CLINICAL RESEARCH STATISTICAL ANALYSIS PLAN
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A Randomised, Double-Blind, Placebo-Controlled Trial of the Safety and Efficacy of Topical Alicaforsen Enema in Subjects with Active, Chronic, Antibiotic Refractory Primary Idiopathic Pouchitis

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1 STATISTICAL CONSIDERATIONS

A detailed and comprehensive Statistical Analysis Plan (SAP) will be prepared and signed-off before database lock. Minor changes to the statistical methods set out in this protocol

need not be reported as a protocol amendment but must be documented in the SAP and in the study report.

The statistical analysis and report will conform to the relevant ICH requirements.

1.1 STATISTICAL METHODS

1.1.1 Summary Tables and Listings

The standard summary statistics for continuous baseline and outcome variables are: N, mean, standard deviation, median, quartiles, minimum and maximum.

The standard summary statistics for categorical baseline and outcome variables are: count and proportion (expressed as percentage of non-missing values).

All efficacy outcomes, vital signs and laboratory assessments will be summarised by treatment group and timepoint. Baseline and demographic data will be summarised by treatment group.

The summary tables and figures will be supported by full subject listings.

1.1.2 Hypothesis Testing and Confidence Intervals

All significance probabilities will be two tailed. Statistical tests will be at a 5% level of significance.

Confidence intervals will be 95% two-sided.

1.1.3 Randomisation and Stratification

The center will dispense the study medication under blinded conditions according to a centrally administered, permuted block randomisation sequence (1:1 ratio for the treatment arms), managed at the study level and implemented using IWRS.

1.1.4 Subject Population/Sets to Be Evaluated

It is expected that there will be an average of 3 to 4 subjects recruited per site, and hence stratification by study site could be potentially unblinding with respect to the block size given the expected difference in response rates between the two study arms. In accordance with recommendations included in ICH E9 guidelines, the randomisation will therefore be stratified by region, i.e. North America (US and Canada) and Rest of World (Europe, including Israel).

All Randomised Set

The All Randomised Set comprises all subjects which have been randomised.

Safety Set

The safety analysis (SAF) set will consist of all randomized subjects who took at least a part of one dose of study drug, and will be used for all analyses of safety.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized subjects with confirmed active pouchitis at baseline, and who took at least one dose of study drug and have at least one post-

baseline assessment of efficacy. The FAS will be the primary analysis set for analyses of efficacy.

Per Protocol Set

The per protocol set (PPS) will comprise all subjects in the FAS who have not violated any major entry criteria and have not deviated significantly from the protocol during the course of the study. Any efficacy analyses from this population will be considered as supportive to those performed on the FAS. Reasons for exclusion from the PPS will be defined prospectively in the statistical analysis plan.

Pharmacokinetically Evaluable Analysis Set

The Pharmacokinetically Evaluable Analysis Set (PKAS) comprises all subjects receiving active treatment with sufficient blood samples taken to establish a pharmacokinetic profile and for whom the time of last dose prior to sampling is known.

The final determination of the membership of analysis sets will be made at the blinded data review meeting.

1.2 SAMPLE SIZE DETERMINATION

The sample size is based on being able to claim superiority of alicaforsen over placebo; at Week 10 separately for each of the two co-primary endpoints.

The primary analysis will be based on a Cochran-Mantel-Haenszel Test (CMH) stratifying by region. In addition, a difference in the proportion of subjects with treatment success (and the associated estimated 95% confidence interval) will be presented based on the Z-test.

For both co-primary end-points, it is assumed in the sample size calculation that 50% of patients on alicaforsen and 20% on placebo will have a successful treatment outcome. Based on a 1:1 design, 63 subjects per treatment group will be required to show superiority (alicaforsen to placebo odds ratio >1) using a two-sided CMH Test at the 5% significance level and with 95% power. For the same design, 69 subjects per treatment group will be required to show superiority (proportion of successes for alicaforsen minus placebo >0) using a two-sided z-test with continuity correction and pooled variance at the 5% significance level and with 95% power.

With 69 subjects per treatment group (138 in total), the study will therefore be powered to show superiority both in terms of the difference of proportions and the odds ratio (both calculations derived using PASS 13 Power Analysis and Sample Size Software (2014). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass).

To demonstrate the superiority of alicaforsen over placebo, both co-primary endpoints must be significant using a two sided CMH test at the 5% significance level. Therefore, a total sample size of 138 subjects will give an overall power of 90% (0.95 * 0.95) assuming independence of the two co-primary endpoints.

The randomization is stratified by region (North America vs Rest of World).

1.3 STATISTICAL ANALYSES

1.3.1 Demographic and Baseline Characteristics

Subject demographic and baseline characteristic data will be summarised using the standard summary statistics.

1.3.2 Efficacy Analysis

All efficacy analyses and summaries will be performed using the FAS. A confirmatory analysis of the primary efficacy parameter will be carried out using the PPS.

All statistical tests comparing the treatment groups will be two-tailed and at the 5% level of significance.

1.3.2.1 Co-Primary Efficacy Analysis

The co-primary measures of efficacy will be:

- The proportion of subjects who are in endoscopic remission (Endoscopic Score ≤1), and
- The proportion of subjects who achieve a Stool Frequency Score ≤1, at Week 10.

The proportion of subjects who achieve each of the co-primary measures of efficacy at Week 10 will be presented and compared between treatment groups using a Cochran-Mantel-Haenszel test accounting for region. The difference in proportions between the two groups and associated 95% confidence interval will also be presented.

Subjects with a missing endoscopic assessment at week 10, or insufficient stool frequency data, will be declared not to have achieved the respective outcomes.

Secondary analyses of the co-primary endpoints will include investigations of the effect of treatment and baseline characteristics (region, treatment, sex, age group, PDAI score) on the co-primary endpoints using logistic regression models.

1.3.2.2 Secondary and Tertiary Efficacy Analyses

Stool Frequency

The mean daily stool frequency (frequency of bowel movement) will be evaluated from the data recorded in the subject's diary using the data from the 7 days prior to each study visit. Mean frequency and change from baseline will be summarized.

The change from baseline at each study visit will be analyzed using a mixed effects repeated measures analysis of covariance (ANCOVA) model of the change from baseline with region, treatment, Visit, treatment*Visit interaction, sex and age group as fixed effects and baseline value as covariate.

The proportion of subjects with a Stool Frequency Score equal to 0 ('Normal number of stools per day for the subject') will be summarized and analyzed in the same way as for the co-primary endpoints.

The time to clinical remission will be summarized using Kaplan-Meier methods. Subjects without remission, (including those who withdrew from the study without remission) will be censored at the time of last assessment of the Stool Frequency Score. Kaplan-Meier curves

will be presented with estimates of the probability of remaining remission-free at Weeks 3, 6 and 10 together with their 95% confidence intervals. The time to clinical remission will be investigated using a Cox proportional hazards regression model including terms for region, treatment, sex, age group, and the baseline mean number of bowel movements per day.

Urgency

Urgency will be recorded for each bowel movement in the subject's diary as none (0), some urgency (1), great urgency (2), and subject soiled themselves, involuntarily (3). The frequency of each score in the 7 diary days prior to a study visit will be evaluated.

Changes from baseline in urgency score at week 6 will be summarized and compared between treatments.

The proportion of subjects recording urgency episodes with score 3 (fecal incontinence) after 3, 6 and 10 weeks of treatment will be analysed using logistic regression models including terms for region, treatment, sex, age group and baseline urgency.

Rectal Bleeding

The frequency of rectal bleeding will be evaluated from the data recorded in the subject's diary for the 7 days prior to each study visit.

Changes from baseline in rectal bleeding at Week 6 will be summarized and compared between treatments.

PDAI

The PDAI will be assessed at Day 1 and Weeks 6 and 10. The separate domain scores and the overall score, together with their change from baseline, will be calculated at each visit and summarized by treatment group.

The change from baseline for each PDAI domain score and for the overall score, will be analysed using the same repeated measures ANCOVA model as for the mean number of bowel movements per day.

Estimates of the following proportions of subjects will be evaluated at Weeks 6 and Weeks 10:

- Those who achieve an overall PDAI score <5
- Those who achieve an overall PDAI score <3
- Histological Improvement, defined as a PDAI Histology Score <=1 (for subjects with a baseline score >1), or with at least a 2-point improvement from baseline
- Histological Healing, defined as a PDAI Histology Score of 0 for subjects that had a baseline subscore above 0.

Each of these proportions will be compared between treatment groups at each visit using a Cochran-Mantel-Haenszel test accounting for region. In addition, they will be analyzed using separate logistic regression models for Week 6 and Week 10. These models will include terms for region, treatment, sex, age group and the corresponding baseline PDAI score.

Quality of Life Questionnaire

Quality of life is assessed using the CGQL (at baseline and Weeks 6, 10, 18 and 26) and EQ-5D-5L (at baseline, Week 6 and Week 10).

The change from baseline for each CGQL and EQ-5D domain score, and for the overall scores, will be summarized by study visit.

The Total CGQL Score and the EQ-5D Index score will be analysed using the same repeated measures ANCOVA model as for the mean number of bowel movements per day.

Pouchitis intervention

The proportion of subjects, by week 26, who have not received any additional pouchitis treatment will be calculated and compared between the two treatment groups.

The time to first intervention (including POUCHITIS related surgery) will be calculated for each subject. The time will be calculated in days from the start of study treatment.

Subjects not requiring a rescue intervention during their treatment period will be censored at the time of stopping study treatment in the analysis. Kaplan-Meier curves will be presented and estimates of the probability of no "rescue" intervention at Weeks 3, 6 and 10 will be provided together with their 95% confidence intervals.

In addition, time to first "rescue intervention" will be analyzed using a Cox's proportional hazards regression model including terms for region, treatment, sex, age group, and the baseline PDAI score.

1.3.2.3 Exploratory

The exploratory analysis will include, but will not be limited to, the exploratory analyses specified in this protocol. Any exploratory analyses performed which were not specified in the protocol or the statistical analysis plan will be clearly described as such in the clinical study report.

Histology

The change from baseline in histological score, assessed using the D'Haens score, will be measured at baseline, Weeks 6 and 10. Changes from baseline will be summarized.

Inflammatory markers

The inflammatory marker CRP and WBC will be measured at Screening, Day 1 and Weeks 3 and 6. The change from baseline to Weeks 3 and 6 will be analysed

Faecal calprotectin will be measured at screening, Weeks 3, 6, 10, 18 and 26. The change from baseline will be summarized.

WPAI

Scores derived from the WPAI (i.e. the percentage of worktime missed due to health issues, percentage of work impairment due to health issues, percentage activity impairment due to health issues, and the percentage productivity loss) will be suitably summarized by treatment group at each study visit.

1.3.2.4 Subgroup analysis

The primary endpoint will also be analysed in the following subgroups for consistency with the overall trial results.

- Gender
- Age group

Subjects who have previously failed antibiotics will be further categorized into those who were "efficacy" failures (failed to respond to initial therapy, or lost response to continued therapy) and those who failed due to intolerance. An appropriate summary/analysis of these subjects groups will be performed.

1.3.3 Safety Analysis

Safety will be assessed using the SAF.

1.3.3.1 The number and proportion of subjects with AEs

Adverse Events will be coded using the MedDRA classification to give a preferred term and primary system organ class for each event. Proportions of subjects with adverse events will be presented by treatment group. Tables of AEs will be presented by system organ class, and preferred term, and include overall totals for AEs within each system organ class. Counting will be done by subject and not by event. A table of counts and percentages will also be made of those subjects with SAEs or AEs which led to withdrawal from the study.

Treatment-emergent AEs are defined as adverse events that had an onset day and time on or after the day and time of the first dose of study drug up to 30 days after the last application of treatment. Adverse Events having missing onset dates will be considered as treatment emergent.

Worsening of pouchitis will be evaluated as a separate event.

1.3.3.2 Laboratory Parameters

The number (%) of subjects with abnormal values post-baseline will be tabulated by treatment group. In addition, summary statistics (i.e., mean, minimum, maximum, standard deviation, and number of subjects) will be presented for each treatment group for each laboratory parameter at each visit. When calculating the summary statistics only, the last observation within a visit window will be used if there are multiple observations. Changes from baseline will be presented in a similar format. An additional listing will be provided of those subjects who have clinically significant laboratory values. The data will also be presented as shift tables and clinically significant abnormalities will also be examined.

1.3.3.3 Vital Signs

Abnormal vital signs will be counted by treatment group. The number (%) of subjects with any post-baseline vital sign readings above and/or below the specified levels will be presented for each treatment group. In addition, summary statistics (i.e., mean, minimum, maximum, standard deviation, and number of subjects) will be presented for each treatment group for each vital sign at each visit, comparing changes from baseline. When calculating the summary statistics only, the last observation within a visit window will be used if there

are multiple observations. The data will also be presented as shift tables and clinically significant abnormalities will also be examined.

1.3.3.4 Withdrawals

The number (%) of subjects who withdraw from the study and their reasons for withdrawal will be tabulated by treatment group. The distribution of withdrawals and reasons will also be displayed at each visit by treatment group

1.3.4 OPEN LABEL ACCESS ANALYSIS

The following safety analysis will be conducted in all subjects receiving at least a part of one dose

1. The number and proportion of subjects with AEs; (worsening of disease will also be evaluated separately)
2. Assessment of clinical laboratory parameters
3. Assessment of vital signs

The following efficacy analysis will be conducted in all subjects receiving at least 80% of a 42-night treatment course

1. Percentage of subjects who report response to open-label access alicaforsen.
2. Interval between previous and current treatment courses

1.4 BLINDED DATA REVIEW MEETING

The Sponsor will convene a Blinded Data Review Meeting after the data has been cleaned but before the study is unblinded. The review will be performed within the framework of the requirements of the ICH Guideline E9.

The terms of reference for the Data Review Meeting will include, but will not be limited to:

- the determination of whether protocol violations are 'major' or 'minor', or not a protocol violation at all;
- the allocation of subjects to analysis sets;
- a review of missing data and of outliers;
- a review of the distribution of the efficacy variables, considering any implications for the proposed method of statistical analysis;
- an assessment of the overall endoscopic remission rate;
- a review of whether additional covariates need to be included in the analyses;
- the finalisation of the SAP.

If the overall endoscopic remission rate indicates that the assumptions of the sample size calculation were not realized during the study then the Sponsor, taking the advice of the Data Review Meeting, may make one or more of the following changes to the primary analysis:

- changing the definition of the analysis set used;
- changing the definition of the primary endpoint;
- any other change deemed by the Sponsor as necessary to preserve the integrity of the study.

All persons taking part in the Data Review Meeting and all persons taking part in any deliberations concerning the format of the primary analysis must remain blinded to treatment allocation until any changes to the protocol specified analyses are documented and signed-off.

Formal records shall be kept of when the statistical analysis plan was finalized as well as when the blind was subsequently broken.

1.4.1 PK Parameters

Plasma PK parameters for alicaforsen will be evaluated as follows. All consenting subjects will provide single time-point plasma samples at Day 1, Week 3 and Week 6.

In addition, full plasma concentration-time curves will be generated from a subset of randomised subjects (24 subjects, approximately 12 on active treatment and 12 on placebo) at Day 1 and Week 6. Samples from subjects in the placebo group will NOT be analyzed.

In this subset of subjects, the following PK parameters, as a minimum, will be calculated for alicaforsen: C_{max}, t_{max}, $t_{½}$, AUC_t, $AUC_{0–24}$, $AUC_{0–\infty}$, and λ_z .

The following pharmacokinetic parameters will be derived:

C_{max} The peak plasma concentration.

t_{max} The time of peak plasma concentration.

$t_{½}$ The terminal half-life calculated from the terminal slope of the log concentration-time curve.

AUC The area under the concentration-time curve will be calculated using the trapezoid rule for the interval 0 to t_n h (time t_n is the time at which the last non-zero level was recorded), plus the area under the exponential curve from t_n h to infinity. The linear trapezoidal method will be used up to t_{max}, and the logarithmic method will be used for the plasma concentrations declining from C_{max}. The area from the time of last measured concentration to infinity will be extrapolated using the following formula:

$$AUC_{t_n-\infty} = \frac{\hat{C}_n}{\lambda_z}$$

where λ_z is the elimination rate constant calculated from non-linear regression of the terminal portion of the plasma concentration time curve, and $\hat{C}_n$ is the predicted value of the concentration at time t_n.

Alicaforsen concentration-time curves will be plotted for individual subjects and will be summarized at Day 1 and Week 6, with concentrations on both log and linear scales. Single time-point PK data will be tabulated and summarized for each subject at Day 1, Week 3 and Week 6. Summary statistics for these data will also be computed across subjects.