A Phase 1b/2a, single-center, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, pharmacokinetic characteristics, and preliminary efficacy of multiple ascending doses of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia.

Protocol Synopsis

Protocol No.: TNP-2092-06

Approval Date: 17 August 2021

Protocol Synopsis of Study TNP-2092-06

Title of study: A Phase 1b/2a, single-center, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, pharmacokinetic characteristics, and preliminary efficacy of multiple ascending doses of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia

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Sponsor: TenNor Therapeutics (Suzhou) Limited.

Investigator: Yanhua Ding, The First Hospital of Jilin University

Study center: The study was conducted in a single center in China.

Study Phase: 1b/2a

Objectives

Primary objectives:

1. To evaluate the safety and tolerability of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia.
2. To evaluate the pharmacokinetic characteristics of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia.

Secondary objectives:

1. To evaluate the preliminary efficacy of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia.
2. To observe the effects of TNP-2092 Capsules on hepatic encephalopathy related clinical symptoms and signs, neuropsychological indicators, and quality of life in liver cirrhosis patients with hyperammonemia.

Investigational Product:

TNP-2092 Capsules: strength 100 mg, Storage condition: stored in a cold place (2-10 °C), sealed, and protected from light.

TNP-2092 Placebo Capsules: strength: NA. Storage condition: stored in a cold place (2-10 °C), sealed, and protected from light.

Study Design

The aim of this study is to evaluate the safety, tolerability, and pharmacokinetic characteristics of TNP-2092 Capsules in liver cirrhosis patients with hyperammonemia; and to preliminarily observe the effects of the study drug on blood ammonia and hepatic encephalopathy related clinical symptoms and signs, neuropsychological indicators, and quality of life in liver cirrhosis patients with hyperammonemia.

A total of 3 dose groups will be set up, i.e., 100 mg BID, 300 mg BID and 600 mg BID groups. Drugs will be orally administered 30 min after breakfast and dinner for 14 consecutive days, and last dose will be administered 30 min after breakfast on the morning of D15. Each dose group will include a study drug TNP-2092 capsule arm and a placebo control arm. Subjects will exit upon completion of the safety and tolerability evaluation on D17.

Twelve liver cirrhosis patients with hyperammonemia are planned to be enrolled in each dose group. The 12 patients will be assigned in a ratio of 2:1 to the TNP-2092 capsule arm and the placebo arm, with 8 patients receiving TNP-2092 Capsules and 4 receiving placebo.

In each dose group, a sentinel-based sequential enrollment design will be applied, and the 12 patients will be sequentially enrolled into two subgroups which will consist of 3 and 9 patients

respectively. If the drug is evaluated as tolerable in the safety and tolerability assessment on D2, D4 and D7 of treatment in the first subgroup, enrollment for the next subgroup may start.

Enrollment for the second dose group may start only after the previous dose group has fully completed the treatment period and passed the safety and tolerability evaluation.

During the study, safety and tolerability will be observed and evaluated in subjects at scheduled time points in the protocol. PK blood samples will be collected from subjects at scheduled time points in the protocol for pharmacokinetic analysis.

Dosing Regimen

The drug is administered orally 30 min after the breakfast and dinner in the treatment period (Days 1-14), and the last dose is administered 30 min after the breakfast on Day 15. Water are not allowed from 1 hour pre-dose to 2 hours post-dose on Day 1 and Day 15.

Screening Evaluations

After signing the written Informed Consent Form, subjects received a series of examinations, including: demographic data (including sex, ethnicity, age, fertility status, weight, height, and BMI), clinical assessment of HE grade, asterixis examination, neuropsychological evaluation, medical history, surgical history, smoking history, alcohol/diet, previous medication, physical examination, vital signs (blood pressure, pulse and body temperature), 12-lead ECG, chest X-ray, abdominal B ultrasound (liver, bile, pancreas and spleen), Fibroscan (transient elastography), clinical laboratory tests (blood ammonia, hematology, blood chemistry, urinalysis, stool routine, coagulation), infectious disease tests (hepatitis B serologic test, hepatitis C antibody, hepatitis C core antigen, HIV antigen/antibody, treponema pallidum antibody, additional RPR test should be performed for those tested positive in treponema pallidum antibody), blood pregnancy test (for females only), to confirm the inclusion criteria and exclusion criteria.

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, alcohol breath test, fasting blood ammonia concentration, asterixis, Neuropsychological evaluation: Number connection test (NCT-A) and Digit symbol test (DST), Quality of life score (quality of life scale for patients with mild hepatic encephalopathy).

Note: Screening results are valid within 7 days for clinical laboratory tests

Diagnosis and main criteria for inclusion

Each subject was required to meet all of the following criteria to be eligible for study enrollment:

1) 18-65 (inclusive) years of age, male or female.
2) Clinically diagnosed with liver cirrhosis.
3) Fasting venous blood ammonia above upper limit of normal (ULN).
4) Organ functions must meet the following criteria:
   a) Peripheral blood: absolute neutrophil count $\geq 0.5*10^9$/L, platelet $\geq 20*10^9$/L, hemoglobin ≥ 8 g/dL.
   b) Liver: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq 5 \times$ ULN; serum total bilirubin (TBL) $\leq 5 \times$ ULN.
   c) Kidney: creatinine clearance ≥ 60 mL/min.
   d) No malabsorption or other gastrointestinal disorders that affect drug absorption.
5) Weight ≥ 45 kg and body mass index [BMI = weight (kg)/height ^{2} (m^2)] between 18 and 34 (inclusive) kg/m^2.
6) Subjects (including their partners) will have no pregnancy plan and voluntarily take effective contraceptive measures within 6 months after drug withdrawal. Refer to Appendix 9 for specific contraceptive measures.

7) Subjects or their legal representatives sign the Informed Consent Form and fully understand the content, procedures, and potential adverse reactions prior to the initiation of the study.
8) Able to complete the study per the requirements in the study protocol.

Subjects meeting any of the following criteria were ineligible for study enrollment:

1) Subjects who are allergic to rifamycin or quinolone antibacterial agents or those with an allergic constitution.
2) Pregnant or lactating women, or women of childbearing age with a positive pregnancy test from the screening period to initiation of the study treatment.
3) Subjects with serious nervous or mental disorders.
4) Subjects with Child-Pugh class C liver cirrhosis.
5) Subjects with Grade 2 or above hepatic encephalopathy.
6) Subjects who have been diagnosed with *Clostridium difficile*-induced pseudomembranous enteritis within 3 months.
7) Subjects who have had systemic infection or gastrointestinal bleeding within 7 days prior to screening.
8) Subjects with clinically significant abnormal clinical laboratory tests or other clinical findings indicative of clinically significant disorders that, in the opinion of the investigator, make them not eligible for this clinical study.
9) Subjects who have used sedatives, probiotics, cathartics or antibacterial agents within 7 days prior to screening.
10) Subjects who have used other study drugs or participated in other drug clinical trials within 1 month prior to screening.
11) Subjects need to use the following concomitant drugs during the study treatment period: cathartics and drugs for ammonia reduction listed in 5.2.1 in the Guidelines on the Management of Hepatic Encephalopathy in Liver Cirrhosis 2018 (e.g., lactulose, lactitol, LOLA, rifaximin, other antibacterial agents, etc.) ; HIV protease inhibitors (e.g., ritonavir boosted or non-boosted saquinavir, atazanavir, darunavir, fosamprenavir, tipranavir, etc.) ; praziquantel; halothane; class IA and III antiarrhythmics (disopyramide, procainamide, quinidine, amiodarone, dofetilide, dronedarone, ibutilide, sotalol, etc.) ; strong inhibitors and inducers of liver metabolic enzymes;
12) Positive HIV antigen/antibody screen; positive Treponema pallidum antibody screen requires the investigator's judgment with the consideration of RPR results.
13) Positive urine drug screen or history of drug abuse within the past 5 years.
14) Positive alcohol breath test.
15) Acute diseases or concomitant medications from screening to study medication.
16) Other circumstances deemed by the investigator to be unsuitable for enrollment in this study.

Safety and Tolerability Evaluation

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 Time Point:

- Day 1: 30-60 min prior to the first dose, and 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h and 12 h post-dose (prior to the next dose).
- Days 3, 5, 7, 9, 11 and 13: 30-60 min prior to the first dose.
- Day 15: 30-60 min pre-dose, and 0.5 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 24 h and 36 h post-dose.

Efficacy Evaluation

Fasting venous blood ammonia concentration and area under the blood ammonia concentration-time curve. Asterixis: elicited (yes/no). Neuropsychological evaluation: Number connection test (NCT-A) and Digit symbol test (DST). Quality of life score (quality of life scale for patients with mild hepatic encephalopathy). Clinical grade of hepatic encephalopathy (HE).

Statistical methods

SAS 9.4 was used for the statistical analysis.

Population for analyses were:

- Full Analysis Set (FAS): all the subjects who were randomized into groups.
- Safety Analysis Set (SS): all the subjects who have been randomized into groups and received at least one dose of study drug.
- PK Concentration Analysis Set (PKCS): all the subjects who have been randomized into groups, received at least one dose of study drug, and have at least one evaluable blood drug concentration.
- PK Parameter Analysis Set (PKPS): all subjects who have been randomized into groups, have received at least one dose of study drug, and have at least one evaluable pharmacokinetic parameter.

Interim analysis:

No interim analysis was planned.

Efficacy analysis:

All efficacy endpoints will be descriptively summarized. Changes at each post-baseline visit from baseline will be calculated for all endpoints except asterixis.

For fasting blood ammonia concentration, if data follow a normal distribution, single-group t-test will be used to determine, by treatment group, whether the change from baseline at each post-baseline time point is 0; and analysis of covariance will be performed for differences between treatment groups and between each treatment group and the control group in changes from baseline at each post-baseline time point. If data do not follow a normal distribution, Wilcoxon signed rank test and Wilcoxon rank sum test will be used. The areas under the blood ammonia concentration-time curve will be calculated, and t-test or Wilcoxon rank sum test will be used for inter-group comparison. Normal fasting venous blood ammonia concentrations across all post-baseline visits in each dose group and the placebo group will be counted, and their percentages in all measurements will be calculated; Logistic regression will be used to compare the difference in the percentage of normal blood ammonia concentrations between each dose group and the placebo group.

With regard to whether asterixis is elicited, baseline, D7 and D15 results will be compared by treatment group using paired chi-square test; Logistic regression (with baseline and group as

independent variables) or non-parametric methods will be used for inter-group comparison of D7 and D15 results.

For NCT-A and DST results, t-test or Wilcoxon signed rank test will be used to determine whether the change from baseline in each treatment group is 0 on D7 or D15. Inter-group t-test or Wilcoxon rank sum test will be used to determine whether changes on D7 and D5 are different between treatment groups and between each treatment group and the control group.

NCT-A and DST results will be analyzed using age-corrected scaled scores.

For the quality of life scale for patients with mild hepatic encephalopathy, the total score of the 30 questions will be calculated for the subject, and paired t-test will be used to determine whether the change from baseline at each post-baseline visit is 0 within each group; and analysis of covariance (baseline as the covariate and group as the fixed factor) to determine whether changes from baseline at each post-baseline visit are different between the groups (including the placebo group).

For HE grades, Wilcoxon signed rank test will be used to determine whether changes from baseline in each treatment group are different on D7 or D15. Wilcoxon rank sum test will be used to determine whether changes on D7 and D15 are different between treatment groups and between each treatment group and the control group.

If D7 or D15 data are missing, the last observation carried forward (LOCF) approach will be used for imputation.

PK analysis:

Plasma drug concentration (c)-time (t) data will be summarized by dose group and time point and c-t curves plotted based on the PK concentration set. PK parameters for the first dose (Day 1) and the last dose (Day 15) will be calculated by a non-compartmental model using Phoenix WinNonLin (version 8.3), including: T_{max}, C_{max}, $t_{1/2}$, $AUC_{0-\infty}$, AUC_{0-last}, AUC_{0-tau} and CL/f. In addition, the accumulation index will also be calculated: AUC_{0-tau} (D15)/AUC_{0-tau} (D1); C_{max} (D15)/C_{max} (D1). Each parameter will be summarized by dose group based on the PK parameter analysis set. PK concentrations and PK parameters for each subject will be listed.

Safety analysis:

Adverse events will be coded using the latest medical dictionary MedDRA. All treatment emergent adverse events, serious adverse events, study drug-related adverse events and adverse events leading to withdrawal will be summarized by treatment group, system organ class and preferred term based on the safety analysis set. The number and percentage of subjects for each category of adverse events and the number of adverse events will be calculated. All adverse events will be listed. Laboratory tests, 12-lead ECG, vital signs and physical examination results will be summarized by treatment group and visit and listed. Where appropriate, cross tabulations will be used to reflect changes from baseline in post-baseline test/examination results.