TEACH-IP-15

FINAL REPORT

PROTOCOL NUMBER:	TEACH-IP-15
PROTOCOL TITLE:	Testing the Effect of Adding CHronic Azithromycin to inhaled Tobramycin. A randomized, placebo-controlled, double-blinded trial of azithromycin 500mg thrice weekly in combination with inhaled tobramycin
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FUNDING AGENCIES:	National Institutes of Health/National Heart, Lung, and Blood Institute (NHLBI) Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT)
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PREFACE

The Statistical Analysis Plan (SAP) as outlined in this document will be finalized prior to the completion of the first comprehensive DSMB interim report. Any modifications to the SAP after finalization will be documented. The SAP contains all modifications and updates to the planned analyses that were outlined in the original study protocol. This plan details all *a priori* specified analyses that will be performed upon study completion and database lock, with detailed specifications for all tables, figures, and statistical models.

Signature Page

Analysis plan approved by:

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1. Overview

1.1 Study Rationale and Design

Patients with cystic fibrosis (CF) commonly acquire a chronic airway infection with *Pseudomonas aeruginosa* (*P.a.*). Both inhaled tobramycin and oral azithromycin have been proven beneficial in these patients and are currently used by the majority of eligible patients. Seventy five percent of US patients prescribed inhaled tobramycin are also prescribed chronic oral azithromycin. We believe that azithromycin is inhibiting the anti-*P.a.* effects and clinical benefits of inhaled tobramycin. This study will investigate whether azithromycin is associated with poorer clinical and microbiologic outcomes as compared to placebo during concurrent administration of inhaled tobramycin.

This is a prospective, randomized, placebo-controlled, double-blinded study of azithromycin in subjects with chronic *Pa* airway infection using inhaled tobramycin. Subjects who have received two (2) or more cycles of inhaled tobramycin within the 24 weeks prior to enrollment will be recruited into the study. Approximately 120 eligible subjects (allowing for 10 withdrawn) will be enrolled and randomized to either azithromycin or placebo at Visit 1 (Day -14), approximately 14 days prior to the start day of their next planned 28-day nebulized solution or dry powder tobramycin (TISP) cycle. Subjects will be randomized in a 1:1 fashion to azithromycin (500 mg three times per week) or matched placebo.

Between Visit 1 (Day -14) and Visit 2 (Day 0), a two-week run-in period will be used to begin administration of either azithromycin or placebo. Among those randomized to azithromycin, the 2-week run-in period will be used to initiate azithromycin and standardize uptake prior to the start of the next TISP cycle. Among those randomized to placebo, the run-in period will be used as a standardized washout prior to the start of the next TISP cycle. Subjects will begin TISP at Visit 2 in addition to their continued dosing of randomized study drug treatment (azithromycin or placebo). Subjects will continue both TISP and study drug dosing for 28 days (up until Visit 3), which corresponds with the end of a clinically prescribed 4-week cycle of TISP. Subjects completing the study through Visit 3 (Day 28) will be offered participation and consent to an 8-week open-label extension period during which azithromycin will be provided. During the open-label period, subjects will be instructed to remain off of TISP for the first 4-week period, followed by a 4-week period of TISP. Total duration of subject participation will be up to 6 weeks for those enrolled in the randomized study and up to 98 days for subjects participating in the optional open-label extension. Subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for the study.

The primary objective of this study is to determine if azithromycin impairs the previously-recognized clinical benefits of inhaled tobramycin by comparing changes in pulmonary function as measured by FEV_1 between subjects randomized to azithromycin versus placebo.

The secondary objectives for the randomized period are to:

- Compare changes in patient-reported quality of life between subjects randomized to azithromycin versus placebo

The secondary exploratory objectives for the randomized period are to:

- Compare the safety profile between subjects randomized to azithromycin versus placebo
- Compare changes in additional spirometry measures, bacterial density, and weight between subjects randomized to azithromycin versus placebo
- Compare rates of pulmonary exacerbations, hospitalizations, and acute antibiotic usage between subjects randomized to azithromycin versus placebo

The objectives for those continuing in the open-label extension:

- Among those randomized to azithromycin and remaining on azithromycin, obtain additional data to determine the long term effect of azithromycin on clinical outcomes
- Among those randomized to placebo and switching to azithromycin, compare differences in clinical outcomes observed during the randomized and open label periods

1.2 Interim Data Safety Monitoring Board Reviews

Safety monitoring and study conduct will be assessed by a DSMB with members appointed by the NHLBI. The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures and for monitoring overall study conduct. Serious adverse events (SAEs) will be monitored on an ongoing basis. In addition to SAE monitoring, quarterly summary reports tabulating SAEs by treatment group will be provided to the DSMB. A comprehensive interim report will be provided to the DSMB when approximately 50% of patients have completed the randomized portion of the trial. The report will include an overview of enrollment by site, detailed summaries of all SAEs, AEs, withdrawals, drug discontinuations, hospitalizations, protocol violations and other clinical safety endpoints. Table shells detailing the results to be presented in the semi-annual reports are included in Appendix A (randomized period) and Appendix B (open-label period), whereas Appendix C will include listings of protocol violations and SAE narratives.

1.3 Statistical Monitoring Guidelines and Stopping Rules

While there are no pre-determined stopping rules for safety, the DSMB may decide to stop the study either temporarily or permanently if there are significant concerns regarding subject safety. There is however pre-specified stopping rule for efficacy/harm and futility with respect to the primary endpoint.

Should the DSMB consider stopping the trial based on the primary endpoint, they will be guided by a formal stopping rule to be assessed when approximately 50% of patients have completed the randomized portion of the trial. The primary endpoint is the difference between the azithromycin and placebo treatment groups in the relative change in FEV_1 (liters) from randomization at Visit 1 (Day -14) to Visit 3 (Day 28). We hypothesize that azithromycin is associated with poorer clinical outcomes (inferiority) as compared to placebo during concurrent administration of inhaled tobramycin and thus there will be a smaller improvement or a worsening in FEV_1 in the azithromycin treatment group as compared to placebo treatment from Visit 1 (Day -14) to Visit 3 (Day 28). Assuming a sample size of 110 subjects, two-sided type 1 error of 0.05, and a standard deviation for the relative change of 13%, the study has approximately 85% power to detect a difference (relative change in FEV_1 in the azithromycin treatment group minus the relative change in FEV_1 in the placebo treatment group) of less than or equal to -7.5% (e.g. azithromycin is clinically inferior to placebo).

For the purposes of calculating formal boundaries for stopping under the null and alternative hypotheses (no difference, and clinical inferiority of azithromycin, respectively), we will use the hypothesized treatment effect of -7.5%. Assuming a conservative O'Brien-Fleming boundary[1], the two-sided stopping rule would suggest that the trial be stopped early in favor of the alternative hypothesis (azithromycin inferiority) if the observed treatment effect is less than -9.8% at the interim review (n=55). Should there be evidence in the opposite direction where azithromycin is clinically superior to placebo, the trial will be stopped at the interim analysis when observed treatment effect is 9.8% or greater. The trial *could* stop under the null hypothesis if the observed treatment effect is greater than 0.05% but less than 9.8% *and the DSMB has simultaneous safety or accrual concerns*. If the trial was to continue to completion, the null hypothesis will be rejected in favor of the alternative if the observed treatment effect is less than or equal to -4.9% at the end of the trial.

The probability of stopping at the interim analysis if the true treatment effect is 0.0% is 0.016 (or less than 2%). Similarly, if the treatment effect is ±3.75% or ± 7.5% the probability of stopping is 0.05 and 0.26, respectively.

The actual stopping boundaries will be computed at the time of the comprehensive interim report to the DSMB. Thus, the actual stopping boundaries may differ from those provided above depending on the number of subjects evaluable for the primary endpoint. R RCTdesign software will be used to calculate the formal stopping boundary and adjust for this interim analysis in the final analysis.

2 Report Generation

2.1 Data Flow

An electronic data capture system, Medidata Rave, will be utilized for collection of study data. The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit or assessment into the protocol-specific electronic Case Report Form (eCRF). Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number and initials. If a correction is required for an eCRF, the time and date stamp will track the person entering or updating eCRF data and creates an electronic audit trail.

The data will be entered into a validated database. The DCC will be responsible for data processing, in accordance with procedural documentation. All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

Once data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

2.2 Screening Spirometry

The study sites were permitted to use their spirometers with their proprietary reference equations to determine study eligibility per FEV_1 (% predicted) inclusion criteria. Because database randomization was built using the FEV_1 (liters) as an input to GLI equations to calculate percent of predicted FEV_1 for stratification, minor discrepancies in resulting % predicted values would potentially prevent some patients from participating in the study.

With this in mind, the lower limit of inclusion criteria (25-50% predicted) was lowered to 20% predicted (as determined by GLI equations) in the database. Because this change was limited to database randomization mechanism and did not affect study inclusion criterion, no protocol amendment was issued. The sites were asked to document all FEV_1 % predicted calculation discrepancies in the investigator log.

2.3 Report Generation

The final statistical report will describe and justify any deviations from the original statistical plan described herein. Analyses will be performed using SAS 9.4 software or most current version of

R. All programs used to produce this report will be documented, tested, and archived and all tables, figures and listings will be validated before considered final.

2.4 Data Sets Analyzed

All analyses will be performed using a modified intent-to-treat (m-ITT) population, which is defined as all randomized participants who received at least one dose of study drug. Participants who are discontinued from study drug temporarily or permanently are encouraged to complete all remaining study visits and will remain in the analyses population according to ITT. The primary efficacy analyses will be repeated in the per-protocol population, which is defined as participants having completed ≥80% of doses (azithromycin and or placebo) and who did not require the use of acute antibiotics or steroids.

2.5 Definitions

Baseline – Baseline refers to Visit 1 (Day -14) when participants were randomized.

Study drug – During the randomized period, the study drug refers to the drug the participant was randomized to (either azithromycin or placebo). During the open-label period, the study drug refers to open-label azithromycin.

Treatment group – During the randomized period, the treatment group refers to participants randomized to either azithromycin or placebo. During the open-label period, the treatment group refers to participants either remaining on azithromycin or switching from placebo to azithromycin.

References

1. Emerson, SS and Fleming, TR. Symmetric group sequential test designs. *Biometrics.* 45:905-923, 1989.

Appendix A. Overview of Planned Analyses

A.1 Summary of Enrollment and Study Visit Completion

The cumulative enrollment of participants randomized into the study is graphically summarized. The number of participants screened, eligible, randomized, treated, withdrawn, and completing the study during the randomized period is summarized by both treatment group and site. Reasons for screen failure are summarized. Screen failures are participants that signed informed consent but did not meet eligibility criteria or chose not to participate in the study. The number of participants who withdrew early from the study is tabulated by treatment group and reason for withdrawal is summarized. Participant disposition figure detailing enrollment, treatment allocation, follow-up, and analyses populations is provided.

A.2 Demographics and Baseline Characteristics

Baseline demographics and clinical characteristics are descriptively summarized by treatment group and overall. Summarized characteristics include age, sex, CFTR genotype, race, height, weight, BMI, FEV_1 (liters and % predicted), delivery method of chronic tobramycin (i.e., dry powdered or inhaled), current use of chronic azithromycin, and use of other chronic medications. Fisher Exact tests are used to test at a two-sided 0.05 level of significance for differences in categorical variables across treatment arms. A two-sided t-test assuming unequal variance is used to test the difference between treatment arms for all continuous variables at a two-sided 0.05 level of significance.

A.3 Summary of Protocol Adherence Measures

The number of participants expected to have completed each visit and the number of participants who have missed a visit during the randomized period are summarized by treatment group. The follow-up time in the randomized period is summarized overall and by treatment group and also as the average time for each participant.

The proportion of participants who discontinued the study drug or experienced a dose modification during the randomized period is shown by treatment group. Reasons for drug discontinuation or other change to study drug regimen are also summarized.

Study drug compliance (azithromycin or placebo) is summarized in terms of the protocol defined treatment regimen during the randomized period. Compliance percentage is calculated for each participant as the number of doses reported taken divided by the number of doses expected (three 500 mg capsules per week over the six-week period, i.e. 18 doses), multiplied by 100. Also summarized is the compliance with TIS/TIP, calculated in a similar manner and assuming that the expected number of doses is 84 (inhaled twice daily over the six-week period). The average drug compliance is summarized in each treatment group. Also shown is the number and percentage of participants with ≥ 80% compliance.

The number of participants included in the per-protocol population is provided by treatment group are reasons for exclusions are listed. Per-protocol population is defined as all randomized

participants having completed ≥80% of doses of study drug (azithromycin or placebo) and who did not require the use of acute antibiotics or steroids.

A.4 Summary of Adverse Events

The safety population is used in all summaries of adverse events (AEs) and serious adverse events (SAEs). Note that all tables summarizing AEs also include SAEs (which are a subset of the AEs) unless otherwise specified.

According to our regulatory reporting criteria, a pulmonary exacerbation requiring a hospitalization results in an SAE (regardless of its expectedness). For the TDN trials, sites are asked to record the symptoms or physical findings associated with the pulmonary exacerbation as individual adverse events and to designate the seriousness of each of these events. Thus a single pulmonary exacerbation can be reported as several SAEs.

The incidence of AEs (including emergent abnormal QTc parameters) between the azithromycin and placebo treatment groups is tabulated by seriousness, severity, and relationship to study drug. The proportion of participants with at least one (S)AE, the average number of (S)AEs per patient, and the rate of (S)AEs per day of follow-up are reported. Poisson regression modeling is used to derive rate ratios and corresponding 95% confidence and to compare groups using a using a two-sided 0.05 level test. The difference in the proportion of participants experiencing at least one (S)AE between treatment groups is also reported, with corresponding 95% confidence intervals calculated using the Newcombe-Wilson method and p-values derived from a Fisher's exact test. Histograms showing the frequency of the number of (S)AEs in each treatment group are included.

All reported SAEs and AEs are coded using MedDRA and grouped by body system. Detailed summary tables of individual (S)AEs grouped by SOC are included to show the number and percent of participants experiencing each (S)AE by treatment group. The number and rate of events for each (S)AE is also reported by treatment group. Poisson regression is used to derive rate ratios and corresponding 95% confidence intervals comparing treatment arms for each SOC. Treatment groups are compared by the proportion of participants experiencing (S)AEs by System Organ Class (SOC) as well as the difference in proportions with corresponding 95% confidence intervals to compare treatment arms.

All SAE narratives are included in a listing appended to this report.

A.5 Summary of Spirometry Results

The primary endpoint is the difference between the azithromycin and placebo treatment groups in the relative change from Visit 1 to Visit 3 in FEV1 (liters). The primary efficacy analysis is conducted on the m-ITT population and repeated on per-protocol population. Predicted values for spirometry measures at each visit are calculated using the Global Lung Initiative reference equations.

A linear regression model adjusted for randomization strata is used to compare the primary endpoint between treatment groups. Least squares means and the treatment effect are presented

along with corresponding 95% confidence intervals and p-values. The primary analysis is adjusted for the group sequential monitoring.

The primary endpoint is also summarized descriptively as a plot of average values in each treatment group at each post-randomization visit accompanied by 95% confidence intervals (using t-distribution approximation).

Additional secondary analysis may be performed to adjust for potential baseline confounders including age, sex, use of CFTR modulators, and baseline FEV1. To address missing data, the least favorable treatment arm imputation method is used to impute that missing value with the mean change from the treatment arm with the worst change in the observed case analysis. In further sensitivity analyses, the primary endpoint is summarized for a select number of baseline covariates including age, sex, baseline FEV1, chronic azithromycin use, and inhaled tobramycin formulation.

The exploratory spirometry endpoints, i.e. absolute change from Visit 1 to Visit 3 in FEV1 (liters and % predicted), absolute and relative change in FVC (liters and % predicted), and absolute and relative change in FEF25-75% are summarized descriptively as plots of mean values and corresponding 95% confidence intervals over time.

Additionally, changes in spirometry measures from Visit 1 to Visit 3 and Visit 2 to Visit 3 are modeled using linear regression adjusted for randomization strata. The estimates of least squares means, differences in the least squares means between the treatment groups, and corresponding 95% confidence intervals and p-values are presented.

All of the spirometry measures are summarized at each study visit during the randomized period and as a change from Visit 1 and Visit 2. The differences between treatment groups are presented with accompanying 95% confidence intervals and p-values calculated using two-sample t-tests.

Post-hoc comparisons of participants responding and not responding to monotherapy are summarized. Several ‘responder’ definitions are considered, e.g. XX% or greater relative increase in FEV1 % predicted from Visit 1 to Visit 3, XX% or greater relative increase in FEV1 % predicted from Visit 2 to Visit 3, 11 or greater point decline in CFRSD-CRISS from Visit 1 to Visit 3, and a XX point increase in CFQ-R respiratory symptom scale.

A.6 Pulmonary Exacerbations, Hospitalizations and Antibiotic Usage

The number of pulmonary exacerbations and hospitalizations is descriptively summarized. Proportions of participants initiating intravenous, inhaled, and oral antibiotics during randomized period are also presented. The differences in the proportions of participants experiencing at least one event between treatment groups are estimated with accompanying 95% confidence intervals calculated using the Newcombe-Wilson method and p-values derived from a Fisher’s exact test.

A.7 Summary of Weight

Mean changes in weight (kg) from Visit 1 to each post-randomization visit for both treatment groups during the randomized period are summarized descriptively in a figure. 95% confidence intervals (using t-distribution approximation) are included at each time point. The number of participants at each time point is included in a legend below the figure.

A linear regression model adjusted for randomization strata is used to compare changes in weight (kg) between treatment groups. Least squares means and the treatment effect are presented along with corresponding 95% confidence intervals and p-values.

The weight (kg) is also summarized at each study visit during the randomized period and as a change from Visit 1 and Visit 2. The differences between treatment groups are presented with accompanying 95% confidence intervals and p-values calculated using two-sample t-tests.

A.8 Summary of Patient-Reported Quality of Life Measures

Mean changes in CFRSD-CRISS and CFQ-R Respiratory Symptom Scale (RSS) from Visit 1 to each post-randomization visit for both treatment groups during the randomized period are summarized descriptively in figures. 95% confidence intervals (using t-distribution approximation) are included at each time point. The number of participants at each time point is included in a legend below the figures.

Linear regression models adjusted for randomization strata are used to compare changes in CFRSD-CRISS and CFQ-R RSS between treatment groups. Least squares means and the treatment effect are presented along with corresponding 95% confidence intervals and p-values.

CFRSD-CRISS and CFQ-R RSS are also summarized at each study visit during the randomized period and as a change from Visit 1 and Visit 2. The differences between treatment groups are presented with accompanying 95% confidence intervals and p-values calculated using two-sample t-tests