A Single-center, Randomized, Open-label Phase Ib/IIa Clinical Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Preliminary Efficacy of Helicobacter Pylori Eradication after Multiple Doses of TNP-2198 Capsules Combined with Rabeprazole Sodium Enteric-Coated Tablets, and Multiple Doses of TNP-2198 Capsules Combined with Rabeprazole Sodium Enteric-Coated Tablets and Amoxicillin Capsules in Asymptomatic Healthy Participants with Positive Helicobacter Pylori Infection

Protocol Synopsis

Protocol No.: TNP-2198-04

Approval Date: 20 June 2021

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Study Title	A Single-center, Randomized, Open-label Phase Ib/IIa Clinical Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Preliminary Efficacy of Helicobacter Pylori Eradication after Multiple Doses of TNP-2198 Capsules Combined with Rabeprazole Sodium Enteric-Coated Tablets, and Multiple Doses of TNP-2198 Capsules Combined with Rabeprazole Sodium Enteric-Coated Tablets and Amoxicillin Capsules in Asymptomatic Healthy Participants with Positive Helicobacter Pylori Infection
Study Objectives	**Primary Objectives:** 1 To evaluate the safety and tolerability of multiple doses of TNP-2198 Capsules combined with Rabeprazole Sodium Enteric-coated Tablets, and multiple doses of TNP-2198 Capsules combined with Rabeprazole Sodium Enteric-coated Tablets and Amoxicillin Capsules in asymptomatic healthy participants with positive Helicobacter pylori infection; 2 To evaluate the pharmacokinetics of multiple doses of TNP-2198 (including major metabolites), rabeprazole, and amoxicillin in each treatment group. **Secondary Objectives:** To evaluate the preliminary efficacy of multiple-dose combination of TNP-2198 Capsules and Rabeprazole Sodium Enteric-coated Tablets and multiple-dose combination of TNP-2198 Capsules, Rabeprazole Sodium Enteric-coated Tablets and Amoxicillin Capsules in the eradication of Helicobacter pylori.
Clinical Study Protocol No.	TNP-2198-04
Sponsor	TenNor Therapeutics
Clinical Trial Notification No.	CXHL1800160/161
Study Facility	Phase I Drug Clinical Study Center of the First Bethune Hospital of Jilin University
Study Phase	Phase Ib/IIa
Investigational Products	**TNP-2198 Capsules** Manufacturer: WuXi STA (Shanghai) Co., Ltd. (entrusted) Strength: 100 mg Storage Condition: Preserve in tightly closed containers, protected from light and stored at ambient temperature (10-30 °C) **Rabeprazole Sodium Enteric-coated Tablets** Manufacturer: Eisai China lnc. Drug Approval No.: GuoYaoZhunZi H20090090 Strength: 20 mg Storage Condition: Preserve in tightly closed containers, protected from light and stored below 25 °C **Amoxicillin Capsules** Manufacturer: Zhuhai United Laboratories (Zhongshan) Co., Ltd. Drug Approval No.: GuoYaoZhunZi H44021351 Strength: 0.25 g Storage Condition: Below 25 °C
Study Design	Participants: 40 asymptomatic healthy participants positive for Helicobacter pylori infection, males and females.

	The study is designed to evaluate the safety, tolerability, and pharmacokinetics of multiple doses of TNP2198 Capsules combined with Rabeprazole Sodium Enteric-coated Tablets, and multiple doses of TNP-2198 Capsules combined with Rabeprazole Sodium Enteric-coated Tablets and Amoxicillin Capsules in asymptomatic healthy participants with positive Helicobacter pylori infection, and to evaluate the preliminary efficacy of each dosage regimen for Helicobacter pylori eradication. A total of 4 treatment groups will be set up in this study and the participants will be randomized at a ratio of 1: 1: 1: 1. • Group A: TNP-2198 Capsules 200 mg twice daily (BID) + Rabeprazole Sodium Enteric-coated Tablets 20 mg BID (n=10); • Group B: TNP-2198 Capsules 400 mg BID + Rabeprazole Sodium Enteric-coated Tablets 20 mg BID (n=10); • Group C: TNP-2198 Capsules 600 mg BID + Rabeprazole Sodium Enteric-coated Tablets 20 mg BID (n=10); • Group D: TNP-2198 Capsules 400 mg BID + Rabeprazole Sodium Enteric-coated Tablets 20 mg BID + Amoxicillin Capsules 1 g BID (n=10). Screening will occur within 14 days of the first dose of study investigational products. All participants will provide informed consent and undergo Screening evaluations to determine their eligibility. Eligible participants will be admitted to the clinical study center on day -1 (the day before dosing, D-1). On Day 1 to Day 14, breakfast and dinner will be completed within 30 minutes prior to dosing, and the investigational products will be administered after meals. On Day 15, breakfast will be completed within 30 minutes prior to dosing, and the last dose will be administered after meals. Pharmacokinetic blood samples will be collected at the time points scheduled in the protocol during the study, and the safety tolerability evaluation and ^{14}C urea breath test (UBT) will be conducted. All participants will be discharged after completion of the safety and tolerability evaluation on Day 17 and return to the clinical study center for a follow-up UBT test between Days 44 and 50 to evaluate the preliminary efficacy of Helicobacter pylori eradication.
Screening Evaluations	Participants will be screened from D-14 to D-2. Screening evaluations: demographic information (including sex, ethnicity, age, weight, height and BMI), medical history, surgical history, smoking history, reproductive status, drinking history/diet, prior medications, physical examination, vital signs (blood pressure, pulse rate, respiratory rate and body temperature), 12-lead ECG, chest plain X-ray, abdominal Dopler ultrasound (digestive system + urinary system), clinical laboratory tests (hematology, blood biochemistry, urinalysis and coagulation function), infectious disease tests (hepatitis B serologic test, hepatitis C antibody, hepatitis C core antigen, HIV antigen/antibody, treponema pallidum antibody [an additional rapid plasma regain (RPR) test is required in patients positive for treponema pallidum antibody), serum pregnancy test (females only) and UBT. Note: The test results of hepatitis B virus, hepatitis C antibody, hepatitis C core antigen, HIV antigen/antibody and treponema pallidum antibody are valid within 3 months; Chest anteroposterior radiograph valid within 1 year; Abdominal Dopler ultrasound valid within 1 month.
Admission (Baseline) Evaluations	Admission (baseline) will be occurred on D-1. Baseline evaluations: Medical history, surgical history, clinical laboratory tests, physical examination, vital signs, 12-lead ECG, serum pregnancy test (females only), urine drug screen (morphine, marijuana), alcohol breath test, UBT. Note: Screening results are valid within 7 days for clinical laboratory tests and UBT.
Dosing Regimen	The investigational products shall be orally administered twice daily after completion of meals, for 14 consecutive days. On Day 15, the participants have breakfast within 30 minutes prior to dosing, and the last dose shall be taken after completion of meals. Each participant takes 29 doses of the investigational product. The dose of the investigational product for each group are as follows: • Group A: TNP-2198 Capsules 200 mg/time, i.e. 2 capsules/time; Rabeprazole Sodium Enteric-coated Tablets, 20 mg/time, i.e., 1 tablet/time;

	• Group B: TNP-2198 Capsules 400 mg/time, i.e., 4 capsules/time; Rabeprazole Sodium Enteric-coated Tablets, 20 mg/time, i.e., 1 tablet/time; • Group C: TNP-2198 Capsule 600 mg/time, i.e. 6 capsules/time; Rabeprazole Sodium Enteric-coated Tablets, 20 mg/time, i.e., 1 tablet/time; • Group D: TNP-2198 Capsules 400 mg/time, i.e., 4 capsules/time; Rabeprazole Sodium Enteric-coated Tablets, 20 mg/time, i.e., 1 tablet/time; Amoxicillin Capsules, 1 g/time, i.e., 4 capsules/time.
Inclusion Criteria	Participants must meet all of the following criteria to be enrolled: 1 Have signed the informed consent form before the study, and fully understand the study contents, process and possible adverse reactions; 2 Participants who are able to complete the study as required by the protocol; 3 Participants are willing to have no pregnancy plan within 6 months after last dose of investigational products and voluntarily take effective contraceptive measures; 4 Male and female participants aged 18-65 years (inclusive); 5 Male participants weighing not less than 50 kg and female participants weighing not less than 45 kg; Body mass index within the range of 18-30 kg/m^2 (inclusive); 6 Health status: participants do not have history of clinically significant cardiovascular, liver, kidney, digestive tract, nervous system, respiratory system, mental disorder and metabolic disorder; 7 Physical examination and vital signs are normal or abnormal but not clinically significant as judged by the investigator; 8 Paticipants whose ^{14}C urea breath test (UBT) results are positive. 9 Clinical laboratory test results are within normal range or abnormal but not clinically significant as judged by the investigator.
Exclusion Criteria	Participants were not to be enrolled if they met any of the following exclusion criteria: **1** History of helicobacter pylori eradication therapy (including participation in other clinical studys for helicobacter pylori eradication); 2 Those who smoked more than 5 cigarettes per day in the 3 months prior to screening; 3 Those who are allergic to the investigational product or or its excipients, or participants with allergic constitution (multiple drug and food allergies); 4 Those who have a history of drug and/or alcohol abuse (average weekly consumption of ≥ 14 units of alcohol: 1 unit = 285 mL of beer, or 25 mL of spirits, or 100 mL of wine); 5 Those who have blood donation or massive blood loss (> 450 mL) within 3 months prior to screening; 6 Those who have taken any drug that changes the activity of liver enzyme within 28 days prior to screening; 7 Those who have taken any prescription drugs, over-the-counter drugs, any vitamin products, or herbal remedies within 14 days prior to screening; 8 Those who have taken special diet (including pitaya, mango, grapefruit, etc.) or have had strenuous exercise within 2 weeks prior to screening, or other factors affecting drug absorption, distribution, metabolism, excretion, etc.; 9 Those who have had a major change in diet or exercise habits recently; 10 Those who have taken the other study drug or participated in the other clinical study that have not been finished within 1 month prior to administration of the investigational product; 11 Those who have a history of dysphagia or any gastrointestinal disease affecting drug absorption; 12 Those who have any disease that increases the risk of bleeding, such as hemorrhoids, acute gastritis or gastric and duodenal ulcers; 13 Those who have abnormal ECG with clinical significance; 14 Female participants who are in lactation or have a positive result in serum pregnancy test at screening or during the course of the study;

	15 Those with symptoms of cardiovascular, digestive, respiratory, urinary, neurological, hematological, immunological, endocrine system or tumor, and mental illness or with past medical history that has not been cured; 16 The following diseases (including but not limited to gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, pulmonary, immunologic, psychiatric or cardiovascular and cerebrovascular diseases) with clinically significant abnormalities in clinical laboratory tests or other clinical findings; 17 Viral hepatitis (including hepatitis B and C), HIV antibody, treponema pallidum antibody positive (those with positive treponema pallidum antibody need additional RPR test); 18 Acute illness or concomitant medication from the time of signing the informed consent form to the time before administration of the investigational product; 19 Consumption of chocolate, any caffeine-containing or xanthine-rich food or beverages within 48 hours prior to administration of the investigational product; 20 Those who have taken any alcohol-containing products within 48 hours prior to administration of investigational product; 21 Those with a positive result in urine drug screening or a history of drug abuse or drug use within the past 5 years; 22 Other conditions considered unsuitable for participation in the study by the investigator.
Safety and Tolerability Evaluation	CTCAE 5.0 criteria are used. All participants are observed for any adverse events occurring during the clinical study, and their clinical manifestations, severity, onset time, end time, treatment measures and outcome are recorded, and their correlation with the investigational product shall be determined. Safety and tolerability evaluation includes adverse events (AE), clinical laboratory tests (hematology, blood biochemistry, coagulation, urinalysis), vital signs (blood pressure, pulse rate, respiratory rate and body temperature), 12-lead electrocardiogram (ECG) and physical examination.
Pharmacokinetics Sample Collection and Processing Methods	The test sample is plasma and the test substances are: TNP-2198 (including major metabolites), rabeprazole and amoxicillin. **Collection time points:** • Day 1: within 30 minutes before breakfast, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h after dosing (prior to the second dose of the day); • Days 3, 5, 7, 9, 11, 13, and 14: within 30 min before breakfast; • Day 15: within 30 minutes before breakfast and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24 (Day 16) and 48 (Day 17) h after dosing. **Processing method:** Whole blood is collected in vacuum blood collection tubes containing anticoagulant, 4 mL per tube, and plasma is obtained by centrifugation after completion of whole blood collection, and the plasma samples are divided into two aliquots and stored in a refrigerator at -80 °C, one for pharmacokinetics analysis and the other as a backup. The process from sample collection to the refrigerator at -80 °C storage needs to be completed within 1 hour. Refer to the Laboratory Operation Manual for specific operation requirements.
PK Parameters	First dose PK parameters include at least: • Time to peak concentration (T_{max}) • Peak concentration (C_{max}) • Elimination half-life ($t_{1/2}$) • Area under the plasma concentration-time curve from the first dose extrapolated to infinity ($AUC_{0-\infty}$) • Area under the plasma concentration-time curve from the first dose to the dosing interval (12 h) (AUC_{0-tau})

	• Apparent volume of distribution (V_d/F) • Apparent clearance (CL/F) Last dose PK parameters include at least: • Time to peak ($T_{max,ss}$) • Maximum plasma concentration at steady state ($C_{max,ss}$) • Minimum plasma concentration at steady state ($C_{min,ss}$) • Mean plasma concentration at steady state（$C_{avg,ss}$) • Elimination half-life ($t_{1/2,ss}$) • Area under the plasma concentration-time curve from the last dose to the last measurable concentration time point ($AUC_{0\text{-last, ss}}$) • Area under the plasma concentration-time curve from the last dose to the dosing interval (12 h) ($AUC_{0\text{-tau,ss}}$) • Area under the plasma concentration-time curve from the last dose extrapolated to infinity ($AUC_{0\text{-}\infty\text{, ss}}$) • Apparent volume of distribution at steady state (V/F_{ss}) • Apparent clearance (CL/F_{ss}) • Accumulation Index (R_{ac}): $AUC_{0\text{-tau,ss}}$ on Day 15/$AUC_{0\text{-tau}}$ on Day 1 • Fluctuation index (DF): Percentage fluctuation at steady state = 100 × ($C_{max,ss}$ - $C_{min,ss}$)/$C_{avg,\ ss}$
Preliminary Evaluation of Helicobacter pylori Eradication	All participants will undergo a total of 4 UBT tests (under fasting conditions before breakfast) at baseline, Day 8, Day 16, and Day 44-50 after last dosing, to evaluate the preliminary efficacy in the eradication of Helicobacter pylori.
Statistical Analysis	**Safety and tolerability analysis** The analysis of safety and tolerability are mainly based on descriptive statistics. AEs, serious adverse events (SAEs), drug-related AEs and AEs leading to withdrawal from the study are summarized. The number of participants, number of AEs and incidence of all AEs will be tabulated and summarized by treatment group, system organ class and preferred term. The incidence of adverse events (occurring separately in each treatment group, both in 2 groups, or in 3 groups) in each treatment group will be tabulated and described according to CTCAE grade by treatment group. The number of participants, number of AEs and incidence of AEs in each treatment group will be tabulated according to severity and relationship to the investigational product by system organ class and preferred term. The laboratory test results that are normal before the test but abnormal after treatment are described by treatment group. The mean, standard deviation, median, minimum and maximum values of vital signs (blood pressure, respiratory rate, pulse rate and body temperature) and laboratory test before and after administration are calculated according to the treatment group, respectively. If necessary, paired t test or non-parametric test can be used for comparison before and after administration. SAE and suspected unexpected serious adverse reactions (SUSARs) are presented separately. **Pharmacokinetic parameter analysis**

	Plasma concentrations will be summarized descriptively by treatment group and time point, including: arithmetic mean, standard deviation, minimum, median, maximum, geometric mean, coefficient of variation, geometric coefficient of variation. Descriptive analysis will be performed for the pharmacokinetic parameters calculated after the first and last administration by treatment group, including: arithmetic mean, standard deviation, minimum, median, maximum, geometric mean, coefficient of variation, geometric coefficient of variation, etc. In addition, the accumulation index $AUC_{0\text{-}tau,ss}$ (D15)/$AUC_{0\text{-}tau}$ (D1) is calculated. Mean and individual concentration-time curves are displayed by linear and semi-logarithmic plotting. Analysis of variance or non-parametric test will be performed on pharmacokinetic parameters between each treatment group. **Preliminary analysis of Helicobacter pylori eradication** The preliminary eradication effect analysis will be based on the full analysis set, which is defined as all participants who are randomized and receive at least one dose of investigational product. The preliminary efficacy of Helicobacter pylori eradication will be analyzed descriptively by frequency (percentage) of 4 UBT test results (negative or positive) at baseline, on Day 8, Day 16 and Day 44-50 after last dosing in different treatment groups, and inter-group comparison will be performed. Fisher's exact test will be used for inter-group comparison. As an auxiliary analysis, the mean, standard deviation, median, minimum, maximum, and change from baseline will be presented for UBT values. Inter-group comparisons of UBT values will be analyzed using the model of analysis of covariance or mixed-effects model repeated-measures (MMRM) with baseline UBT values as a covariate, treatment group and dose group as a fixed variable. If the model of analysis of covariance was used, the last observation carried forward (LOCF) was used to impute missing values. There are no clear statistical hypotheses in this study and all statistical tests are exploratory test with a two-sided 5% nominal significance level. The statistical analysis method and plan are detailed in the statistical analysis plan.

Schedule of Activities

Study Procedures		Screening Visit (D-14-D-2)	D-1	D1	D2-14	D15	D16	D17	Follow-up (during D44-50)
Screening	Signing of ICF	X							
	Demographics	X							
	Medical and surgical history	X	X						
	Concomitant medications	X	X	X	X	X	X	X	X
	Chest plain X-ray	X							
	Abdominal Doppler ultrasound (digestive system + urinary system)	X							
	Infectious disease screening (hepatitis B serologic test, hepatitis C core antigen, hepatitis C antibody, HIV antigen/antibody, treponema pallidum antibody)	X							
	Breath alcohol test		X						
	Urine drug screening (morphine, marijuana)		X						
	Blood pregnancy test	X	X					X	
	Verification of inclusion and exclusion criteria	X	X						
Enrollment	Admission		X						
	Randomization		X						
Study treatment administration	Dosing			X	X	X			
	Clinical laboratory tests (blood routine, blood biochemistry, coagulation, urine routine)[1]	X	X		X			X	

Safety and tolerability Assessment	**Physical examination[2]**	X	X		X			X	
	12-lead ECG[3]	X	X	X		X		X	
	Vital signs[4]	X	X	X	X	X	X	X	
	Monitoring and recording of adverse events			X	X	X	X	X	X
PK sample collection	**Blood sample[5]**			X	X	X	X	X	
Preliminary efficacy of Hp eradication	**^{14}C-urea breath test[6]**	X	X		X		X		X
End of study	**Discharge**							X	
	Follow-up, study completion								X

Note:

1 Clinical laboratory tests will be performed at the screening period, baseline period, D4, D6, D8, D12 (before the first dose), and D17 (48 h after dosing on D15). The results of screening examination within 7 days can be used for the baseline period, and no additional test will be required.

2 Physical examination will be performed at the screening period, baseline period (D-1), D4, D6, D8, D12 and D17.

3 12-lead ECG will be performed at the screening period, baseline period (D-1), D1 (2 h after the first dose), D15 (within 60 min before and 2 h after dosing) and D17 (48 h after dosing on D15).

4 Vital signs will be examined at the screening period, baseline period (D-1), D1 (within 60 min before the first dose and 0.5 h, 2 h, 4 h, 8 h, 12 h after dosing), D2-14 (within 60 min before the first dose), D15 (within 60 min before dosing and 0.5 h, 2 h, 4 h, 8 h, 12 h after dosing), D16 (24 h after dosing on D15) and D17 (48 h after dosing on D15).

5 PK blood samples will be collected on D1 (within 30 min before breakfast, and 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h after dosing), within 30 min before breakfast on D3, D5, D7, D9, D11, D13 and D14, on D15 (within 30 min before breakfast, and 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h after dosing), D16 (24 h after dosing on D15) and D17 (48 h after dosing on D15).

6 UBT will be performed in fasting state before breakfast and will be performed on D8 during D2-14; the results of screening examination within 7 days can be used for the baseline period, and no additional test will be required