STATISTICAL ANALYSIS PLAN

TEMOCILINA VS MEROPENEM FOR THE TREATMENT OF BACTEREMIA DUE TO THIRD-GENERATION CEPHALOSPORIN-RESISTANT ENTEROBACTERALES: A PRAGMATIC, RANDOMIZED TRIAL

ACRÓNIMUM: ASTARTÉ

Nº EUDRACT:2020-000064-39

VERSION 2.0, 4th April 2021

1. INTRODUCTION

This is the Statistical Analysis Plan (SAP) for the "ASTARTÉ" trial, a pragmatic, investigator-driven, randomised, multicentre, open label clinical trial comparing temocillin with meropenem as targeted treatment of bacteremia due to third-generation cephalosporin-resistant Enterobacterales (3GCRE).

This SAP was developed and registered before the initiation of the study.

1.1 Study hypothesis

Targeted treatment of bacteremia due to 3GCRE with temocillin is not inferior in clinical efficacy to treatment with meropenem, and may therefore represent a therapeutic alternative in these infections that makes it possible to reduce the use of carbapenems.

1.2 Primary Study Objective

To demonstrate the non-inferiority in terms of efficacy of temocillin compared to meropenem in the targeted treatment of bacteremia due to 3GCRE Enterobacterales, measured as: clinical and microbiological at the test of cure (TOC), survival at day 28, no need to stop or change the treatment due to adverse events, perceived failure or superinfection; no need to prolong treatment beyond 14 days; and no recurrence until day 28.

1.3 Secondary Study Objectives

- To provide information about the efficacy and safety of temocillin and carbapenems in Proporcionar información específica sobre la eficacia y seguridad de temocilina y carbapenemes in patients with the target Infections in subgroups of patients frequently underrepresented in randomized trials, such as those with severe condition, elderly, immunodepressed and renal insufficiency.

- To provide Information about the pharmacokinetics and pharmacodynamics of temocillin in patients with bacteremia.
- To provide Information about the MIC distribution among 3GCRE Enterobacterales and their association with mechanisms of resistence.

1.4 Primary endpoint

The primary endpoint is “overall success”, composed by all of the following: (a) Clinical cure at TOC; (b) survival at day 298; (c) no need to stop or change the assigned treatment because of adverse Events, failure or superinfection; (d) no need to continue with the assigned drug after day 14; and (e) no recurrence until day 28.

The definitions for all the outcomes are specified in the study protocol. The TOC will be performed 7-10 days after the last day of antibiotic therapy.

1.5 Secondary endpoints

- 28-day all-cause mortality.
- Length of hospital stay and length of IV and total antibiotic therapy duration.
- Adverse Events (rate and severity).
- Development of resistance during therapy to the study drugs.
- Development of superinfections.
- Recurrence rate (relapses and reinfections) until day 28..
- Changes in SOFA scale.
- Among patients <70 year-old patients, changes in Barthel scale.
- Exploratory: temocillin serum levels (only at coordinating site), temocillin MIC in Enterobacterales according to mechanisms of resistance to 3GC

1.6 Interim Analyses

Interim analyses for safety are planned after recruitment of the first 50 and 150 patients.

The ability to recommend study termination is solely within the DSMB's discretion and judgement, without any influence of the trial investigators or any other party The DSMB may make a binding recommendation to terminate the study if there is substantial ethical concern as a result of any of the following:

a) Excessive AE in the intervention arm (in terms of frequency and/or severity). Trial termination can be recommended if it is considered that continuation may expose participants to unacceptable risk of harm.

b) Interim analysis providing statistical evidence of positive effect; the interim analysis performed with 50 or 150 participants should shows a difference in the rate of the primary endpoint in favor of the intervention at a type I error <0.003.

e) Interim analysis providing statistical evidence of futility. A conditional power approach calculated using Mehta and Pocock method; A conditional power ≤20% will be considered low enough to recommend termination of the trial on the basis of futility.

2. STUDY POPULATIONS

2.1 Intent-to-treat (ITT) population (ITT): all randomized patients. No specific analysis will be performed in this population.

2.2 Modified intention-to-treat population (mITT): all randomized patients who had been adequately included and have received at least one intravenous dose of antibiotics. The primary analysis and safety analysis will be performed in this population.

2.3 Per protocol population (PP): all randomized patients who received 4 full days of therapy with the assigned study drug.

2.4 Clinically evaluable population (CEP): patients with clinical evaluation at the test of cure (TOC) and assessment of mortality at day 28, or had had clinical failure or died before TOC.

3. ASSIGNMENT TO POPULATIONS AND PROTOCOL VIOLATIONS

Randomized patients considered to be as potentially inadequately included by the monitoring team will be reviewed by 2 investigators blinded to the assignment and outcome for a final decision. All protocol violations occurring after randomisation will be collected. The final assignment of participants to the mITT, PP and CEP will be made after reviewing the protocol violations prior to database lock, with assessors blinded to the outcomes.

4. DATABASE AND MISSING DATA

4.1 Database archive and validation

The study database will be exported from the electronic CRF to SPSS software, and will be maintained at FISEVI files. The Spanish Clinical Research Network (SCReN) team will monitor the data for coherence, missing values and outliers, according to their standard monitoring procedures. All corrections will be performed by the study team together with the external monitoring team before database lock.

4.2 Missing Data

Missing data for key study variables, if any, will be presented and compared across study arms. Missing data for secondary exposure or variables will be considered as such and presented. No imputation of missing data is planned.

5. STATISTICAL ANALYSIS

5.1 Primary endpoint

The primary endpoint will be collected as a dichotomus variable (yes/no). The absolute difference in the proportion of overall success in the study arms (with one-sided 95% CIs) in the mITT population will be calculated, with the meropenem arm as the reference group. The specific reasons for not reaching overall success (failure, withdrawals, recurrence, death, etc.) will be specified in both study arms.

5.2 Secondary endpoints

The absolute difference with one-sided 95% CI in rates of 28-day all-cause mortality and adverse events will be evaluated in the mITT population. Additionally, these endpoint and cure rate will be evaluated in the PP population. The rates of resistance development, superinfections, and recurrences will be evaluated in the CE populations; the absolute difference with 95% CI in mean length of hospital stay and length of IV and total antibiotic therapy duration, and of Barthel scale (in patients >70 years) will be calculated in the CE populations. The absolute difference with 95% CI in mean change of SOFA scale from recruitment day 1 to day 3, and of Barthel scale from recruitment to TOC (among patients <70 years) will be assessed in the CE population.

5.3 Subgroup analyses

The primary endpoint will be analysed in the following subgroups:

- Age, ≤80 and >80 years
- Males and females
- In vitro active/inactive empirical treatment
- Charlson index ≤2 and >2 points
- Source of bacteremia (urinary tract, biliary tract, other intraabdominal infections, skin and skin structure infections, unknown source, vascular catheters, others)
- Non/severe sepsis
- Community/non-community acquired infection
- Temocillin MIC (breakpoint for subgroups not established, will be settled according to distribution found)

5.4 Multivariate analysis

A multivariable analysis using logistic regression will be performed to estimate the impact of treatment on the primary endpoint, including sites as fixed or random effects and all other covariables with a bivariable 2-sided P <0.20.

Variables not improving the model fit as assessed by using Akaike's information criteria were excluded using a stepwise method.

5.5 DOOR/RADAR analysis

In order to provide additional information of the different components of the primary endpoint, an analysis with desirability of outcome ranking (DOOR) and response adjusted for duration of antibiotic risk (RADAR) will be performed. First, patients will be classified according to the following ordinal system:

Category	Death	Severe adverse event	Failure or hospital stay >10 days after randomization	Recurrence	Mild or moderate adverse event
1	No	No	No	No	No
2	No	No	No	No	Yes
3	No	No	No	Yes	Yes or no
4	No	No	Yes	Yes or no	Yes or no
5	No	Yes	Yes or no	Yes or no	Yes or no
6	Yes	Yes or no	Yes or no	Yes or no	Yes or no

Second, in case of tie, the duration of total antibiotic duration will be considered; patients with a shorter duration will be given a better category. Finally, the probability of having a worse ranking among patients treated with temocillin than among patients with meropenem will be calculated; the upper bound of the 95% CI of the probability should be >50% to suggest non-inferiority of temocillin.