A Multicenter, Randomized, Double-blind, Bismuth-containing Quadruple Active Comparator-controlled Phase 3 Clinical Trial to Evaluate the Efficacy and Safety of Rifasutenizol (TNP-2198) in Combination with Rabeprazole and Amoxicillin in Treatment-Naïve Participants with H. pylori Infection

Protocol Synopsis

Protocol No.: TNP-2198-07

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SYNOPSIS OF PROTOCOL

Study Title: A Multicenter, Randomized, Double-blind, Bismuth-containing Quadruple Active Comparator-controlled Phase 3 Clinical Trial to Evaluate the Efficacy and Safety of Rifasutenizol (TNP-2198) in Combination with Rabeprazole and Amoxicillin in Treatment-Naïve Participants with H. pylori Infection

Indication: H. pylori infection

Development Phase： Phase 3

Study Objectives

Primary objective

- To evaluate whether the eradication rate of Rifasutenizol (TNP-2198) in combination with rabeprazole sodium and amoxicillin is non-inferior to bismuth quadruple regimen in treatment-naïve participants with Helicobacter pylori infection (if non-inferiority conclusion is established, it will be further assessed whether it is superior to bismuth quadruple regimen).

Secondary objectives

- To evaluate the efficacy of Rifasutenizol (TNP-2198) in combination with rabeprazole and amoxicillin in treatment-naïve participants with H. pylori infection based on the results of H. pylori culture and drug susceptibility testing.
- To evaluate the safety of Rifasutenizol (TNP-2198) in combination with rabeprazole and amoxicillin in treatment-naïve participants with H. pylori infection.
- To evaluate the pharmacokinetic profile of Rifasutenizol (TNP-2198) when administered in combination with rabeprazole and amoxicillin.

Study Endpoints

Primary endpoint:

- Eradication rate of H. pylori (based on the test results of ^{13}C-UBT) 4-6 weeks after the last dose of the study drugs.

Secondary endpoints:

- Safety: incidence of adverse events during the study, relationship with the study drugs and severity, laboratory tests (blood chemistry, hematology, and urinalysis), vital signs, electrocardiogram (ECG), physical examination.
- Pharmacokinetic (PK): PK parameters of Rifasutenizol (TNP-2198):
  - PK parameters of the first dose: $T_{max,}$ C_{max}, $t_{1/2,}$ $AUC_{0\text{-}tau,}$ $AUC_{0\text{-}\infty,}$ Vd/F, and CL/F;
  - PK parameters of the multiple-dose: $T_{max,\ ss}$; $C_{max,\ ss}$; $t_{1/2,\ ss}$; $AUC_{0\text{-}tau,\ ss}$; $AUC_{0\text{-}\infty,\ ss}$; Vd/F_{ss}; CL/F_{ss}; and R_{ac}.

Methodology/Study Design

This is a multi-center, randomized, double-blind, bismuth-containing quadruple active comparator-controlled Phase 3 clinical study to evaluate the efficacy and safety of Rifasutenizol (RSZ, formally known as TNP-2198) in combination with rabeprazole and amoxicillin in treatment-naïve participants with H. pylori infection using an adaptive design with sample size re-estimation. The status of H. pylori infection during the Screening Period is determined by ^{13}C-urea breath test (^{13}C-UBT) and confirmed by gastroscopic biopsy histology.

Eligible subjects will be randomly assigned to test group or control group at a 1:1 ratio stratified by study site, and will receive RSZ capsules, rabeprazole sodium enteric-coated tablets, amoxicillin capsules combined with clarithromycin placebo tablets and bismuth potassium citrate placebo capsules (test group), or bismuth-containing quadruple regimen of amoxicillin capsules, clarithromycin tablets, rabeprazole sodium enteric-coated tablets and bismuth potassium citrate capsules combined with RSZ placebo capsules (control group) for 14 consecutive days. ^{13}C-UBT will be performed 4-6 weeks after the last dose to evaluate the eradication effect of H. pylori.

This study will also conduct a PK study at one study site to evaluate the PK profile of RSZ when administered in combination with rabeprazole and amoxicillin. A minimum of 20 subjects and a maximum of 40 subjects will be enrolled in the PK study at this study site, and randomly assigned to the test group and the control group in a 1:1 ratio. For the enrolled subjects, PK blood samples will be collected before and after the morning administration on Day 1 (D1) and D14 (1-3 day(s) earlier if necessary).

Number of Subjects

According to the study results of clinical trials for H. pylori eradication in China and abroad, it is estimated that the eradication rate of H. pylori with 14-day bismuth-containing quadruple therapy (amoxicillin, clarithromycin, rabeprazole and bismuth potassium citrate) is about 85%. If the eradication rates of H. pylori in the test group and the control group are both 85%, Type I error is 0.025 (one-sided), power is 90%, a non-inferiority margin is -10%, and a dropout rate is about 15%, the required sample size is about 700 subjects (350 and 350 subjects in the test group and the control group, respectively, including the subjects participating in the PK study). Considering the uncertainty of the treatment effect, an adaptive design with sample size re-estimation is planned in this trial. One interim analysis is planned will be performed by an independent data monitoring committee (IDMC) when the primary endpoint data of 50% of the subjects are obtained. If the eradication rate of H. pylori observed in the test group is less than 10% in the control group, the trial can be terminated due to futility. In addition, sample size re-estimation will be performed during the interim analysis, and the re-estimation of sample size will be based on the Promising-Zone method (2011). The actual sample size will depend on the effect size or p-value observed during the interim analysis. Up to 1200 subjects are planned to be enrolled in this study. If the eradication rate of H. pylori in the test group 4-6 weeks after the end of treatment is non-inferior to that in the control group, the superiority comparison between the test group and the control group will be further performed.

A Promising-Zone method is used for sample size re-estimation and power calculation to strictly control the Type I error. Statistical simulation results indicate that the adaptive design will provide 97.3% power to test non-inferiority if the efficacy (eradication rate of H. pylori) is 85% in the test group and 85% in the control group at a non-inferiority margin of -10%. If the efficacy is 82% in the test group and still 85% in the control group, the adaptive design will provide 77.1% power to test for non-inferiority. If the efficacy of the test group is higher than the estimated value, such as 90% eradication rate in the test group and 83% eradication rate in the control group, the design will provide approximately 78% power to test for superiority. If the eradication rate is 90% in the test group and 84% in the control group, the design will still provide approximately 66.1% power to test for superiority.

Inclusion and Exclusion Criteria

All of the following criteria must be met for inclusion:

1) Voluntarily sign the informed consent form.
2) Age 18-65 years (inclusive), male or female.
3) The result of ^{13}C-UBT is positive (≥ 4 Delta Over Baseline), and the infection of H. pylori are confirmed by gastroscopic biopsy histology.
4) Subjects agree to refrain from taking any antibiotics or traditional Chinese medicines with antibacterial effect, bismuth, and antacids (such as PPI, H_2 receptor blocker, P-CAB) other than the study drugs during the Screening Period until the end of the study (Visit 5, i.e., Efficacy Evaluation Visit).
5) Subjects and their heterosexual partners must agree to have no pregnancy plan and voluntarily take effective contraceptive measures during the trial and for at least 6 months after the end of the study medication.
6) Willing to follow and able to complete all trial procedures.

Subjects will not be enrolled in the study if they meet any of the following exclusion criteria:

1) Allergy to any of the study drugs (rabeprazole, amoxicillin, clarithromycin, bismuth potassium citrate), allergic constitution (multiple drug and food allergies); or any contraindication to the use of rifamycin, nitroimidazoles or study drugs.
2) History of H. pylori eradication therapy (including participation in other clinical trials for H. pylori eradication).
3) Subjects with confirmed tuberculosis (TB) or Mycobacterium avium complex (MAC) infection or a history of TB or MAC infection.
4) History of dysphagia or any gastrointestinal disorder affecting drug absorption.

5) History of obstruction pyloric; or excessive gastric acid secretion (such as Zollinger-Ellison syndrome).
6) History of gastric cancer.
7) History of neoplasm malignant within 5 years prior to screening, with the exception of basal cell carcinoma or carcinoma cervix in situ treated without evidence of recurrence.
8) History of esophageal or gastric surgery, except for simple repair of the perforated ulcer.
9) History of substance abuse or drug use within 5 years prior to screening.
10) Alcohol abuse or a history of alcohol abuse within 5 years prior to screening (average weekly consumption of ≥ 14 units of alcohol: 1 unit = 285 mL of beer, or 25 mL of spirits, or 100 mL of wine/Chinese rice wine/rice wine);
11) Presence of active gastric and/or duodenal ulcer.
12) Anticoagulant therapy or long-term treatment with nonsteroidal anti-inflammatory drugs.
13) Treatment with any other investigational new drugs within 4 weeks prior to the Screening Period.
14) Any prohibited medications or non-drug therapies as specified in the protocol.
15) White blood cell count or neutrophil count below the lower limit of normal range.
16) Anemia (hemoglobin < 90 g/L).
17) Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, or serum creatinine above the upper limit of normal range.
18) Test positive for hepatitis B surface antigen, hepatitis C antibody, AIDS antibody, or microspironema pallidum antibody.
19) Abnormal ECG with clinical significance.
20) Female subjects who are pregnant, lactating, or have a positive urine pregnancy result during the Screening Period.
21) Inability to communicate with the Investigator and to comply with the study requirements.
22) Other conditions considered inappropriate to participate in this study by the Investigator, e.g., the subject has a history of severe central nervous system, cardiovascular, pulmonary, hepatic, renal, metabolic, gastrointestinal, urological, endocrine, or hematological diseases, or has clinical manifestations of these diseases.

Study Drug, Dose, Dosage Form and Mode of Administration

Test group

Administered for 14 consecutive days as follows:

- RSZ capsules, 400 mg, BID, taken orally within half an hour after breakfast and dinner
- Rabeprazole sodium enteric-coated tablets, 20 mg, BID, taken orally within half an hour before breakfast and dinner
- Amoxicillin capsules, 1 g, BID, taken orally within half an hour after breakfast and dinner
- Clarithromycin placebo tablets, BID, taken orally within half an hour after breakfast and dinner
- Bismuth potassium citrate placebo capsules, BID, taken orally within half an hour before breakfast and dinner

Control Group

Administered for 14 consecutive days as follows:

- Amoxicillin capsules, 1 g, BID, taken orally within half an hour after breakfast and dinner
- Clarithromycin tablets, 500 mg, BID, taken orally within half an hour after breakfast and dinner
- Rabeprazole sodium enteric-coated tablets, 20 mg, BID, taken orally within half an hour before breakfast and dinner
- Bismuth potassium citrate capsules, 240 mg, BID, taken orally within half an hour before breakfast and dinner
- RSZ placebo capsules, BID, taken orally within half an hour after breakfast and dinner

Duration of Subject Participation in the Study

Planned total duration of study participation: up to approximately 70 days, i.e., 14 days of Screening, 14 days Treatment Period, and up to 42 days of Follow-up.

Analysis Population

Intention-to-treat (ITT): includes all randomized subjects and will be analyzed according to scheduled treatment groups.

Modified intention-to-treat (mITT): includes all subjects in the ITT who received at least one dose of study drugs and will be analyzed according to scheduled treatment groups.

Micro-ITT: includes all subjects in the mITT set who have a positive pre-dose H. pylori culture and a drug susceptibility testing result and will be analyzed according to scheduled treatment groups.

Per-protocol (PP): includes all subjects without major protocol violations affecting efficacy analysis, who are randomized and receive more than 75% (including 75%) of the total amount of each study drugs and have ^{13}C-UBT performed 4-6 weeks after the last dose of study drugs and will be analyzed according to scheduled treatment groups.

Safety set (SS): includes all randomized subjects who received at least one dose of study drug and will be analyzed according to actual treatment groups.

Pharmacokinetic concentration set (PKCS): includes all randomized subjects who have received RSZ capsules and have at least one valid observed PK concentration data and will be analyzed according to actual treatment groups.

Pharmacokinetic parameter set (PKPS): includes all randomized subjects who have received RSZ capsules and have at least one valid PK parameter data and will be analyzed according to actual treatment groups.

Efficacy Evaluation

Efficacy analysis and estimands:

The efficacy endpoint of the study is the eradication rate of H. pylori 4-6 weeks after the last dose of the study drug, and the analysis of the mITT population is the primary analysis of the study.

Depending on the study objectives and study design, the following primary estimands are specified:

	Primary estimand
Population	Treatment-naïve participants with H. pylori infection who meet the inclusion and exclusion criteria
Variable	Eradication of H. pylori (based on the test results of ^{13}C-UBT) 4-6 weeks after the end of treatment
Treatment	RSZ capsules (400 mg) administered BID in combination with rabeprazole sodium enteric-coated tablets (20 mg), amoxicillin capsules (1 g), clarithromycin placebo tablets, and bismuth potassium citrate placebo capsules, or bismuth-containing quadruple regimen plus RSZ placebo capsules; Continuous treatment for 14 days
Intercurrent events	Intercurrent events affecting the assessment of the primary endpoint include, but are not limited to: • Discontinuation of study treatment • Events of concomitant medications and non-drug therapy during the study that may affect the test results of the post-treatment ^{13}C-UBT Detailed descriptions of intercurrent events, missing data, related to the estimands will be provided in the statistical analysis plan.
Population level summary	Eradication rate of H. pylori at 4-6 weeks after the end of treatment in the test group and the control group

In addition, subgroup analysis of efficacy endpoints will also be performed based on drug susceptibility testing results: based on the baseline H. pylori culture and drug susceptibility testing results, the patients will be grouped according to the resistance status (resistant or sensitive) to rifabutin, amoxicillin, clarithromycin, metronidazole, tetracycline, furazolidone and levofloxacin, and the eradication rates of H. pylori at 4-6 weeks after the last dose of the test group and the control group in each subgroup will be analyzed.

Safety Evaluation

Safety endpoints: incidence of adverse events (AEs) during the study, relationship with the study drugs and severity, laboratory tests (hematology, urinalysis, blood chemistry), vital signs, ECG, physical examination.

Pharmacokinetics (PK)

For subjects participating in the PK study, PK blood samples (4 mL each) will be collected in the ethylenediamine tetraacetic acid (EDTA) anticoagulant tubes within 30 minutes before the first study drugs administration in the morning and 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 hours (before the second RSZ capsules/placebo administration on the same day) after RSZ capsules/placebo administration on D1 and D14 (1-3 day(s) earlier if necessary). The allowable time window for blood sampling is ± 0. 5 hours.

- PK parameters of the first dose include: $T_{max,}$ $C_{max,}$ $t_{1/2}$, $AUC_{0\text{-}tau,}$ $AUC_{0\text{-}\infty}$, Vd/F, and CL/F.
- PK parameters of the multiple-dose include: $T_{max,\ ss;}$ $C_{max,\ ss}$; $t_{1/2,\ ss}$; $AUC0_{\text{-}tau,\ ss}$; $AUC_{0\text{-}\infty,\ ss}$; Vd/F_{ss}; CL/F_{ss}; and R_{ac}.

Statistical Method

Demographical analysis

Demographic analysis is conducted based on the ITT. Descriptive statistics will be performed for all demographic (age, sex, weight, height, ethnicity, etc.) and baseline characteristics, taking the last valid measurement before the administration of the study drugs as the baseline.

Efficacy analysis

Efficacy analysis is mainly based on the test results of ^{13}C-UBT at 4-6 weeks after the last dose of study drugs to evaluate the effect of H. pylori eradication. Analysis will be mainly based on mITT set, and sensitivity analysis or supplementary analysis will be performed for the primary endpoints based on other efficacy analysis sets (ITT, Micro-ITT, and PP). Descriptive analysis is performed using frequency (percentage) according to ^{13}C-UBT test results (negative or positive) in different groups. Farrington-Manning test is used as the primary analysis for non-inferiority comparison between the test group and the control group, with non-inferiority margin of -10%; If the non-inferiority test is statistically significant, the Farrington-Manning method is continued to be used for superiority test (null hypothesis is that the difference in eradication rate of H. pylori between the test group and the control group is ≤ 0, and alternative hypothesis is that the difference in eradication rate of H. pylori between the test group and the control group is > 0). As a supportive analysis, the point estimate and two-sided 95% confidence interval (CI) of the difference in eradication rate of H. pylori between the test group and the control group will be calculated using the Miettinen-Nurminen method. If the lower limit of the 95% CI is greater than -10%, the eradication rate of H. pylori in the test group is considered to be non-inferior to that in the control group. If the test group is non-inferior to the control group and the lower limit of 95% CI is > 0, the eradication rate of H. pylori in the test group is considered superior to that in the control group. In addition, the efficacy of RSZ in combination with rabeprazole and amoxicillin in treatment-naïve participants with H. pylori infection is evaluated in a subgroup analysis based on the results of H. pylori culture and drug susceptibility testing.

Safety analysis

The evaluation and analysis of safety and tolerability are mainly based on descriptive statistics. Aes, treatment-emergent adverse events (TEAE), serious adverse events (SAE), drug-related TEAE, and TEAE leading to withdrawal from the trial will be summarized.

The number and incidence of TEAE will be summarized and described by system organ class and preferred term in each group.

The number and incidence of TEAE will be summarized and described by system organ class, preferred term, and severity in each group.

The number and incidence of drug-related TEAE will be summarized and described by system organ class and preferred term in each group.

Vital signs (blood pressure, respiration, pulse, and body temperature), laboratory tests and ECG results and changes from baseline will be summarized and described in each group, and clinically significant abnormal results and all test results post-baseline will be listed.

SAE, TEAE leading to discontinuation of study treatment, and TEAE leading to premature withdrawal from the study will be presented separately.

Pharmacokinetic analysis

Pharmacokinetic parameters (non-compartmental models) will be estimated and analyzed for plasma concentration data using Phoenix WinNonlin software, and the main pharmacokinetic parameters will be calculated using PKPS data.

PK concentration data will be statistically summarized and tabulated for RSZ at each sampling time point as defined in the protocol. Mean and median drug concentration-time profiles (linear and semi-logarithmic plots) will also be plotted by planned sampling time for each subject. The PK concentration data for individual subjects will be used to plot the drug concentration-time curve (linear and semi-logarithmic plots) based on the actual sampling time.

For samples with concentrations below the quantification limit, samples sampled before C_{max} should be calculated as zero and samples sampled after C_{max} should be calculated as not detectable when performing pharmacokinetic analysis. According to the treatment groups, the analysis results of main pharmacokinetic parameters are summarized and displayed by sample size, arithmetic mean, standard deviation, coefficient of variation, median, minimum, maximum, geometric mean, and geometric coefficient of variation.

PK parameters of the first dose include: T_{max}, C_{max}, $t_{1/2,}$ $AUC_{0\text{-}tau,}$ $AUC_{0\text{-}\infty}$, Vd/F, and CL/F. PK parameters of the multiple-dose include: $T_{max,\ ss}$; $C_{max,\ ss}$; $t_{1/2,\ ss}$; $AUC_{0\text{-}tau,\ ss}$; $AUC_{0\text{-}\infty,\ ss}$; Vd/F_{ss}; CL/F_{ss}; and R_{ac}. For subjects with $AUC\_{}_{\%\ Extrap} > 20\%$, descriptive statistical analysis will not be performed for $AUC_{0\text{-}\infty}$, $t_{1/2}$, CL/F, Vd/F, and $AUC\_{}_{\%\ Extrap}$.

The statistical analysis method and plan are detailed in the statistical analysis plan.