 consecutive monthly negative sputum cultures by Month 6) will remain in study for 12 months, starting from the first negative culture that defines culture conversion.

*All non-converters and subjects who experienced a relapse or recurrence after culture conversion will discontinue the study at Month 8 and may be eligible to enter a separate Open-Label study (INS-312).

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LIST OF ABBREVIATIONS

Abbreviation	Term
6MWT	six-minute walk test
AE	adverse event
AESI	adverse events of special interest
AFB	Acid-Fast Bacteria/Bacterium
ANCOVA	Analysis of Covariance
ATS	American Thoracic Society
BR	Bronchiectasis
CF	Cystic Fibrosis
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practices
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
CRO	Clinical Research Organization
CRP	C-Reactive Protein
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DPPC	Dipalmitoylphosphatidylcholine
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOS	End of Study
EOT	End of Treatment
EP	Evaluable Population
EQ-5D-3L	formerly EuroQol 5D, a generic health-status classification instrument
FDA	Food and Drug Administration of the United States of America
FEV ₁ (L)	Forced Expiratory Volume in 1 second in liters
FEF _(25-75%)	Forced Expiratory Flow rate during mid portion of expiration
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
hCG	human Chorionic Gonadotropin
IB	Investigator's Brochure

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Abbreviation	Term
ICH	International Conference on Harmonisation
IDSA	Infectious Diseases Society of America
EC	Ethics Committee
IL-6	Interleukin-6
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	intravenous
LS	Least Square
LAI	Liposomal Amikacin for Inhalation
MAC	<i>Mycobacterium avium</i> complex
MIC	Minimum inhibitory concentration
mm	millimeter
MMRM	Mixed-effects Model for Repeated Measures
MNAR	Missing Not at Random
NTM	Nontuberculous Mycobacteria
<i>Pa</i>	<i>Pseudomonas aeruginosa</i>
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
QD	Once Daily
rhDNase	recombinant human Deoxyribonuclease
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SGRQ	St. George's Respiratory Questionnaire
SpO2	Pulse Oximetry
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOBI	Tobramycin for Inhalation
ULN	Upper Limit of Normal
w:w	Weight to Weight

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1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Nontuberculous Mycobacterial Lung Infections

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment. The pulmonary infection caused by these organisms has features that overlap with tuberculosis, but disease definition can be more complex as recovery of a single isolate from the airway secretions does not necessarily indicate disease. In contrast to tuberculosis, there is no convincing evidence of person-to-person spread (Griffith, et al. 2007; Olivier, et al. 2003). It appears that the prevalence of human disease attributable to these organisms over the past 2 decades is increasing (Khan, et al. 2007; Marras, et al. 2007; Khan et al, 2008). 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, certain disease and demographic populations seem to be particularly susceptible to nodular bronchiectatic pulmonary disease with predominant infection of the anterior aspect of the mid-lung. In cystic fibrosis (CF), the prevalence of NTM in the lower airways is 13%, and increases with age (Olivier, et al. 2003). Elderly, Caucasian women without apparent predisposing conditions have been reported with increasing frequency to have pulmonary disease associated with *Mycobacterium avium* complex (MAC), and one community pulmonary practice reported this to be a prominent cause of chronic cough with infiltrates (Prince, et al. 1989).

Mycobacterium avium and *Mycobacterium avium* complex, a symbiotic infection of *M. avium* and *M. intracellulare*, are the predominant infective species in NTM pulmonary disease in US, Japan, European countries, and elsewhere, followed by *M. abscessus* and *M. kansasii*. There is a perception that *M. abscessus* as a causative species is on the rise, which is a concern because these infections are especially difficult to treat (Leung 2013 and Olivier 2003). In CF, the rates of NTM lung infection caused by *M. abscessus* often exceed those caused by MAC.

Signs and symptoms of NTM pulmonary infection are variable and nonspecific. They include chronic cough, sputum production, and fatigue. Less commonly, malaise, dyspnea, fever, hemoptysis, and weight loss can also occur, usually with advanced NTM lung

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infection. Evaluation is often complicated by the symptoms caused by co-existing lung diseases. These conditions include chronic obstructive airway disease (COPD) associated with smoking, bronchiectasis, previous mycobacterial diseases, CF, and pneumoconiosis (Wilson, et al. 1997).

Current treatment of NTM lung infection is primarily with multi-drug regimens developed for the treatment of tuberculosis. This approach is not optimal, and the morbidity and mortality associated with NTM infection is significant. A study by Andrejak et al in Denmark demonstrated that mortality after 5 years in those who were infected according to the American Thoracic Society /Infectious Diseases Society of America (ATS/IDSA) criteria was 40%. In this study, *M. xenopi* was associated with a particularly poor prognosis (Andrejak, et al 2010).

1.1.2 Liposomal Amikacin for Inhalation (LAI)

LAI is a sterile aqueous liposomal formulation for inhalation via nebulization. LAI is comprised of amikacin sulfate encapsulated in liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and cholesterol; other inactive ingredients include sodium chloride, sodium hydroxide for pH adjustment and water for injection. LAI is supplied in a single use 10 mL vial to deliver 590 mg amikacin to the nebulizer. LAI is administered by inhalation via a PARI eFlow® nebulizer, a small machine that delivers medication in the form of a mist inhaled into the lungs, which is approved by the European Medicines Agency (EMA) for use in European Union (elsewhere it is an investigational medical device that is not yet commercially approved).

1.1.3 Prior Clinical Experience

LAI is a novel formulation of amikacin that is encapsulated in liposomes comprised of DPPC and cholesterol (naturally occurring lipids in the lung) to allow for effective delivery in the lung, leading to high concentrations of the drug at the target sites of infection, with low systemic levels. Clinical studies to date have evaluated LAI in a total of 519 subjects, including 6 healthy subjects, 383 subjects with CF, 43 subjects with bronchiectasis (BR), and 87 subjects with NTM lung disease in Canada, Europe, and the United States. Early studies in the clinical development program established that LAI was effective in improving lung function in CF patients with chronic *Pseudomonas aeruginosa* (*Pa*) lung infection. The

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pivotal phase 3 study that supports efficacy in the management of chronic *Pa* in patients with CF is TR02-108 in which LAI was non-inferior to tobramycin for inhalation (TOBI). An open-label extension to TR02-108, the TR02-110 study, established a sustained effect of LAI in these subjects for up to 30 months. These data are supported by earlier studies assessing LAI against placebo for short cycles of treatment (TR02-105 and TR02-106) in which LAI demonstrated statistical superiority over placebo. All controlled comparator studies (TR02-105, 106, 108, and 110) established an additional benefit in quality of life measures for these subjects.

The program also included a study in patients with NTM lung infection (TR02-112) that randomized and dosed 89 subjects with LAI or placebo added to a background multi-drug regimen. The key secondary endpoint assessed sputum cultures and demonstrated that LAI was statistical superiority at the end of the double-blind period (Day 84) for achieving a negative sputum culture. At Day 84, 25.0% of subjects with add-on LAI had a negative sputum culture compared to 6.7% of subjects with add-on placebo. This is an important result in this population as subjects were refractory to therapy for a minimum of 6 months to more than 2 years prior to enrollment. Furthermore, 12 subjects demonstrated sustained negative culture results with add-on LAI to the end of the open-label phase (Day 168) including the 28 days off LAI follow-up period. The change in subject functional status was assessed using the 6 minute walk test and a statistically significant difference in favor of LAI (a mean increase from Baseline [Day 1] of 23.859 meters vs. a mean decrease of 25.032 meters in the LAI vs. placebo arms, respectively ($p = 0.0134$)).

Additional results of clinical studies of LAI are summarized in the Investigator's Brochure (IB).

1.2 RATIONALE FOR THE STUDY

Treatment guidelines for patients with MAC lung infection in the published ATS/ IDSA consensus document were based primarily on small uncontrolled or non-comparative studies in patients with predominantly severe or refractory MAC disease (Griffith, et al. 2007).

Overall, there remains a lack of sufficiently powered, prospective clinical trials aimed at the treatment of pulmonary MAC infection. Many of the drugs used in the prolonged, recommended multi-drug regimens are expensive and poorly tolerated (Ballarino, et al.

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2009). It is clear from these single-site studies that there is an unmet need for more effective, less-toxic therapeutic options.

Amikacin solution for parenteral administration is an established drug that is effective against a variety of NTM. However, its use is limited by poor tissue penetration into lung tissue and the need to administer it intravenously (IV) in high enough doses that cause toxicity to hearing, balance, and kidney function. In the case of bacterial infections of the lung, the inhalation route of administration is advantageous over the IV route in that the aminoglycoside is delivered directly to the effect-site with neither significant systemic absorption nor the associated systemic toxicities. Disadvantages of aerosolized aminoglycoside solutions include rapid clearance from lung tissue, which necessitates frequent dosing (Geller, et al. 2002) and the length of time required to inhale sufficient amounts of drug. Both factors place a high daily treatment burden on patients and may limit patient compliance. Thus, LAI was developed to overcome these limitations.

Lipid/liposome delivery systems similar to that used to deliver LAI in this study have been used to improve the therapeutic index of several injectable therapeutic agents. In a liposome drug delivery system, a bioactive agent entrapped in a liposome is administered to the patient. Alternatively, drugs can be combined with lipids to form nonencapsulated lipid drug complexes having therapeutic advantages. Therapeutic properties of several commercial products, such as anticancer compounds liposomal doxorubicin (Doxcil®) and liposomal daunorubicin (Daunosome®) and the antifungal lipid complex of amphotericin B (Abelcet®, Ambisome®), have improved dramatically after encapsulation or complexing with a lipid. Although these commercial products are administered IV, inhaled Ambisome is being evaluated for prophylactic use.

Study TR02-112 was a randomized double-blind placebo controlled that dosed 89 subjects with LAI or placebo added to a background multi-drug regimen. At Day 84, 25.0% of subjects with add-on LAI had a negative sputum culture compared to 6.7% of subjects with add-on placebo. This is an important result in this population as subjects were refractory to therapy for a minimum of 6 months to more than 2 years prior to enrollment. Furthermore, 12 subjects demonstrated sustained negative culture results with add-on LAI to the end of the open-label phase (Day 168) including the 28 days off LAI follow-up period.

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The change in subject functional status was assessed using the 6 minute walk test and a statistically significant difference in favor of LAI (a mean increase from Baseline [Day 1] of 23.859 meters vs. a mean decrease of 25.032 meters in the LAI vs. placebo arms, respectively ($p = 0.0134$)).

Study INS-212 will evaluate whether the signal identified in TR02-112 is further confirmed in a longer duration of LAI treatment in patient with NTM MAC lung infections who are refractory to a stable multi-drug regimen for at least 6 months.

1.3 RISK-BENEFIT ASSESSMENT

The safety of LAI has been evaluated in 13 studies, 11 of which are completed and 2 are ongoing. The 6 main safety studies conducted in CF and NTM included a total of 434 unique treated subjects who received LAI at doses ranging from 70 mg QD to 590 mg QD or TOBI or placebo. In all 6 of the main safety studies, most subjects were Caucasian and the majority were female.

In each of the 6 main safety studies, the majority of the subjects in all treatment groups experienced at least 1 treatment-emergent adverse event (TEAE), with most events related to the subjects' underlying diagnosis. Across the 6 main safety studies, the most common serious adverse event (SAE) in all groups was hospitalization due to pulmonary exacerbation. Most SAEs were not considered by the investigator to be treatment-related. In TR02-112, in the double-blind phase, SAEs were reported for a greater proportion of subjects in the LAI arm than in the placebo arm (18.2% versus 8.9%, respectively). The incidence of SAEs did not increase in the LAI arm after additional exposure to the study drug in the open-label phase of TR02-112 compared with the double-blind phase (14.3% vs. 18.2%, respectively).

In preclinical studies, treatment-related lung tumors were observed at the end of 2 years of dosing in 2 of 120 rats. The theoretical risk of lung tumors must be weighed against the potential benefits of LAI for patients, and will depend on a number of factors, including severity of disease, comorbidities and the availability of alternative treatments.

Additional details of clinical studies and potential AEs evidenced from preclinical studies are presented in the IB.

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2 STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective is to evaluate the efficacy of LAI (590 mg) administered QD, when added to a multi-drug regimen, for achieving culture conversion (3 consecutive monthly negative sputum cultures) by Month 6 compared to a multi-drug regimen alone. The date of conversion is defined by the date of the first of 3 negative cultures.

2.2 SECONDARY OBJECTIVES

1. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the six minute walk test (6MWT) at Month 6 compared to a multi-drug regimen alone
2. To evaluate the efficacy of LAI (590 mg) QD when added to a multi-drug regimen on the durability of treatment success 3 months after the end of total treatment course (negative sputum culture after 3 months off treatment)
3. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen for time to culture conversion compared to a multi-drug regimen alone
4. To evaluate the efficacy of LAI (590 mg) administered QD, when added to a multi-drug regimen, for achieving sustainability (consecutive negative sputum cultures [with no more than 2 consecutive monthly broth positive cultures] for 12 months on treatment) compared to a multi-drug regimen alone
5. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the 6MWT at EOT compared to a multi-drug regimen alone
6. To evaluate patient reported symptoms of NTM and change from Baseline (Day 1) in quality of life scores on the St. George's Respiratory Questionnaire (SGRQ) at Month 6.

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2.3 EXPLORATORY OBJECTIVES

The exploratory objectives are:

1. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters within the LAI (590 mg) administered QD added to a multi-drug regimen arm
2. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters for all subjects
3. To evaluate the change from Baseline to Month 6 on the 6MWT for converters versus non-converters within the multi-drug regimen alone arm
4. To evaluate the change from Baseline to Month 6 on Body Mass Index (BMI) for those randomized to LAI (590 mg) administered QD added to a multi-drug regimen compared to a multi-drug regimen alone
5. To evaluate the efficacy of LAI (590 mg) administered QD when added to a multi-drug regimen on the 6MWT at Month 8 and 3 months off-treatment compared to a multi-drug regimen alone
6. To evaluate the proportion of subjects achieving culture conversion with durability after 12 months off treatment (EOS) in the LAI arm compared to a multi-drug regimen alone
7. To compare patient reported symptoms of NTM and change from Baseline (Day 1) in quality of life scores on the SGRQ – Part 2 (Activities of Daily Living) at Month 6
8. To compare change from Baseline (Day 1) in the EQ 5D patient reported health outcomes at Month 6 and EOT
9. To evaluate the number of subjects in each treatment arm who develop a new strain of MAC during the study at EOS
10. To compare all-cause mortality between treatment arms 12 months after treatment (EOS).

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2.4 SAFETY OBJECTIVE

The safety objective is to evaluate the safety and tolerability of LAI (590 mg) QD added to a multi-drug regimen arm.

2.5 PHARMACOKINETIC OBJECTIVES

1. To evaluate the PK of LAI via a population PK approach (Months 1, 3 and 6).
2. To evaluate the concentration of amikacin in sputum after 2 days interruption of LAI dosing in a subset of subjects (Months 4, 5 and 6)
3. To evaluate the concentration of amikacin in sputum after 28 days off-LAI in a subset of subjects
4. To evaluate the concentration of amikacin in sputum after 3 months off-LAI in a subset of subjects

2.5.1 Comprehensive Pharmacokinetic Sub-Study Objectives

1. To characterize systemic amikacin exposure, including approximate systemic bioavailability in Japanese subjects, using a population PK model to describe the disposition of amikacin in the serum
2. To compare population PK modeling between Japanese subjects to historical population PK modeling for LAI
3. To evaluate the amikacin concentration in sputum

3 INVESTIGATIONAL PLAN

3.1 STUDY DESIGN

The Screening window allows time for sputum culture results, susceptibility by 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. Once Screening assessments are complete and sputum culture results are known, eligible

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subjects will be randomized 2:1 to LAI administered QD plus a multi-drug regimen or a multi-drug regimen alone. The primary endpoint will assess culture conversion by Month 6.

Sputum culture results will only be available to the site after the Month 6 sputum result is known, in time for the Month 8 visit. At Month 8 (-28 to +7 days), after all sputum culture results are known, up to and including Month 6, subjects will be assessed as converters or non-converters.

All converters will remain in Study INS-212 (Section 4.6). All converters who, after culture conversion, subsequently have MAC-positive sputum cultures in liquid broth media (agar negative) for 1 or 2 consecutive months only, will also remain in Study INS-212. These subjects will continue on their randomized treatment regimen until they complete a total of 12 months of treatment (EOT), starting from the first of 3 negative cultures that defines culture conversion. These subjects will return after the EOT visit for 28 day, 3, 6 and 12 months off-treatment follow-up visits. The 12 months off-treatment follow-up visit will be the end of study (EOS) visit. No NTM treatment will be administered during the off-treatment phase.

At Month 8, all non-converters will be discontinued from INS-212 (Section 4.6). All subjects who experienced a relapse or recurrence (Section 4.6) by Month 6 will also be discontinued from INS-212 at their Month 8 visit. These subjects may be eligible to enter a separate open-label study of LAI (Study INS-312), provided all entry criteria have been met for that study.

Expectorated sputum (spontaneous or induced e.g., with nebulized hypertonic saline solution as needed) will be collected at Day 1 and every month through Month 6, and at Months 8, 12, at EOT, at 28 days, 3, 6 and 12 (EOS) months off-treatment.

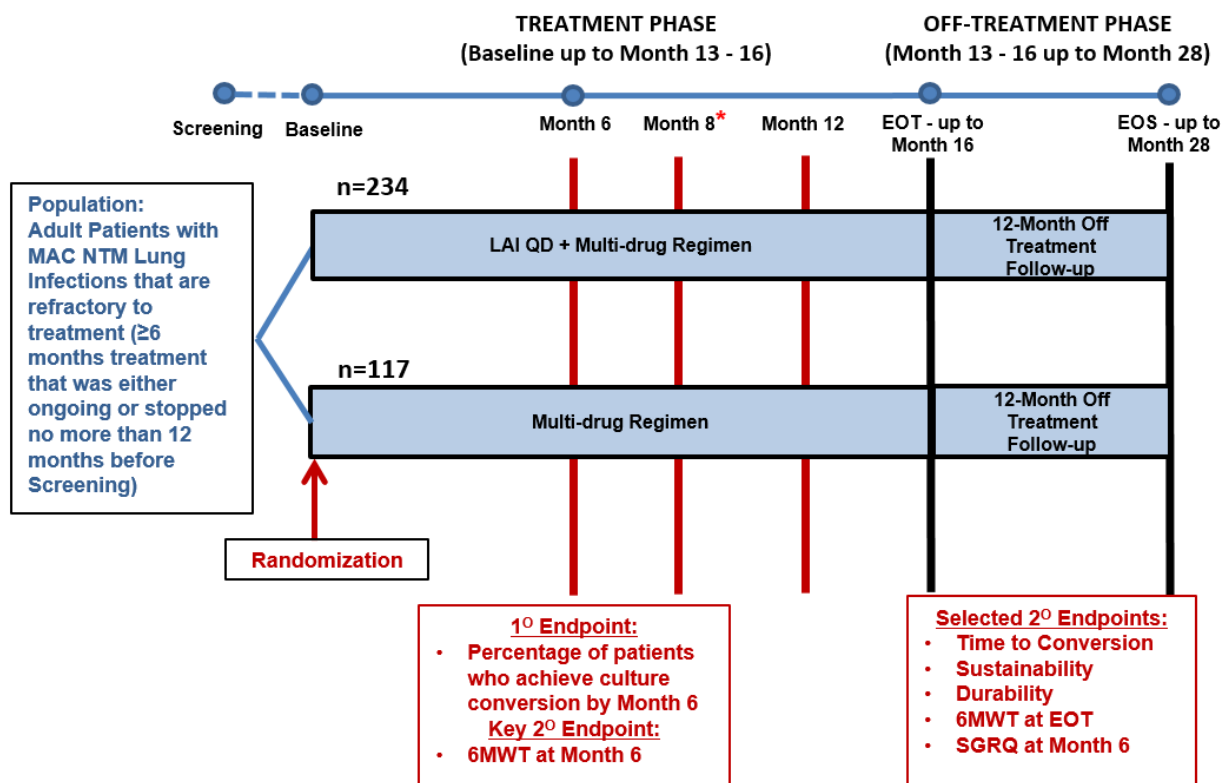
All subjects will come in monthly for routine visits through Month 6, at Months 8, 10, 12, 14, EOT, and at 28 days, 3, 6 and 12 (EOS) months off-treatment. Home Healthcare visits may be available at Months 1, 2, and 5 for sites with IRB/EC approval to conduct Home Healthcare visits, and qualifying subjects who may have difficulty attending clinic visits. (At Months 10 and 14 subjects randomized to LAI will return to the clinical site for drug dispensation, and AE and concomitant medication assessments). Unscheduled visits will occur as needed should subjects' symptoms worsen between visits.

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Figure 3-1 Study Design



*All converters (at least 3 consecutive monthly negative sputum cultures by Month 6) will remain in study for 12 months, starting from the first negative culture that defines culture conversion.

*All non-converters and subjects who experienced a relapse or recurrence after culture conversion will discontinue the study at Month 8 and may be eligible to enter a separate Open-Label study (INS-312).

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3.2 PRIMARY ENDPOINT

The primary endpoint is the proportion of subjects achieving culture conversion (3 consecutive monthly negative sputum cultures) by Month 6 in the LAI arm compared to multi-drug regimen alone.

3.3 SECONDARY ENDPOINTS

Secondary efficacy endpoints are:

1. Change in 6MWT distance at Month 6 in the LAI arm compared to multi-drug regimen alone
2. Proportion of subjects achieving culture conversion with durability at 3 months off treatment in the LAI arm compared to multi-drug regimen alone
3. Time to culture conversion in the LAI arm compared to multi-drug regimen alone at Month 6
4. Proportion of subjects achieving culture conversion with sustainability at the EOT in the LAI arm compared to multi-drug regimen alone
5. Change in 6MWT distance at EOT in the LAI arm compared to multi-drug regimen alone
6. Change from Baseline (Day 1) at Month 6 in the SGRQ.

3.4 EXPLORATORY ENDPOINTS

1. Change in 6MWT distance at Month 6 for converters versus non-converters in LAI arm
2. Change in 6MWT distance at Month 6 for converters versus non-converters for all subjects
3. Change in 6MWT distance at Month 6 for converters versus non-converters in the multi-drug regimen alone arm
4. Change in BMI at Month 6 in LAI arm compared to a multi-drug regimen alone
5. Change in 6MWT distance at Month 8 and 3 months off-treatment in the LAI arm compared to multi-drug regimen alone

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6. Proportion of subjects achieving culture conversion with durability after 12 months off treatment (EOS) in the LAI arm compared to a multi-drug regimen alone
7. Change from Baseline (Day 1) at Month 6 in the SGRQ – Part 2 (Activities of Daily Living)
8. Change from Baseline (Day 1) at EOT in the EQ-5D-3L
9. Proportion of subjects who develop a new strain of MAC in the LAI arm compared to multi-drug regimen alone
10. Radiological changes in CT scan, within a sub-set of subjects, at EOT in the LAI arm compared to multi-drug regimen alone, where appropriate
11. Mortality at EOS

3.5 SCHEDULE OF EVENTS

The schedule of events is presented in [Table 3-1](#). Detailed procedures and assessments performed at each scheduled study visit are presented in [Section 6](#).

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Table 3-1 Schedule of Events

INS-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)	(±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	–	–	-

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INS-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)	(±3)	(-28; +7)	(±5)	(±5)	(±5)	(±5)	(±5)	(±7)	(±7)	(±7)
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	–	–	–	–	

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INS-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)	(±3)	(-28; +7)	(±5)	(±5)	(±5)	(±5)	(±5)	(±7)	(±7)	(±7)
Serum for Biomarkers (CRP and IL-6)	–	X	–	–	–	–	–	X	–	–	–	–	X	–	–	–	–	
<i>Sub-Study:</i> CT scan of chest ⁱ	–	X	–	–	–	–	–	–	–	–	–	–	X	–	–	–	–	
<i>Sub-Study:</i> CT scan of chest (Japan)	See APPENDIX 5: JAPAN SPECIFIC CT SCAN SUB-STUDY																	
<i>Sub-Set:</i> Population PK sampling ^j (blood and sputum)	–	–	X	–	X	–	–	X	–	–	–	–	–	–	–	–	–	
<i>Sub-Set:</i> Sputum only PK Collection	–	X	–	–	–	X	X	X	–	–	–	–	–	X	X	–	–	
<i>Sub-Study:</i> Comprehensive PK sampling ^k (Japan)	X	X	–	X	–	–	X	–	–	–	–	–	–	–	–	–	–	

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INS-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)
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)
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 ^l	–	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 ^m									

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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; FEV₁, 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 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. See [Section 7.2.4](#).
- ^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 Only for subjects who agreed to participate in the CT Scan sub-study. A prior chest CT scan may be used as a subject's Baseline (Day 1) measurement if this CT scan was obtained within 6 months from the subject's Baseline (Day 1) visit. Subjects will have their follow-up chest CT scan at the EOT visit provided the last chest CT scan was not obtained within the prior 6 months. High resolution CT scan is preferred, if available. Please refer to [APPENDIX 4](#) for additional details on the CT Scan sub-study.
- ^j Only for subjects who agreed to participate in the population PK sub-set (US and Japan only). Blood and sputum samples will be collected from 0 to 1 hours before and from 1 to 4 hours after study drug administration ([Section 7.4.1](#)).
- ^k Only for Japanese subjects from sites in Japan who agreed to participate in the comprehensive PK sub-study. Please refer to [APPENDIX 5](#) for additional details on the comprehensive PK sub-study.
- ^l 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 ([Section 3.1](#), [Section 4.6](#)). 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.
- ^m 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

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3.6 DISCUSSION OF STUDY DESIGN

3.6.1 Number of Subjects

The study is designed to enroll enough subjects to ensure at least 261 evaluable subjects reach Month 6. Accounting for discontinuation, it is anticipated that up to approximately 351 randomized subjects may be required. An evaluable subject is one who has at least 1 sputum culture result at Baseline (Day 1) and for each month through Month 6.

3.6.2 Study Duration

This study has an anticipated recruitment period of 18-24 months, with each subject receiving treatment in the study for up to 16 months (excluding Screening and observational follow-up). Subjects will remain in the study for up to 31 months, approximately 2.5 months for Screening plus up to 16 months in the randomized open-label treatment phase plus 12 months in the off-treatment phase.

3.6.3 Premature Termination of Study Site

For reasonable cause, either an individual Investigator may terminate participation prematurely or Insmmed may terminate this study prematurely. Written notification of the termination to each affected investigational site is required. Some conditions that may warrant termination include the following:

- discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study
- failure of the Investigator to enter study subjects at an acceptable rate
- insufficient adherence to protocol requirements (e.g., noncompliance) by the subject and/or the study site
- decision on the part of the Sponsor to suspend or discontinue development of the drug
- decision by a regulatory authority or the Sponsor to stop the study at any time, where applicable

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In the event of study discontinuation, subjects will discontinue the investigational treatments. Refer to [Section 5.12.1](#) for follow-up procedures.

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

3.6.4 Assignment to Study Drug

Eligible subjects will be stratified at Screening according to smoking status (current smoker or not) and prior multi-drug regimen (on treatment or off treatment for at least 3 months) and then randomly assigned in a 2:1 ratio to treatment with either LAI (590 mg) QD plus multi-drug regimen or to multi-drug regimen alone, using an interactive web response system (IWRS). Treatment randomization codes will be generated using SAS®.

3.6.5 Data Monitoring Committee

In order to ensure the safety of subjects enrolled in INS-212, Insmmed plans to implement a Data Monitoring Committee (DMC). The Committee will consist of experts outside of Insmmed who are not involved in the study conduct. The DMC will provide a centralized review function independent of Insmmed clinical team and all other individuals associated with the conduct of the study. The DMC will be comprised of at least two physicians with pulmonary expertise who are not investigators in the clinical study and a statistician who is experienced in the evaluation of safety data. Further details are provided in the DMC charter.

4 STUDY POPULATION

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

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4.1 INCLUSION CRITERIA

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

1. be male or female, 18 years or older (20 years or older in Japan)
2. be positive for MAC on culture as defined in inclusion criterion No. 4 while being treated with a multi-drug treatment regimen (at least 2 antibiotics) for a minimum duration of 6 consecutive months that is either ongoing or was stopped no more than 12 months before Screening (exceptions to multi-drug treatment regimen for 6 consecutive months include treatment with doses or frequencies below those recommended by guidelines and/or short interruptions of therapy, both occurring due to safety/tolerability issues)
3. be diagnosed with MAC NTM lung infection with evidence of underlying lung disease such as nodular bronchiectasis and/or fibrocavitary disease by chest radiography or chest computed tomography. High resolution CT (HRCT) scan is preferred, if available
4. have 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 one obtained within 6 months prior to Screening and one positive culture at Screening (cultures to be at least 1 month apart). Cultures may be obtained from sputum or bronchoscopy.
5. have a MAC-positive sputum at Screening
6. be willing to adhere to multi-drug treatment regimen during the course of the study
7. be able to produce approximately 3 mL of sputum or be willing to undergo an induction that produces approximately 3 mL of sputum for mycobacteriology
8. female of child bearing potential agrees to practice an acceptable method of birth control (e.g., true abstinence [refraining from heterosexual intercourse during the entire study], hormonal or barrier methods, partner sterilization, or intrauterine device [IUD]) while participating in the trial. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of the study, and withdrawal are not acceptable methods of contraception.

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9. the patient will provide written informed consent before performing any study related procedure
10. be willing to have serum specimens stored
11. be able to comply with study drug use, study visits, and study procedures as determined by the investigator.

4.2 EXCLUSION CRITERIA

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

1. patients with cystic fibrosis
2. patients whose MAC NTM infection is resistant to amikacin (as identified by MIC susceptibility $>64 \mu\text{g/mL}$)
3. patients who are not able to perform the 6MWT
4. positive pregnancy test or lactation at Screening. All women of child bearing potential will be tested. Women not of child bearing potential are defined as postmenopausal (i.e., amenorrheic 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 (e.g., high dose ibuprofen, inhaled anti-inflammatory agents including steroids, low dose maintenance steroids, rhDNase) within 28 days before Day 1
10. administration of any investigational drug within 8 weeks before Screening

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11. prior exposure to LAI (including clinical study)
12. known hypersensitivity to aminoglycosides
13. use of inhaled or systemic aminoglycosides with activity against MAC (e.g., amikacin, kanamycin, or streptomycin) within 28 days before Day 1
14. acquired and primary immunodeficiency syndromes (e.g. 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 or alanine aminotransferase ≥ 3 times the upper limit of normal (ULN) or total bilirubin ≥ 2 times ULN at Screening
17. absolute neutrophil count $\leq 500/\mu\text{L}$ 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, interferes 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 are not expected to survive the duration of the study
23. Patients with disseminated MAC infection

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4.3 REMOVAL OF SUBJECTS

A subject may be withdrawn from the study for any of the following reasons:

1. the event of the subject's death
2. the subject experiences an AE and the Investigator or the patient determines that withdrawal from the study is appropriate
3. a major protocol deviation that interferes with the integrity of the study data for this subject
4. the subject is noncompliant (does not comply with dosing instructions provided by the Investigator or does not comply with the study visit schedule) and the Investigator determines that withdrawal of the subject is appropriate
5. the subject withdraws consent to participate in the study
6. the site is unable to contact the subject after all reasonable efforts have been exhausted (Lost to Follow-Up)
7. any condition that, in the judgment of the Investigator, would compromise the ability of the subject to comply with the study protocol or complete the study
8. any subject who has to have "rescue" medication will be discontinued

A subject may decide to withdraw from the study at any time, for any reason, without prejudice to subsequent care or treatment by the Investigator.

Subjects who discontinue the study prematurely should be assessed in accordance with the end-of-treatment visit in [Section 6.8](#) and the follow-up procedures in [Section 5.12.1](#).

4.4 RE-SCREENING OF SUBJECTS

Subjects may be re-screened once, if approved by Sponsor, if the subjects' Screening culture is not positive for MAC or there is a substantial delay (longer than the Screening period) in obtaining the results from the microbiology labs. 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),

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the Screening window of 10 weeks must be maintained. Subject will only be allowed to be re-screened only provided that all other entry criteria have been satisfied. Re-screened subjects will be assigned a new subject number within the EDC system. All Screening assessments must be repeated when a subject is re-screened.

4.5 REPLACEMENT OF SUBJECTS

Subjects may be replaced to ensure 261 subjects will be evaluable subjects by Month 6.

4.6 DEFINITIONS OF CONVERTER AND COMPLETER

A converter is defined as a subject who has 3 consecutive monthly MAC-negative sputum cultures at any time within the first 6 months of the study.

Relapse or recurrence is defined as having MAC-positive sputum cultures in liquid broth media (agar negative) for 3 or more consecutive months, or having 1 MAC-positive sputum culture on solid media (agar positive) after achieving culture conversion.

A non-converter is defined as a subject who does not have 3 consecutive monthly MAC-negative sputum cultures at any time within the first 6 months of the study.

A completer for this study will be defined as follows:

1. A converter who successfully completes 12 months on the same treatment regimen beginning from the first of 3 negative cultures that defined conversion and successfully completes the 12 months off-treatment EOS follow-up visit.
2. A non-converter who successfully completes all dosing and protocol requirements up to and including the Month 6 study visit.

5 STUDY TREATMENTS

5.1 INVESTIGATIONAL STUDY DRUG

Liposomal amikacin sulfate is encapsulated in liposomes composed of DPPC and cholesterol (2:1 w:w lipid ratio) formulated as a suspension at a targeted concentration equivalent to 70 mg

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amikacin/mL in 10 mL of water for injection (**Error! Reference source not found.**5-1). The total lipid to drug ratio is 0.57 to 0.77 w:w (weight to weight).

Table 5-1 Composition of Liposomal Amikacin Sulfate

Ingredient	Quality Grade	Mg/mL
Amikacin base (amikacin sulfate)	EP/USP	70
Cholesterol	HP ^a	15.7
Dipalmitoylphosphatidylcholine (DPPC)	In-house	31.5
Sodium chloride	USP/EP/JP	12
Sodium hydroxide	USP/EP/JP	QS to adjust pH
Water for injection	USP/EP/JP	QS

Abbreviations: EP, European Pharmacopeia; HP, High Purity; JP, Japanese Pharmacopeia; NF, National Formulary, USP, United States Pharmacopeia.

^a HP grade meets or exceeds all requirements for cholesterol USP/NF/EP.

5.2 REFERENCE STUDY DRUG

None

5.3 ADMINISTRATION OF STUDY DRUG

LAI is administered daily by inhaling drug product that has been aerosolized in an eFlow nebulizer over approximately 14 minutes. Study drug will be administered daily by the subject except two days prior to a scheduled study visit when sputum is collected (refer to [Section 7.1.1](#)). The day of study visit, when sputum is collected, the study drug will be administered at the study

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site by study personnel. Written directions for preparing and administering the study drug will be provided to subjects.

Dose Interruption

If a subject experiences local respiratory events such as, but not limited to, dysphonia, oropharyngeal pain, and cough while taking LAI that may be distressing to the subject, short interruptions of LAI may be necessary and are permitted. As the frequency of these local respiratory events decreased with continued use in prior LAI studies, it is recommended that LAI is reintroduced after a short interruption of dosing when symptoms subside. Please contact the medical monitor to discuss.

5.4 SELECTION OF DOSES

It is intended that all subjects randomized to the LAI plus multi-drug regimen will receive the contents of 1 vial, 590 mg of LAI, of nebulized study drug.

Subjects who cannot tolerate 590 mg of LAI QD will be discontinued from LAI (with the exception of short interruptions as discussed in [Section 5.3](#)); the dose of LAI will not be changed during the study.

5.5 SELECTION OF TIMING OF ADMINISTRATION

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

5.6 BLINDING

None

5.7 STUDY KIT

Subjects will receive kits containing vials of LAI, the handset/nebulizer, and controller.

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5.8 DRUG SUPPLY

The active ingredient, amikacin sulfate, is manufactured in accordance with cGMPs by ACS DOBFAR S.p.A.

5.8.1 Packaging

LAI is provided as a unit dose consisting of a 10 mL Type I clear borosilicate glass vial with a 20 mm finish using a closure system consisting of a 20 mm bromobutyl stopper and a 20 mm aluminum flip-off tear-off combination seal.

5.8.2 Labeling

Vials of study drug will be labeled as shown in [APPENDIX 2](#).

5.8.3 Storage

At the site, LAI must be stored at 2°C to 8°C (36°F to 46°F) in a secured place with restricted access. Do not freeze.

5.8.4 Compliance and Drug Accountability

Drug accountability will be recorded at each study visit by count of study drug at each study visit.

A subject will be considered non-compliant with study drug if treatment adherence is less than 80% or more than 120% unless instructed by the Investigator to interrupt dosing for safety reasons ([Section 5.3](#)).

5.8.5 Dispensing of Study Drug

Study drug (LAI) will be shipped to the study site after the first subject is screened. Patients randomized to the LAI treatment will be dispensed adequate study drug at each visit to allow for daily dosing, including extra supply for potential study visit scheduling delays.

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5.8.6 Disposition and Reconciliation of Study Drug

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

The pharmacy will maintain detailed documentation of the number and identification of vials with copies of these documents to be provided to the Sponsor at the end of the study. All used and unused boxes of study drug will be maintained by the site until inventoried by the study monitor. Upon completion of the drug inventory by the study monitor, used and any unused vials will be disposed of in accordance with instructions provided to sites and according to site destruction policies. Documentation of destruction should be provided to the Sponsor.

Accountability of the PARI eFlow[®] nebulizer will be maintained and tracked at the site. Subjects must return the nebulizers to the study site at the end of treatment. Additional instructions for handling the disposal or return of the devices will be provided to the sites and according to site destruction policies.

5.9 CONCOMITANT MEDICATIONS

Throughout the duration of the study, subjects should continue on the same, multi-drug (at least 2 antibiotics), anti-mycobacterial regimen. The regimen should be based on the 2007 ATS/IDSA Guidelines or respective local guidelines, and should not change during the treatment period except for safety concerns or if “rescue” anti-mycobacterial therapy is required. Any subject who has to have “rescue” medication will be discontinued. All NTM treatment should stop at the EOT visit. No NTM treatment should be given during the safety follow-up period.

Where allowed, and if approved by IRB/EC, the sponsor will provide reimbursement of the multi-drug anti-mycobacterial regimen for as long as the patient participates in the study and is adherent to all protocol requirements. These drugs may include, but are not limited to: azithromycin, clarithromycin, clofazimine, ethambutol, ethionamide, rifabutin, and rifampicin.

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5.10 PROHIBITED MEDICATIONS

Subjects are prohibited from using any inhaled antibiotics and any aminoglycosides with activity against MAC from 28 days before Day 1, and should not initiate these drugs during the study period unless clinically indicated as determined by the Investigator. If a subject requires an inhalational antibiotic for medical management of an acute pulmonary exacerbation secondary to Gram-negative bacteria during the study, then LAI should be interrupted during the course of that inhaled antibiotic treatment and until the acute event has resolved. Inhaled antibiotics such as tobramycin that do not have activity against MAC are permitted. Administration of LAI should begin again after the inhaled antibiotic is stopped and the acute event has resolved. During the course of an inhaled antibiotic treatment, the subject will remain in the study and complete all remaining study visits if possible. Exceptions include treatment with IV amikacin (or nebulized IV amikacin solution) and other aminoglycosides with activity against MAC, such as streptomycin or kanamycin. Such aminoglycosides for the treatment of NTM lung infection will be considered a “rescue” medication. If the Investigator believes that any of the aforementioned prohibited medications are necessary they must discuss these with the medical monitor and the subject will discontinue study drug and/or the study. Any questions about prohibited medications should be discussed with the medical monitor for the study.

5.11 PRECAUTIONARY MEDICATIONS

Treatment with oral or IV antibiotics (other than the subject’s stable anti-mycobacterial regimen) for acute pulmonary exacerbations or acute infection is allowed as determined by the Investigator; the reason for use must be documented in the eCRF (electronic case report form). Intra-muscular antibiotics that do not have activity against MAC (see prohibited medications) are permitted and must be documented in the eCRF.

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

Although systemic exposure to amikacin is low after LAI administration, precaution should be taken if subjects require the following systemic medications that may have possible interactions

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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 is allowed. Subjects who develop bronchospasm may be pre-treated with a bronchodilator before study drug administration.

Table 5-2 List of Examples of Precautionary Medications

Name of Drug, etc.	Clinical Symptoms/ Treatment	Mechanism/ Risk Factor(s)
Potentially nephrotoxic blood substitutes such as Dextran Hydroxyethyl starch, etc.	Since there is a risk that nephrotoxicity occurs or is aggravated, concomitant use should be avoided. When nephrotoxicity occurs, discontinue drug and implement appropriate treatment such as renal dialysis, etc.	The mechanism of action is not completely clear but there are reports that the combination leads to deposits of aminoglycoside antibiotics in blood and to vacuolization of the proximal tubular epithelium.
Loop diuretics Ethacrynic acid Furosemide Azosemide, etc.	Since both drugs may cause or aggravate nephro- and ototoxicity, concomitant use should be avoided	The mechanism of action is not completely clear but there are reports that the combination leads to increased blood concentration and renal deposits of aminoglycoside antibiotics
Nephrotoxic and ototoxic drugs such as Vancomycin, Enviomycin, Platinum-containing anti- cancer drugs (cisplatin, carboplatin, nedaplatin), etc.	Since both drugs may cause or aggravate nephro- and ototoxicity, concomitant use should be avoided	Both drugs are nephrotoxic/ ototoxic but the mechanism of action of the interaction is not known.

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Anesthetics Muscle relaxants Tubocurarine Pancuronium bromide Tolperisone Botulinum type A toxin products, etc.	Risk of respiratory suppression. If respiratory suppression occurs, administer choline esterase inhibitor or calcium preparation, etc. as appropriate.	Both drugs have a neuro-muscular inhibitory effect. Concomitant use will aggravate this effect.
Nephrotoxic drugs Cyclosporine A Amphotericin B, etc.	Risk that renal impairment occurs or is aggravated	Both drugs are nephrotoxic but the mechanism of the interaction is not known.

5.12 DISCONTINUATION OF STUDY DRUG AND/OR STUDY PARTICIPATION

1. The Investigator may discontinue study drug and/or study participation in the interest of subject safety. The Investigator must identify specific AEs (laboratory abnormality, intercurrent illness, other medical condition or situation) that result in premature discontinuation of study drug and/or study participation in the eCRF.
2. A subject who withdraws consent to receive study drug may be followed up according to the protocol visit schedule. All attempts must be made to conduct an EOT visit ([Section 6.7](#)).
3. Subjects who meet the following criteria established by Hy's law will be discontinued from the study: alanine aminotransferase or aspartate aminotransferase ≥ 3 ULN AND total bilirubin > 2 ULN.
4. Subjects who need "rescue" medication as described in [Section 5.9](#) will be discontinued.
5. Subjects who become pregnant will be discontinued from the study and followed until the pregnancy is concluded.
6. Administration of study drug will be discontinued to all subjects seen by an Investigator who terminates participation in the study prematurely.

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7. Administration of study drug will be discontinued to all subjects if the study is terminated.

The Investigator must contact the medical monitor immediately before discontinuing a subject from study drug and/or study participation if possible, or no later than 1 business day after the event. When a subject is prematurely discontinued from study drug, the subject will be given the option to continue in the study, off study drug, until their Month 8 visit (except for subjects enrolled at sites in Japan. All subjects at sites in Japan who discontinue study drug will also discontinue study participation). When a subject is prematurely discontinued from study drug and/or study participation, the Investigator will clearly document the reason in the medical record and complete the appropriate eCRF page describing the reason for discontinuation. If the subject completes all study procedures (including those who discontinued study drug but remain in the study), up to and including the Month 6 study visit, and sputum results indicate non-conversion or relapse/recurrence, they will be invited to participate in a separate open-label study of LAI (Study INS-312), provided all entry criteria have been met for that study. Any subject who discontinues the study prior to the Month 6 study visit will not be eligible to participate in the separate open-label study of LAI (INS-312).

In the event that a subject is withdrawn from the study treatment because of an AE, the subject 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 subject fails to complete 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 subject by the Investigator or other staff members were unsuccessful.

5.12.1 Procedures for Discontinuation

If a subject prematurely discontinues the study prior to the Month 12 (Visit 11) visit (excluding subjects enrolling in INS-312), the subject will have:

- EOT visit

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- Telephone contact 28 days after the EOT visit for safety follow-up
- Telephone contact at Month 12 (time point from Baseline) for safety follow-up (if not coinciding with the 28 day after EOT telephone contact)
- Telephone contact 12 months after the EOT visit for vital status and safety follow-up

If a subject prematurely discontinues the study on or after the Month 12 visit, the subject will have:

- EOT visit
- Telephone contact 28 days after the EOT visit for safety follow-up
- Telephone contact 12 months after the EOT visit for vital status and safety follow-up

If a subject prematurely discontinues the study during the off-treatment phase (after completion of 12 months of treatment), the subject will have:

- Telephone contact at the 12 Month Safety Follow-Up visit for vital status and safety follow-up

Subjects who are part of the sputum PK subset who discontinue LAI at any time point will have to return for clinic visits 28 days and 3 months off-LAI for sputum PK and sputum culture collection.

6 STUDY PROCEDURES

6.1 SCREENING VISIT (VISIT 1)

None of the following procedures or assessments can be made until the subject has provided informed consent as documented on the Informed Consent Form (ICF).

After informed consent has been obtained, all of the following will be performed:

- 12-lead ECG (see [Section 7.2.5](#))
- obtain medical history (this can be done anytime between Screening and Baseline and the information will only be entered into the EDC system once a subject has been enrolled into the study)
- administer physical examination, including vital signs and pulse oximetry (see [Section 7.2.2](#) and [Section 7.2.3](#))

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- administer audiology test (may be administered either at the Screening or Baseline [Day 1] visit, not both; see [Section 7.2.9](#))
- soliciting of concomitant medications (see [Section 7.2.8](#)) (this can be done anytime between Screening and Baseline and the information will only be entered into the EDC system once a subject has been enrolled into the study)
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect blood or urine specimens for clinical chemistry, hematology, and urinalysis testing and, for women of child-bearing, a human chorionic gonadotropin (hCG) pregnancy test (see [Section 7.2.7](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- subjects will collect 2 sputum samples at home during the week immediately following the Screening visit.

6.2 BASELINE VISIT (DAY 1, VISIT 2)

All of the following should be performed or obtained within 70 days after Screening unless the results of the sputum culture analysis are delayed. 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.

- administer SGRQ before study drug administration (see [Section 7.3.1](#))
- administer EQ-5D-3L before study drug administration (see [Section 7.3.2](#))
- collect information on healthcare resource utilization
- update medical history
- administer physical examination, including vital signs and pulse oximetry. Only vital signs and pulse oximetry will be assessed both before and after study drug administration (see [Section 7.2.2](#) and [Section 7.2.3](#))
- administer 6MWT (see [Section 7.2.1](#))

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- administer the pulmonary function test prior to study drug administration (see [Section 7.2.4](#))
- administer audiology test (may be administered either at the Screening or Baseline [Day 1] visit, not both; see [Section 7.2.9](#))
- obtain CT chest scan if part of CT Scan Sub-Study and has not been acquired within 6 months before Day 1 (see [Section 7.3.4](#), [APPENDIX 4](#))
- obtain CT chest scan if part of Japan specific CT Scan Sub-Study and has not been acquired within 6 months before Day 1 (see [APPENDIX 5](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect sputum specimen for sputum-only PK sub-set analyses (see [Section 7.4.1](#))
- collect blood or urine specimens for clinical chemistry, hematology, and urinalysis testing and, for women of child-bearing, a urine pregnancy test (see [Section 7.2.7](#))
- collect blood (serum) specimen for biomarker assessment (CRP and IL-6) (see [Section 7.2.7](#))
- collect blood (serum) and sputum specimens for Comprehensive PK Sub-Study analysis in Japanese subjects only (see [Section 7.4.3](#) and [APPENDIX 3](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- administer study drug at the study site (see [Section 5.3](#))
- dispense study drug.

6.3 VISITS AT MONTHS 1 THROUGH 6 AND 8 (VISITS 3 THROUGH 9) DURING THE RANDOMIZED, TREATMENT PHASE

All of the following should be performed or obtained at Visits 3 through 9, unless noted, within 3 days of the scheduled visit. For Visits 3 (Month 1), 4 (Month 2), and 7 (Month 5), Home

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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:

- administer SGRQ before study drug administration at the Months 3, 6, and 8 visits only (see [Section 7.3.1](#))
- administer EQ-5D-3L before study drug administration at the Months 3, 6, and 8 visits only (see [Section 7.3.2](#))
- collect information on healthcare resource utilization
- At Months 2, 4, 6, and 8 only: administer physical examination, including vital signs and pulse oximetry. Vital signs and pulse oximetry will be assessed both before and after study drug administration (see [Section 7.2.2](#) and [Section 7.2.3](#))
- administer 6MWT at the Months 4, 6 and 8 visits only (see [Section 7.2.1](#))
- administer the pulmonary function test at Month 6 only prior to study drug administration (see [Section 7.2.4](#))
- administer audiology test at the Months 3 and 6 visits only (see [Section 7.2.9](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect blood and urine specimens for clinical chemistry, hematology, and urinalysis testing at the Months 1, 3, 6, and 8 visits only and, for women of child-bearing, a urine pregnancy test at Months 1-6 and 8 (see [Section 7.2.7](#))
- collect blood (serum) and sputum specimen for biomarker assessment at the Month 6 visit only (CRP [c-reactive protein] and IL-6 [interleukin 6]) (see [Section 7.2.7](#))
- collect blood and sputum specimens for population PK sub-set analyses at the Months 1, 3, and 6 visits only (see [Section 7.4.1](#))
- collect sputum specimens for sputum-only PK sub-set analyses at the Months 4, 5 and 6 visits only, 2 days interruption of LAI dosing (see [Section 7.4.1](#))

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- collect blood (serum) and sputum specimens for Comprehensive PK Sub-Study analysis at the Month 1, 3 and 6 visits for Japanese subjects only (see [Section 7.4.3](#) and [APPENDIX 3](#))
- (Japan only) at Month 6 obtain CT chest scan as part of Japan specific CT Scan Sub-Study (see [APPENDIX 4](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- administer study drug at the study site (see [Section 5.3](#))
- dispense study drug
- collect previously dispensed study drug vials from subject.

Beginning with Month 3 and extending throughout the study, subjects will be assessed for culture conversion by the central microbiology laboratory ([Section 3.1](#)). Beginning with Month 4, relapse or recurrence will be assessed by the central microbiology laboratory ([Section 3.1](#)).

6.4 MONTH 10 (VISIT 10) DURING THE RANDOMIZED, TREATMENT PHASE

All of the following should be performed or obtained within 5 days of the scheduled visit:

- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- dispense study drug
- collect previously dispensed study drug vials from subject.

For subjects who were randomized to the multi-drug regimen only arm, this visit may take place as a telephone contact as no drug dispensation will be performed.

6.5 MONTH 12 (VISIT 11) DURING THE RANDOMIZED, TREATMENT PHASE

All of the following should be performed or obtained within 5 days of the scheduled visit:

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- administer SGRQ before study drug administration (see [Section 7.3.1](#))
- administer EQ-5D-3L before study drug administration (see [Section 7.3.2](#))
- collect information on healthcare resource utilization
- administer physical examination, including vital signs and pulse oximetry. Only vital signs and pulse oximetry will be assessed both before and after study drug administration (see [Section 7.2.2](#) and [Section 7.2.3](#))
- administer audiology test (see [Section 7.2.9](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect blood and urine specimens for clinical chemistry, hematology, and urinalysis testing and, for women of child-bearing, a urine pregnancy test (see [Section 7.2.7](#))
- (Japan only) obtain CT chest scan as part of Japan specific CT Scan Sub-Study (see [APPENDIX 4](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- administer study drug at the study site (see [Section 5.3](#))
- dispense study drug
- collect previously dispensed study drug vials from subject.

6.6 MONTH 14 (VISIT 12) DURING THE RANDOMIZED, TREATMENT PHASE

All of the following should be performed or obtained within 5 days of the scheduled visit:

- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- dispense study drug
- collect previously dispensed study drug vials from subject.

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For subjects who were randomized to the multi-drug regimen only arm, this visit may take place as a telephone contact as no drug dispensation will be performed.

6.7 END OF TREATMENT – UP TO MONTH 16 (VISIT 13) DURING THE RANDOMIZED, TREATMENT PHASE

All of the following should be performed or obtained within 5 days of the scheduled visit:

- obtain 12-lead ECG (see [Section 7.2.5](#))
- administer SGRQ (see [Section 7.3.1](#))
- administer EQ-5D-3L (see [Section 7.3.2](#))
- collect information on healthcare resource utilization
- administer physical examination, including vital signs and pulse oximetry. (see [Section 7.2.2](#) and [Section 7.2.3](#))
- administer 6MWT (see [Section 7.2.1](#))
- administer audiology test (see [Section 7.2.9](#))
- obtain CT chest scan if part of CT Scan Sub-Study and no less than 6 months have elapsed since the previous CT scan was performed (see [Section 7.3.4](#), [APPENDIX 4](#))
- (Japan only) at Month 16, obtain CT chest scan as part of Japan specific CT Scan Sub-Study (see [APPENDIX 45](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect blood and urine specimens for clinical chemistry, hematology, and urinalysis testing and, for women of child-bearing, a urine pregnancy test (see [Section 7.2.7](#))
- collect blood (serum) specimen for biomarker assessment (CRP and IL-6) (see [Section 7.2.7](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- collect previously dispensed study drug vials from subject

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For subjects who do not convert, the last visit will occur no later than at the Month 8 visit. For subjects who do convert, the last visit will be at the visit corresponding to 12 months following their conversion dates. All subjects who discontinue study drug early should have all procedures listed in the end of treatment (EOT) visit performed, with the exception of the chest CT scan. A chest CT scan should only be performed if the subject is participating in the CT scan sub-study but only if 6 months or more have elapsed since the subject's last CT scan has been performed. Please refer to [APPENDIX 5](#) for Japan specific CT Scan Sub-Study study procedures and study schedule. High resolution CT scan is preferred, if available.

6.8 OFF-TREATMENT SAFETY FOLLOW-UP VISITS (VISIT 14, 15, AND 16) DURING THE OFF-TREATMENT PHASE FOR SUBJECTS WHO COMPLETE 12 MONTHS OF TREATMENT

Visit 14 (28 day off-treatment safety follow-up)

For any subject who completes treatment, all of the following should be performed or obtained within 5 days of the 28 day safety follow-up scheduled visit:

- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect sputum specimen for sputum-only PK assessment (see [Section 7.4.1](#))

Visits 15 and 16 (3 and 6 months off-treatment safety follow-up)

All of the following should be performed or obtained within 7 days of the scheduled visit:

- administer SGRQ at the 3 month safety follow-up only (see [Section 7.3.1](#))
- administer EQ-5D-3L at the 3 month safety follow-up only (see [Section 7.3.2](#))
- administer physical examination, including vital signs and pulse oximetry at the 3 month safety follow-up only (see [Section 7.2.2](#) and [Section 7.2.3](#))

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- administer 6MWT at the 3 month safety follow-up only (see [Section 7.2.1](#))
- administer the pulmonary function test at the 6 month safety follow-up only (see [Section 7.2.4](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- dispense sputum collection containers (see [Section 7.2.7](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))
- collect sputum specimen for sputum-only PK assessment for the 3 month safety follow-up off-LAI only (see [Section 7.4.1](#))

6.9 END OF STUDY -12 MONTHS OFF-TREATMENT (VISIT 17)

All of the following should be performed or obtained within 7 days of the scheduled visit:

- administer physical examination, including vital signs and pulse oximetry (see [Section 7.2.2](#) and [Section 7.2.3](#))
- solicit concomitant medications (see [Section 7.2.8](#))
- assess AEs (see [Section 8](#))
- collect sputum specimen for microbiological assessment (see [Section 7.1.1](#))

The EOS visit is scheduled to occur 12 months after EOT and is for all subjects who complete 12 months of treatment from their first of 3 negative cultures that classified them as a converter.

7 STUDY VARIABLES AND METHODS OF ASSESSMENT

7.1 PRIMARY ENDPOINT VARIABLES

7.1.1 Sputum Collection, Transport, and Assessment

Pre-dose expectorated or induced sputum specimens (approximately 3 mL) are required at Screening, Day 1, at Months 1 through 6, at Months 8 and 12, at EOT, at the 28 day, the 3, 6, and 12 months off-treatment visits. To improve the probability of obtaining a good sputum specimen, at least 2 and preferably 3 sputum samples will be obtained from each subject at each

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assessment. If a subject is unable to produce sputum spontaneously, one induced sputum specimen at the site will be acceptable. With the exception of the Screening samples (collected during the week following the Screening visit), each sputum sample will be collected on consecutive days prior to a scheduled visit. Subjects will refrain from administering LAI on the days sputum is obtained, starting 2 days prior to the scheduled visit, even if a spontaneous sputum cannot be obtained by the subject at home. At the scheduled visit, once sputum is collected (spontaneously or by induction), the administration of LAI can recommence. Sites are encouraged to notify subjects by telephone of this procedure 3 days prior to their scheduled visit. If a subject is unable to produce sputum independently, sputum may be induced as described in [APPENDIX 1](#), if these suggestions are safe for the subject. If a subject is still unable to produce sputum despite reasonable efforts, and they have already met the definition of converter, this will be recorded as a negative culture result at that time point.

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

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

7.1.2 Microbiological Assessment

Sputum specimens will be cultured in liquid media in addition to solid media (agar). If results are negative on agar, the liquid media will be held for 6 weeks before reporting as culture negative.

Standard antibiotic sensitivity testing using MICs will be routinely performed on bacterial isolates (Clinical and Laboratory Standards Institute [CLSI] M48-A and NCCLS M24-A)

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(Forbes 2008). All mycobacterial isolates will be banked for subsequent determination of amikacin susceptibility and selective molecular typing (Prammananan, et al. 1998).

Isolates of MAC will be identified to complex, using a commercial RNA probe (AccuProbe, Gen Probe, Inc.) and subsequently identified to species (*M. avium*, *M. intracellulare*, MAC “X” group) using molecular methodology (Cousins, et al. 1996).

7.2 SECONDARY ENDPOINT VARIABLES

7.2.1 Six-minute Walk Test

7.2.1.1 Methodology

A 6MWT of exertional capability will be performed at Baseline (Day 1) and at Months 4, 6, 8 and at EOT and 3 months off-treatment according to ATS guidelines (<http://ajrccm.atsjournals.org/cgi/content/full/166/1/111>). Overall fatigue and dyspnea (Borg scale) will be measured before and after subjects walk a prescribed course as far as they can in 6 minutes.

7.2.1.2 Assessment

The 6MWT must be conducted by a site member who is blinded to the subject’s open-label treatment assignment. The maximum distance achieved will be recorded in the eCRF.

7.2.2 Physical Examination

7.2.2.1 Methodology

A physical examination of the head (external), eyes, ears, nose and throat, lungs, cardiovascular system, abdomen, musculoskeletal system, skin, lymph nodes, central nervous system, and, as appropriate, other body systems will be performed before the administration of study drug at every scheduled visit except at Months 1, 3, 5, 10, 14, at 28 days and 6 months off-treatment. The physical examination will also include weight; the measurement of height, without shoes, will only be done at Screening. All significant findings must be recorded in the eCRF as described below.

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7.2.2.2 Assessment

Significant findings at Baseline (Day 1) and any changes from Baseline (Day 1) prior to first dose of LAI or multi-drug regimen at Day 1 must be recorded in the Medical History/Current Medical Conditions pages of the eCRF. Significant findings made after the Baseline (Day 1) visit that meet the definition of an AE must be recorded on the Adverse Event page of the eCRF.

7.2.3 Vital Signs

7.2.3.1 Methodology

Five-minute sitting blood pressure, pulse rate, body temperature, respiratory rate, and oxygen saturation will be recorded in the eCRF at every scheduled visit except at Months 1, 3, 5, 10, 14, at 28 days and 6 months off-treatment.

At visits when the 6MWT is administered (at Baseline [Day 1], at Months 4, 6, and 8 and at EOT and 3 months off-treatment) vital signs will be measured before and after subjects walk a prescribed course as far as they can in 6 minutes.

7.2.3.2 Assessment

Significant findings observed at any time after the first dose of LAI or multi-drug regimen that meet the definition of an AE must be recorded on the Adverse Event page of the eCRF.

7.2.4 Pulmonary Function Tests

Pulmonary function tests (FEV₁[L], FEF_(25-75%), FVC) will be performed at sites with access to spirometers and trained personnel to perform spirometry at Baseline (Day 1), Month 6 for all subjects and at the 6-months off-treatment visit only for subjects completing LAI treatment. All subjects undergoing PFTs should be optimally treated for their underlying lung disease before these assessments are performed. Additionally, for subjects randomized to the LAI plus multi-drug regimen arm they will be performed prior to the administration of LAI. If necessary, per the discretion of the Investigator, subjects randomized to LAI may have pre- and post-dose PFTs performed to determine the degree of sub-clinical bronchospasm and airway obstruction so

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appropriate management can be commenced prior to LAI dosing. . More information will be provided in a separate manual provided by the Sponsor.

7.2.4.1 Assessment

The pulmonary function parameters of forced expiratory volume in 1 second (FEV₁[L]), forced expiratory flow rate during mid portion of expiration (FEF_(25-75%)) and forced vital capacity (FVC) based on local assessments will be recorded on the specific page within the eCRF. Any clinically significant findings observed must be recorded on the Adverse Event page of the eCRF.

7.2.5 12-lead Electrocardiogram

7.2.5.1 Methodology

An ECG will be recorded at Screening and EOT.

7.2.5.2 Assessment

The Screening ECG will be considered as Baseline. The date and interpretation (Normal; Abnormal – not clinically significant; Abnormal – clinically significant) must be recorded within the ECG page of the eCRF. Any abnormal – clinically significant findings at Baseline (Day 1) and any changes from Baseline (Day 1) prior to first dose of LAI or multi-drug regimen at Day 1 must be recorded in the Medical History/Current Medical Conditions pages of the eCRF. Any abnormal – clinically significant findings made after the Baseline (Day 1) visit that meet the definition of an AE must be recorded on the Adverse Event page of the eCRF.

7.2.6 Adverse Events

7.2.6.1 Methodology

AEs will be identified at every contact with a subject throughout the study period beginning with Day 1 and after first dose of LAI or multi-drug regimen by direct observation, from assessments of safety parameters, and by asking open-ended, non-leading questions such as “How have you felt since your last visit?” See [Section 8](#) for definitions and a fuller description of the assessment

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and reporting requirements of AEs. Any AE that is observed prior to first dose of LAI or multi-drug regimen will be classified as medical history.

7.2.6.2 Assessment

All AEs will be recorded on the Adverse Event page of the eCRF. Attempts will be made to determine the start and stop dates of the event, the intensity, causality, treatment, and outcome of the event. Any AE that is observed prior to first dose of LAI or multi-drug regimen must be recorded on the Medical History page of the eCRF.

7.2.7 Clinical Laboratory Evaluations

The following clinical chemistry, hematology, and urine parameters comprise the clinical laboratory evaluations:

Clinical chemistry	Sodium, chloride, potassium, bicarbonate (CO ₂), magnesium, calcium, glucose, phosphate, total bilirubin (with direct and indirect fractionation, if total bilirubin is elevated >2 ULN), alkaline phosphatase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, creatinine, urea-nitrogen, uric acid, estimated glomerular filtration rate from the Cockcroft-Gault method, and, at Screening only, human chorionic gonadotropin (hCG) pregnancy test for women of child-bearing potential
Hematology	Hemoglobin, erythrocytes, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), leukocytes, differential blood count of neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets.
Urinalysis	Qualitative analysis of glucose, ketones, nitrites, protein, pH, leukocytes, blood, bilirubin, specific gravity; microscopic examination for cells, casts, and bacteria Urine pregnancy test for women of child-bearing potential. Performed at the site and results will be entered into the eCRF

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Other

Biomarkers CRP and IL-6

Assessments made only before the first administration of study drug are not considered safety parameters.

7.2.7.1 Methodology

Blood samples will be drawn with a minimum number of needle insertions to determine clinical chemistry and hematology parameters at Screening, and before administering study drug on Day 1, at Months 1, 3, 6, 8, and 12, and at EOT. Lab values that meet exclusionary criteria based on results from the central laboratory may be repeated at the Investigator's discretion and sent to the central laboratory provided that the re-evaluation is completed within the protocol defined Screening period and can only be repeated once. Only those labs that need to be re-evaluated should be performed as an unscheduled visit, not the entire laboratory panel. Details for collecting, handling, storing, and transporting blood samples will be provided in the site laboratory manual.

Urine samples will be collected at the same times as blood is drawn.

7.2.7.2 Assessment

Clinical chemistry and hematology analyses will be performed by a central laboratory; qualitative urinalyses will be performed by a central laboratory, and evaluated microscopically for cells, casts, and bacteria.

7.2.8 Concomitant Medications

The dosage of all concomitant medications should be recorded on the eCRF at every contact with a subject throughout the study period beginning with Day 1.

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7.2.9 Audiology

7.2.9.1 Methodology

Audiology testing will be performed either at Screening or before administration of study drug at Baseline (Day 1), before study drug administration at Months 3, 6 and 12, and the end of treatment visit. Frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz will be evaluated for each ear using air conduction. If post-Baseline (Day 1) audiometry findings are an AE Grade 2 or higher (according to the Common Terminology Criteria for Adverse Events [CTCAE] Version 4.0 guideline), an additional audiometric evaluation should be conducted at the next scheduled visit.

7.2.9.2 Assessment

Significant Baseline (Day 1) findings and all post-Baseline (Day 1) findings (including hearing loss of a CTCAE Grade 2 or higher) prior to first dose of LAI or multi-drug regimen on Day 1 must be recorded on the medical history page of the eCRF. Significant findings that meet the definition of an AE (including hearing loss of a CTCAE Grade 2 or higher) must be recorded on the AE page of the eCRF.

7.3 EXPLORATORY ENDPOINT VARIABLES

7.3.1 St. George's Respiratory Questionnaire

7.3.1.1 Methodology

The SGRQ is a self-administered questionnaire that has been validated in subjects with airways disease, specifically in subjects with BR (Jones 1991; Jones, et al. 1991; Wilson, et al. 1997). The SGRQ assesses health-related quality of life in subjects with chronic pulmonary disease by evaluating 3 health domains: symptoms (distress caused by respiratory symptoms); activity (effects of disturbances to 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). The SGRQ will be completed before administration of study drug on Day 1, at Months 3, 6, 8, and 12, and at EOT and 3 months off-treatment.

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7.3.1.2 Assessment

A composite total score is derived as the sum of domain scores for symptoms, activity, and impact, with 0 the best possible score and 100 the worst possible score. A reduction in score of 4 units is generally recognized as a clinically meaningful improvement in quality of life.

7.3.2 EQ-5D-3L

7.3.2.1 Methodology

The EQ-5D-3L, is a preference-based, (multi-attribute) generic health-status classification instrument that generates a composite score reflecting the preference value associated with a given health state. The system has 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 3 levels: no health problems, moderate health problems, and extreme health problems. Current general health is also scored using a visual analog scale. The EQ-5D-3L (Version 1.0 - 01Oct2014) will be completed before administration of study drug on Day 1, at Months 3, 6, 8, and 12, and at EOT and 3 months off-treatment.

7.3.2.2 Assessment

The health state is reported with a 5-digit descriptor ranging from 11111, representing the best case, to 33333, representing the worst case. The score for each domain of the EQ-5D-3L will be reported in the eCRF.

7.3.3 Identification of New or Different MAC Strains

7.3.3.1 Methodology

In subjects who convert (i.e., who have 3 consecutive monthly negative MAC sputum cultures) and who subsequently have a positive MAC sputum culture, analyses will be conducted to confirm whether the positive MAC culture is due to a different MAC species or strain acquired by the subject, or due to a relapse of the same MAC species or strain as observed at Baseline (Day 1). The MAC species will be identified by sequencing the gene for the 16S rRNA subunit.

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If the MAC isolates identified after conversion are a different species, it will be determined that the subject acquired a different MAC species, rather than having a relapse. If the same species as seen at Baseline (Day 1) is observed, then DNA fingerprinting (based on a variable number of tandem repeat) will be conducted to identify the strain to determine whether the subject has acquired a different MAC strain.

7.3.3.2 Assessment

Different species or strains of MAC than those that are identified post Baseline (Day 1) after culture conversion will be recorded in source data.

7.3.4 Computed Tomography Scan of Chest

Chest CT scans will be evaluated as part of the chest CT Scan Sub-Study at sites that are willing and have been approved for the sub-study. The objective this sub-study will be to compare assessments of the chest CT scans, read by a trained medical professional, from Baseline (Day 1) to the (EOT) visit (± 14 days) provided a chest CT scan was not obtained within the prior 6 months. High resolution CT scan is preferred, if available. Details regarding the chest CT Scan Sub-Study are described in [APPENDIX 4](#). Details of the Japan specific CT Scan Sub-Study are described in [APPENDIX 5](#).

7.3.5 All-cause Mortality

7.3.5.1 Methodology

Study staff will make every effort to determine the vital status of subjects who do not complete the EOS visit.

7.3.5.2 Assessment

The cause of death, if available, for subjects that do not survive 12 months beyond EOT or early discontinue during the study will be recorded in the eCRF.

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7.4 PHARMACOKINETICS

7.4.1 Methodology

Population PK Sub-Set (US and Japan Only): Blood and sputum samples will be collected to determine amikacin concentrations, one from 0 to 1 hour before and one from 1 to 4 hours after study drug administration at Months 1, 3, and 6 in at least 16 evaluable US subjects randomized to the LAI treatment arm at pre-selected sites. In addition, at least 8 evaluable Japanese subjects randomized to the LAI treatment arm will participate in the population PK assessment.

Sputum-Only PK Sub-Set: An additional sub-set of subjects in the LAI added to a multi-drug regimen arm (at least 60 subjects) will have sputum samples collected to assess residual amikacin concentration in sputum after interrupting/stopping LAI treatment at the various different time points: Baseline, Months 4, 5 and 6 (2 days interruption of LAI dosing), 28 days and 3 months off-treatment.

All samples will be stored at or below -20°C before shipping to a central PK laboratory. Details for collecting, handling, storing, and transporting blood and sputum samples for PK analyses will be provided in the site laboratory manual.

7.4.2 Sample Collection

All sample handling procedures, including the date and time of each sample collection will be documented in the eCRF. The time of placement into storage (at the end of the sample work-up), and the date of transfer or shipment of the samples to the responsible analyst will be collected in the source documents. .

7.4.3 Comprehensive Pharmacokinetic Sub-Study (Japanese sites only)

PK of LAI in serum and sputum will be determined at pre-defined time points for the first 6 months in at least 8 evaluable Japanese subjects who have been randomized to the LAI plus a multi-drug regimen arm. The objectives will be to characterize the PK, including bioavailability, and systemic exposure of LAI, compare to historical population PK results, and to evaluate the

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concentration of LAI in the sputum for Japanese subjects with NTM lung disease. Refer to [APPENDIX 3](#)

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COMPREHENSIVE PHARMACOKINETIC SUB-STUDY for details.

7.4.3.1 Comprehensive Pharmacokinetic Sub-Study Sample Collection

All sample handling procedures, including the date and time of each sample collection. The time of placement into storage (at the end of the sample work-up), and the date of transfer or shipment of the samples to the responsible analyst will be collected in the source documents.

7.5 HEALTH CARE RESOURCE UTILIZATION

The number of days missed from work, NTM related and non-NTM related visits to healthcare providers, Emergency Room (ER) visits, and hospitalizations including days in the hospital, and days in Intensive Care Units (ICU) will be captured at every visit except Month 10, 14, and off-treatment visits.

7.6 APPROPRIATENESS OF MEASUREMENTS

Data will be collected using standard methods, widely used and generally regarded as reliable accurate, and relevant.

8 ADVERSE EVENTS

The Investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE or SAE as provided in this protocol.

8.1 DEFINITION OF AN ADVERSE EVENT

Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Examples of AEs include one of the following or a combination of 2 or more of these factors:

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- A new sign, symptom, illness, or syndrome
- Worsening of a concomitant illness
- An effect of investigational product, including comparator or concomitant medication
- An effect of an invasive procedure required by the protocol
- An accident or injury
- Laboratory abnormality that the Investigator considers clinically relevant

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Surgical measures planned prior to study enrollment are permitted by the clinical study protocol and the condition(s) leading to these measures are not AEs.

8.2 DEFINITION OF A SERIOUS ADVERSE EVENT

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
 - NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require

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intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. . The IB serves as the Reference Safety Information for the determination of expectedness of AEs. Investigators should reference the current IB when assessing the expectedness of adverse reactions for reporting to Health Authorities/IRB/Independent or Institutional Ethics Committee (EC)/Investigators.

8.2.1 Assessment of Intensity

Each AE will be graded according to CTCAE v4.0, as applicable. All other laboratory and clinical AEs that occur in a subject will be assessed for severity and classified using the categories below.

- **Grade 1 (Mild):** Event requires minimal or no treatment and does not interfere with the subject's daily activities.
- **Grade 2 (Moderate):** Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Grade 3 (Severe):** Event interrupts a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- **Grade 4 (Life threatening):** Any adverse drug experience that places the subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).
- **Grade 5 (Death)**

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8.2.2 Assessment of Causality

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

Definitely Related: A reaction that follows a reasonable temporal sequence from administration of study drug; that follows a known or expected response pattern to the study drug; it disappears or decreases on cessation or reduction in study drug dose; and/or it reappears or worsens when the study drug is administered.

Probably Related: A reaction that follows a reasonable temporal sequence from administration of study drug; that follows a known or expected response pattern to the study drug; and/or that could not be reasonably explained by other factors such as underlying disease, complications, concomitant drugs, or concurrent treatments.

Possibly Related: A reaction that follows a reasonable temporal sequence from administration of study drug; that follows a known or expected response pattern to the study drug, but that could reasonably have been produced by a number of other factors including underlying disease, complications, concomitant drugs, or concurrent treatments.

Not Related: A reaction for which sufficient data exist to indicate that the etiology is unrelated to the study drug.

8.2.3 Assessment of Outcome

The Investigator will record the outcome of the AE as either resolved or ongoing on the AE page of the eCRF. AEs of unknown outcome will be considered as ongoing for purposes of AE reporting.

8.3 ADVERSE EVENTS OF SPECIAL INTEREST

Adverse events of special interest (AESI) are those known to be attributed to parenteral amikacin:

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- nephrotoxicity, including elevation of serum creatinine, albuminuria, presence of red and white cells, casts, azotemia, and oliguria
- ototoxicity affecting the eighth cranial nerve resulting in hearing loss, loss of balance, or both.
- neuromuscular disorders
- allergic alveolitis
- bronchospasm
- hemoptysis

Cough is often associated with the drug products that are nebulized. In order to properly characterize AEs of cough, specific details regarding AE of cough will be reported in separate eCRF pages.

It is expected that patients in this study will experience a large number of pulmonary exacerbation events due to their underlying lung condition(s). Pulmonary exacerbation will be defined based on the Investigators best clinical judgment. Details of the type of exacerbation should be provided. These events should not be reported on the AE eCRF unless they meet the criteria for an SAE. Instead, these events should be reported in the “pulmonary exacerbation not SAE” page of the eCRF.

8.4 REPORTING REQUIREMENTS

8.4.1 Adverse Events

All AEs will be reported on the Adverse Events Form of the eCRF. Adverse events that occur between the time subject signs the informed consent form for the study and the time when subject receives his/her first dose of LAI or multi-drug regimen on Day 1 will be summarized as medical history and not as a treatment emergent AE (TEAE) unless the event meets the definition of an SAE as defined below.

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8.4.2 Serious Adverse Events

All SAEs, regardless of causality, must be reported to organization delegated by the Sponsor (Primevigilance will handle SAE reporting for INS-212) on a Serious Adverse Event Report Form within 24 hours of becoming aware of the event; corrections and additions are required to be submitted within 24 hours. Study-specific email, phone, and fax number for SAE reporting are presented in the Study Reference Manual.

Unexpected drug-related SAEs as assessed by Sponsor or authorized person qualify for expedited reporting and will be reported to the IRB/EC, regulatory authorities, participating Investigators and, if cross reporting is required for suspected unexpected serious adverse reactions (SUSARs), in accordance with all applicable global laws and regulations. A SUSAR is a Serious Adverse Reaction, which is suspected to be caused by the investigational medicinal product and which is unexpected i.e. its nature or severity is not consistent with the information in the relevant source documents. SAEs, including those that do not meet requirements for expedited reporting, and all other AEs will be reported to the regulatory agencies as appropriate (i.e. for the FDA these are reported in the Investigational New Drug [IND] annual report and for EMA these are reported in the Drug Safety Update Report ([DSUR]).

8.4.2.1 Pregnancy

Any pregnancy that occurs during any phase of the study must be reported to Prime Vigilance within 24 hours of learning of the pregnancy using a Clinical Study Pregnancy Form.

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

8.4.2.2 Overdose

An overdose is defined as a dose greater than the dose level evaluated in this study as described in [Section 5](#) that results in clinical signs and symptoms. In the case of an overdose of study drug,

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the Investigator should use clinical judgment in treating the overdose and contact the study Medical Monitor. The Investigator should refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, adverse events, and other significant data pertaining to the study drug used in the study. Such document(s) may include, but not limited to, the Investigator's brochure for LAI and approved product labeling for Amikacin.

8.5 FOLLOW-UP OF ADVERSE AND SERIOUS ADVERSE EVENTS

All SAEs, including those ongoing at end-of-study, must be followed until resolution or stabilization or until otherwise explained.

8.6 REGULATORY ASPECTS

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

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

9 STATISTICS

9.1 SAMPLE SIZE

The sample size was determined for the primary comparison between the treatment arms LAI plus a multi-drug regimen and a multi-drug regimen alone with respect to the proportion of subjects achieving culture conversion by Month 6 using nQuery Advisor[®] 7.0. Assuming a culture conversion rate by Month 6 of no less than 20% for the LAI plus a multi-drug regimen treatment arm, the rate by Month 6 of 5% for the multi-drug regimen alone treatment arm, and a 2:1 randomization ratio, a sample size of 261 subjects (174 for LAI plus a multi-drug regimen and 87 for the multi-drug regimen) will provide at least 90% power for the continuity-corrected Chi-square test at the 2-sided significance level of 0.05.

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

Due to lack of durability data, it is difficult to estimate the power with a total sample size of 261 subjects for the comparison between treatment arms with respect to the proportion of subjects achieving culture conversion with durability after 3 months off-treatment. Assuming the proportion of subjects achieving culture conversion with durability is 16% and 4% for LAI plus a multi-drug regimen and a multi-drug regimen respectively, and a 2:1 randomization ratio, a sample size of 261 subjects (174 for LAI plus a multi-drug regimen and 87 for the multi-drug regimen) will provide at least 83% power for Fisher's exact test at the 2-sided significance level of 0.05.

9.2 STRATIFICATION

Subjects will be stratified by smoking status (current smoker or not) and prior multi-drug regimen (on treatment or off treatment for at least 3 months) at Screening.

9.3 RANDOMIZATION AND BLINDING

Eligible subjects will be randomized 2:1 to open-label treatment with LAI plus a multi-drug regimen or a multi-drug regimen alone.

9.4 STATISTICAL METHODOLOGY

A statistical analysis plan (SAP) will be developed describing details regarding all analyses, tables, figures, and data listings. All analyses will be performed using SAS®.

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The final analyses of the primary efficacy endpoint, the change from Baseline to Month 6 in 6MWT distance, and time to culture conversion by Month 6 will be performed when the last subject completes Month 6 and data for all subjects who have completed Month 6 are available. These final analyses will be performed prior to the completion of the study. Interim analysis of some safety data will be included.

9.5 ANALYSIS POPULATIONS

9.5.1 Intent-to-treat Population

The intent-to-treat (ITT) population is the set of all randomized subjects.

9.5.2 Per-protocol Population

The per-protocol (PP) population is a subset of the ITT subjects who received at least 1 dose of either LAI plus a multi-drug regimen or a multi-drug regimen, met the inclusion/exclusion criteria, and did not have major protocol violations.

9.5.3 Safety Population

The safety population is the set of all subjects who received at least 1 dose of either LAI plus a multi-drug regimen or a multi-drug regimen.

9.5.4 Pharmacokinetic Population

The PK population is the set of all randomized subjects who received at least 1 dose of study drug and provided at least 1 PK sample.

9.6 EFFICACY ANALYSIS

9.6.1 Primary Endpoint Analysis

The primary efficacy endpoint is the proportion of subjects achieving culture conversion by Month 6. The primary efficacy analysis will be performed for the ITT population using the Cochran-Mantel-Haenszel test stratified by smoking status and prior multi-drug regimen (on treatment or off treatment for at least 3 months) at the 2-sided significance level of 0.05. The

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null hypothesis assumes that the culture conversion by Month 6 is independent of treatment, and the alternative hypothesis assumes that the culture conversion by Month 6 is associated with treatment. Subjects with missing sputum culture results for which culture conversion cannot be evaluated (3 consecutive monthly negative sputum cultures) will be considered as non-converters.

A supportive analysis of the primary efficacy endpoint will be performed for the PP populations similarly using the Cochran-Mantel-Haenszel test stratified by smoking status and prior multi-drug regimen.

9.6.2 Secondary Endpoint Analyses

The change from Baseline (Day 1) to Month 6 in 6MWT distance will be analyzed for the ITT population (assuming Missing-Not-at-Random [MNAR]) using a model under the pattern-mixture model framework. This model assumes that subjects on the LAI plus a multi-drug regimen arm with missing data follow the response distribution of a multi-drug regimen alone arm. It involves 3 steps:

1. The posterior mean and covariance estimates from the SAS MI procedure using the available non-missing data of a multi-drug regimen alone arm will be utilized to multiply impute missing data.
2. The endpoint will then be analysed for each complete data set with imputed data using an analysis of covariance (ANCOVA) with treatment arm and the randomization strata as factors, and the Baseline 6MWT distance as a covariate.
3. The treatment LSMEAN differences will be averaged and the associated standard errors will be summarized based on within-imputation and between-imputation variances using the SAS MIANALYZE procedure, to yield a final estimate of treatment contrast with associated 95% confidence interval (CI) and p-value.

A sensitivity analysis of the change from Baseline (Day 1) to Month 6 in 6MWT distance will be performed using a mixed-effects model for repeated measures (MMRM) with an unstructured covariance matrix implemented with SAS MIXED procedure over Months 4 and 6. This model

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will include treatment, month, the treatment-by-month interaction, smoking status and prior multi-drug regimen (on treatment or off treatment for at least 3 months) as fixed factors, the Baseline (Day 1) 6MWT distance as a covariate, as well as the Baseline 6MWT distance-by-month interaction. The Kenward-Rogers correction for denominator degrees of freedom, standard errors, and test statistics will be utilized. The between-treatment difference in Least Squares (LS) mean change from Baseline to Month 6, the corresponding 95% CI for the true between-treatment difference, and the p-value will be presented.

Supportive ANCOVA analyses of the change from Baseline to Month 6 in 6MWT distance with treatment arm and the randomization strata as factors, and the Baseline 6MWT distance as a covariate will also be performed for both the ITT and the PP populations. In the ANCOVA analysis for the ITT population, the last-observation-carried-forward method will be used to handle the missing data.

The changes from Baseline to Month 8 (exploratory endpoint), EOT, and 3 months off-treatment (exploratory endpoint) in 6MWT distance will be analyzed for the ITT population using the similar mixed-effects model for repeated measures (MMRM) with an unstructured covariance matrix implemented with SAS MIXED procedure over all post-randomization visits.

Kaplan-Meier estimates for the distribution of time to culture conversion will be constructed for treatment arms. The treatment comparison will be made using the stratified log rank test for the ITT population. The estimated median time to culture conversion for each treatment arm will be presented. The time to culture conversion will also be analyzed using Cox regression model to estimate hazards ratio.

The proportion of subjects achieving culture conversion with sustainability at the EOT and the proportion of subjects achieving culture conversion with durability after 3 months off-treatment will be analyzed for both the ITT and PP populations similarly using the Cochran-Mantel-Haenszel test stratified by smoking status and prior multi-drug regimen. Subjects who relapse, have “rescue” medication and/or die before reaching EOT for sustainability and 3 months off-treatment for durability will be considered as failure to achieving culture conversion.

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The change from Baseline (Day 1) to Month 6 in SGRQ total score and the SGRQ – Part 2 subdomain (exploratory endpoint) will be analyzed for the ITT population similarly using the same MMRM method as the analysis of the change from Baseline to EOT in 6MWT distance.

9.6.3 Exploratory Endpoint Analyses

The change in 6MWT distance at Month 6 for converters compared with non-converters overall for ITT population, within LAI plus a multi-drug regimen, within the multi-drug regimen alone, the change in BMI at Month 6 for LAI plus a multi-drug regimen compared with a multi-drug regimen alone, and the changes in chest CT scan at EOT for LAI plus a multi-drug regimen compared with a multi-drug regimen alone in a sub-set of subjects will be analyzed using the ANCOVA models with the comparison arm (converters vs. non-converters or the treatment arm) and the randomization strata as factors, and the corresponding Baseline measure as a covariate. If a CT scan is not performed at EOT for any subject, then prior CT scan will be used in the analysis.

The change from Baseline (Day 1) to Month 6 and EOT in the EQ-5D-3L patient reported health outcomes will be analyzed for the ITT population similarly using the same MMRM method as the analysis of the change from Baseline to EOT in 6MWT distance. Kaplan-Meier estimates for the distribution of time to death will be constructed by treatment arm. The treatment comparison will be made using the stratified log rank test for the ITT population. Incidence of new MAC strains will be summarized by treatment arms.

9.7 SAFETY ANALYSIS

Safety data will be summarized for the Safety Population.

Adverse events will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). For non-converters entering the single arm LAI study (Study INS-312), safety monitoring will continue as defined in that protocol. The number and percent of subjects with treatment emergent AEs by system organ class and preferred term will be presented by treatment arm. The severity of the AEs, the relationship to the investigational

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product, AEs causing study discontinuation, and all treatment emergent SAEs will be similarly presented. Fatal SAEs will also be presented separately in a similar manner.

Vital signs (temperature, systolic and diastolic blood pressure, pulse, pulse oximetry, and respiratory rate) results will be descriptively summarized by treatment arm.

Clinical laboratory test and pulmonary function results will be descriptively presented. Shifts of laboratory results from Baseline (Day 1) to Month 6 and EOT will be presented by treatment arm.

10 DATA MANAGEMENT

10.1 SOURCE DOCUMENTS

Study data will be collected on source documents. The Principal Investigator (PI) is responsible for assuring that collected data are complete and accurate. Source documentation (the point of initial recording of a piece of data) should support data collected on the eCRF. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical study.

10.2 DATA COLLECTION AND CASE REPORT FORM MONITORING

All data obtained for this study will be entered into a local regulation (i.e. 21 CFR Part 11 in the USA) compliant Data Management System provided by Insmmed or its designee. These data will be recorded with an Electronic Data Capture (EDC) system using eCRFs. The Investigator will ensure the accuracy and completeness of the data reported to the Sponsor. All data entry, modification or deletion will be recorded automatically in an electronic audit trail.

The Investigator will provide access to his/her original records to permit a representative from the Sponsor to verify the proper transcription of data. Data reported in the eCRFs should be consistent with and substantiated by the subject's medical record and original source documents. The eCRF data will be monitored by the Sponsor or designee. The final, completed eCRF Casebook for each subject must be electronically signed and dated by the PI within the EDC

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system to signify that the Investigator has reviewed the eCRF and certifies it to be complete and accurate.

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

10.3 STUDY RECORDS RETENTION

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

11 ETHICAL CONSIDERATIONS

A copy of the protocol, informed consent forms, other information to be completed by subjects, such as questionnaires and any proposed advertising or recruitment materials, will be submitted to the regulatory authority(ies) and ECs in accordance with country-specific requirements.

All subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above must be submitted and approved in accordance with country-specific requirements.

Periodic study status reports will be submitted to the ECs in accordance with country-specific regulations. Where applicable, the Investigator will be responsible for obtaining IRB/EC approval of the annual continuing review throughout the duration of the study.

The PIs will notify the local IRBs/ECs of violations from the protocol and SAEs.

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Subjects will be informed that medical care will not be affected by their agreement or refusal to participate in this study, and that they are free to withdraw from the study at any time without prejudice to the clinician patient relationship.

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013), and the ICH E6 Guideline for Good Clinical Practice (GCP). The Sponsor will ensure that the study complies with all local, federal, or country regulatory requirements as applicable. Throughout the study, the Sponsor and its designee will work with the Investigator(s) to ensure proper study protocol implementation and adherence to regulatory requirements as listed in the study protocol.

11.1 GOOD CLINICAL PRACTICE

This study is to be conducted according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice), in agreement with the Declaration of Helsinki and in keeping with local regulations.

11.2 DELEGATION OF INVESTIGATOR DUTIES

The Investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The Investigator should maintain a list of sub-Investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

11.3 PATIENT INFORMATION AND INFORMED CONSENT

Before being enrolled in the clinical study, patients must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to them.

An informed consent document that includes both information about the study and the consent form will be prepared and given to the patient. This document will contain all the elements required by the ICH E6 Guideline for Good Clinical Practice and any additional elements

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required by local regulations. The document must be in a language understandable to the patient and must specify who informed the patient. Where required by local law, the person who informs the patient must be a physician.

12 ADMINISTRATIVE PROCEDURES

12.1 FINANCIAL DISCLOSURE BY INVESTIGATOR

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

12.2 STUDY REGISTRATION AND RESULTS DISCLOSURE

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

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

12.3 STUDY FILES AND MATERIALS

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

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

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The study may be audited by qualified delegates from the Sponsor or a competent regulatory authority ([Section 12.11](#)).

12.4 USE OF STORED SAMPLES AND DATA

Serum and sputum samples collected for PK analysis will be stored for a period of up to 2 years after the completion (termination) of the study, or longer if required by the institution participating in the study. Serum samples from the IL-6 analysis will be stored at the aforementioned laboratory for a period of up to 2 years after the completion (termination) of the study, or longer if required by the institution participating in the study to see whether there may be indicators associated with NTM lung infections and for other exploratory analyses for an NTM lung infection (no genetic testing or analysis will be performed on blood samples collected). Mycobacterial isolates will be stored for a period of up to 2 years after the completion (termination) of the study, or longer if required by the institution participating in the study and used for future selective susceptibility testing for correlation with microbiologic and clinical response and for molecular typing of serial isolates to look for multiple strains within individuals that might influence treatment outcome. Stored samples will be labeled with study and subject information and kept in a locked room with limited access. Electronic data will be kept in password-protected computers at the laboratory and then transferred to the Sponsor or Clinical Research Organization (CRO), as applicable, for data analysis. Samples and corresponding data will be tracked using the laboratory's specimen tracking system.

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

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

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

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12.5 Disposition of Stored Samples and Data

Access to stored samples will be limited by using a locked room. Samples stored by the central laboratories will be labeled with the subject's study identification information. Data will be kept in password-protected computers at the laboratory and then transferred to the vendor for data analysis. Samples and corresponding data acquired will be tracked using the laboratory's specimen tracking system.

In the future, other Investigators may wish to study these samples and/or data. In that case, IRB/EC approval and Sponsor approval must be obtained before any sharing of samples and/or data. Any clinical information shared about the sample would similarly require prior Sponsor and IRB/EC approval.

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

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

12.6 INITIATION OF STUDY

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

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

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12.7 PATIENT REIMBURSEMENT, LIABILITY, AND INSURANCE

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

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

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

12.8 SUBJECT IDENTIFICATION AND CONFIDENTIALITY

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

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

12.9 STUDY MONITORING

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

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The Investigator must give the monitor access to relevant hospital or clinical records to confirm their consistency with the eCRF entries. No information in these records about the identity of the subjects will leave the study center. The study Sponsor monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of primary activity and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan.

12.10 STUDY PLAN AMENDMENTS

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

The requirements for approval should in no way prevent any immediate action from being taken by the Investigator or by the Sponsor in the interests of preserving the safety of all subjects included in the study. If an immediate change to the protocol is felt to be necessary by the Investigator and is implemented by him/her for safety reasons, the study Sponsor or its agent should be notified and the applicable regulatory authority(ies)/central ECs/local IRBs/ECs should be informed within 10 working days. Any other regional reporting requirements must be adhered to.

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

12.11 AUDITS AND INSPECTIONS

Domestic and foreign regulatory authorities, the IRB/EC, and an auditor authorized by the sponsor may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be

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guaranteed by the Investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection provided that subject names are obliterated on the copies to ensure confidentiality.

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

12.12 USE OF DATA AND PUBLICATIONS

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

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

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

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14 APPENDICES

APPENDIX 1 SPUTUM INDUCTION SUGGESTIONS

Introduction

Collection of good sputum samples is critical to the success of this study. Sputum samples will be collected at the clinical site and also by the subject at home. To facilitate obtaining good sputum samples during the study visits at the clinic, sputum induction must be performed if the subject is unable to expectorate approximately 3.0 ml of sputum.

Purpose

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

Suggested Equipment

- Standard handheld nebulizer used in the clinic or the subject can be asked to bring the nebulizer they use at home for pulmonary hygiene
 - The nebulizer should be thoroughly disinfected to ensure no cross-contamination
 - **DO NOT** use the subject's PARI eFlow® nebulizer for sputum induction
- Sputum specimen containers with label – sputum collection tube provided by central laboratory
- Sodium chloride solution (Saline)
- Standard clinic supplies (e.g., disinfectant/germicidal/alcohol wipes, tissues, paper towels, etc)

Procedure

General Instructions:

- At the clinic, sputum induction should occur before administration of study drug.

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

Inhalation and Collection Procedures:

- The induction procedure should start by utilizing lower concentrations of saline (e.g. 3%) based on the Investigator's preference.
- Approximately 3-6 mL of the selected saline should be placed in the nebulizer.
- The subject should be sitting up or in a semi-fowler position.
- The subject may wear a nose clip during the nebulization.
- The subject should breathe slowly and deeply through the nebulizer mouthpiece inhaling the salt water mist. Remind the subject to not breathe quickly but to have slow, deep breaths pausing at peak inspiration to allow deposition of particles.
- The nebulization time is 10 minutes.
- At the end of this time, the subject should take a few deep breaths, swallow the extra saliva in his/her mouth and try to cough up a sputum sample.
- The subject should be encouraged to cough forcefully using the deep coughing method and/or "huffing" cough method.
- All sputum should be deposited in the container. The specimen container should not be opened until the specimen is ready to be deposited. The container should be closed immediately after depositing the sample.
- The sputum sample should be approximately 3 mL - slightly below the bottom line (5 mL) on the collection container.

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- If a sufficient sputum sample is not collected and the subject appears to be tolerating the induction procedure well, the subject can complete another 10 minute nebulization period.
 - If a second 10-minute nebulization period is required, the recommendation is to increase the sodium chloride concentration (i.e., if 3% was used first then 7% should be used for the subsequent nebulization; if 7% was used first then 10% should be used for the subsequent nebulization).
 - Closely monitor the subject for tolerability issues or side effects.
 - No more than two 10-minute nebulization periods should be completed.
- The sputum sample should be refrigerated until it is sent to the microbiology laboratory.

Side Effects

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

Miscellaneous

- If the subject needs to expectorate during nebulization, turn off the nebulizer and allow the subject to cough up sputum into the container. If a sufficient specimen is not collected, the subject should then resume the nebulization to complete the 10 minute nebulization duration.
- The subject should be encouraged to blow his/her nose as often as needed during the induction procedure to help prevent nasal secretions from becoming mixed with sputum specimen.

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APPENDIX 2 CONTENT OF STUDY DRUG LABEL**Table 14-1 Content of Study Drug Label**

Number	Label requirements	Specific Label information
1	Pharmaceutical dosage form, route of administration, and in the case of open study, the name/identifier and strength/potency	590 mg Liposomal Amikacin (aminoglycoside) nebulizer suspension for inhalation using PARI eFlow® nebulizer
2	Quantity of the dosage unit	590 mg
3	Protocol number	INS-212
4	Patient Identifier	Patient specific
5	Human patient randomization number	Patient specific
6	Lot number	Patient specific
7	Re-assay/expiration date	Patient specific
8	Caution statement	For clinical trial use only.
9	Storage conditions	Store between 2-8 °C. Do not freeze. Protect from heat.
10	Sponsor identification, name, address and phone number	GLOBAL CLINICAL AND REGULATORY AFFAIRS Insmed Incorporated 10 Finderne Avenue Bridgewater, NJ 08807
11	Direction for use	This product should only be used after appropriate training. Shake well.
12	Statement: <i>“Keep out of sight and reach of children”</i>	Keep out of sight and reach of children.

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APPENDIX 3

COMPREHENSIVE PHARMACOKINETIC SUB-STUDY

Sub-study of INS-212:

Comprehensive Pharmacokinetic Sub-Study (Japan)

Sub-Study Protocol

Version Date: 22 February 2016

Name of Approver

[REDACTED]

Title of Approver

[REDACTED]

Signature of Approver _____

[REDACTED]

Date: 22 February 2016

Insmmed Incorporated
10 FINDERNE AVENUE, BUILDING 10
BRIDGEWATER, NJ 08807-3365

Protocol Amendment #3 version: 22 February 2016

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SIGNATURE PAGE FOR THE INVESTIGATOR**Protocol Number****INS-212**

I have read the INS-212 Comprehensive Pharmacokinetic Sub-Study protocol and agree that it contains all necessary details for carrying out this sub-study. I will conduct the sub-study as outlined herein and will complete the sub-study within the time designated, in accordance with all stipulations of the sub-study protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the INS-212 Comprehensive Pharmacokinetic Sub-Study protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this sub-study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the sub-study.

I will use only the informed consent approved by the Institutional Review Board/Independent Ethics Committee (IRB/EC) and will fulfill all responsibilities for submitting pertinent information to the IRB/EC responsible for this sub-study.

I agree that the Sponsor, Insmmed Incorporated, or its representatives shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Insmmed Incorporated, or study drug code in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this sub-study protocol, to any amendment to the sub-study protocol, or to the performance of this sub-study, without the prior written consent of Insmmed Incorporated.

Name of Approver

Type or print name of signer.

Signature of Approver _____

Date: mm/dd/yyyy

Protocol Amendment #3 version: 22 February 2016

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Introduction

LAI is a novel formulation of amikacin that is encapsulated in liposomes comprised of DPPC and cholesterol (naturally occurring lipids in the lung) to allow for effective delivery in the lung, leading to high concentrations of the drug at the target sites of infection, with low systemic levels. In clinical study TR02-112 LAI in addition to standard background therapy was statistically superior at the end of the double-blind period (Day 84) for achieving a negative sputum culture compared to those who received placebo in addition to standard background therapy. Population PK modeling in the TR02-112 study revealed that systemic bioavailability of LAI is sufficiently low resulting in minimal systemic exposure relative to an IV administration of amikacin. The median estimated AUC in the subjects included in this analysis was approximately 13 times lower than the mean daily AUC observed in a published study of subjects with CF who received amikacin IV 30 mg/kg once daily. In general, the AUC₂₄ at steady-state was slightly higher than that observed on Day 1. However, the difference was small (percent difference in the median values is 19.0%) indicating that very little accumulation of amikacin occurs with repeat dosing up to approximately 6 months.

Study INS-212 will evaluate whether the signal identified in TR02-112 is further confirmed in a longer duration of LAI treatment in patient with NTM MAC lung infections who are refractory to a stable multi-drug regimen for at least 6 months. This Comprehensive PK sub-study is being conducted at Japanese sites in Japan only and will only include subjects of Japanese descent. This sub-study will characterize the system exposure of amikacin after inhalation of LAI and evaluate the concentration of LAI in the sputum for these Japanese subjects. This will be compared to historical data from non-Japanese subjects in lieu of a Phase I bridging study and in agreement with PMDA regulators.

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Sub-Study Objectives

1. To characterize systemic amikacin exposure, including approximate systemic bioavailability in Japanese subjects, using a population PK model to describe the disposition of amikacin in the serum
2. To compare population PK modeling between Japanese subjects to historical population PK modeling for LAI
3. To evaluate the amikacin concentration in sputum

Sub-Study Design

At Baseline (Day 1), Japanese subjects at Japanese sites who are randomized to the LAI plus multi-drug regimen arm and signed the Comprehensive PK Sub-Study informed consent will be able to participate. PK blood and sputum samples will be collected from PK subjects at Baseline (Day 1) and at Months 1, 3, and 6. Details on sample collection timepoints are listed in the Sub-Study Procedure section below. The Comprehensive PK Sub-Study will enroll subjects until 8 subjects complete all required assessments and have evaluable data. .

Sub-Study Endpoints

1. Characterize systemic amikacin exposure, including approximate systemic bioavailability in Japanese subjects
2. Compare Japanese subjects to historical population using PK modeling
3. Evaluate the amikacin concentration in sputum

Sub-Study Population

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Only Japanese sites will participate in this sub-study once they have IRB/EC approval for the main INS-212 study and for this sub-study. All subjects must meet eligibility criteria for the main INS-212 study, must give written informed consent for that study, and must also give written informed consent for this sub-study. Eight evaluable subjects are expected to participate in the Comprehensive PK sub-study. There are no separate inclusion or exclusion criteria for this sub-study besides the requirement for written informed consent.

Sub-Study Procedures

Either at Screening or at Baseline (Day 1), subjects will be asked to provide informed consent for this sub-study. Blood and sputum samples will be collected to determine amikacin concentrations. The Schedule of Events for the Comprehensive PK Sub-Study is provided in [Table 14-2](#).

PK sputum sample collection timepoints

On Day 1, two to three (2-3) sputum samples will be collected at the following timepoints: 0 to 1 hour prior to study drug administration and at 8 (optional) and 24 hours after study drug administration. At Months 1, 3, and 6, PK sputum will be collected 0 to 1 hour prior to study drug administration and 1 to 4 hours after study drug administration. Day 1 and Month 3 PK sputum samples must be collected in conjunction with the 8 PK blood draws on the same day and time (within 30 minutes of blood being collected) as indicated below.

PK blood sample collection timepoints

On Day 1 and at Month 3, subjects will have 8 PK blood sample collections at the following timepoints: 0 to 1 hour prior to study drug administration, and at 1, 2, 4, 6, 8, 12, and 24 hours after study drug administration (± 5 minutes at each time point is allowed). At Months 1 and 6, PK blood samples will be collected 0 to 1 hour prior to study drug administration and 1 to 4 hours after study drug administration.

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All samples will be stored at or below -20°C before shipping to a central PK laboratory. Details for collecting, handling, storing, and transporting blood and sputum samples for PK analyses will be provided in the site laboratory manual.

Table 14-2 Schedule of Events for INS-212 Comprehensive Pharmacokinetic Sub-Study

INS-212 Comprehensive Pharmacokinetic Sub-Study				
	Baseline	Month 1	Month 3	Month 6
	(V2)	(V3)	(V5)	(V8)
Visit Window	Day 1	(± 3 days)	(± 3 days)	(± 3 days)
Informed Consent ^a	X	–	–	–
PK Blood Sampling ^b	pre-dose: 0-1 hr	pre-dose: 0-1 hr	pre-dose: 0-1 hr	pre-dose: 0-1 hr
	post dose: 1, 2, 4, 6, 8, 12, 24 hrs	post dose: 1-4 hrs	post dose: 1, 2, 4, 6, 8, 12, 24 hrs	post dose: 1-4 hrs
PK Sputum Sampling	pre-dose: 0-1 hr	pre-dose: 0-1 hr	pre-dose: 0-1 hr	pre-dose: 0-1 hr
	post dose: 8 (optional), 24 hrs	post dose: 1-4 hrs	post dose: 1-4 hrs	post dose: 1-4 hrs
Administer Study Drug at Site	X	X	X	X
^a The subject will be asked to provide informed consent for this sub-study at either Screening or at Baseline (Day 1). Informed consent must be obtained prior to any procedures for this sub-study can be performed. ^b blood samples have a window of (± 5 minutes at each time point)				

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APPENDIX 4

CT SCAN SUB-STUDY

Sub-study of INS-212:

CT Scan Sub-Study

CT Scan Sub-Study Protocol

Version Date: 22 February 2016

Name of Approver

[REDACTED]

Title of Approver

[REDACTED]

Signature of Approver

[REDACTED]

Date: 22 February 2016

Insmed Incorporated
10 FINDERNE AVENUE, BUILDING 10
BRIDGEWATER, NJ 08807-3365

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SIGNATURE PAGE FOR THE INVESTIGATOR**Protocol Number**

INS-212

I have read the INS-212 CT Scan Sub-Study protocol and agree that it contains all necessary details for carrying out this sub-study. I will conduct the sub-study as outlined herein and will complete the sub-study within the time designated, in accordance with all stipulations of the sub-study protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the INS-212 CT Scan Sub-Study protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this sub-study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the sub-study.

I will use only the informed consent approved by the Institutional Review Board/Independent Ethics Committee (IRB/EC) and will fulfill all responsibilities for submitting pertinent information to the IRB/EC responsible for this sub-study.

I agree that the Sponsor, Inmed Incorporated, or its representatives shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Inmed Incorporated, or study drug code in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this sub-study protocol, to any amendment to the sub-study protocol, or to the performance of this sub-study, without the prior written consent of Inmed Incorporated.

Name of Approver

Type or print name of signer.

Signature of Approver _____

Date: mm/dd/yyyy

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Introduction

LAI is a novel formulation of amikacin that is encapsulated in liposomes comprised of DPPC and cholesterol (naturally occurring lipids in the lung) to allow for effective delivery in the lung, leading to high concentrations of the drug at the target sites of infection, with low systemic levels. In clinical study TR02-112 LAI in addition to standard background therapy was statistically superior at the end of the double-blind period (Day 84) for achieving a negative sputum culture compared to those who received placebo in addition to standard background therapy.

Study INS-212 will evaluate whether there are any radiographical changes that occur in subjects who have achieved culture conversion and continued on treatment.

Sub-Study Objective

1. To compare assessments of radiographs, read by a trained medical professional, from Baseline (Day 1) in CT scans at EOT in a subset of subjects within each treatment arm

Sub-Study Design

Eligible subjects must be enrolled into the INS-212 study. At randomization (Day 1), any subject who has signed the informed consent for this chest CT Scan Sub-Study will participate; there is no maximum number of subjects that can participate. At the latest, the informed consent and the subject's Baseline chest CT scan must be collected no later than 2 weeks after the subject's first dose of LAI or multi-drug regimen in the INS-212 study. A prior chest CT scan may be used as a subject's Baseline measure if this CT scan was obtained within 6 months from the subjects Day 1 study visit. All subjects in this chest CT Scan Sub-Study will have their follow-up chest CT scan on the (EOT) visit (± 14 days) provided the last chest CT scan was not obtained within the prior 6 months. High resolution CT scan is preferred, if available.

Sub-Study Endpoint

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1. Radiological changes in CT scan, within a sub-set of subjects, at EOT in the LAI arm compared to multi-drug regimen alone, where appropriate

Sub-Study Population

Only sites that have agreed to and have the ability to perform the chest CT scans will participate in this sub-study once they have IRB/EC approval for the main INS-212 and for this chest CT Sub-Study. Subjects must provide written informed consent for both the main INS-212 study and for this sub-study. It is expected that at least 60 subjects will participate in this CT Scan Sub-Study.

Sub-Study Procedures

On Day 1 (± 14 days) subjects will be asked to provide informed consent for this sub-study. Once the informed consent has been signed the subject should have a chest CT scan performed (a prior CT scan that was obtained within 6 months prior to signing the informed consent may be used for Baseline). A CT scan should not be performed during Screening for this CT-Scan Sub-Study. It will be performed at Baseline (Day 1) once the subject is randomized. A second chest CT scan will be performed when a subject has completed treatment ($EOT \pm 14$ days), which includes any subject that prematurely discontinues study drug provided that more than 6 months have elapsed since their last CT scan. High resolution CT scan is preferred, if available.

All chest CT scans will be read locally and a description of findings for each scan will be entered into the eCRF page. An overall assessment of improved, worsening, or no change from previous scan will be reported for all scans after the subject's Baseline scan. All chest CT scans must be made available to the Sponsor to be stored as study source documentation.

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Table 14-3 Schedule of Events for INS-212 CT Scan Sub-Study

INS-212 CT Scan Sub-Study		
	Baseline	EOT
	(V2)	
Visit Window	(-6 months to +14 Days)	(±14 days)
Informed Consent ^a	X	—
CT Scan of Chest	X	X
^a informed consent for this sub-study must take place after the subject has been enrolled and randomized into the INS-212 study. This will be a separate informed consent from the INS-212 main study. ^b Computed tomography of the chest at Baseline (Day 1) is performed only for those subjects whom have signed the CT Scan Sub-Study informed consent if not completed within 6 months before the first dose of LAI or multi-drug regimen at the Baseline (Day 1) visit. EOT scan is required if no less than 6 months have elapsed since the previous CT scan was performed.		

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APPENDIX 5

JAPAN SPECIFIC CT SCAN SUB-STUDY

Sub-study of INS-212:
Japan Specific CT Scan Sub-Study

CT Scan Sub-Study Protocol

Version Date: 22 February 2016

Name of Approver

[REDACTED]

Title of Approver

[REDACTED]

Signature of Approver

[REDACTED]

Date: 22 February 2016

Insmmed Incorporated
10 Finderne Avenue, Building 10
Bridgewater, NJ 08807-3365

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SIGNATURE PAGE FOR THE INVESTIGATOR

Protocol Number

INS-212

I have read the INS-212 Japan Specific CT Scan Sub-Study protocol and agree that it contains all necessary details for carrying out this sub-study. I will conduct the sub-study as outlined herein and will complete the sub-study within the time designated, in accordance with all stipulations of the sub-study protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the INS-212 Japan Specific CT Scan Sub-Study protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this sub-study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the sub-study.

I will use only the informed consent approved by the Institutional Review Board/Independent Ethics Committee (IRB/EC) and will fulfill all responsibilities for submitting pertinent information to the IRB/EC responsible for this sub-study.

I agree that the Sponsor, Insmmed Incorporated, or its representatives shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Insmmed Incorporated, or study drug code in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this sub-study protocol, to any amendment to the sub-study protocol, or to the performance of this sub-study, without the prior written consent of Insmmed Incorporated.

Name of Approver

Type or print name of signer.

Signature of Approver _____

Date: mm/dd/yyyy

Introduction

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LAI is a novel formulation of amikacin that is encapsulated in liposomes comprised of DPPC and cholesterol (naturally occurring lipids in the lung) to allow for effective delivery in the lung, leading to high concentrations of the drug at the target sites of infection, with low systemic levels. In clinical study TR02-112 LAI in addition to standard background therapy was statistically superior at the end of the double-blind period (Day 84) for achieving a negative sputum culture compared to those who received placebo in addition to standard background therapy.

Study INS-212 will evaluate whether there are any radiographical changes that occur in subjects who have achieved MAC culture conversion and continued on treatment.

Sub-Study Objectives

1. To compare assessments of radiographs, read by a trained medical professional, from Baseline (Day 1) in CT scans at EOT in Japanese subjects from sites in Japan
2. To assess the safety of LAI by CT scans (at 6 month intervals) in subjects from sites in Japan

Sub-Study Design

Eligible subjects must be enrolled into INS-212: A Randomized, Open-Label, Multicenter Study of Liposomal Amikacin for Inhalation (LAI) in Adult Patients with Nontuberculous Mycobacterial (NTM) Lung Infections Caused by *Mycobacterium avium* complex (MAC) That are Refractory to Treatment. The informed consent for this Japan specific CT Scan Sub-Study is included in the informed consent for the main study. All Japanese subjects in Japan will participate in this sub-study. There is no maximum number of subjects that can participate. The subject's Baseline chest CT scan must be collected no later than 2 weeks after the subject's first dose of LAI or multi-drug regimen in the INS-212 study. A prior chest CT scan may be used as a subject's Baseline measure if this CT scan was obtained within 6 months from the subject's Baseline (Day) 1 study visit in the INS-212 study. All subjects will have CT scans at their Months 6, 12, and EOT visits, and at 18 and 24 months after EOT.

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Sub-Study Endpoint

1. Radiological changes in CT scan at EOT in the LAI arm compared to multi-drug regimen alone, where appropriate
2. Six-month interval safety assessment for subjects enrolled at sites in Japan

Sub-Study Population

All Japanese subjects at all study sites in Japan will participate in this sub-study once they have IRB/EC approval. The informed consent for this Japan specific CT Scan Sub-Study is included in the informed consent for the main study. All Japanese subjects in Japan will participate in this substudy. There are no separate inclusion or exclusion criteria for this sub-study.

Sub-Study Procedures

The subject should have a chest CT scan performed (a prior CT scan that was obtained within 6 months prior to Day 1 may be used for Baseline). The first chest CT scan should be performed at Day 1 or up to 14 day after first dose of LAI or multi-drug regimen. Additional CT scans will be obtained at their Months 6, 12, EOT months (if at least 6 months have elapsed since the previous CT scan), and at 18 and 24 months after EOT.

All chest CT scans will be read locally and a description of findings for each scan will be entered into the eCRF page. An overall assessment of improved, worsening, or no change from previous scan will be reported for all scans after the subject's Baseline scan. All chest CT scans must be made available to the Sponsor to be stored as study source documentation.

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Table 14-4 Schedule of Events for INS-212 Japan Specific CT Scan Sub-Study

	INS-212 Japan Specific CT Scan Sub-Study						
	Screening	Baseline (Day 1)	Month 6	Month 12	EOT	Month 18	Month 24
	(V1)	(V2)	(V8)	(V11)	(V13)	—	—
Visit Window		(-6 months to +14 Days)	(±3 days)	(±3 days)	(±14 days)	(±14 days)	(±14 days)
Informed Consent ^a	X	-	—	—	—	—	—
CT Scan of Chest		X ^b	X	X	X ^b	X	X
^a In Japan, all subjects will participate in the CT scan sub-study. Informed consent for this sub-study is included in the informed consent of INS-212 main study. ^b A prior chest CT scan may be used as a subject's Baseline (Day 1) measurement if this CT scan was obtained within 6 months from the subject's Baseline (Day 1) visit. Subjects will have their follow-up chest CT scan at the EOT visit provided the last chest CT scan was not obtained within the prior 6 months. High resolution CT scan is preferred, if available.							

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