NOVARTIS

Clinical Development

TBM100/Tobramycin

CTBM100CDE02 / NCT02248922

An 8 week open-label interventional multicenter study to evaluate the lung clearance index as endpoint for clinical trials in cystic fibrosis patients ≥ 6 years of age, chronically infected with *Pseudomonas aeruginos*a

Statistical Analysis Plan (SAP)

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Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
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23-Nov-2016	Prior to DBL	Creation of updated final version	Updated version as per suggestion received from clinical team	Section 2.13

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List of abbreviations

AE	Adverse event
ALT	Alanine aminotransferase/glutamic pyruvic transaminase/GPT
AST	Aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
b.i.d.	bis in die/twice a day
CF	Cystic fibrosis
CFU	Colony-forming units
CRF	Case Report/Record Form
CPO	Country Pharma Organization
CRO	Contract Research Organization
CSR	Clinical Study Report
DS&E	Drug Safety and Epidemiology
ECG	Electrocardiogram
ECFS	European Cystic Fibrosis Society
FEV1	Forced expiratory volume at 1 second
FRC	Functional residual capacity
HRCT	High-Resolution Computer tomography
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
i.v.	Intravenous(ly)
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
LCI	Lung clearance index
MBW	Multiple breath inert gas washout
MIC	Minimal inhibitory concentration
o.d.	omnia die/once a day
PAES	Post-authorization Efficacy Studies
PASS	Post-authorization safety study
p.o.	per os/by mouth/orally
TEAE	Treatment Emergent Adverse Event
SAE	Serious adverse event
S_{acin}	N2 slope acinar airways
S_{cond}	N2 slope conductive airways
SOP	Standard operation procedure
SPCS	Study Protocol Concept Sheet
TAQ	Treatment adherence questionnaire
WOCBP	Women of child-bearing potential

1 Introduction

This document contains details of the statistical methods which will be used in the phase IV clinical trial of the clinical study protocol CTBM100CDE02. This statistical analysis plan (SAP) module is prepared based on amended protocol version 02, CRF version 3.0. Mock tables and listing mocks are included in TLF. The purpose of the study is to evaluate LCI by a

standardized procedure in a well characterized study setting and to assess feasibility of LCI as a more sensitive method than FEV1 to measure effectiveness of antibiotic therapy in patients with CF aged 6 years and older with mild to moderate lung disease.

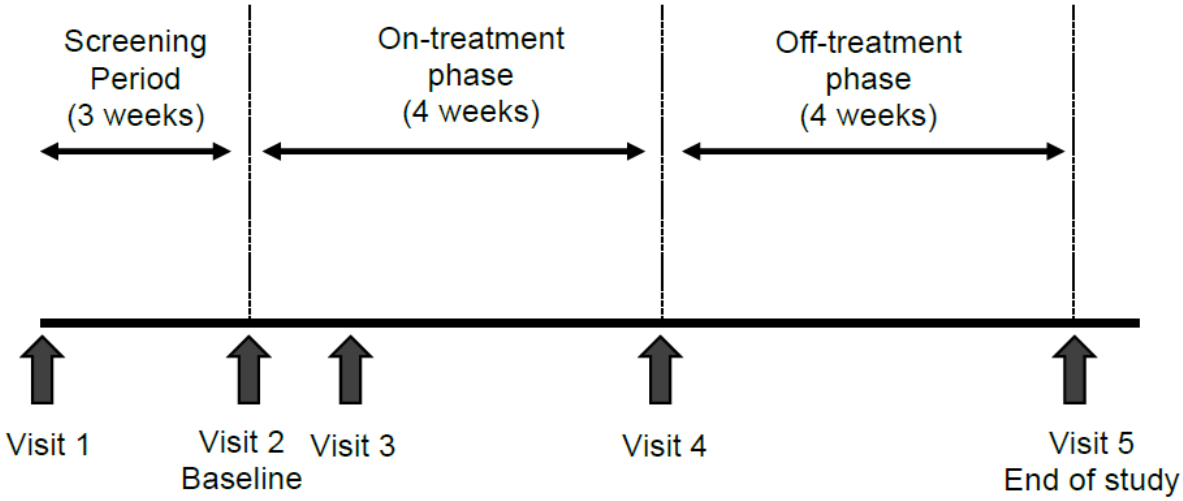
Data will be analyzed by statistical software SAS version 9.4 according to the data analysis section 10 of the study protocol which is available in Appendix 16.1.1 of the CSR. Important information is given in the following sections and details are provided, as applicable, in Appendix 16.1.9 of the CSR.

1.1 Study design

This study uses an open-label, single arm design in patients suffering from CF, aged 6 years and older, with an elevated LCI of ≥ 7.5 at screening, who have a chronic pulmonary infection with *P. aeruginosa* (confirmed within the last 12 month and at screening), and who are receiving inhaled Tobramycin therapy in a 28 days on / off regime in the past 3 month before screening. It is intended that 35 patients (children, adolescents and young adults) will be recruited in the DACH region at approximately 8 study sites.

The study will consist of 5 – 26 days screening period to test the presence of *P. aeruginosa*, a baseline visit, followed by the treatment phase of 28 days on-treatment period and subsequently 28 days off-treatment period. During the study a total of 5 visits are designated.

The structure of study design is as



Assessment schedule is as

Visit	1	2	3	4	5*
days / Procedure	-26 to -5 Screening	1 Baseline	7 Week 1 (+/- 2 days)	28 Week 4 (- 3 days)	56 End of Study (- 3 days)
Inclusion / exclusion criteria	x	x			
Demographic data	x				
Obtain informed consent	x				
Physical examination	x	x			x
Vital signs	x	x	x	x	x
Height and weight / BMI	x	x	x	x	x
Clinical chemistry [a]	x				x
Sputum (microbiology) [b]	x	x	x	x	x
Lung function (spirometry)[c]	x	x	x	x	x
FRC (bodyplethysmography)		x	x	x	x
LCI/ MBW	x	x	x	x	x
Serum pregnancy test	x				x
Urine pregnancy test		x			
Adverse events		x	x	x	x
Serious Adverse events	x	x	x	x	x
Concomitant medication	x	x	x	x	x

*When a patient prematurely discontinues the study before study completion, all procedures at Visit 5 should be performed.

a. Clinical chemistry: urea, creatinine, CRP

b. Pre-treatment sputum to be collected, preferably the first morning specimen; if the patient is unable to produce a sputum specimen, a deep cough throat swab or an induced sputum sample will be collected, at the investigator's discretion.

c. Spirometry should be done using a bodyplethysmograph to allow for FRC_{pleth} assessment in parallel

1.2 Study objectives and endpoints

The objectives of study are as

Primary Objectives

The primary objective is to assess the change of LCI after 4 weeks following onset of study drug inhalation versus Baseline.

Secondary objectives

- Change of FEV1 % predicted after 4 weeks following onset of study drug inhalation versus Baseline.
- Change in CFU after 4 weeks following onset of study drug inhalation versus Baseline
- Change of LCI after 1 week following onset of study drug inhalation versus Baseline.
- Change of LCI, FEV1 % predicted and CFU between Week 4 (end of Study drug inhalation in the current treatment cycle) and Week 8 (prior to start of Study drug inhalation in the following treatment cycle).
- Correlation between the changes of LCI, FEV1 % predicted and CFU after 1 week, 4 weeks, and 8 weeks versus Baseline, respectively.

Exploratory objectives

- To explore the change in LCI between Baseline and Week 4 (week 0-4), and Week 4 and Week 8 (week 4-8), respectively, in patients using Tobramycin inhalation powder compared to patients using Tobramycin inhalation solution.
- To assess and compare S_{acin}(N2 slope acinar airways) and S_{cond}(N2 slope conductive airways) at baseline and at Visit 4 in patients using Tobramycin inhalation powder vs. patients using Tobramycin inhalation solution.
- To assess the changes in FEV1 % predicted and CFU from Baseline to Visit 3 (week 1) and to compare the change in FEV1 % predicted with the change in CFU and the change in LCI from baseline to Visit 3 (week 1) with the corresponding changes in FEV1 % predicted and CFU.
- To explore the change in air trapping (FRCples - FRC_{MBW}) after 1 week, 4 weeks, and 8 weeks versus Baseline, respectively.
- To explore the association of additional markers of disease progression (need for i.v. antipseudomonal therapy, hospitalization due to exacerbations of lung disease) on LCI at Baseline.
- To assess and compare the ratio of patients with pathological LCI and pathological FEV1 and their mean deviation from normal LCI and FEV1 at Baseline.
- To assess and compare the ratio of patients with pathological LCI and pathological FEV1 and their mean deviation from normal LCI and FEV1 at week 4.

2 Statistical methods

2.1 Data analysis general information

Following standards are applied for analysis unless otherwise specified.
The data will be analyzed by Novartis. All analyses will be performed by using SAS Version 9.4. It is planned that the data from all centers that participate in this protocol will be used, so that an adequate number of patients will be available for analysis.

All continuous variables will be summarized by simple descriptive statistics (n, mean, standard deviation, median, minimum, maximum). Number and percentage will be presented for categorical data. All statistical tests will be applied at 5% significance level. All presented data will be presented in the form of by-patient listings sorted with treatment dose form and visit (if applicable).

2.1.1 General definitions

Study treatment/ Study drug: The investigational drug whose properties are being tested in the study.

Date of first administration of study drug/treatment: It is defined as the date of first dose of study drug administration to the patient.

Date of last administration of study drug/treatment: It is defined as the date of last dose of study drug administration to the patient.

Study day: Study day is defined as the number of days since the date of first dose of study drug administration. For a particular date, study day is calculated as:

Study day = Assessment date – Date of first dose of study drug administration + 1.

Baseline: Baseline is the last assessment obtained on or before the date of first dose of study drug administration. All assessments obtained after the date of first dose of study drug administration are considered as post-baseline unless otherwise specified.

On-treatment period: The period where the patients are exposed to the study treatment. For this study the treatment phase consists of 28 days of on-treatment period.
Lost to follow-up: The patients whose study completion status is unclear because they fail to appear for study visits without stating an intention to withdraw.

2.2 Analysis sets

In the study protocol, the safety population was predefined as consisting of all patients that enter the study (provide Informed Consent). This was motivated by the fact that the primary focus of this study was not to describe effects of a drug intervention, but to compare the accuracy of two different assessment methods, namely LCI and FEV1. However, a valid assessment of these methods is very difficult in patients without any treatment exposure. Therefore, it was decided to change the definition of the Safety Set

Safety Set: The safety set will consist of all patients that enter the study (provide Informed Consent) and have been exposed to at least one dose of study drug. The Safety set will be used for all analyses.

Table 1-1 Patient classification criteria

Analysis set	Population codes that cause patient to be excluded	Non-PD criteria that cause patient to be excluded
Safety set	1	Did not provide informed consent Did not exposed to at least one dose of study drug

Population code: 0=Include in everything, 1=Exclude from safety set.

2.2.1 Subgroup of interest

All safety analysis will be performed by treatment dose form as Tobramycin inhalation solution (TIS, 300mg/5mL or 300mg/4mL) to be nebulized or as Tobramycin inhalation powder (TIP, 112mg), as prescribed by the treating physician.

2.3 Patient disposition, demographics and other baseline characteristics

Appropriate descriptive statistics for patient disposition, demographic, disease history and baseline characteristics will be presented to describe the study population.

2.3.1 Patient disposition

Total number of patients who were screened, re-screened will be presented by number and percentage for safety set. The summary will be presented for screening phase, end of treatment phase completion and study completion phase. The number and percentage will be presented for patients who treated and entered in the study, who completed the study, who discontinued the study and treatment phase along with reason of discontinuation for safety set by treatment dose form and overall.

Patient identification number and whether they completed the study or discontinued from the study will be listed, along with date of last dose, date of discontinuation/study completion and primary reason of discontinuation by age group and treatment dose form. A Separate by patient listing of all patients along with inclusion and exclusion criteria status will also be provided.

2.3.2 Protocol Deviation

The number and percentage of patients with protocol deviations will be tabulated by category and deviation for the Safety set; overall and by treatment dose form. Protocol deviations will be listed with date and study day of occurrence, deviation code by age group and treatment dose form for Safety set.

Patient exclusion from analysis set will be listed for all patients with reasons of exclusion (i.e., both protocol and non-protocol deviation).

2.3.3 Demographic characteristics

The following demographic characteristics collected in the CRF at screening will be summarized using the Safety set.

Continuous variables:

- Age (years)
- Height (cm)
- Weight (Kg)
- BMI (Kg/m^2)

Categorical variables:

- Age categories(6 to 12 years, >12 to 17years, >17years)
- Sex (Male, Female)
- Race (Caucasian, Black, Asian, Other)
- Ethnicity (Hispanic or Latino, East Asian, Southeast Asian, South Asian, West Asian, Russian, Mixed Ethnicity, Not Reported, Unknown, Other)

Descriptive statistics (n, mean, median, standard deviation, minimum and maximum) will be presented for continuous variables for all patients in the Safety set; overall and by treatment dose form. The number and percentage of patients in each category will be presented for categorical variables. For categorical variables, the patients in the missing category will be taken into account for denominator for calculating the percentage.

Patient demographic data will be listed with date of assessment for safety set by age group and treatment dose form.

2.3.4 Medical history

Medical history will be coded using the Medical Dictionary for Regulatory Activities terminology (MedDRA version 18.1 and above or version used for coding). History/conditions will be summarized for the safety set by primary system organ class and preferred term; overall and by treatment dose form. Verbatim recorded history term will be listed together with the coded terms, date of diagnosis and whether the problem was ongoing by age group and treatment dose form for safety set at start of the study. Separate listing of cystic fibrosis will be presented with date of diagnosis by age group and treatment dose form for safety set.

2.3.5 Surgical and Medical Procedures

All surgical and medical procedures will be summarized for safety set by treatment dose form and overall. Patient data listing will be provided with name of surgery, amount, reason, start date, and end date or ongoing at final exam, sorted with age group and treatment dose form and visit(if applicable).

2.4 Treatments (study treatment, rescue medication, concomitant therapies, compliance)

2.4.1 Study drug Administration

Tobramycin, either as Tobramycin inhalation solution (TIS, 300mg/5mL or 300mg/4mL) to be nebulized or as Tobramycin inhalation powder (TIP, 112mg), as prescribed by the treating physician will be administered to each patient twice daily. Neither TIS nor TIP will be provided to the patient.

The extent of exposure will be examined to determine the degree to which safety can be assessed for Tobramycin, either TIS or TIP. The extent of exposure to study drug will be characterized according to the duration of exposure and the number of patients exposed.

Duration of exposure to the treatment will be calculated as the number of days between the first dose date and the last dose date exposed to that treatment over the specified period, expressed as: Duration of exposure = ((Date of last known dose of study drug – Date of first dose of study drug) + 1. If a substantial number of interruptions occur, additional tables accounting for interruptions may be produced.

The duration of exposure (in days) will be summarized by treatment dose form and overall for the safety set as

- a continuous variable with the standard descriptive statistics, and
- a categorical variable classified into ≤ 14days, > 14 – 21days, > 21 – 28days, >28 days with number and percentage of patients in each category.

Patient data listing will be provided along with dose administered(unit), total daily dose(unit), regimen, frequency, start date, study day, end date, dose permanently discontinued, and reason of dose discontinuation by age group and treatment dose form for safety set.

2.4.2 Compliance

Compliance will be assessed by the investigator and/or study personnel at each visit using information provided by the patient via a patient diary. Assessment of compliance will be based on:

- The number of dosing sessions per day (e.g. morning and evening)
- The number of actual days on treatment.

The compliance is defined as the percentage of doses taken by the patient and will be calculated using the following formula:

Compliance (%) = 100 x total study dose consumed by patient/ total study dose patient supposed to consume.

Compliance will be summarized by treatment form and overall for the safety set as

- a continuous variable with the standard descriptive statistics, and
- a categorical variable classified into $< 80\%$, $\geq 80\%$ to $< 100\%$, ≥ 100 to $< 120\%$, $\geq 120\%$, with number and percentage of patients in each category.

Patient data for compliance will listed along with date of first/last dose, treatment duration, total dose consumed by age group and treatment dose form for safety set.

2.4.3 Prior, concomitant and post therapies

Rescue medication is any on demand medication at acute clinical deterioration of the patient, independent of the currently used standard therapy.

Prior medications are those concomitant medications which were taken and stopped prior to the first dose of study drug.

Concomitant medications are those concomitant medications which were taken prior to and continued after the first dose of the study drug or those medication which were given at least once between the day of first dose of study treatment and the date of the last study visit.

All prior and concomitant medication will be coded using WHO drug dictionary with updated version. All prior and concomitant medications will be summarized in one table.
The number and percentage of patients taking prior medication, concomitant medications will be presented by ATC class and preferred term; overall and by treatment dose form.
Subject data for prior and concomitant medication will be provided in the form of listing along with medication name, dose, start date/End date of medication, route, frequency, ongoing at final examination and reason by treatment dose form for safety set.

2.5 Analysis of the primary objective

Primary effectiveness analysis will be performed on safety set.

2.5.1 Primary endpoint

The primary variable of this study is the LCI.

The primary effectiveness variable is the time course and changes in LCI from baseline (Visit 2) to Week 4 (Visit 4).

2.5.2 Statistical hypothesis, model, and method of analysis

The primary objective of the study is to evaluate the effectiveness of antibiotic therapy TBM100 in patients with CF aged 6 years and older with mild to moderate lung disease. The effectiveness will be assessed by changes in LCI from baseline to Week 4. Baseline LCI is the assessment taken prior to the start of TBM100, which is typically the value at Visit 2. If baseline value is missing then change is taken from the data recorded at screening. The primary analysis will include patients with a valid observation at baseline and post-baseline visit.

The summary of LCI at baseline, at each post-baseline visit and it's change from baseline to each post-baseline visit will be presented using descriptive statistics (n, mean, standard deviation, median, minimum and maximum). Change from baseline will be calculated as value at post-baseline visit minus value at baseline.

Between visits comparison for changes in LCI from baseline will be analyzed using an ANOVA model with factors patient and visit. Estimates over time will be presented as LS-Means, pairwise LS-Mean differences along with 95% confidence intervals and two sided p-values for the pairwise differences between visits.

Additionally, for accessing trend of change in LCI over time, mean values over time and the row LCI values over time for each patient will be displayed graphically for each respective visit. All analysis mentioned in this section will be done for safety set at 5% significance level.
All efficacy data will be listed with date of assessment by age group and treatment dose form.

2.5.3 Handling of missing values/censoring/discontinuations

There will be no imputation for missing data at Week 4, and the primary analysis will be based on only observed data.

2.5.4 Supportive analyses

Not Applicable.

2.6 Analysis of the key secondary objective

Not Applicable.

2.6.1 Key secondary endpoint

Not Applicable.

2.6.2 Statistical hypothesis, model, and method of analysis

Not Applicable.

2.6.3 Handling of missing values/censoring/discontinuations

Not Applicable.

2.7 Analysis of secondary efficacy objectives

All secondary efficacy analysis will be provided for safety set.

2.7.1 Secondary endpoints

The secondary effectiveness variables are the changes in FEV1 % predicted, CFU and LCI and it's comparison among themselves at respective visit will be performed for safety set.

2.7.1.1 Changes in FEV_1 % predicted from baseline to Week 4

The changes from baseline in FEV_1 % predicted to each post-baseline visit will be analyzed in a similar manner as the primary variable, i.e.

- The FEV_1 % predicted and it's mean change from baseline to each post-baseline visit will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum and maximum).
- Between visits comparison for changes in FEV_1 % predicted from baseline will be analyzed in similar manner as primary variable. Estimates change over time will be presented as LS-Means, pairwise LS-Mean differences along with 95% confidence intervals and two sided p-values for the pairwise differences between visits.

2.7.1.2 Changes in CFU (/mL) from baseline to Week 4

The changes from baseline in CFU (/mL) to each post-baseline visit will be analyzed in a similar manner as the primary variable, i.e.

- The CFU and it's mean change from baseline to each post-baseline visit will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum and maximum).
- Between visits comparison for change in CFU from baseline will be analyzed in similar manner as primary variable. Estimates change over time will be presented as LS-Means with 95% confidence intervals and two sided p-values for the pairwise differences between visits.

2.7.1.3 Change in LCI from baseline to Week 1

This analysis is contained in the ANOVA model for LCI described above.

2.7.1.4 Changes in LCI, FEV_1 % predicted, CFU between Week 4 and Week 8

This analysis is contained in the ANOVA models described above.

2.7.1.5 Correlation between change from baseline in LCI, FEV_1 % predicted, CFU at Week 1, Week 4 and Week 8

Correlation based on change in FEV1 % predicted, CFU and LCI will be evaluated for each pair using Karl Pearson correlation coefficient. Visit 2 will be considered as baseline. If baseline value is missing then change is taken from the data recorded at screening. Change from baseline will be calculated as value at post-baseline minus value at baseline. Estimates as Karl Pearson correlation coefficient with their 95% confidence intervals and p-values will be presented. Additionally, the correlation among each pairs will be explored using scatterplots for raw values as well as for changes at respective visit.

All subject efficacy data including FEV1, predicted FEV1, FRC, LCI by multiple breath washouts, CFU (quantitative density) will be listed by age group, treatment dose form and visit(as applicable) along with date of assessment for safety set.

2.7.2 Handling of missing values/censoring/discontinuations

Missing data will not be imputed and the secondary analysis will be based on only observed data.

2.8 Safety analyses

Safety analysis including TEAE, vital sign, lab parameter and it's change from baseline will be evaluated based on safety set. All listings will be ordered according to age categories 6 to 12, >12 to 17 and >17 for safety set.

2.8.1 Adverse events (AEs)

The verbatim term recorded on CRF will be identified as adverse event and will be coded by primary system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) version 18.1 and above, and other version used for coding. Adverse events starting on or after the time of the first inhalation of study drug but not later than 7 days (30 days in the case of a SAE) after the date of last dose of study drug taken or those AE present at baseline with increased severity will be classified as a treatment emergent adverse event and will be included in all summaries. AEs which started and ended prior to baseline and AEs which started prior to baseline but whose severity does not change post baseline will not be included in treatment emergent adverse event.

2.8.1.1 Adverse events of special interest / grouping of AEs

Treatment emergent adverse events by primary system organ class and preferred term

The number and percentage of patients who reported treatment emergent adverse events will be summarized for each primary system organ class and preferred term; overall and by treatment dose form. Primary system organ classes will be sorted alphabetically and, within each primary system organ class, the preferred terms will be sorted in descending order of frequency.

If a patient reported more than one adverse event with the same preferred term, the adverse event will be counted only once at the preferred term level. If a patient reported more than one

adverse event within the same primary system organ class, the patient will be counted only once at the system organ class level.

Treatment emergent adverse events by severity

All treatment emergent adverse events will be summarized with maximum severity for each primary system organ class and preferred term; overall and by treatment dose form. If a patient reports more than one adverse event within the same primary system organ class, only one adverse event will be counted for that patient at the highest severity level in the total row for each primary system organ class. If a patient reported more than one adverse event with the same preferred term, the highest (maximum) severity will be presented.

Treatment emergent adverse events suspected to be related to study drug

An adverse event related to study drug is defined as one considered by the investigator to have a suspected relationship with the diagnostic method or the study drug. The treatment emergent adverse events suspected to be related to the diagnostic method or study drug (according to the investigators) will be summarized by primary system organ class and preferred term; overall and by treatment dose form. Relationship to study drug is considered as suspected for those events where "Relationship to study drug" is answered by the investigator as "Suspected".

Treatment emergent adverse events leading to permanent study drug discontinuation

Treatment emergent adverse events leading to permanent study drug discontinuation will be summarized by primary system organ class and preferred term using; overall and by treatment dose form.

Treatment emergent adverse events requiring action taken with study treatment

Treatment emergent adverse events requiring action to be taken with study treatment will be summarized by primary system organ class and preferred term; overall and by treatment dose form.

Treatment emergent adverse events requiring significant additional medication or therapy

Treatment emergent adverse events requiring significant additional therapy (concomitant medication, non- drug therapy) will be summarized by primary system organ class and preferred term; overall and by treatment dose form.

Treatment emergent adverse events outcome

Treatment emergent adverse events with outcome will be summarized by primary system organ class and preferred term; overall and by treatment dose form.

Treatment emergent serious adverse events

Number and percentage of patients with treatment emergent serious adverse events, regardless of study drug relationship, will be presented by primary system organ class and preferred term; overall and by treatment dose form for safety set.

The patient data listing includes, SOC/PT/Verbatim term, start date/study day, end date/study day, the severity of an AE, whether or not an AE is related to MBW or study drug, and whether or not it is a serious AE, action taken with study drug, outcome, duration. Duration will be calculated as end date – start date+1 and for ongoing last visit date – start date+1.

The following patient data listings will be provided by age group and treatment dose form.

1. Treatment emergent serious adverse event
2. Discontinuation due to treatment emergent adverse events

Additionally, listing of all adverse events and treatment emergent adverse event will be provided for safety set by age group and treatment dose form.

TEAE of special interest

Treatment emergent adverse events of special interest(Elevated BUN and Creatinine, Tinnitus and Hearing loss) will be summarized by primary system organ class and preferred term; overall and by treatment dose form.

2.8.2 Deaths

All the deaths in the clinical database will be listed with the last dose date/visit, date of death and investigator-reported principal cause by age group and treatment dose form. Treatment emergent adverse event leading to death will be summarized with numbers and percentages; overall and by treatment dose form for safety set.

Laboratory data

Clinical laboratory parameters include creatinine, urea, C Reactive protein, Burkholderia complex, Pseudomonas aeruginosa, Pseudomonas aeruginosa Tobra MIC, blood urea nitrogen (BUN), Choriogonadotropin beta. All laboratory data (Hematology, chemistry and urinalysis) will be summarized for safety set by treatment dose form, overall and visit. For continuous data the summary statistics (number of observations, mean, standard deviation, median, minimum and maximum) will be provided for safety set for both actual value and change from baseline. Visit 2 will be considered as baseline. If baseline value is missing then change is taken from the data recorded at screening. Number and percentage will be provided for categorical data.

Newly occurring or worsening notably abnormal laboratory values to each post-baseline time point will be listed for the selected parameters creatinine (>=2mg/dl, above normal), blood urea nitrogen (BUN) (>=40mg/dl, above normal). A case will be considered as newly occurring if all the components are not clinically notable at baseline but clinically notable thereafter. If patients have a missing baseline value for at least one component, post-baseline values meeting the notable criterion will be considered as newly occurring.

Observed value of all laboratory parameter will be listed with abnormal values flagged (if applicable) for safety set for all patient along with date of assessment, sorted with age group, treatment dose form and visit. A separate listing for pregnancy test (serum and urine) will be provided with date of assessment by age group and treatment dose form for safety set.

2.8.3 Other safety data

2.8.3.1 ECG and cardiac imaging data

Not Applicable.

2.8.3.2 Vital signs

Vital sign including sitting systolic/diastolic blood pressure, sitting radial pulse rate (over a 30 second interval), respiratory rate, body temperature, height, weight, and body mass index (BMI) will be summarized for safety set by descriptive statistics (number of observations, mean, standard deviation, median, minimum and maximum) for both actual value and change from baseline by treatment dose form, overall and visit. If baseline value is missing then change is taken from the data recorded at screening.

Vital signs data will be listed along with date of assessment, sorted with age group, treatment dose form and visit (if applicable), notable values will be flagged(if applicable), and any other information collected will be listed as appropriate.

2.8.3.3 Physical Examination

Not Applicable

2.9 Pharmacokinetic endpoints

Not Applicable

2.10 PD and PK/PD analyses

Not Applicable

2.11 Patient-reported outcomes

Not Applicable

2.12 Biomarkers

Not Applicable

2.13 Other Exploratory analyses

2.13.1.1 Comparisons of change in LCI from baseline to Week 4(Week 0-4) and Week 4 to Week 8 (Week 4-8) between the patients using Tobramycin inhalation powder and patients using Tobramycin inhalation solution

Change in LCI from baseline to Week 4(week 0-4) for patients using Tobramycin inhalation powder and patients using Tobramycin inhalation solution will be calculated as Change=LCI at Week 4 – LCI at baseline. Visit 2 will be considered as baseline. If baseline value is missing then change is taken from the data recorded at screening.

Addition to that, change in LCI from Week 4 to Week 8 (week 4-8) for patients using Tobramycin inhalation powder and patients using Tobramycin inhalation solution will be calculated as change=LCI at Week 8 – LCI at Week 4.

- Summary of LCI at baseline, at Week 4 and at Week 8 will be presented standard descriptive statistics (n, mean, standard deviation, median, minimum and maximum) for both groups.
- The mean change from baseline to Week 4 (week 0-4) and Week 4 to Week 8 (week 4-8) will be estimated for both groups.

Analysis will be done for safety population based on observed data.

2.13.1.2 Comparison of S_{acin}(N2 slope acinar airways) and S_{cond}(N2 slope conductive airways) at baseline and at Visit 4 in patients using Tobramycin inhalation powder vs. patients using Tobramycin inhalation solution.

The S_{acin} and S_{cond} values at baseline and at each post-baseline visit in the patients using Tobramycin inhalation powder and patients using Tobramycin inhalation solution will be summarized by descriptive statistics (n, mean, standard deviation, median, minimum and maximum). Comparison between the patients using Tobramycin inhalation powder and patients using Tobramycin inhalation solution for both parameters i.e. S_{acin} and S_{cond} at baseline and at Visit 4 will be performed using a descriptive statistics.

Additionally, for accessing trend of change in S_{acin} (N2 slope acinar airways) and S_{cond} (N2 slope conductive airways) over time, mean change from baseline over time will be displayed graphically to each respective visit.

Analysis will be done for safety set based on observed data.

2.13.1.3 Comparisons of changes in FEV1 % predicted, CFU and LCI from baseline to visit 3 (Week1)

The change in FEV1 % predicted, CFU and LCI from baseline to Visit 3(Week1) will be calculated using the formula as Change= value at Week 1 – value at baseline. Visit 2 will be considered as baseline. If baseline value is missing then change is taken from the data recorded at screening. Analysis will be performed based on observed data.

- Summary of FEV1 % predicted, CFU and LCI at baseline and at Visit 3(Week1) for both groups will be presented standard descriptive statistics (n, mean, standard deviation, median, minimum and maximum).

- The mean changes in FEV1 % predicted, CFU, and LCI from baseline to visit 3 (week 1) will be displayed for both groups.

Comparisons of changes in FEV1 % predicted, CFU and LCI from baseline to visit 3 will be done based on descriptive statistics. Analysis will be done on safety set.

2.13.1.4 Change in air trapping (FRC_{ples} - FRC_{MBW}) from baseline to Week 1, Week 4 and Week 8.

Air trapping will be assessed by the difference in FRC measured by bodyplethysmography (FRCples) and FRC measured by MBW (FRC_{MBW}). The air trapping (FRC_{ples} - FRC_{MBW}) and its change from baseline to each post-baseline visit will be summarized by descriptive statistics (n, mean, standard deviation, median, minimum and maximum). The change in air trapping from baseline will be calculated using as Change= Value at post-baseline – value at baseline. Visit 2 will be considered as baseline. If baseline value is missing then change is taken from the data recorded at screening. Analysis will be performed based on observed data for safety set.

2.13.1.5 Association of additional markers of disease progression on LCI at baseline

Number and percentage will be presented for the patients who took i.v. antipseudomonal therapy, and who were hospitalized due to exacerbations of lung disease.
Association between i.v. antipseudomonal therapy and LCI at baseline will be based on standard descriptive statistics (n, mean, standard deviation, median, minimum and maximum). Similarly, the association between hospitalization due to exacerbations of lung disease and LCI at baseline will be evaluated in the same way.
Analysis will be done for safety population based on observed data.

2.13.1.6 Patients with pathological LCI and pathological FEV1 at Baseline and at Week 4 and it's deviation from normal LCI and normal FEV1.

Number of patients with pathological LCI (i.e. LCI above 7.5) and normal LCI will be summarized by frequency and proportion. The summary of normal LCI and pathological LCI at baseline and at Week 4 will be presented by descriptive statistics (n, mean, standard deviation, median, minimum and maximum) for safety set.

Predicted FEV1 value will be calculated using below formula (Knudson)
Predicted FEV1 for Male subject aged <=24 yrs = 0.1168* Height (in Inches) + 0.0450*Age -4.808;
Predicted FEV1 for Male subject aged >24 yrs = 0.1321* Height (in Inches) - 0.0270*Age -4.203;
Predicted FEV1 for Female subject aged <=19yrs = 0.0686* Height (in Inches) + 0.0850*Age -2.703;
Predicted FEV1 for Female subject aged >19yrs = 0.0686* Height (in Inches) - 0.0210*Age -0.794;
Height in inches will be converted as: Height (in inches) =0.39*height (in cm).
And percent predicted FEV1 will be calculated as: % predicted FEV1 = FEV1/Predicted FEV1*100.
Number of patients with pathological FEV1 (i.e. FEV1 below 75% predicted) and normal FEV1 will be summarized by frequency and proportion. Normal FEV1 and pathological FEV1 at baseline and at Week 4 will be summarized by descriptive statistics (n, mean, standard deviation, median, minimum and maximum) for safety set.

All by-subject exploratory efficacy data including air trapping (FRC_{ples} - FRC_{MBW}), S_{acin} (N2 slope acinar airways) and S_{cond} (N2 slope conductive airways) will be listed by age group, treatment dose form and visit(as applicable) along with date of assessment for safety set.

2.14 Interim analysis

No interim analysis planned for this study

3 Sample size calculation

The primary effectiveness outcome measure of the study will be the change from baseline to 4 weeks in LCI in patients treated with TBM100. Therefore, sample size calculations for this study are based on the primary objective which produces a precise estimate of these changes in LCI. The precision of the estimated mean change in LCI at 4 weeks, as assessed by the 95% confidence interval

Since LCI is expected to be a more sensitive measure (compared to FEV1), thus the number of patients required to detect changes in FEV1 should also be sufficient to detect relevant changes in LCI and to explore the correlation between LCI and FEV1.

For FEV1, a change of 5% with an intraindividual SD of 9% is expected between on- and off-treatment cycles. Under these assumptions, 28 patients will be required to detect this change with 80% power on a two-sided, 5% significance level. To compensate for some protocol violations and drop-outs, a total of 35 patients should be recruited into this trial.

4 Change to protocol specified analyses

The following changes from protocol have been implemented at the SAP development stage.

Definition of safety set

1. As per protocol: The safety set will consist of all patients that enter the study (provide Informed Consent).
   Changes in SAP: The safety set will consist of all patients that enter the study (provide Informed Consent) and have been exposed to at least one dose of study drug.
2. As per protocol: Changes in FEV1 over time-point following onset of study drug inhalation versus Baseline were analyzed.
   Changes in SAP: Based on comments on Dry run output from clinical team, changes in FEV1 % predicted instead of changed in FEV1 (L) over time-point following onset of study drug inhalation versus Baseline will be analyzed.

5 Appendix

All statistical output required for drafting CSR were outlined RAP module 7.

5.1 Imputation rules

5.1.1 Study drug

Not Applicable

5.1.2 AE date imputation

AE date imputation is based only on a comparison of the partial AE start date to the treatment start date as mentioned in the Table 1-2 below.

1. If the AE start date year value is missing, the date uncertainty is too high to impute a rational date. Therefore, if the AE year value is missing, the imputed AE start date is set to NULL.
2. If the AE start date year value is less than the treatment start date year value, the AE started before treatment. Therefore:
    a. If the AE year is less than the treatment year and the AE month is missing, the imputed AE start date is set to the mid-year point (01JulYYYY).
    b. Else if the AE year is less than the treatment year and the AE month is not missing, the imputed AE start date is set to the mid-month point (15MONYYYY).
3. If the AE start date year value is greater than the treatment start date year value, the AE started after treatment. Therefore:
    a. If the AE year is greater than the treatment year and the AE month is missing, the imputed AE start date is set to the year start point (01JanYYYY).
    b. Else if the AE year is greater than the treatment year and the AE month is not missing, the imputed AE start date is set to the month start point (01MONYYYY).
4. If the AE start date year value is equal to the treatment start date year value:
    a. And the AE month is missing or the AE month is equal to the treatment start month, the imputed AE start date is set to one day after treatment start.
    b. Else if the AE month is less than the treatment start month, the imputed AE start date is set to the mid-month point (15MONYYYY).
    c. Else if the AE month is greater than the treatment start month, the imputed AE start date is set to the start month point (01MONYYYY).

Table 1-2: AE date imputation

	MON	**MON < CFM**	**MON = CFM**	**MON > CFM**
	MISSING			
YYYY MISSING	NULL	NULL	NULL	NULL
	Uncertain	Uncertain	Uncertain	Uncertain
YYYY < CFY	(D) = 01JULYYYY	(C)= 15MONYYYY	(C)= 15MONYYYY	(C)= 15MONYYYY
	Before Treatment Start	Before Treatment Start	Before Treatment Start	Before Treatment Start
YYYY = CFY	(B)= TRTSTD+1	(C)= 15MONYYYY	(A)= TRTSTD+1	(A)= 01MONYYYY
	Uncertain	Before Treatment Start	Uncertain	After Treatment Start
YYYY > CFY	(E)= 01JANYYYY	(A)= 01MONYYYY	(A)= 01MONYYYY	(A)= 01MONYYYY

	After Treatment Start	After Treatment Start	After Treatment Start	After Treatment Start
Before Treatment Start		Partial indicates date prior to Treatment Start Date		
After Treatment Start		Partial indicates date after Treatment Start Date		
Uncertain		Partial insufficient to determine relationship to Treatment Start Date		
LEGEND:				
(A)		MAX(01MONYYYY,TRTSTD+1)		
(B)		TRTSTD+1		
(C)		15MONYYYY		
(D)		01JULYYYY		
(E)		01JANYYYY		

Adverse event end date will not be imputed.

5.1.3 Concomitant medication date imputation

Concomitant medication (CMD) date imputation uses both a comparison of the partial CMD start date to the treatment start date, and the value of the CMDTYP1C flag (1, 2, or 3). Event date comparisons to treatment start date are made based on the year and month values only (any day values are ignored) in Table 1-3 below.

1. If the CMD start date year value is missing, the date will be imputed based on the CMDTYP1C flag value. If the flag value is 1 or 3, the imputed CMD start date is set to one day before the treatment start date. Else, if the flag value is missing or 2, the imputed CMD start date is set to one day after the treatment start date. (Note that for some legacy data, the CMDTYP1C variable may not exist in the data. When this happens and the CMD start date year value is missing, the imputed date value will be NULL.)
2. If the CMD start date year value is less than the treatment start date year value, the CMD started before treatment. Therefore:
    a. if the CMD year is less than the treatment year and the CMD month is missing, the imputed CMD start date is set to the mid-year point (01JulYYYY).
    b. Else if the CMD year is less than the treatment year and the CMD month is not missing, the imputed CMD start date is set to the mid-month point (15MONYYYY).

If the CMD start date year value is greater than the treatment start date year value, the CMD started after treatment. Therefore:

    a. If the CMD year is greater than the treatment year and the CMD month is missing, the imputed CMD start date is set to the year start point (01JanYYYY).
    b. Else if the CMD year is greater than the treatment year and the CMD month is not missing, the imputed CMD start date is set to the month start point (01MONYYYY).

3. If the CMD start date year value is equal to the treatment start date year value:
    a. and the CMD month is missing or the CMD month is equal to the treatment start month,

i. If the flag value is 1 or 3, the imputed CMD start date is set to one day before the treatment start date.

ii. Else, if the flag value is missing or 2, the imputed CMD start date is set to one day after the treatment start date.

a. Else if the CMD month is less than the treatment start month, the imputed CMD start date is set to the mid-month point (15MONYYYY).

b. Else if the CMD month is greater than the treatment start month, the imputed CMD start date is set to the start month point (01MONYYYY).

Table 1-3: CMD date imputation

	MON MISSING	**MON < CFM**	**MON = CFM**	**MON > CFM**
YYYY MISSING	**(F)**	**(F)**	**(F)**	**(F)**
	Uncertain	Uncertain	Uncertain	Uncertain
YYYY < CFY	**(D)=01JULYYYY**	**(C)=15MONYY**	**(C)=15MONYY**	**(C)=15MONYY**
	Before Treatment Start	Before Treatment Start	Before Treatment Start	Before Treatment Start
YYYY = CFY	**(B)**	**(C)=15MONYY**	**(B)**	**(A)=01MONYYYY**
	Uncertain	Before Treatment Start	Uncertain	After Treatment Start
YYYY > CFY	**(E)= 01JANYYYY**	**(A)=01MONYYYY**	**(A)=01MONYYYY**	**(A)=01MONYYYY**
	After Treatment Start	After Treatment Start	After Treatment Start	After Treatment Start

Before Treatment Start	Partial indicates date prior to Treatment Start Date
After Treatment Start	Partial indicates date after Treatment Start Date
Uncertain	Partial insufficient to determine relationship to Treatment Start Date
LEGEND:	
(A)	MAX(01MONYYYY,TRTSTD+1)
(B)	IF CMDTYP1C IN (1,3) THEN TRTSTD-1 ELSE IF CMDTYP1C in(. , 2) THEN TRTSTD+1
(C)	15MONYYYY
(D)	01JULYYYY
(E)	01JANYYYY
(F)	IF CMDTYP1C IN (1,3) THEN TRTSTD-1 ELSE IF CMDTYP1C in (. , 2) THEN TRTSTD+1

Concomitant medication end date will not be imputed.

5.1.3.1 Prior therapies date imputation

Not Applicable

5.1.3.2 Post therapies date imputation

Not Applicable

5.1.3.3 Other imputations

Not Applicable

5.2 AEs coding/grading

The verbatim term recorded on CRF will be identified as adverse event and will be coded by primary system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) version 18.1 and above or versions used for coding.

5.3 Laboratory parameters derivations

Table 1-4: Criteria for clinically notable laboratory values

Laboratory Variable	Clinically notable abnormal value(range)
Blood urea nitrogen	> 39 mg/dL
Creatinine	> 1.99 mg/dL

5.4 Statistical models

5.4.1 Primary analysis

Mixed model for LCI comparison between visit

The SAS procedure PROC MIXED will be used for this analysis with the following code:

Model: LCI = Visit + patients + error.

```
proc mixed data=<....>  method=reml;
    where visit in (2, 3, 4, 5);
        class visit usubjid;
        model lci= visit ;
        repeated visit/subject=usubjid type=un;
        lsmeans visit /pdiff cl;
        estimate "LCI at Baseline vs LCI at Visit 3"  -1 1 0 0 / cl alpha=0.05;
        estimate "LCI at Baseline vs LCI at Visit 4"  -1 0 1 0 / cl alpha=0.05;
        estimate "LCI at Baseline vs LCI at Visit 5"  -1 0 0 1 / cl alpha=0.05;
        estimate "LCI at Visit 3 vs LCI at Visit 4"  0 1 -1 0 / cl alpha=0.05;
        estimate "LCI at Visit 3 vs LCI at Visit 5"  0 1 0 -1 / cl alpha=0.05;
        estimate "LCI at Visit 4 vs LCI at Visit 5"  0 0 1 -1 / cl alpha=0.05;
    ods output lsmeans=lsm diffs=diff1;
run;
```

where,

lci = LCI at each visit,

subjid=Patient ID.

5.4.2 Secondary analysis

The SAS procedure PROC MIXED will be used for this analysis of FEV1 and CFU with the following code:

Model:FEV1 = Visit + patients + error.

```
proc mixed data=<....> method=reml;
    where visit in (2, 3, 4, 5);
        class visit usubjid;
        model fev1= visit ;
        repeated visit/subject=usubjid type=un;
        lsmeans visit /pdiff cl;
        estimate "FEV1 at Baseline vs FEV1 at Visit 3"  -1 1 0 0 / cl alpha=0.05;
        estimate "FEV1 at Baseline vs FEV1 at Visit 4"  -1 0 1 0 /cl alpha=0.05;
        estimate "FEV1 at Baseline vs FEV1 at Visit 5"  -1 0 0 1 /cl alpha=0.05;
        estimate "FEV1 at Visit 3 vs FEV1 at Visit 4"  0 1 -1 0 /cl alpha=0.05;
        estimate "FEV1 at Visit 3 vs FEV1 at Visit 5"  0 1 0 -1 /cl alpha=0.05;
        estimate "FEV1 at Visit 4 vs FEV1 at Visit 5"  0 0 1 -1 /cl alpha=0.05;
  ods output lsmeans=lsm diffs=diff1;
run;
```

where,

fev1= FEV1 at each visit,

subjid=Patient ID.

Pearson correlation coefficient among the pairs based on the change from baseline in LCI, FEV1 and CFU after 1 week, 4 weeks, and 8 weeks

The SAS procedure CORR will be used to compute this coefficient with the following code:

```
proc corr data= <..... > pearson fisher;
        var var1;
        with var2;
  ods output FisherPearsonCorr=cor;
  ods output PearsonCorr=correl;
run;
```

where, var1 and var2 are two different variables measured on the same patient.

5.5 Rule of exclusion criteria of analysis sets

All protocol deviations defined at the start of the study are listed with associated population codes in below table.

Deviation code	Text description	Severity code
INCL01	Patient has not provided the written informed consent.	1
INCL02	Cystic fibrosis diagnosis is not confirmed by the study protocol specified tests.	0

INCL03	Patient age is outside protocol specified range. Please verify Birth Year and Informed Consent date.	0
INCL04	Elevated LCI is < 7.5 at screening, confirmed by a central MBW specialist.	0
INCL05	FEV1 of < 50% predicted at screening.	0
INCL06	*P. aeruginosa* is not present in two sputum or deep cough throat swab cultures or bronchoalveolar lavage (BAL) (only for BAL, a threshold level of 103 CFU/mL is required) within 12 months prior to screening or in one culture within 12 months prior to screening and in the sputum or deep cough throat swab culture at screening	0
INCL07	No use of inhaled Tobramycin in 28 days on / off regimen in the past 3 months before screening.	0
INCL08	Patient is not clinically stable in the opinion of the investigator and unlikely to be able to participate in the study until the end of the study (Visit 5).	0
EXCL01	History of sputum culture or deep cough throat swab (or BAL) culture yielding *Burkholderia cenocepacia* complex within 2 years prior to screening and / or sputum culture yielding *B. cenocepacia* complex at screening (Visit 1).	0
EXCL02	Hemoptysis more than 60 mL at any time within 30 days prior to screening (Visit 1).	0
EXCL03	Patient has history of hearing loss or chronic tinnitus.	0
EXCL04	Serum creatinine 2mg/dL or greater, BUN 40 mg/dL or greater, known local or systemic hypersensitivity to aminoglycosides.	0
EXCL05	Patients who are regularly receiving more than 1 class of inhaled anti-pseudomonal antibiotic during the study or in the past 56 days (8 weeks) prior to baseline visit (Visit 2).	0
EXCL06	Patients who have used oral or intravenous anti-pseudomonal antibiotics within 28 days prior to on-phase of study drug (Visit 2). These patients may be rescreened after 1 month following stop of i.v. treatment.	0
EXCL07	Change in dose, formulation or strength of the study drug in the past treatment cycle before screening.	0
EXCL08	Patients following onset or discontinuation of therapy with macrolides, chest physiotherapy, nebulized hypertonic saline, dornase alpha, long acting bronchodilators, inhaled steroids or inhaled mannitol during the study and within 56 days (8 weeks) prior to baseline visit (V2).	0
EXCL09	Use of loop diuretics within 7 days prior to first study medication administration (Visit 2).	0
EXCL10	Administration of any investigational drug within 30 days or 5 half-lives, whichever is longer, prior to screening (Visit 1).	0
EXCL11	Signs and symptoms of acute pulmonary disease, e.g. pneumonia, pneumothorax.	0
EXCL12	Body mass index less than 12 kg/m^2	0
EXCL14	History of malignancy of any organ system, treated or untreated, regardless of whether there is evidence of local recurrence or metastases.	0
EXCL15	Patients with known or suspected neuromuscular disorders, e. g. Parkinson´s	0

	disease, Myasthenia gravis.	
EXCL16	Patients or caregivers who are considered potentially unreliable or considered unlikely to be compliant within the trial.	0
EXCL17	Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin (hCG) laboratory test (> 5 mIU/mL).	0
EXCL19	Study personnel or first degree relatives of investigator(s) must not be included in the study.	0
EXCL 13	Body Weight of less than 15kg.	0
COMD01	Use of loop diuretics during administration of study drug.	0
COMD02	Use of iv or oral antipseudomonal antibiotic in the 23 days prior and of study visit (V5).	0
COMD03	Use of short acting ß-agonists (SABA) in the 6 hours prior to each FEV1/Spirometry or LCI assessment.	0
COMD04	Use of long acting ß-agonists (LABA) in the 12 hours prior to each FEV1/Spirometry or LCI assessment.	0
COMD05	Use of long acting muscarinic antagonists (LAMA) in the 12 hours prior to each FEV1/Spirometry or LCI assessment.	0
COMD06	Inhalation of study drug is interrupted for more than 3 days during the "on-phase".	0
COMD07	Any use of iv antipseudomonal antibiotic in the absence of an AE during the study.	0
COMD08	Any inhaled antipseudomonal antibiotics other than tobramycin during the study.	0
EXCL 18	Women who are menstruating and capable of becoming pregnant and either not practicing a medically approved method of contraception or not practicing total abstinence during and up to at least 4 weeks after the end of treatment.	0
OTH10	Spirometry was not conducted according to ATS criteria.	0
Med	Patient did not receive at least one dose of study drug	1

6 Reference

Not Applicable.