A Phase 1/2, randomized, controlled, open-label, Proof-of-Concept study to evaluate the safety and tolerability, pharmacokinetics, and efficacy of TNP-2092 administered via intra-articular injection (IA) in adult participants with early or acute hematogenous prosthetic joint infection (PJI) requiring or not requiring debridement, antibiotics, and implants retention (DAIR) after total knee arthroplasty (TKA)

Protocol

Protocol No.: TNP-2092-IA-01

Approval Date: 3 DEC 2024

PROTOCOL OF TNP-2092-IA-01

Study title:

A Phase 1/2, randomized, controlled, open-label, Proof-of-Concept study to evaluate the safety and tolerability, pharmacokinetics, and efficacy of TNP-2092 administered via intra-articular injection (IA) in adult participants with early or acute hematogenous prosthetic joint infection (PJI) requiring or not requiring debridement, antibiotics, and implants retention (DAIR) after total knee arthroplasty (TKA).

Study Population:

Participants with early (within 1 month of total knee arthroplasty [TKA]) or acute hematogenous (within 3 weeks of infection symptoms) PJI requiring or not requiring DAIR treatment after TKA.

Investigational Site:

The study is a clinical study conducted in China.

Phase of Study: 1/2

Study objectives:

Primary objective

- To evaluate the safety and tolerability of TNP-2092 administrated IA in adult participants with early (within 1 month of TKA) or acute hematogenous (within 3 weeks of infectious symptoms) PJI requiring or not requiring DAIR treatment after TKA, on the basis of vancomycin intravenous [IV] treatment combined with oral antibiotics.

Secondary objectives

- To evaluate the systemic and local pharmacokinetic (PK) profile of TNP-2092 administered IA for PJI.
- To evaluate the efficacy of TNP-2092 administered IA for the treatment of PJI.

Study Endpoints:

1 Safety and tolerability:

Adverse events reported during the study, vital signs, physical examination, laboratory tests, etc.

2 Pharmacokinetic endpoints:

Including TNP-2092 concentrations in synovial fluid and TNP-2092 PK parameters in plasma.

- Synovial fluid
  - Drug concentrations at 12 h, 24 h after the first dose, before dosing on Day 7, and before the last dose, and at 12 h, 24 h, 48 h, 72 h after the last dose.
- Plasma
  - First dose of TNP-2092: time to maximum concentration (T_{max}), maximum observed concentration (C_{max}), elimination half-life ($t_{1/2}$), area under the curve from the time of dosing to infinity ($AUC_{0-\infty}$), area under the curve from the time of dosing to the last measurable concentration (AUC_{0-t}), etc.
  - Last dose of TNP-2092: time to maximum concentration at steady state ($T_{max, ss}$), maximum observed concentration at steady state ($C_{max, ss}$), elimination half-life at steady state ($t_{1/2, ss}$), area under the curve from time of dosing to the last measurable concentration at steady state ($AUC_{0-t, ss}$), area under the curve from time of dosing to infinity at steady state ($AUC_{0-\infty, ss}$), area under the curve over the dosing interval at steady state ($AUC_{0-tau, ss}$), accumulation ratio (R_{ac}), etc.

3 Efficacy Endpoints:

a) Primary Efficacy Endpoint:

i. Early Assessment (EA) response rate, after 2 weeks of IV + IA treatment, participants who met all the following criteria will be judged as responders:

- At least 2 body temperature measurements within the last 24 hours separated by more than 6 hours were ≤ 37.6ºC.
- Peripheral white blood cell (WBC) count returned to normal range (as determined by local laboratory reference range).

- Synovial fluid WBC < 3000 cell/μL and polymorphonuclear leukocytes percentage (PMN%) < 80%.
- Inflammatory manifestations (pain, erythema, edema, wound exudate) at the primary infection site resolved, with pain requiring resolution or tolerance.
- Inflammatory markers (ie, C-reactive protein [CRP]) improved to 50% of normal or baseline values.

b) Secondary Efficacy Endpoints:

i. End of treatment (EOT) response rate, after 8 weeks of oral antibiotic therapy, participants who met all the following criteria will be judged as responders:
- Joint pain was tolerable.
- Joint function improvement.
- Inflammatory markers (ie, CRP) returned to ≤ 10 mg/L.

ii. Treatment failure rate within 6 months after the start of study treatment, and those who meet any of the following criteria are treatment failures:
- No response at EA assessment.
- No response at EOT assessment.
- Receiving systemic antibiotics for infected joints after the end of study treatment.
- Additional surgical treatment of infected joints is required during the study.
- Death due to primary joint infection.

Study Design:

This is a Phase 1/2, randomized, controlled, open-label, proof-of-concept study to evaluate the safety and tolerability, pharmacokinetics, and efficacy of TNP-2092 administered IA on the basis of vancomycin IV and oral antibiotics therapy in participants with early (within 1 month of TKA) or acute hematogenous (within 3 weeks of infectious symptoms) PJI requiring or not requiring DAIR therapy after TKA.

The study population is participants with confirmed or suspected Gram-positive bacteria causing early (ie, within 1 month of TKA) or acute hematogenous (within 3 weeks of infection symptoms) PJI requiring or not requiring DAIR therapy after TKA. Participants will undergo screening assessments within 7 days prior to study start.

First, three eligible participants will be enrolled as sentinel arms to receive TNP-2092 50 mg IA once daily on the basis of vancomycin IV treatment (1 g every 12 h [q12 h], dose adjusted according to renal function), both IV and IA treatment will be last for 14 days, and participants will be assessed for EA after IA administration of TNP-2092 on D14. Participants who met the protocol-specified criteria for transfer to oral therapy will be transferred to oral antibiotics on D15 and continued to receive oral rifampicin capsules 0.45 g and levofloxacin tablets 0.5 g once daily, and participants who are intolerant to rifampicin and/or levofloxacin, or whose pathogens are resistant to rifampicin and/or levofloxacin could be switched to minocycline hydrochloride capsules orally every 12 hours, with the first dose doubled and 200 mg orally, followed by 100 mg each dose. Oral therapy will be administered for a total of 8 weeks (56 days). After oral treatment, participants will be evaluated by EOT from D71 to D77. Participants who met the protocol-specified stopping criteria for oral antibiotics will be to enter the follow-up period. The investigator follows up the participants by telephone once a month and decides whether they needed to return to the study site for follow-up according to the situation until 6 months after the start of study treatment for efficacy evaluation (to confirm whether the participant 's disease status met the protocol-defined "treatment failure"), that is, the end-of-study (EOS) assessment. During the sentinel arm study, the volume and frequency of TNP-2092 IA administration may be adjusted case-by-case after the investigator has fully discussed the decision with the sponsor. Participants in the sentinel arm will be discharged on D17 after all PK samples had been collected.

During the study, if criteria for intravenous transfer to oral antibiotics are not met at the EA assessment or criteria for discontinuation of oral antibiotics are not met at the EOT assessment, the investigator will decide the subsequent treatment regimen and the participant will be withdrawn from the study.

After all sentinel participants are tolerable safety at EA assessment and complete the PK study, an additional 20 participants will be enrolled and randomize in 1:1 ratio to the experimental arm (TNP-2092 IA + vancomycin IV + oral antibiotics) and the control arm (vancomycin IA + vancomycin IV + oral antibiotics). The experimental group will be treated the same as the sentinel group (TNP-2092 dose volume, dose, frequency can be adjusted according to the sentinel group synovial fluid TNP-2092 concentration, PK characteristics, and safety results at the EA visit); the control group will receive vancomycin IA + vancomycin IV + oral antibiotics. The study process of the two groups is the same as that of the sentinel group. Participants in the test group will be discharged on D17 after all PK samples are collected; participants in the control group will be discharged on D15 after completing EA assessments and IV + IA administration. During the screening period, samples of joint cavity puncture fluid and blood on the affected side will be obtained for Gram staining, culture and drug sensitivity test, and second-generation gene sequencing (NGS) detection will also be performed to determine the pathogen and drug resistance to the investigational product. After enrollment, if the participant has a negative culture result (i.e., no pathogenic microorganism is cultured), pathogenic bacteria will be determined by reference to NGS results, otherwise pathogenic bacteria will be determined based on culture results; if the participant requires DAIR treatment, samples such as blood, synovial fluid, infected joint tissue (if needed), knee spacer ultrasonic concussion fluid (if needed), and surgical area ultrasonic concussion fluid (if needed) will be collected during DAIR surgery for Gram staining, culture, and drug susceptibility testing, and pathogenic bacteria will be confirmed by reference to culture results. If no pathogenic bacteria can be identified (eg, culture, NGS results are negative), or PJI is confirmed to be caused by Gram-negative bacteria infection, fungal infection, or Enterococcus species infection, or mycobacterial infection, or Gram-positive bacteria mixed with Gram-negative bacteria and/or fungal infection, or blood culture shows systemic infection (sepsis), local intra-articular injection therapy will be discontinued, the investigator will treat the patient with appropriate antibiotics as appropriate, and the participant will withdraw from the study and will not be included in the efficacy assessment.

Inclusion and exclusion criteria:

Criteria for inclusion:

Participants who met all the following criteria were eligible for this study:

1 Early (within 1 month of TKA) or acute hematogenous (within 3 weeks of infectious symptoms) PJI requires or does not require DAIR therapy after TKA.
2 Suspected or confirmed PJI was caused by a Gram-positive bacterial infection, including methicillin-resistant and ciprofloxacin-resistant Staphylococcus aureus and Staphylococcus epidermidis, as judged by the investigator.
3 Agree to be hospitalized for 2 weeks with local intra-articular injection.
4 18 years of age or older (of either sex) at the time of signing the informed consent form (ICF).
5 The implanted prosthetic joint was well fixed.
6 No sinus tract that communicates with the prosthesis.
7 Body mass index (BMI) $\geq$ 18 kg/m ^{2} and $\leq$ 34 kg/m ^{2}.
8 Agree to voluntarily use effective contraception from signing the ICF through 8 weeks after the last dose of investigational product (in case of premature withdrawal from the study) or through completion of the end-of-study visit. Male participants must refrain from donating sperm during this period.

Exclusion Criteria:

Participants who met any of the following criteria were not included in the study:

1 History of hypersensitivity or intolerance to any of the following agents: vancomycin or TNP-2092.
2 Definite PJI of Gram-negative infection, fungal infection, or Enterococcus infection, or Mycobacterium infection, or Gram-positive mixed Gram-negative and/or fungal infection.
3 Definite systemic infection (sepsis).
4 Expected survival less than 2 years.
5 Female participant is pregnant, lactating, or has a positive screening/baseline pregnancy test.
6 Surgical or medical conditions that, in the opinion of the investigator, could affect the participant's ability to participate in the study, or affect the administration of investigational product, or affect the interpretation of study results, including but not limited to active malignancy, metabolic disease, alcohol or drug abuse, or clinically significant laboratory abnormalities.
7 Presence of serious liver, blood, or immune system disorders as evidenced by the following:

a) Acute hepatitis of any cause within the past year.
b) Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), or total bilirubin levels > 2 times the upper limit of normal (ULN).
c) Presence of end-stage liver disease-related manifestations such as ascites or hepatic encephalopathy.
d) Current or anticipated neutropenia (ie, neutrophil count < 0.5 x 10^9/L).
e) Chemotherapy for cancer, radiation therapy, or potent noncorticosteroid immunosuppressants (eg, cyclosporine, azathioprine, tacrolimus, immunomodulatory monoclonal antibody therapy, etc) within the past 3 months or corticosteroids (≥ 40 mg prednisone/day) for more than 14 days within 30 days prior to randomization.

8 Positive AIDS antibody screening.

9 History or evidence of severe renal disease or creatinine clearance < 30 mL/min based on the Cockcroft-Gault formula.

10 Systemic antibiotics for more than 3 days within 2 weeks prior to enrollment, except for infections other than PJI that are treated with non-systemic or narrow-spectrum anti-gram-negative antibiotics.

11 Rifampicin within 4 weeks prior to enrollment.

12 Treatment with an investigational agent within 30 days or 5 half-lives (whichever is longer) prior to the first dose of study intervention.

13 Participants who, in the opinion of the investigator, were unable to comply with the protocol and study drug administration procedures or complete the clinical study.

Study drug, dosage form, administration and dosage:

The dosing schedule for the sentinel and randomized trial arms is as follows:

Intravenous and intra-articular administration period:

Intra-articular administration: TNP-2092 for injection, 100 mg/vial. A vial of lyophilized TNP-2092 (100 mg) will be dissolved with 6 mL of normal saline, and 3 mL of the drug solution (containing 50 mg TNP-2092) will be withdrawn and diluted with 7 mL of normal saline to finally prepare 10 mL of normal saline containing 50 mg TNP-2092. Administered by intra-articular injection, 10 mL (50 mg) once daily for 14 days at the same time of day (up to 1 hour earlier or later the basis of the first dose). If the participant 's joint had a drainage tube in place, the drainage tube will be closed for 12 hours after intra-articular administration, depending on the investigator' s actual situation. If participant D1 requires DAIR surgery, the first dose should be administered intraoperatively.

Intravenous infusion: Vancomycin Hydrochloride for Injection, 0.5 g/vial. Vancomycin 1 g will be dissolved in 20 mL normal saline and then diluted in 230 mL normal saline to finally prepare vancomycin 1 g in 250 mL normal saline, and the intravenous drip time needed to be more than 60 min, q12h ± 1h per day, for 14 days. If participant D1 requires DAIR surgery, the first dose should be administered after surgery.

During the sentinel arm study, the volume and frequency of TNP-2092 IA administration may be adjusted after the investigator has fully discussed the decision with the sponsor, depending on the participant 's actual situation. During the randomized study, the volume, dose, and frequency of TNP-2092 IA administration could be adjusted in the experimental group based on synovial fluid concentrations and PK profiles in the sentinel group and safety results at the EA visit.

Oral Phase:

Rifampicin capsules: 0.15 g/capsule. 0.45 g (3 capsules) orally once daily within 1 h before breakfast for 8 weeks (56 days).

Levofloxacin Tablets: 0.5 g/tablet. 0.5 g (1 tablet) orally once daily within 1 hour before breakfast for 8 weeks (56 days).

If susceptibility testing results show resistance to rifampicin and/or levofloxacin, or intolerance by the patient's participant, treatment with oral minocycline hydrochloride capsules will be substituted as follows:

Minocycline hydrochloride capsules: 100 mg/capsule. Administered orally q12h ± 1h daily, doubling the first dose, 200 mg (2 capsules) orally, then 100 mg (1 capsule) each time for 8 weeks (56 days).

The total duration of oral treatment during the oral administration period was 8 weeks (56 days) regardless of whether oral medication will be changed (eg, intolerance)

Control treatment, dosage form and dosage and administration:

The dosing regimen for the control group after randomization was as follows:

Intravenous and intra-articular administration period:

Intra-articular: Vancomycin Hydrochloride for Injection, 0.5 g/vial. Vancomycin 0.5 g will be dissolved in 10 mL of normal saline and administered as an intra-articular injection once a day, at the same time as possible (up to 1 hour earlier or later the basis of the first dose), 10 mL (0.5 g) per dose for 14 days. If the participant's joint had a drainage tube in place, the drainage tube will be closed for 12 hours after intra-articular administration, depending on the investigator' s actual situation of the participant. If participant D1 requires DAIR surgery, the first dose should be administered intraoperatively.

Intravenous infusion: Vancomycin hydrochloride for injection: 1 g. Dosage and administration are the same as those in the sentinel group.

Oral Phase:

Drug selection, usage and dosage are the same as those in the sentinel group.

Duration of participant participation:

The entire study duration consists of a screening period of up to 1 week, an antibiotic treatment period of approximately 10 weeks (approximately 2 weeks IV + IA treatment and 8 weeks oral antibiotic treatment), and a follow-up period of approximately 3.5 months for approximately 6.25 months.

Number of participants:

Twenty-three evaluable participants are planned to be enrolled in this study, with the first 3 participants serving as sentinel groups, followed by 20 participants, 10 participants in the test group and 10 participants in the control group. Evaluable participants are those who completed Visit 5 (EA visit) and PK study.

Primary Statistical Analysis Methods:

Analysis Population

All analysis populations will be confirmed prior to study database lock.

Full Analysis Set (FAS): includes all enrolled participants who received at least 1 dose of investigational product. Analyses were performed by planned treatment group.

Microbiological Intent-to-Treat Set (micro-ITT): includes all enrolled participants with etiologically confirmed Gram-positive bacterial infections (except Enterococcus spp., Mycobacterium spp.) who received at least 1 dose of investigational product. Analyses were performed by planned treatment group.

Safety set (SS): includes all participants who received at least one dose of investigational product after enrollment and have safety evaluation. Analyses were performed by actual treatment group.

PK Concentration Analysis Set (PKCS): All enrolled participants who had used TNP-2092 and had at least one observed PK concentration comprised the PK concentration analysis set for this study. Analyses were performed by actual dose group.

PK Parameter Analysis Set (PKPS): All enrolled participants who had used TNP-2092 and had at least one PK parameter observed comprised the PK parameter analysis set for this study. Analyses were performed by actual dose group.

Safety and tolerability analysis

Safety and tolerability of TNP-2092 IA treatment will be evaluated by treatment-emergent adverse events, serious adverse events, laboratory tests, vital signs, and physical examination results summarized by treatment group based on the SS analysis set. Descriptive statistics will be mainly performed for safety and tolerability.

Pharmacokinetic analysis

Based on the PKCS analysis set, TNP-2092 plasma and synovial fluid concentrations will be summarized by time point with descriptive statistics including the number of observations, arithmetic mean, standard deviation, arithmetic coefficient of variation (% CV), geometric mean, median, geometric% CV, minimum, and maximum. Plasma concentration-time data will be used to derive the following PK parameters using a noncompartmental model: first-dose PK parameters included T_{max}, C_{max}, $t_{1/2}$, $AUC_{0-\infty}$, AUC_{0-t}, etc.; steady-state PK parameters included $T_{max, ss}$, $C_{max, ss}$, $t_{1/2, ss}$, $AUC_{0-t, ss}$, $AUC_{0-\infty,ss}$, $AUC_{0-tau, ss}$, R_{ac}, etc. Descriptive statistics for the PK parameters described above will be performed based on the PKPS analysis set and included the number of observations, arithmetic mean, standard deviation, %CV, geometric mean, median, geometric% CV, minimum, and maximum. Descriptive statistical analysis will not be performed for $AUC_{0-\infty}$, $t_{1/2}$, $AUC_{\_\% Extrap}$ if the residual area under the plasma concentration-time curve after dosing ($AUC_{\_\% Extrap}$) is > 20% in participants.

Efficacy Analysis

EA response rate, EOT response rate, 6-month treatment failure rate after start of IV + IA treatment, and other categorical variables will be described by frequency and percentage by dose group (if any) and treatment group based on the micro-ITT analysis set. Fisher 's exact test will be used to compare the test group with the control group in the randomization phase, and the 95% confidence interval (CI) for the rate difference between the test group and the control group will be estimated with the exact method. Continuous variables (e.g., laboratory test indicators) will be described by mean, standard deviation, median, maximum and minimum in different dose groups (if any) and treatment groups and compared between groups using t test or Wilcoxon rank sum test. Comparisons before and after treatment will be performed using paired t-test or signed rank sum test. Unless otherwise specified, all statistical tests will use a 2-sided test with alpha = 0.05 to calculate the 2-sided 95% CI. All statistical tests are exploratory.

Sentinel participants and randomized participants will be analyzed separately, and sentinel participants will also be combined into trial arms for pooled analysis.