A Multicenter, Randomized, Double-Blind, Parallel-Controlled, Dose-Finding Phase 2 Study to Compare the Anti-tetanus Neutralizing Antibody Titers and Safety of TNM002 Injection with Human Tetanus Immunoglobulin or Placebo Following a Single Intramuscular Injection in Chinese Adult Volunteers

Version No.: V2.2 Version Date: 20-June-2022

NCT No.:	NCT05625477
Protocol No.:	TNM002-P2-CH01
Investigational Product:	TNM002 Injection
Study Phase:	2
Study Site:	PKUCare Luzhong Hospital
Sponsor:	Trinomab Biotech Co., Ltd.
Compliance Statement:	This study will be conducted in strict compliance with Good Clinical Practice

Study Protocol

Title	A Multicenter, Randomized, Double-Blind, Parallel-Controlled, Dose-Finding Phase II Study to Compare the Anti-tetanus Neutralizing Antibody Titers and Safety of TNM002 Injection with Human Tetanus Immunoglobulin or Placebo Following a Single Intramuscular Injection in Chinese Adult Volunteers
Protocol No.	TNM002-P2-CH01
Study Phase	II
Objectives	**Primary:** • To compare the anti-tetanus neutralizing antibody titers of TNM002 with human tetanus immunoglobulin (HTIG) following a single intramuscular (IM) injection in Chinese adult volunteers. **Secondary:** • To compare the safety and tolerability of TNM002 with HTIG and placebo following a single IM injection in Chinese adult volunteers. • To assess the pharmacokinetic (PK) properties of TNM002 in Chinese adult volunteers. • To assess the immunogenicity of TNM002 in Chinese adult volunteers. **Exploratory:** • To explore the population pharmacokinetic/pharmacodynamic (PopPK-PD) properties of TNM002 in Chinese adult volunteers.
Endpoints	**Primary:** • Proportion of volunteers with an increase of anti-tetanus neutralizing antibody titers from baseline (ΔTiters) ≥ 0.01 IU/mL at 24 hours post-dose *In this study, serum anti-tetanus neutralizing antibody titers is a pharmacodynamic (PD) parameter.* **Secondary:** • Safety endpoints, including type and incidence of adverse events (AEs) and serious adverse events (SAEs), physical examinations, vital signs, 12-lead ECGs, and laboratory tests (hematology, blood chemistry, and urinalysis); • The anti-tetanus neutralizing antibody ΔTiters at 24 hours, 48 hours (Day 3), and on Days 7, 21, 30, and 90 post-dose; • Proportion of volunteers with anti-tetanus neutralizing antibody ΔTiters ≥0.01 IU/mL at 48 hours (Day 3) and on Days 7, 21, 30, and 90 post-dose; • Proportion of volunteers with anti-tetanus neutralizing antibody ΔTiters ≥0.01 IU/mL at both 24 hours and Day 30 post-dose; • Proportion of volunteers with anti-tetanus neutralizing antibody ΔTiters ≥0.1 IU/mL at 24 hours, 48 hours (Day 3) and on Days 7, 21, 30, and 90 post-dose; • Duration of anti-tetanus neutralizing antibody ΔTiters ≥0.01 IU/mL post-dose; • Duration of anti-tetanus neutralizing antibody ΔTiters ≥0.1 IU/mL post-dose; • The anti-tetanus neutralizing antibody titers at 24 hours, 48 hours (Day 3), and on Days 7, 21, 30, and 90 post-dose; • Proportion of volunteers with anti-tetanus neutralizing antibody titers ≥0.01 IU/mL at 24 hours, 48 hours (Day 3), and on Days 7, 21, 30, and 90 post-dose; • Proportion of volunteers with anti-tetanus neutralizing antibody titers ≥0.1 IU/mL at 24 hours, 48 hours (Day 3), and on Days 7, 21, 30, and 90 post-dose; • PK parameters of TNM002 including maximum concentration (C_{max}), time to maximum concentration (T_{max}), elimination half-life ($t_{1/2}$), area under the plasma concentration-time curve from time 0 to t (AUC_{0-t}), and area under the plasma concentration-time curve from time 0 to ∞ ($AUC_{0-\infty}$); and apparent clearance (CL/F) and apparent volume of distribution

<table>
<tr><td></td><td>(V_d/F) if data permitted;
• Positive rate of anti-drug antibody (ADA) in the TNM002 group.</td></tr>
<tr><td>Study Design</td><td>This study is designed as a multicenter, randomized, double-blind, active- and placebo-controlled study to compare the anti-tetanus neutralizing antibody titers and safety of TNM002 Injection with HTIG or placebo following a single IM injection, and determine the dose of TNM002 Injection for tetanus prophylaxis in phase III clinical study. The study will be conducted at approximately 5-10 study centers, and approximately 240 Chinese male or female adult volunteers are planned to be enrolled. Eligible volunteers will be randomized in a ratio of 2: 2: 1: 2: 1 to TNM002 Injection 5 mg, TNM002 Injection 10 mg, TNM002 Injection 15 mg, HTIG 250 IU, and placebo groups. At randomization, the volunteers will be stratified by the rapid test results of tetanus antibody IgG (negative and positive), and the proportion of volunteers with negative test results is at least 50%. The study drug and number of volunteers in each treatment group are shown in the table below.
<table>
<tr><th>Group</th><th>Number of Volunteers</th><th>Drug for Injection</th><th>Dose</th><th>Dose Volume</th></tr>
<tr><td>1</td><td>60</td><td>TNM002 Injection</td><td>5 mg</td><td>0.25 mL</td></tr>
<tr><td>2</td><td>60</td><td>TNM002 Injection</td><td>10 mg</td><td>0.5 mL</td></tr>
<tr><td>3</td><td>30</td><td>TNM002 Injection</td><td>15 mg</td><td>0.75 mL</td></tr>
<tr><td>4</td><td>60</td><td>HTIG Injection</td><td>250 IU</td><td>2.5 mL</td></tr>
<tr><td>5</td><td>30</td><td>Placebo</td><td>NA</td><td>0.5 mL</td></tr>
</table>
This study will consist of a screening period (D-28 to D-2), an in-patient period (D-1 to D4), and a follow-up period (D5 to D106). Eligible volunteers will be admitted to the study site on D-1. After completing the relevant tests required by the protocol and randomization, all volunteers will receive the study drug on D1. Dispensing and administration of the study drug will be performed by unblinded study personnel, and the injection time of all study drugs should be controlled within 30 (± 3) seconds. After dosing, volunteers will undergo the safety assessment at the study site, including physical examination, vital signs, 12-lead ECGs, and laboratory tests (hematology, blood chemistry, and urinalysis). At the same time, the investigator will also collect serum samples at specified time points for anti-tetanus neutralizing antibody titers, PK, and immunogenicity analysis. Safety results on D-1 are defined as the baseline of safety assessment, and anti-tetanus neutralizing antibody titers, PK, and immunogenicity analysis results of serum samples collected prior to dosing on D1 are defined as the baseline of the relevant assessment. Volunteers will be discharged from the hospital after completing safety assessment and serum sample collection on D4. After completing the blood collection at 48 hours post-dose and safety assessment during the in-patient period, the subject can be discharged early at the comprehensive judgment of the investigator.
Volunteers will return to the study site on Days 7, 14, 21, 30, 60, 90, and 106 for safety assessment and/or serum sample collection for anti-tetanus neutralizing antibody titers, PK, and immunogenicity analysis. The visit on D106 is considered as the end of study (EOS) visit, during which the volunteers will undergo a comprehensive safety assessment.</td></tr>
</table>

Study Population	Chinese adult volunteers
Inclusion Criteria	**Each subject must meet the following criteria to be enrolled in this study:** 1) Chinese male or female adults aged ≥ 18 years; 2) Healthy volunteers with normal or abnormal but not clinical significant results on physical examination, vital signs and clinical laboratory tests at screening or volunteers with stable chronic diseases and who are not expected to be at significant risk for participating in the clinical study as judged by the investigator; The chronic diseases include hypertension, diabetes mellitus, hyperlipidemia, hyperuricemia, etc. A volunteer with stable chronic disease is defined as follows: • A volunteer without hospitalization or emergency visits due to worsening chronic disease/state within 12 months prior to screening; • A volunteer who maintains a stable regimen for chronic diseases within 3 months prior to screening; • A volunteer with no acute change in chronic disease/status within 1 month prior to screening and who is not expected to worsen or have significant change in treatment during the study as judged by the investigator. 3) Women of childbearing potential and at risk of pregnancy and men of childbearing potential must agree to use at least one of the following highly effective contraceptive methods throughout the study and for 150 days after dosing, including: • Intrauterine device (IUD); • Oral, injectable, or implantable hormonal contraceptives; • Barrier contraception + spermicide; • Surgical contraceptive methods (e.g., vasectomy, salpingectomy, hysterectomy, etc.); Women of no childbearing potential will be authorized to participate in this study if at least one of the following criteria are met: • Documented hysterectomy and/or bilateral oophorectomy; • Medically confirmed ovarian function failure; • Postmenopausal, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; and have a serum follicle stimulating hormone (FSH) level confirming the post-menopausal status. 4) Volunteers who are able to well communicate with investigator as well as understand and adhere to the requirements and procedures of this study; 5) Volunteers who voluntarily provide signed written informed consent form.

Exclusion Criteria	**Volunteers who meet any of the following criteria will be excluded from the study:** 1) History of allergy to the investigational product, human immunoglobulin preparation or any component of other therapeutic monoclonal immunoglobulins; 2) History of chronic eczema or urticaria or multiple drug allergies; 3) History of blood phobia or needle sickness; 4) History of acute infection within 1 week prior to screening; 5) Previously diagnosed or suspected immunodeficiency or autoimmune disease; 6) Exposure to tetanus vaccine within the 10 years; 7) Receipt of an immunoglobulin or blood products within 6 months prior to dosing; 8) Exposure to any live attenuated or inactivated vaccines (including COVID-19 vaccine) within 4 weeks prior to dosing or plan to receive such vaccines within 3 months after dosing; 9) Use of any new drug therapy within 2 weeks or 5 drug half-lives (whichever is longer) prior to screening; 10) Known or suspected history of drug abuse within the past 5 years or a previous history of drug addiction or with positive urine drug test; 11) History of significant alcohol abuse within 6 months prior to screening, or any indication of regular use of more than 14 units of alcohol per week (1 unit = 360 mL of beer or 45 mL of alcohol 40% or 150 mL of wine); 12) Participation in any clinical studies with drugs or device within 3 months prior to screening, or ≤ 5 elimination half-lives (whichever is longer) from the last dose of the investigational drug, except for participation in clinical studies that do not use the investigational drug or device after screening or the observational, non-interventional studies; 13) History of clinically significant bleeding disorders (e.g., coagulation factor deficiency, coagulation defects, or platelet disorder); 14) Scars, tattoos, rash at the injection site that affect the administration or assessment; 15) Positive for human immunodeficiency virus (HIV) or hepatitis C virus (HCV) antibodies, or treponema pallidum-specific antibody or hepatitis B surface antigen (HBsAg) at screening; 16) Ear temperature > 37.5 °C, pulse rate > 100 beats/min or < 50 beats/min at screening; 17) For volunteers with no prior chronic disease or age < 60 years, systolic blood pressure (SBP) ≥ 140 mmHg or < 90 mmHg, and/or diastolic blood pressure (DBP) ≥ 90 mmHg or < 50 mmHg or ECG QTcF (heart rate-corrected) > 450 ms and/or other clinically significant abnormalities expected to be at significant risk for participating in the clinical study; 18) Laboratory tests: alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2 ULN, total bilirubin > 1.5 ULN, decreases in haemoglobin (< 120 g/L in males and < 100 g/L in females), platelet count < 120×10^9/L, white blood cell count < 3×10^9/L, neutrophil count < 1.8×10^9/L or abnormal and clinically significant test results as judged by the investigator (except for the abnormal and clinically significant test results that can be explained by chronic disease); 19) Pregnant or lactating women; 20) Study personnel involved in the design and/or conduct of this study; 21) Other conditions that are considered to be not suitable for the study by the investigator.

<table>
<tr><td>Study Drugs</td><td>
<table>
<tr><th>Drug Name</th><th>TNM002 Injection</th><th>Human Tetanus Immunoglobulin</th><th>Placebo</th></tr>
<tr><td>Dosage Form</td><td>Injection, solution</td><td>Injection, solution</td><td>Injection, solution</td></tr>
<tr><td>Strength</td><td>20.0 mg/1.0 mL/vial</td><td>250 IU/2.5 mL/vial</td><td>1 mL/vial</td></tr>
<tr><td>Route of Administration</td><td>IM gluteal injection</td><td>IM gluteal injection</td><td>IM gluteal injection</td></tr>
</table>
</td></tr>
<tr><td>Sample Size Estimation</td><td>Approximately 240 Chinese adult volunteers are planned to be enrolled in this study. Since this study is a phase II dose-finding study, the sample size is not estimated based on formal statistical assumptions.</td></tr>
</table>

Study Flow Chart

Period	Screening Period	In-Patient Period[a]					Follow-up Period							Premature Withdrawal
Study Day	**D-28~D-2**	**D-1**	**D1**	**D2**	**D3**	**D4**	**D7**	**D14**	**D21**	**D30**	**D60**	**D90**	**D106**	
Time window	**/**	**/**	**/**	**/**	**/**	**/**	**±1d**	**±2d**	**±2d**	**±2d**	**±3d**	**±5d**	**±7d**	**±7d**
Informed consent	X													
Inclusion/exclusion criteria	X	X												
Randomization		X												
Demographics	X													
Medical/treatment history	X													
Weight/height	X													
Vital sign[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X					X		X			X		X	X
12-lead ECG	X		X[2]			X		X			X		X	X
Rapid test of tetanus antibody IgG	X													
Serum pregnancy test[3]	X	X								X	X	X	X	X
Serum FSH test[4]	X													
Viral serology test[5]	X													
Laboratory tests (including hematology, blood chemistry, and urinalysis)	X	X[6]				X		X			X		X	X
Urine drug test	X	X												
Admission		X	X	X	X	X								
Study drug administration			X											
Injection site assessment[7]			X											
Serum sample collection for anti-tetanus neutralizing antibody titers test[8]			X	X	X	X	X	X	X	X	X	X	X	X
Serum sample collection for PK analysis[8]			X	X	X	X	X	X	X	X	X	X	X	X
Serum sample collection for immunogenicity analysis[8]			X				X	X	X	X	X		X	X

Recording of concomitant medications	X
Recording of AEs	X

Notes:

a. After completing the blood collection at 48 hours post-dose and safety assessment during the in-patient period, the subject can be discharged early at the comprehensive judgment of the investigator.

1. A vital sign test will be performed within 2 hours prior to dosing on Study Day D1. For other visits, a vital sign test should be performed on the same day.
2. A 12-lead ECG will be performed within 2 hours prior to dosing on D1. Before 12-lead ECG measurements, the volunteers should have a rest for at least 5 minutes and smoking, alcohol consumption, strenuous exercise, and mood fluctuation will be not allowed.
3. A serum (beta human chorionic gonadotropin [β-HCG]) pregnancy test will be performed for women of childbearing potential only.
4. Female volunteers who have not undergone hysterectomy or bilateral oophorectomy and have been menopausal for at least 1 year without other pathological or physiological reasons will undergo the serum FSH test to determine their post-menopausal status. The serum FSH test is not required for female volunteers of childbearing potential. Female volunteers with amenorrhea for less than 1 year should be considered fertile and do not require FSH test.
5. Viral serology tests include HBsAg, HCV antibody, HIV and treponema pallidum-specific antibodies.
6. If laboratory tests have been performed within 7 days prior to admission (D-8 to D-1), then the tests do not need to be repeated on D-1.
7. On D1 of the in-patient period, an injection reaction assessment will be performed 30 minutes (± 5 minutes) after IM injection of the study drug, and the local reactions at the injection site will be closely monitored and recorded.
8. The collection time of serum samples for anti-tetanus neutralizing antibody titers, PK and immunogenicity analysis are detailed in Section 8.2-8.4 of the text.

Statistical Analysis Plan

All statistical analysis will be performed using SAS version 9.4 or above.

The statistical analysis is mainly descriptive analysis. The continuous variables will be statistically described using the number of volunteers, mean, median, standard deviation (SD), minimum and maximum. The categorical and grade variables will be statistically described using frequencies and percentages for each category or grade, and missing values will not be included in the calculation of percentages unless otherwise stated.

No formal hypothesis testing will be performed on the data, but two-sided 95% confidence interval (CI) will be calculated based on $\alpha = 0.05$.

Statistical Analysis Population

- **Full Analysis Set (FAS):** all randomized volunteers who receive at least one dose of the study drug will be included in the FAS, which will be used for the analysis of volunteer disposition, demographics and baseline characteristics.
- **Anti-tetanus Neutralizing Antibody Titers Analysis Set:** all randomized volunteers who receive at least one dose of the study drug with at least one valid post-dose serum anti-tetanus neutralizing antibody titers test data will be included in the anti-tetanus neutralizing antibody titers analysis set. If an event occurred that could affect the anti-tetanus neutralizing antibody titer or the detection (e.g., major violation of protocol inclusion criteria, use of prohibited drugs during the study, etc.), all or partial test data of corresponding volunteers will not be included in the anti-tetanus neutralizing antibody titers analysis.
- **Safety Set (SS):** all randomized volunteers who receive at least one dose of the study drug with at least one post-dose safety evaluation will be included in the SS. SS is the safety evaluation population for this study.
- **PK Concentration Set (PKCS):** all randomized volunteers who receive at least one dose of the study drug with at least one analyzable PK concentration point during the study will be included in the PKCS. If an event occurred that could affect the PK concentration or the detection (e.g., major violation of protocol inclusion criteria, use of prohibited drugs during the study, etc.), all or partial PK data of corresponding volunteers will not be included in the PKCS. The PKCS will be used for the PK concentration analysis.

PK Parameter Set (PKPS): all randomized volunteers who receive at least one dose of the study drug with at least one analyzable PK parameter during the study will be included in the PKPS. If an event occurred that could affect the PK parameters (e.g., major violation of protocol inclusion criteria, use of prohibited drugs during the study, etc.), all or partial PK data of corresponding volunteers will not be included in the PKPS. The PKPS will be used for the PK parameter analysis.

- **Immunogenicity Analysis Set:** all randomized volunteers who receive TNM002 with at least one post-dose immunogenicity data will be included in the immunogenicity

analysis set.

The anti-tetanus neutralizing antibody titers, PK, immunogenicity, and safety analysis will be based on the actual treatment group, and the remaining analysis will be based on the randomized treatment group unless otherwise specified.

Demographic Data and Other Baseline Characteristics

Demographic data and other baseline characteristics will be analyzed descriptively by treatment group using descriptive statistics, and data will be tabulated and summarized.

Anti-tetanus neutralizing antibody titers Analysis

The number, percentage, 90% CI and 95% CI (based on the Clopper-Pearson method) of volunteers with an increase of anti-tetanus neutralizing antibody titers (Δ titers) ≥ 0.01 IU/mL from baseline at 24 hours post-dose will be calculated. The difference in the percentage of volunteers with Δ titers ≥ 0.01 IU/mL as well as 90% CI and 95% CI (based on the stratified Miettinen-Nurminen method, stratified by the rapid tetanus antibody IgG test results of at randomization stratification) of the difference between groups will be provided. Volunteers who withdraw from the study prematurely without detectable anti-tetanus neutralizing antibody titers at the respective time points will not be included in the analysis.

The anti-tetanus neutralizing antibody titers will be summarized by treatment group at each scheduled time point using the descriptive statistics, including number, median, minimum, maximum, geometric mean titer (GMT), geometric SD, 90% CI and 95% CI of GMT. In addition, the anti-tetanus neutralizing antibody titers at each scheduled time point after dosing will be compared and analyzed between treatment groups based on the mixed-effect model for repeated measures (MMRM). The MMRM model uses the log-transformed anti-tetanus neutralizing antibody titers (referred to as "log antibody titer") at each scheduled time point as the dependent variable, the treatment group, the rapid tetanus antibody IgG test results at randomization stratification, time point, and treatment group-time point interaction as the independent variables, and the intra-subject variance-covariance structure as the nonstructured variance structure (UN). The difference in log antibody titer and the 90% CI and 95% CI of the difference between different treatment groups will be calculated. The difference and corresponding CI of the difference will be antilog-transformed to obtain the ratio of GMT and CI of ratio between different treatment groups. If the model does not converge when the covariance structure is UN, other possible covariance structures, such as Toelitz, autoregression (1) (AR (1)), etc., can be selected, and the covariance structure with the smallest final AIC value will be used for the final model.

The change from baseline in anti-tetanus neutralizing antibody titers at different time points post-dose and the proportion of volunteers with anti-tetanus neutralizing antibody titers ≥ 0.01 IU/mL and ≥ 0.1 IU/mL, ≥ 0.01 IU/mL and ≥ 0.1 IU/mL increase from baseline in anti-tetanus neutralizing antibody titers, ≥0.01 IU/mL increase from baseline in anti-tetanus neutralizing antibody titers at 24 hours and on Day 30 post-dose in different treatment groups will be summarized and compared using a similar statistical analysis method.

The duration of Δ titers≥ 0.01 IU/mL post-dose from baseline is defined as the time interval from the first to the last observed increase of Δtiters ≥ 0.01 IU/mL from baseline. Subjects whose Δ titers remain ≥ 0.01 IU/mL at the last test time point of the study will be considered

censored in duration calculation. The median duration, corresponding two-sided 90% CI, 95% CI, minimum and maximum will be estimated using the Kaplan-Meier method. The duration between different treatment groups will be compared using the Cox proportional hazards model, which will include the treatment groups and the rapid tetanus antibody IgG test results at randomization stratification. If the censoring proportion is low, a descriptive summary of duration will be considered and the duration between different treatment groups will be analyzed based on an analysis of covariance (ANCOVA) model, which will include the treatment groups and the rapid tetanus antibody IgG test results at randomization stratification. The duration of ΔTiters≥ 0.1 IU/mL post-dose from baseline will be analyzed using the same analytical method.

If data are applicable, the subjects will be divided into two subgroups (＜0.01 IU/mL and ≥ 0.01 IU/mL) based on the test results of anti-tetanus antibody in serum samples collected prior to dosing to repeat the above analysis.

The anti-tetanus neutralizing antibody titers at baseline, the post-baseline anti-tetanus neutralizing antibody titers at each time point and the change from baseline in anti-tetanus neutralizing antibody titers will be tabulated by treatment group.

Safety Analysis

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0 or higher. The analysis of AE will be based on treatment-emergent adverse events (TEAEs), while AEs before treatment will be tabulated. All TEAEs, TEAEs related to the study drug, and SAEs will be summarized and analyzed by system organ class (SOC) and preferred term (PT), and the number and incidence will be calculated.

The quantitative safety variables such as laboratory tests, vital signs, 12-lead ECGs, and their changes from baseline will be summarized using descriptive statistics. The changes from baseline in clinical significance of safety variables such as laboratory tests, 12-lead ECGs, and physical examinations will be described using the shift tables.

Pharmacokinetics Analysis

The plasma concentration of the study drug at each scheduled sampling time point for each treatment group will be summarized descriptively, and the mean plasma concentration-time curves (both linear and semi-log scales) will be plotted. The PK concentration data at different time points will be tabulated separately by treatment group.

The PK parameters will be summarized by treatment group using appropriate descriptive statistics including number of volunteers, mean, geometric mean, SD, minimum, median, maximum, and coefficient of variation (CV), and the PK parameters will be tabulated.

Immunogenicity Analysis

The positive rate of ADA in TNM002 group at baseline and at different post-baseline time points will be summarized. For ADA-positive volunteers, the number and percentage of Nab positive volunteers will also be further calculated if data permit. The impact of ADA and/or Nab production on the efficacy, PK profile, and safety of TNM002 will be assessed, as applicable.

PopPK-PD Analysis

PK/PD analysis will be performed using NONMEM (version 7.3 or higher) and/or R software (version 4.0.5 or higher).