STATISTICAL ANALYSIS PLAN (SAP)

Short course antibiotic treatment in Gram-negative bacteremia:

A multicenter, randomized, non-blinded, non-inferiority interventional study

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1 INTRODUCTION

This document describes the Statistical Analysis Plan (SAP) for GNB5 trial, a multicenter, randomized, non-blinded clinical trial comparing shortened antibiotic treatment (5 days) with 7 days or longer antibiotic treatment in patients hospitalized with Gram-negative bacteremia with a urinary tract source of infection. It details the statistical method to be used and outlines the planned analyses for the main study.

2 ANALYSIS OBJECTIVES

These analyses will assess the efficacy and safety of shortened antibiotic treatment (5 days) in treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized immunocompetent adults compared to ≥7 days of antibiotic treatment and will be included in the clinical study report (CSR).

3 STUDY METHOD

3.1 Trial design

Investigator initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. Randomization will occur in equal proportion (1:1) no later than day 5 of efficacious antibiotic treatment as determined by antibiogram. Participants are stratified by center and etiology.

Intervention group will receive antibiotic treatment for 5 days. The control group will receive antibiotic treatment for a minimum of 7 days at the discretion of their treating physician.

3.2 Sample size

We anticipate short-term mortality to be 8% and failure to be 4%. This corresponds to an estimated event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.

Non-inferiority is defined as a difference or margin in the primary endpoint of up to 10%. Given an α of 5% and a β of 90% then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95% confidence interval will exclude a difference in favor of the longer course of antibiotics of more than 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.

A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the overall event rate falls outside the expected event rate of 12%, an SSR based upon blinded review of overall

data (i.e. without knowledge of the group-specific event rates) will be performed. If the overall event rate is lower than expected, then the final sample size will be reduced, using the original sample size formula and replacing the initial estimate with the observed rate. The non-inferiority margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event rate is higher than expected, the sample size will be increased correspondingly. Any sample size adjustment will be reported to the regulatory authorities as a protocol amendment.

3.3 Interim analysis

We will perform interim analyses after the recruitment of every 100 participants. This serves to evaluate primary endpoints and potential adverse events by an independent data and safety monitoring board (DSMB).

The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in the interim analyses will correspond to a p-value of 0.05 in the final analysis.

3.4 Framework

The primary outcome will be tested for non-inferiority. The secondary outcomes will be tested for superiority.

4 ANALYSIS SETS

Analyses will be conducted on the following data sets:

4.1 Intention-to-treat (ITT) Analysis Data Set

The ITT data set will include all randomized study participants who received at least one dose of study drug regardless of their compliance with the rules of the study. The ITT data set will be used for the analysis of all primary and secondary end points, and all safety-related analysis will be based on the ITT population.

4.2 Per protocol (PP) Analysis Data Set

The PP data set will include all randomized participants who received the full duration of study medication according to protocol. Participants with significant variations from the study protocol (e.g. ceasing study drugs early or withdrawal from study for any other reason) will be excluded from the PP

population. Minor procedural variations (e.g. failure to collect additional blood samples at inclusion) will not prelude patients from the PP analysis.

4.3 Protocol violations

All protocol violation occurring after randomization will be listed in the Clinical Study Report, tabulated by study ID and investigating center. Dropouts will be included in the ITT population.

5 ENDPOINTS

Definitions of study endpoints:

5.1 Primary Study Outcome

90-day survival without clinical or microbiological failure to treatment as defined:

1. All-cause mortality from day of randomization and until day 90, with day 1 defined as the date of the initiation of appropriate empiric antibiotic treatment.
2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by sequence analysis occurring from day of randomization and until day 90
3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48 hours due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected from the day of randomization and until day 90
    a. Distant complications of initial infection, defined by growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)
    b. Local suppurative complication that was not present at infection onset (e.g. renal abscess in pyelonephritis)

5.2 Secondary Study Outcomes

To compare shortened antibiotic treatment with longer antibiotic treatment on:

- All-cause mortality at days 14, 30 and 90
- Total duration of antibiotic treatment
- Duration and type of antibiotic treatment
- Total length of hospital stay
- Hospital re-admission within 30 and 90 days
- Antibiotic adverse events
- Use of and type of antimicrobials after discharge

- Severe adverse events grade ≥3
- Acute kidney injury
- *Clostridum difficile* infection
- Multidrug-resistance organism

6 STATISTICAL METHODOLOGY

6.1 Data validation

Data will be examined for missing values and outliers. Measures of central tendency and dispersion for continuous study parameters will be portrayed. Extreme or unexpected values will be examined individually for authenticity and data discrepancies addressed where appropriate. Additional audit and statistical checks will be performed as necessary.

6.2 Missing data

No imputation of missing data will be conducted. Only observed data will be included in the analyses.

6.3 Analyses on continuous variables

For continuous variables (e.g. age, duration of antibiotic therapy and hospital stay) results within the treatment arm will be summarized with the number of observations, medians and interquartile ranges or means and standard deviations, depending on distribution. Differences between the control group and interventional group will be calculated using the Wilcoxon Rank-sum test for nonparametric distribution or student's t test for parametric distribution.

6.4 Analyses on categorical variables

For categorical variables (e.g. gender, readmissions, mortality) results within the treatment group will be summarized with subject counts and percentages. For endpoints, risk ratios (RR) and the absolute risk difference will be calculated (with 95% confidence intervals). The control group, receiving longer antibiotic treatment, will be used as the reference group. P-values will be based on either Pearson's Chi-square tests or Fischer's exact test. Results may also be represented using forest plots in comparison to the non-inferiority margin, using the Miettinen-Nurminen method.

6.5 Endpoint analyses

Both ITT and PP population will be used for both primary and secondary efficacy analyses.

Non-inferiority must be met for the primary analysis of the ITT population for the shortened antibiotic treatment to be regarded as non-inferior to the longer antibiotic treatment. The findings in the PP population must be seen to be consistent in terms of direction and effect size estimates.

The secondary efficacy analyses will be adjusted for multiple testing.

6.6 Subgroup analyses

An analysis of the primary and secondary efficacy endpoints is proposed in the following subgroups:

1. Disease severity (given by qSOFA-score and Pitt bacteremia score)
2. Antibiotic group
3. Day of achieved clinical stability (defined as systolic blood pressure $\geq$ 90 mm Hg, heart rate $\leq$100 beats/min., respiratory rate $\leq$24/minute, peripheral oxygen saturation $\geq$ 90 %)
4. Resistant pathogens
5. Investigating center

7 STATISTICAL SOFTWARE

Statistical analyses will be performed using SAS Statistical Software and R Studio.