Study Protocol

A 4-week Repeat Dose Toxicity Study of Y-3 by Intravenous Injection in Sprague-Dawley Rats

**Test Facility**

|  |  |
| --- | --- |
| Name: | CTI Biotechnology (Suzhou) Co., Ltd. |
| Address: | No. 166, Yuanfeng Road, New & Hi-tech Industrial Development Zone, Kunshan City, Jiangsu Province |
| Telephone: | 0512-36801688 |

**Sponsor**

|  |  |
| --- | --- |
| Name: | NeuroDawn Pharmaceutical Co., Ltd. |
| Address: | Building E2, 4th floor, Jiangsu LifePark , No.9, Weidi Road, Qixia District, Nanjing |
| Telephone: | +86-13851758287 |

# Protocol Signature Page

Study Name: A 4-week Repeat Dose Toxicity Study of Y-3 by Intravenous Injection in Sprague-Dawley Rats

Study Number: A2021003-T011-01

Shiyu Xing 2021-06-23

Shiyu Xing Date

Study Director

Lian Meng 2021-06-23

Lian Meng Date

QA

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# Sponsor's Signature Page

I have understood the relevant requirements of GLP regulations with which this study complies and have evaluated the test facility to ensure that the features of the test article and/or reference standard provided are true and accurate. I have also informed the test facility of the safety of the test article and/or reference standard under this study.

I have confirmed the contents of this protocol and grant my permission for its implementation.

Fang Fang 2021-06-21

Fang Fang Date

Head of the Sponsor (or Representative)

**This page is translated from the following Signature Page in Chinese.**

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# General Information

* 1. **Study Name and Number**

Study Name: A 4-week Repeat Dose Toxicity Study of Y-3 by Intravenous Injection in Sprague-Dawley Rats;

Study Number: A2021003-T011-01.

* 1. **Study Objective**

Sprague-Dawley (SD) rats will be treated with Y-3 once daily by intravenous injection for 4 consecutive weeks, followed with a 4-week recovery phase, to observe the nature, degree, dose-effect, time-effect relationship and reversibility of the toxic reaction that may be caused by the test article, and to preliminarily determine the toxic target organ or target tissue, and its toxicokinetic characteristics, so as to provide references for clinical trials.

* 1. **Test Facility**

Name: CTI Biotechnology (Suzhou) Co., Ltd..

Address: No. 166, Yuanfeng Road, New & Hi-tech Industrial Development Zone, Kunshan City, Jiangsu Province.

Postcode: 215300.

Contact person: Yuye Xia.

Tel: 0512-36801688.

Fax: 0512-36802288.

E-mail: xiayuye@cti-cert.com.

* 1. **Sponsor**

Name: NeuroDawn Pharmaceutical Co., Ltd..

Address: Building E2, 4th floor, Jiangsu LifePark , No.9, Weidi Road, Qixia District, Nanjing;

Postcode: 210042.

Contact: Fang fang.

Tel: +86-13851758287.

E-mail: fangfang@simovay.com.

* 1. **Study Personnel**
     1. **Study Director**

Name: Shiyu Xing.

Address: No. 166, Yuanfeng Road, New & Hi-tech Industrial Development Zone, Kunshan City, Jiangsu Province.

Tel: 0512-36801688.

Fax: 0512-36802288.

E-mail: xingshiyu@cti-cert.com.

* + 1. **Key Study Personnel**

Study procedures: Min Shen.

Veterinarian: Haiyang Wang.

Animal husbandry: Zhihong Wu.

Test article management: Xiaoyan Chen.

Preparation of test article: Jiaping Weng.

Dose formulation analysis: Ping Li.

Clinical examination: Sheng Li.

TK sample analysis: Pei Zhang, Shuangshuang Ye.

Necropsy personnel: Yong Chen.

Gross observation: Fang Zhou, Qi Liu, Yanyan Han.

Histopathological examination: TBD.

Data collection and statistical analysis: Shiyu Xing.

Note: The above personnel are the main responsible persons, and specific operators will be truthfully reflected in the original records.

* 1. **Quality Assurance Personnel**

Name: Lian Meng.

Tel: 0512-36801688.

E-mail: cti-btc-qa.list@cti-cert.com.

Note: If any of the above personnel has changed, the specific information shall be faithfully reflected in the study report.

* 1. **Regulations and Technical Guidelines to be Followed**

The regulations and technical guidelines to be followed in this study include, but not limited to:

FDA Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58, FDA)；

OECD Principles on Good Laboratory Practice [ENV/MC/CHEM (98)17, OECD].

Provisions for Drug Registration (NMPA, July 2020);

Guidance on Repeat Dose Toxicity Studies for Pharmaceuticals (former CFDA, May 2014);

Guidance on Toxicokinetic Studies for Pharmaceuticals (former CFDA, May 2014).

Q&A for Non-clinical Safety Evaluation of Test Articles (former CFDA, May 2014).

ICH M3 (R2): Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. June, 2009.

ICH S4: Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing). June 1998.

This study will be conducted in accordance with the Standard Operating Procedures (SOPs) of the facility, except as otherwise specified in the protocol.

* 1. **Quality Assurance**

In accordance with the former China Food and Drug Administration (former CFDA) Good Laboratory Practice (Order No. 34), the United States Food and Drug Administration (FDA) Good Laboratory Practice for Nonclinical Laboratory Studies (21 CFR 58), the Organization for Economic Cooperation and Development (OECD) OECD Principles of Good Laboratory Practice [ENV/MC/CHEM (98) 17], and the SOPs of the test facility, the Quality Assurance Unit (QAU) will supervise, investigate and review the study protocol, protocol amendments (if any), study process, raw data and final report to ensure the credibility of the study process and study results.

* 1. **Study Schedule**

Scheduled study start date: 2021-06-24.

Scheduled date of first dose: 2021-06-30.

Scheduled necropsy date:

Necropsy date at the end of the dosing phase: 2021-07-28.

Necropsy date at the end of the recovery phase: 2021-08-25.

Scheduled study end date: 2021-08-25.

Scheduled report completion date: 2021-11.

# Study Materials

2. 1. **Test Article**
      1. **General Information**

Name/Code: Y-3.

Facility code: W2021005.

Characterization: light yellow powder.

Strength: 300 g/bag

Content: 96.5%.

Batch No.1193-15-08.

Storage conditions: 2-8℃, protected from excessive light and sealed.

Expiry date: 2023-01-06.

Manufacturer: Jiangsu Simovay Pharmaceutical Co., Ltd (Simovay).

Supplier: NeuroDawn Pharmaceutical Co., Ltd.

Note: If any other batch numbers are used in the study, the relevant information will be truthfully recorded and reflected in the original records and final report, and the final report shall prevail.

* + 1. **Preparation of Test Article**

Preparation method of Y-3 dose formulation: The amount of test article required will be calculated based on the most recent body weight of the animal, administered dose and content (96.5%) [Amount of test article = body weight × administered dose/content (96.5%)]. The concentration of test article required will be calculated based on the amount of test article, content of the test article (96.5%) and preparation volume [Preparation concentration = amount of test article × content (96.5%)/preparation volume]. Measure the appropriate amount of propylene glycol corresponding to 6% of the final volume and add it to a calibrated container, add Kolliphor® HS 15 in an amount corresponding to 3% of the final volume and mix well, add a specific amount of the test substance and stir until dissolved, add a sufficient amount of sodium chloride injection to achieve clarity, further, add mannitol corresponding to 5% of the final volume and stir until completely dissolved ,then adjust the pH to 8.0 using a 0.1 mol/L NaOH solution. Finally, add sodium chloride injection to reach the calibrated scale, thereby obtaining the required concentration of the dose formulation.

On the day of administration, the sub packaged test article to be distributed will be filtered and sterilized with a 0.22 μm PES filter membrane under sterile conditions, the test article shall be protected from light and in room temperature and prepare and use it immediately.

Preparation method: 100 mL dose formulation is used as an example. The preparation method is shown in the following table:

| **Group** | **Dosage**  **（mg/kg）** | **Concentration**  **（mg/mL）** | **Preparation method** |
| --- | --- | --- | --- |
| Y-3  Low Dose Group | 10 | 0.5 | Weigh 6 g of propylene glycol into a calibrated 100 mL container. After adding 3 g of Kolliphor® HS 15 and stirring until well mixed, add 51.8 mg of the test article. Stir until dissolved and then add a suitable amount of sodium chloride injection until the solution is clear. Next, add 5 g of mannitol and stir until completely dissolved. Adjust the pH to 8.0 using a 0.1 mol/L NaOH solution. Finally, add sodium chloride injection to the calibrated mark (100 mL) to obtain the Y-3 low dose group formulations at 0.5 mg/mL. |
| Y-3  Medium Dose Group | 20 | 1.0 | Weigh 6 g of propylene glycol into a calibrated 100 mL container. After adding 3 g of Kolliphor® HS 15 and stirring until well mixed, add 103.6 mg of the test article. Stir until dissolved and then add a suitable amount of sodium chloride injection until the solution is clear. Next, add 5 g of mannitol and stir until completely dissolved. Adjust the pH to 8.0 using a 0.1 mol/L NaOH solution. Finally, add sodium chloride injection to the calibrated mark (100 mL) to obtain the Y-3 medium dose group formulations at 1.0 mg/mL. |
| Y-3  High Dose Group | 40 | 2.0 | Weigh 6 g of propylene glycol into a calibrated 100 mL container. After adding 3 g of Kolliphor® HS 15 and stirring until well mixed, add 207.3 mg of the test article. Stir until dissolved and then add a suitable amount of sodium chloride injection until the solution is clear. Next, add 5 g of mannitol and stir until completely dissolved. Adjust the pH to 8.0 using a 0.1 mol/L NaOH solution. Finally, add sodium chloride injection to the calibrated mark (100 mL) to obtain the Y-3 high dose group formulations at 2.0 mg/mL. |

Preparation condition: Room temperature and avoid light.

Identification method: The prepared Y-3 low medium and high dose groups will be marked with green, blue and red labels, respectively, indicating the study number, name, concentration, quantity, preparation date, preparer, storage conditions, expiry date and finished product number.

Temporary storage conditions after preparation and expiry date: Room temperature, protected from light for use within 24 hours; 2 ~ 8 oC, protected from light for use within 8 days.

* + 1. **Handling of Retention Samples and Remaining Dose Formulations/Test Articles**

Test article retention samples: The sample reserved shall be at least three times the amount required to complete the purity/content test once.

Handling of remaining dose formulations: The remaining dose formulations will be returned to the Test Article Management Department for disposal as drug/chemical waste.

Handling of retained test articles: After the end of the project, the retained test articles will be transferred from the Test Article Management Department to the Archive Management Department for storage in the retention sample archive of the facility.

Handling of remaining test articles: After all studies of the project completed, they will be returned to the sponsor.

* 1. **Vehicle**
     1. **Vehicle 1 General Information**

Name: 1, 2- Propylene Glycol.

Characterization: Colorless viscous liquid.

Batch No.: K2026198.

Expiry date: 2023-03-31.

Storage conditions: Room temperature.

Manufacturer: Aladdin.

If other Batch No. is used in the study, the specific information shall be truthfully recorded and reflected in the original and final report, and the final report shall prevail.

* + 1. **Vehicle 2 General Information.**

Name: Kolliphor ® HS 15.

Characterization: Yellow to cream color paste.

Batch No.: 30744347 G0.

Expiry date: 2022-09-07 (retest date).

Storage conditions: Room temperature.

Manufacturer: BASF SE.

If other Batch No. is used in the study, the specific information shall be truthfully recorded and reflected in the original and final report, and the final report shall prevail.

* + 1. **Vehicle 3 General Information.**

Name: Mannitol.

Characterization: White powder.

Batch No.: E243K.

Expiry date: 2023-05-30.

Storage conditions: Room temperature.

Manufacturer: ROQUETTE.

If other Batch No. is used in the study, the specific information shall be truthfully recorded and reflected in the original and final report, and the final report shall prevail.

* + 1. **Vehicle 4 General Information.**

Name: Sodium chloride injection.

Characterization: Clear and colorless liquid.

Strength: 500 mL: 4.5 g.

Batch No.: B20102104A.

Expiration date: 2022-09-30.

Storage conditions: Airtight.

Manufacturer: Shandong Kelun Pharmaceutical Co., Ltd.

If other Batch No. is used in the study, the specific information shall be truthfully recorded and reflected in the original and final report, and the final report shall prevail.

* + 1. **Preparation of Vehicle Control Group [6% Propylene Glycol + 3% Kolliphor ® HS 15 + 5% Mannitol Solution]**

Preparation method: The preparation of 100 mL dose formulation as an example: under the conditions of room temperature and avoid light, 6 g propylene glycolwill be weighed and take into a container calibrated 100 mL, 3 g Kolliphor ® HS 15 will be added, stirred and mixed, then appropriate amount of sodium chloride injection will be added to dissolve, and then add 5 g mannitol. After stirring and dissolving, the pH will be adjusted to 8.0 with 0.1 mol/L NaOH solution and add sodium chloride injection to the scale (100 mL) to obtain the dose formulation of vehicle control group.

On the day of administration, the subpackaged vehicle control to be distributed will be filtered and sterilized with a 0.22 μm PES filter membrane under sterile conditions, the vehicle control shall be protected from light and in room temperature and prepare and use it immediately.

Identification method: The prepared vehicle control group dose formulations will be labeled with white labels, and marked with study number, name, concentration, amount, preparation date, preparation person, storage conditions, expiry date, and finished product number.

Temporary storage conditions after preparation and expiry date: Room temperature for using within 24 hours; 2 ~ 8 oC for using within 8 days.

* + 1. **Handling of Retention Samples and Remaining Vehicles/Dose Formulations for Vehicle Control Group**

Vehicle retention samples: Samples will be uniformly retained by the Test Article Management Department.

Handling of remaining vehicle of vehicle control group: They will be returned to Test Article Management Department and disposed as drug/chemical waste.

Handling of retained vehicle: They will be transferred to the Archives Management Department and stored in the retained sample archives of the facility.

Handling of remaining vehicle: After all studies of the project completed, vehicle 2 and vehicle 3 will be returned to the sponsor.

* 1. **Dose formulation analysis**

The test article’s content and stability data are provided by the sponsor. The dose formulation analysis method of the test article has been validated in “Validation Study for Analytical Method of Y-3 Dose Formulation Analysis (Study Number: A2021003-FA01)”, and the results showed that Y-3 dose formulations (concentration: 0.1 and 2.0 mg/mL) prepared in vehicle (propylene glycol+ Kolliphor® HS 15+ mannitol+ sodium chloride injection) were stable when stored at room temperature for 1 day and at 2 ~ 8oC, protected from excessive light for 8 days, the stability of the test article will not be tested in this study.

The concentration analysis will be performed on the dose formulation of each Y-3 dose group and vehicle control group on the day of the first and last administration. Another formulation analysis will be added in the first study where a new batch number of test article (if any) is used.

Sampling method: Duplicate samples are taken from each dose formulation to be tested, 700 μL per sample, and store the Y-3 dose group at room temperature, protected from excessive light. One sample is used for analysis and the other for standby. The analytical sample number consists of the finished dose formulation number - position abbreviation + 2-digit serial number (e.g., finished dose formulation number-R01).

Example of treatment method of dose formulations: the dose formulations to be tested will be diluted to volume with diluent (60% acetonitrile aqueous solution) and then loaded for analysis.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Group | Nominal Concentration of Dose Formulation (mg/mL) | Step 1 Dilution (S1) | | Step 2 Dilution (S2) | | Design Concentration (μg/mL) |
| Dose Formulation (μL) | Calibrated Volume (mL) | S1  （μL） | Calibrated Volume (mL) |
| Vehicle Control Group | - | 500 | 10 |  | - | - |
| Y-3  Low Dose Group | 0.5 | 100 | 10 | - | - | 5.0 |
| Y-3  Medium Dose Group | 1.0 | 500 | 10 | 500 | 5 | 5.0 |
| Y-3  High Dose Group | 2.0 | 500 | 10 | 500 | 10 | 5.0 |

Note: “-” means not applicable.

Acceptance criteria of results: The accuracy (ratio of detected concentration to nominal concentration) of the dose formulations in Y-3 group should be within between 90% and 110%. For the dose formulations for vehicle control group, there should be no interfering peak at the retention time of Y-3, or the interfering peak area at the retention time of Y-3 should be ≤ 10% of the peak area of the lower limit of quantification sample.

Handling of remaining dose formulation samples: They will be returned to Test Article Management Department and disposed as drug/chemical waste after the analysis.

* 1. **Operation/Safety Measures**

The test facility will operate in accordance with the Occupational Health Safety and Protection Manual. Appropriate personal protective equipment (PPE) will be worn during the performance of the test procedure.

* 1. **Transfer of Dose Formulations within the Test Facility**

From Test Article Management Department to animal husbandry room, the dose formulations of each group and will be transferred under the conditions of room temperature and protected from light. For the dose formulations of each group received from Test Article Management Department will be stored under the same conditions as transfer when not in use.

* 1. **Other Major Reagents**

| **Name** | **Source** | **Grade** |
| --- | --- | --- |
| Tropicamide Phenylephrine Eye Drops | Santen Pharmaceutical Co., Ltd | Pharmaceutical grade |

Note: If any reagent is used but not listed in the above table, or if any reagent information is changed during the study, the relevant information will be truthfully reflected in the original record and study report, and the original record and study report shall prevail.

* 1. **Main Instruments and Equipment**

| **Instrument Name** | **Manufacturer** | **Model** |
| --- | --- | --- |
| Pure water meter | Millipore | ELIX® Advantage 5 |
| Electronic balance | Mettler Toledo | ML203/02 |
| Electronic balance | Mettler Toledo | ML2001/02 |
| Stainer | Leica | Leica ST5020 |
| Tissue embedding machine | Leica | Leica EG1150C+ EG1150H |
| Automatic dehydrator | Leica | Leica ASP300S |
| Automatic hematology analyzer | Siemens | ADVIA 2120i |
| Automatic biochemistry analyzer | Roche | cobas 6000 |
| Automatic coagulation analyzer | Sysmex | CA-7000 |
| Urine sample analyzer | arkray | AX-4030 |
| Binocular indirect ophthalmoscope | Suzhou 66 Vision Technology Co., Ltd. | YZ25B |

Note: Any changes in the instrument information will be shown in the final report.

# Test System

* 1. **Species/Strain/Grade**

Strain: SD rat.

Grade: SPF grade.

* 1. **Sex and Number**

Number and sex of animals scheduled for purchase: 200, half male and half female.

Number and sex of animals intended for use: 184, half male and half female.

Handling of remaining animals: The remaining animals in this study will be transferred to the Toxicology Operation Department within 1 week after the first dose, and only general observation shall be conducted before the transfer.

* 1. **Source**

Supplier: Zhejiang Vital River Laboratory Animal Technology Co. Ltd..

Production license No: SCXK (Zhejiang) 2019-0001.

Animal quality certificate No.: Original record and final report shall prevail.

Note: If there is any change in the information of specific animals, the original record shall prevail.

* 1. **Body Weight and Age**

Body weight: 190 ~ 220 g for males at purchase and 150 ~ 190 g for females at purchase, 190 ~ 240 g for males at group assignment and 160 ~ 240 g for females at group assignment, and the individual animal weights shall be within the range of ±20% of the average body weight of animals of the same sex at group assignment.

Age: 6 ~ 7 weeks old at purchase, and 6 ~ 8 weeks old at group assignment.

Note: If any animal’s age or body weight exceeds the above range, it can be included in the study after being confirmed by the study director.

* 1. **Animal Identification**

Each animal will be identified by tail marking and cage card.

* 1. **Justification for Selection and Number of Animals in the Study**

Justification for selection of animals: According to the “Guidance on Repeat Dose Toxicity Studies for Pharmaceuticals” (former CFDA, May 2014), repeat-dose toxicity studies need to be carried out on rodents and non-rodents. Rats are recommended to be used as rodents. Previous studies demonstrated that SD rats have been used as sensitive animals of the test article, and they have a relatively clear genetic and biological background.

Justification for determining the number of animals: The minimal number of animals shall be used on the premise of meeting the study objectives, scientific standards, and regulatory requirements. Accordingly, for each dose group in this study, the number of animals will be 46 animals/group [30 animals/group in the main study and 16 animals/group in the toxicokinetic study (alternated for blood collection, 8 animals/group in reality)]. In order to prevent the purchased animals from being unable to meet the study requirements due to unknown reasons, additional 16 animals will be purchased, half male and half female.

# Animal Husbandry and Management

* 1. **Animal Management and Use**

CTI Biotechnology (Suzhou) Co., Ltd. is a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International), and the use of animal has been approved by Jiangsu Science and Technology Department.

This study will be not a simple replicate of any previous study and may not be replaced by any alternative study; by literature searches, there will be no alternative method that can substitute for the method described herein and cause less pain, tension or disease.

The contents and procedures related to animal experiments in the study will comply with the relevant laws and regulations on the management and use of laboratory animals as well as the relevant regulations of Institutional Animal Care and Use Committee (IACUC) of the facility. The number of animals, study design and treatment of animals should be approved by the IACUC of the facility (approval number: IACUC-A2021003-T011-01), and the contents approved by the IACUC should be strictly followed.

* 1. **Animal Receipt and Acclimation**

Animals need to acclimatize to the environment for at least 5 days after the reception.

* 1. **Animal Housing**

Animal use license number of the facility: SYXK (Jiangsu) 2018-0051.

Husbandry location: Inside the barrier system on the 2nd floor of Building 1 in CTI Biotechnology (Suzhou) Co., Ltd..

Husbandry cage type: Polysulfone rat cage, size (L × W × H): 50 cm × 36 cm × 20 cm.

Husbandry density: ≤ 4/cage.

* 1. **Housing Environment**

Husbandry environment standard: National Standards of the People's Republic of China, GB14925-2010.

Husbandry environment control system: MSEA-MVE 6.0 Johnson Animal Room Environment Monitoring System.

Temperature: 20 ~ 26 oC (daily temperature difference ≤ 4 oC);

Relative humidity: 40% ~ 70%.

Lighting: Artificial lighting, 12-hour alternative cycle of light and dark.

Ventilation: No less than 15 times per hour.

* 1. **Environmental Enrichment**

Animals will be provided with toys as an environmental enrichment.

* 1. **Feed**

Type: Maintenance feed for SPF rats and mice.

Lot number of feed: Actual used feed will be reflected in original record and final report.

Manufacturer: Beijing Keao Xieli Feed Co., Ltd..

Production license number: SCXK (Beijing) 2019-0003.

Feeding method: *ad libitum* (unless otherwise specified in the study).

Feed test: The supplier shall provide the quality certificate of the feed. The feeds should be subject to routine tests for nutritional components, chemical contaminants and microbiological parameters by a qualified third party once a year and the test results shall meet the requirements of National Standards of the People's Republic of China. Feeds should be free of known contaminants that may interfere with the study results and animal health.

Note: If there is any change in the feed information, the final report and the original record shall prevail.

* 1. **Bedding**

Bedding type: Corncob;

Lot number of bedding: Actual used bedding will be reflected in original record and final report;

Manufacturer: Beijing Keao Xieli Feed Co., Ltd.;

Production license number: SCXK (Beijing) 2020-0010;

Storage and use: Keep at low temperature, under dry and clean conditions; Spread the sterilized bedding at the bottom of the rat cage to cover the entire bottom;

Bedding test: A third-party qualified professional testing unit is commissioned to test the chemical pollutant parameters on the bedding once a year.

Note: If there is any change in the bedding information, the final report and the original record shall prevail.

* 1. **Drinking Water**

Type: Reverse osmosis water (drinking water for animals);

Method for water supply: Provided in water basin, *ad libitum*;

Testing for conventional water indices: According to the relevant requirements of the National Standard GB5750-2006 of the People's Republic of China, the qualified unit as the third party is commissioned to test the water quality at least once a quarter.

* 1. **Animal Selection**

Healthy animals (female, non-pregnant and non-fertile) will be selected as laboratory animals. During the pretest phase, the animals will be mainly subjected to general observation, and all the animals qualified in the inspection will be included in this study.

# Study Design

Group design: Vehicle Control Group, Y-3 Low Dose Group, Y-3 Medium Dose Group and Y-3 High Dose Group;

Number of animals: 46 animals/group [main study: 30 animals/group, TK study: 16 animals/group (alternated for blood collection, 8 animals/group in reality)], 184 animals in total;

Sex ratio: Half male and half female;

Grouping method: 184 animals with even body weight will be selected from the qualified animals of pretest phase, half male and half female, and were grouped according to body weight by Pristima version 7.4.0 Data collection system.

Specific grouping information is shown in the table below:

| **Group** | **Test article/reference item** | **Dosage**  **（mg/kg）** | **Concentration**  **（mg/mL）** | **Animal Number** | |
| --- | --- | --- | --- | --- | --- |
| **Female** | **Male** |
| Vehicle Control Group | 6% propylene glycol + 3% Kolliphor HS ® 15 + 5% mannitol solution | - | - | 1F001~1F023 | 1M001~1M023 |
| Y-3  Low Dose Group | Y-3 | 10 | 0.5 | 2F001~2F023 | 2M001~2M023 |
| Y-3  Medium Dose Group | Y-3 | 20 | 1.0 | 3F001~3F023 | 3M001~3M023 |
| Y-3  High Dose Group | Y-3 | 40 | 2.0 | 4F001~4F023 | 4M001~4M023 |

Note: The first digit of animal number represents the group (1, 2, 3, and 4 represent Vehicle Control Group, Y-3 Low Dose Group, Y-3 Medium Dose Group, and Y-3 High Dose Group, respectively). The second letter represents sex (F is female, M is males), the last 3 digits represent the animal serial number. Animals with 001-015 in each group are in the main study group and animals with 016-023 are in the TK satellite group. "—" means not applicable.

The TK blood sampling points and animal numbers for each dose group are shown in the following table:

| **Group** | **Blood sampling points and animal numbers** | |
| --- | --- | --- |
| Pre-dose and post-dose 5 min, 1 h, 8 h | Post-dose instant, 30 min, 3 h, 24 h |
| Vehicle Control Group | (♀4/♂4)  1F016 ~ 1F019  1M016 ~ 1M019  (Collect pre-dose only) | (♀4/♂4)  1F020 ~ 1F023  1M020 ~ 1M023  (Collect post-dose 1 h only) |
| Y-3  Low Dose Group | (♀4/♂4)  2F016 ~ 2F019  2M016 ~ 2M019 | (♀4/♂4)  2F020 ~ 2F023  2M020 ~ 2M023 |
| Y-3  Medium Dose Group | (♀4/♂4)  3F016 ~ 3F019  3M016 ~ 3M019 | (♀4/♂4)  3F020 ~ 3F023  3M020 ~ 3M023 |
| Y-3  High Dose Group | (♀4/♂4)  4F016 ~ 4F019  4M016 ~ 4M019 | (♀4/♂4)  4F020 ~ 4F023  4M020 ~ 4M023 |
| Totally | 32 | 32 |

Note: first dose (D1) and last dose (D28) are subject to TK blood collection respectively.

* 1. **Dosing Information**

Dosage: The dosages of Y-3 low, medium and high dose group are 10 mg/kg, 20 mg/kg, and 40 mg/kg, respectively.

Dose volume: The dose volume of each group is 20 mL/kg, the dosage of each animal will be adjusted according to the latest body weight, and the vehicle control group will be administrated the same dose volume of vehicle.

Dose concentration: The dose concentrations of Y-3 low, medium and high dose group are 0.5 mg/mL, 1.0 mg/mL and 2.0 mg/mL, respectively.

Route of administration: Intravenous injection (infusion).

Rate of administration: 2 mL/min.

Site of administration: Tail vein.

Justification for selection: It is consistent with the clinical route of administration.

Frequency and cycle of administration: Once daily for 4 consecutive weeks.

Recovery phase: 4 weeks.

Animals for scheduled necropsy: 20 animals/group in each group of main study group will be dissected at the end of dosing phase (R1) and 80 animals in total. 10 animals/group in each group of main study group at the end of recovery phase (R29) and 40 animals in total.

The day on which animals entered the study is defined as Day 1 of the pretest phase (P1); the day of the first dose is defined as Day 1 of the dosing phase (D1), and the day after the last dose (D28) was defined as Day 1 of the recovery phase (R1).

* 1. **Justification of Dose Selection**

According to the data provided by the sponsor, Y-3 is intended to be used for the treatment of acute ischemic stroke in clinic, and the pharmacodynamic effective dose of rat is 1 mg/kg. According to the results of the preliminary study of the Test Facility, the rats in low dose group, medium dose group and high dose group were treated with Y-3 at 10, 20 and 40 mg/kg by intravenous injection, respectively, and once daily for 3 consecutive weeks. There was no remarkable finding in the general observation and food consumption after the administration. The increase rate of body weight in male animals of medium and high dose group was of the male animals was lower than that of the animals in the vehicle control group on D6. Clinical Pathology parameters fluctuated within normal range. The TK results showed that there was no gender difference in the systemic exposures of each dose group of Y-3 after the administration on D1 and D21, and the systemic exposure was positively correlated with the dosage. There was no obvious accumulation after 21 days of continuous administration. No pathological findings related to test article were found in organ weight, gross observation and histopathological examination at the end of dosing phase. Therefore, under the condition of this study, SD rats were treated with Y-3 at the doses of 10, 20 and 40 mg/kg via gavage, and MTD was ≥ 40 mg/kg.

Based on the above results and the highest solubility of Y-3 was 2 mg/mL, the dosage of the high dose group of Y-3 in this study will be 40 mg/kg, and the medium and low doses will be 20 mg/kg and 10 mg/kg respectively. The dosages of Y-3 low dose group, Y-3 medium dose group, and Y-3 high dose group will be 10, 20, and 40 times of the pharmacodynamically equivalent dose (1 mg/kg), respectively. The vehicle control group will be administrated with the same dosage volume [6% propylene glycol + 3% Kolliphor HS 15 + 5% mannitol].

# Observation and Examination

4. 1. **General Observation**

Observation time: The animals will be observed by the veterinarian for at least 5 days, at least once daily during the pretest phase after arrival. Observation will be conducted once in the morning and afternoon after the group assignment during the pretest phase. Observation will be conducted at least once daily before dosing, after dosing and in the afternoon during the dosing phase. Observation will also be conducted at least once daily in the morning and afternoon during the recovery phase. In the event of obvious abnormal symptoms, the frequency of observation may be increased, and the time should be recorded (animals of scheduled necropsy will be observed once before dissection),

Observation items: Including but not limited to general conditions, behavioral status, eyes, oral cavity, muzzle, ears, hair and skin, feces, urine, genitalia and other abnormal symptoms,

Observation of injection site at the same time of general observation: The details refer to the study protocol " A 4-Week Local Irritation Test of Beagle Dogs Intravenously Infused with YK1169 (Study Number: A2021003-T072-01)".

Animals to be observed: All surviving animals (only results of animals of the main study group will be included in the final report).

* 1. **Body Weight**

Measurement time: The rats will be measured at least twice during the pretest phase, twice a week during the dosing phase, once weekly during the recovery phase and once the day of scheduled necropsy (the first weight during the pretest phase will be only used to calculate the organ-body ratio and organ-brain ratio, and will be not included in the statistical analysis of the body weight parameters). The animals in TK group will be weighted for calculating dosage, and will be not included in the statistical analyses of body weight.

Animals to be measured: All surviving animal (on the day of necropsy, only the animals to be dissected will be measured).

* 1. **Food Consumption**

Measurement time: Food consumption will be measured once before group assignment, once weekly during the dosing phase and once weekly during the recovery phase.

Animals to be measured: All surviving animals in main study;

Measurement method: The amount of feed per cage will be measured on the first day, and the amount remaining will be measured at approximately the same time on the second day. The difference between the two will be the total food consumption of animals in each cage for 24 hours, which will be divided by the number of animals per cage to calculate the mean food consumption per animal (the frequency of measurement can be adjusted appropriately according to the characteristics of test article).

* 1. **Ophthalmologic Examination**

Measurement time: Animals will be examined once during the pretest phase, and once on the necropsy day at the end of the dosing phase (R1) and on the necropsy day at the end of the recovery phase (R29), respectively;

Animals to be measured: All animals in main study during the pretest phase, and animals to be dissected in the main test group on the day of schedule necropsy.

Measurement method: Animals will be examined with a binocular indirect ophthalmoscope by veterinarian.

Examination items: Fundus (optic papilla, retina and retinal vessels).

* 1. **Clinical Pathology**
     1. **Blood Samples**

Sample type: Samples for hematology, coagulation and blood biochemistry;

Test time: On the necropsy day at the end of the dosing phase (R1) and on the necropsy day at the end of the recovery phase (R29);

Animals to be tested: Animals to be dissected in the main study;

Collection method: The animals shall be fasted for 8 ~ 18 hours before sample collection (without water deprivation). Blood will be collected through the abdominal aorta of rats to be dissected after anesthesia with sodium pentobarbital (intraperitoneal injection, 60 mg/kg and the dosage can be adjusted appropriately according to the animal's anesthetic state);

Processing method: The samples for hematology will be directly loaded for analysis. The samples for coagulation will be centrifuged at 1500 g and 20℃ for 15 minutes, and then loaded for analysis. The samples for blood biochemistry will be centrifuged at 2000 g and 20℃ for 10 minutes, and then loaded for analysis.

* + 1. **Urine samples**

Test time: The urine will be collected two days before schedule necropsy (D28 and R27);

Animals to be tested: Animals to be dissected in the main study;

Collection method: Urine samples will be collected by metabolic cages. The urine will be collected for 4 hours. If collection is unsuccessful for some animals in 4 hours, the collection will be continued. And if the collection is unsuccessful before the necropsy, the bladder urine can be collected if necessary. During the collection, the animals will be fasted but without water deprivation;

Processing method: The samples for urinalysis will be directly loaded for analysis. After centrifugation of samples for urine sediment testing at 400 g for 5 minutes at 20℃, the supernatant will be discarded, approximately 0.2 mL of sediment will be left and mixed well, and approximately 20 μL urine sediment will be used for microscopic examination.

* + 1. **Sample Use, Collection Volume, and Collection Tube Type**

| **Test Items** | **Collection Volume** | **Collection Tube Type** |
| --- | --- | --- |
| Hematology | Not less than 1 mL | EDTA • K 2 anticoagulant tube |
| Coagulation | Approximately 2 mL (1.8 mL whole blood + 0.2 mL anticoagulant) | Sodium citrate anticoagulant tube |
| Blood biochemistry | Not less than 2 mL | Inert separation gel vacuum procoagulant collective tube |
| Urinalysis | Not less than 2 mL | Ordinary sample tube |

* + 1. **Clinical Pathological Parameters and Methods**
       1. **Hematology Parameters**

| **Test Items** | **Test Method** |
| --- | --- |
| Red blood cell count (RBC) | Two-dimensional laser scanning |
| Hemoglobin (HGB) | Cyanide hemoglobin |
| Hematocrit (HCT) | Calculation: MCV × RBC |
| Mean corpuscular volume (MCV) | Two-dimensional laser scanning |
| Mean corpuscular hemoglobin (MCH) | Calculation: HGB/RBC |
| Mean corpusular hemoglobin concentration (MCHC) | Calculation: HGB/ (MCV × RBC) × 1000 |
| Reticulocyte count (# RETIC) | Oxazine 750 staining method |
| Reticulocyte percentage (% RETIC) | Calculated: (RET/RBC) × 100 |
| White blood cell count (WBC) | Two-dimensional laser scanning |
| Neutrophil count (# NEUT) and percentage (% NEUT) | Peroxidase staining |
| Lymphocyte count (# LYMPH) and percentage (% LYMPH) | Peroxidase staining |
| Monocyte count (# MONO) and percentage (% MONO) | Peroxidase staining |
| Eosinophil count (# EOS) and percentage (% EOS) | Peroxidase staining |
| Basophil count (# BASO) and percentage (% BASO) | Peroxidase staining |
| Large unstained cells count (# LUC) and percentage (% LUC) | Peroxidase staining |
| Platelet count (PLT) | Two-dimensional laser scanning |

* + - 1. **Coagulation Parameters**

| **Test Items** | **Test Method** |
| --- | --- |
| Prothrombin time (PT) | Coagulation method |
| Activated partial thromboplastin time (APTT) | Coagulation method |
| Thrombin time (TT) | Coagulation method |
| Fibrinogen (FIB) | Coagulation method |

* + - 1. **Blood Biochemistry Parameters**

| **Test Items** | **Test Method** |
| --- | --- |
| Total bilirubin (TBIL) | Diazotization method |
| Total protein (TP) | Colorimetry |
| Albumin (ALB) | Colorimetry |
| Globulin (GLOB) | Calculation: TP-ALB |
| ALB to GLOB (A/G) | Calculation: ALB/GLOB |
| Alanine aminotransferase (ALT) | Calculation: ALB/GLOB |
| Aspartate aminotransferase (AST) | Colorimetry |
| Alkaline phosphatase (ALP) | Colorimetry |
| γ-Glutamyl transpeptidase (GGT) | Enzymatic colorimetry |
| Lactate dehydrogenase (LDH) | Colorimetry |
| Creatine kinase (CK) | Colorimetry |
| Urea (UREA) | Colorimetry |
| Creatinine (CREA) | Enzymic method |
| Glucose (GLU) | Hexokinase method |
| Triglyceride (TG) | Colorimetry |
| Cholesterol (CHOL) | Enzymatic colorimetry |
| Sodium ion concentration (Na + ) | ISE method |
| Potassium ion concentration (K + ) | ISE method |
| Chloride ion concentration (Cl - ) | ISE method |
| Calcium (Ca) | Colorimetry |
| Phosphorus (P) | Colorimetry |

* + - 1. **Urine Parameters**

| **Test Items** | **Test Method** |
| --- | --- |
| Color | Transmitting light determination |
| Turbidity (TURB) | Scattered light determination |
| Glucose (GLU or GLUC) | Dual-wavelength reflection method |
| Protein (PRO) | Dual-wavelength reflection method |
| Bilirubin (BIL) | Dual-wavelength reflection method |
| Urobilinogen (URO) | Dual-wavelength reflection method |
| Power of hydrogen (pH) | Dual-wavelength reflection method |
| Occult blood (BLD) | Dual-wavelength reflection method |
| Ketone body (KET) | Dual-wavelength reflection method |
| Nitrite (NIT) | Dual-wavelength reflection method |
| Leukocyte (LEU) | Dual-wavelength reflection method |
| Specific gravity (SG) | Reflection-type zigzag rate determination method |
| Urine sediment (epithelial cell, cast and crystal) | Microscopic examination |

* + 1. **Storage and Shipment of Sample**

Samples for hematology, coagulation, blood biochemistry and urine will be placed in a sample transport box after collection and send to the Clinical Laboratory within 1.5 h at room temperature.

* 1. **Bone Marrow Examination**

Bone marrow smear time: On the necropsy day at the end of the dosing phase (R1) and on the necropsy day at the end of the recovery phase (R29);

Animals to be smeared: Animals to be necropsied of each group;

Preparation of bone marrow smear: Bone marrow smears will be prepared from sternum or femoral bone marrow at necropsy. The smears will be stained with Liu’s stain solution, and dried in air for examination;

Bone marrow examination parameters: Percentage of granulocytic series, erythrocytic series, lymphocytes, monocytes and other cells, granulocyte-erythrocyte ratio and megakaryocyte count, etc.;

Bone marrow examination should be conducted when hematological parameters are abnormal.

* 1. **Toxicokinetic**
     1. **Sample Collection and Processing Method**

Blood sampling: For animals of Y-3 each dose group, the samples will be collected once before administration and at once after the administration (± 1 min), 5 min (± 1 min), 30 min (± 2 min), 1 h (± 5 min), 3 h (± 5 min), 8 h (± 5 min) and 24 h (±5 min) after administration on D1 and D28;

Vehicle Control Group: The samples will be collected once before administration and once at 1 h (± 2 min) after administration on D1 and D28;

If the sampling time errors are within the above pre-specified range, the toxicokinetic parameters will be calculated with the pre-specified time points. If the sampling time errors are out of the above pre-specified range, the toxicokinetic parameters shall be calculated based on the actual time point (when temporary sampling is required due to poor general conditions of the animal, the actual sampling time points and number of samples shall be used);

Sampling method: Jugular vein or other suitable vein;

Sampling volume: Approximately 0.3 mL of whole blood will be collected into an EDTA-K2 anticoagulant tube and gently mixed;

Blood sample processing: The whole blood samples will be placed in the ice box before centrifugation and transported in the ice box, then centrifuged at 2000 g and 2 ~ 8℃ for 10 minutes in 2 h after collecting, then the plasma will be sub-packed into labeled EP tubes, in which "plasma-1" will be the first testing sample, and the volume of the subpackaged plasma will be 60 μL. The remaining plasma samples (if any) will be stored in "plasma-2" tubes as backup and stored at ≤ -60 ℃. Examples of the EP tubes label formats are as follow:

A2021003-T011-01

Subject：1M001

Timepoint：Day1 0h

Bio.Matrix: Plasma-1

A2021003-T011-01

Subject：1F001

Timepoint：Day1 0h

Bio.Matrix:Plasma-1

The remaining blood samples after TK testing shall be destroyed or sent to the sponsor after the study.

The concentration of Y-3 will be analyzed using the validated LC-MS/MS method. AUC0-t, Cmax,AUC 0-t ratio and C max ratio will be calculated to evaluate the drug accumulation and systemic exposure with dose linear relationship.

The animals in TK group will be conducted euthanasia by CO2 after the collection of the blood sample.



# Gross Necropsy, Organ Weighing and Histopathological Examination

* 1. **Necropsy Time**

At the end of dosing phase (R1): 20 animals/group will be dissected (animals numbered 001 to 010 of each gender in each group), half male and half female and totaling 80 animals. At the end of recovery phase (R29): The remaining 10 animals/group will be dissected (animals numbered 011 ~ 015 of each gender in each group), half male and half female and totaling 40 animals. If the scheduled necropsy animal is moribund or has died before dissection, the actual number of dissections shall prevail.

* 1. **Animals to be Dissected**

All animals to be dissected in the main study group.

* 1. **Anesthesia and Euthanasia Method**

Animals will be fasted for 8 ~ 18 hours before anesthesia (without water deprivation), and pentobarbital sodium will be used to anesthetize by intravenous injection. The dosage will be 60 mg/kg, the concentration will be 20 mg/mL and the volume will be 3 mL/kg (the dosage can be adjusted according to the actual condition). The animals shall be euthanized by abdominal aorta exsanguination after anesthesia.

* 1. **Gross Observation**

Firstly, perform general examination of the external surfaces of the body, including body shape, nutritional status, hair, skin, external genitals, orifices and injection site. Then check the head, neck, chest cavity, abdominal cavity, pelvic cavity and the organs/tissues of the body. Record the findings of the necropsy.

* 1. **Organ Weight**

The organs/tissues required weighting are listed in 7.7. The paired organs will be weighed together, and calculate the organ-to-body ratio and organ-to-brain ratio:

Organ-to-body ratio = organ weight (g)/body weight (g) × 100%;

Organ-to-brain ratio = organ weight (g)/brain weight (g) × 100%.

* 1. **Tissue Fixation**

The bilateral eyeballs, optic nerves, testes and epididymis will be preserved in modified Davidson's fixative solution, and the other tissues/organs will be preserved in 10% neutral buffer formalin fixative solution. Organs/tissues requiring fixation are listed in 7.7.

* 1. **Histopathological Examination.**

The tissues/organs of rats in each group will be fixed and preserved. The collected tissue/organs of main study animals in the vehicle control group and Y-3 high dose group will be processed with routine histological methods, such as embedding in paraffin, sectioning and staining with hematoxylin and eosin (HE), etc. Histopathological examinations will also be performed. If any organ/tissue of animals in Y-3 high dose group has pathological finding, the same organ/tissue of animals in Y-3 medium and low dose groups will be conducted with histopathological examinations.

Tissues/organs requiring extraction, weighing, fixation, and histopathological examination are shown in the following table:

| **Tissue/Organ** | **Weigh** | **Fixative** | **Reserved** | **Histopathology**  **Examination** |
| --- | --- | --- | --- | --- |
| Animal Identification | - | F | Y | - |
| Aorta (thoracic) | - | F | Y | Y |
| Bone and bone marrow (sternum) | - | F | Y | Y |
| Bone (femur, including knee joint) (1) | - | F | Y | Y |
| Brain | Y | F | Y | Y |
| Epididymis (2) | Y | MD | Y | Y |
| Esophagus (cervical segment) | - | F | Y | Y |
| Eyeballs (2) | - | MD | Y | Y |
| Adrenal glands (2) | Y | F | Y | Y |
| Harderian glands (2) | - | F | Y | Y |
| Pituitary | Y | F | Y | Y |
| Prostate | Y | F | Y | Y |
| Salivary gland, submandibular salivary gland (2) | - | F | Y | Y |
| Salivary gland, ‎sublingual‎ gland (2) | - | F | Y | Y |
| Seminal vesicles (2) | - | F | Y | Y |
| Thyroid gland (2) and parathyroid gland (2) \* | Y | F | Y | Y |
| Heart | Y | F | Y | Y |
| Kidney (2) | Y | F | Y | Y |
| Large intestine (cecum) | - | F | Y | Y |
| Large intestine (colon) | - | F | Y | Y |
| Large intestine (rectum) | - | F | Y | Y |
| Liver | Y | F | Y | Y |
| Lung and main bronchus | - | F | Y | Y |
| Lymph node ( inguinal) | - | F | Y | Y |
| Lymph node (mesenteric) | - | F | Y | Y |
| Skeletal muscle (biceps femoris) (1) | - | F | Y | Y |
| Optic nerve (2) \* | - | MD | Y | Y |
| Sciatic nerve (2) | - | F | Y | Y |
| Ovaries (2) | Y | F | Y | Y |
| Fallopian tube (2) | - | F | Y | Y |
| Pancreas | - | F | Y | Y |
| Peyer's patch | - | F | Y | Y |
| Skin and mammary gland, groin (1) | - | F | Y | Y |
| Small intestine (duodenum) | - | F | Y | Y |
| Small intestine (ileum) | - | F | Y | Y |
| Small intestine (jejunum) | - | F | Y | Y |
| Spinal cord (cervical, thoracic and lumbar) | - | F | Y | Y |
| Spleen | Y | F | Y | Y |
| Stomach |  | F | Y | Y |
| Testes (2) | Y | MD | Y | Y |
| Thymus, or thymus area | Y | F | Y | Y |
| Trachea (cervical segment) | - | F | Y | Y |
| Bladder | - | F | Y | Y |
| Uterus (including cervix) | Y | F | Y | Y |
| Vagina | - | F | Y | Y |
| Dosing site | - | F | Y | Y |
| Macroscopic lesions | - | F | Y | Y |

Note: (1) indicates unilateral collection and microscopic examination; (2) indicates bilateral collection and microscopic examination; "\*" indicates at least unilateral microscopic examination; "Y" indicates collection required; "F" indicates 10% neutral buffered formalin fixative; "MD" indicates modified Davidson's fixative; "-" indicates not applicable.

* 1. **Handling of Moribund Animals**

The moribund animals in the main study group will be treated as follows: The status and observation time of rats will be recorded; an appropriate amount of sodium pentobarbital will be used for anesthesia according to their conditions after body weight measurement; examination and dissection as well as various tests will be performed according to the requirements of sections 6.4 ~ 7.7 (if the above parameters are not collected successfully, they will be truthfully recorded and will not be handled as protocol deviations from the study protocol).

For the moribund animals are not in the main study group, the status and observation time of rats will be recorded, euthanasia and gross observations will be performed and histopathological examination will be performed on macroscopic lesions.

* 1. **Handling of Dead Animals**

The dead animals in the main study group will be treated as follows: The time of death or the time of death found will be recorded, and the animals will be promptly dissected after body weight measurement for gross observations to ascertain the cause of death. Tests will be performed for the items required in section 7.4 ~ 7.7 (except for body weighing). If the procedures could not be performed in time, the dead animals will be temporarily stored in a refrigerator (2 ~ 8℃), and dissected as soon as possible within 24 h.

The dead animals are not in the main study group will be treated as follows: The time of death or the time of death found will be recorded, only gross observations will be performed and histopathological examination will be performed on macroscopic lesions.

# Data Collection and Analysis

All raw data in the facility will be collected manually or using a data collection system according to the protocol and SOPs of CTI Biotechnology (Suzhou) Co., Ltd. Manually collected data will be transcribed into Excel sheets for analysis and reporting.

The collection systems for collecting and reporting electronic data are as follows:

| **System** | **Version** | **Use** |
| --- | --- | --- |
| Johnson Control | MSEA-MVE 6.0 | Environmental control and testing of animal rooms |
| Pristima | 7.4.0 | Data collection |
| Phoenix WinNonlin | 7.0 | To calculate toxicokinetic parameters and draw Concentration-Time curves |
| Empower | 3 | Dose formulation analysis |

Note: If other electronic data collection or analysis systems are used in the test, the relevant information will be reflected in the original records and final report. If necessary, paper records can be used for data recording (as raw data).

# Statistical Analysis

Quantitative parameters will be expressed as mean ± standard deviation. When the number of samples is less than 3, the data of this group shall not be included in the statistical comparison.

All quantitative parameters will be collected and statistically analyzed by Pristima data acquisition system, and SPSS or STATA. The LEVENE test of homogeneity of variance is used at first. When the variances are homogeneous (P ≥ 0.05), the one-way analysis of variance (ANOVA) will be used for statistical test. When the variances are not homogeneous (P < 0.05), the rank-sum test (Kruskal-Wallis H test) will be used for statistical analysis. When the one-way analysis of variance (ANOVA) shows that the overall difference is statistically significant (P ≤ 0.05), the Dunnett's LSD test will be used to compare the differences between groups. When the one-way analysis of variance (ANOVA) shows that the overall difference is not statistically significant (P > 0.05), the statistical analysis will end. When the rank-sum test (Kruskal-Wallis H test) shows that the overall difference is statistically significant (P ≤ 0.05), the Mann-Whitney U test will be used to compare the differences between groups. When the rank-sum test (Kruskal-Wallis H test) shows that the overall difference is not statistically significant (P > 0.05), the statistical analysis will end.

T test or t's test will be performed when the number of groups is more than 3.

The comparison between groups will be carried out between each Y-3 dose group and the vehicle control group. There is statistical significance when Dunnett's LSD test shows P < 0.05, the Mann-Whitney U test shows P *≤* 0.05, or t/t 'test shows P < 0.05.

In this study, different calculation models (regression models) and computer programs will be used to analyze and summarize the data. Because different models round or retain the date in different ways (for example: mean, standard deviation) in some tables may be slightly different from the data in other tables, separately calculated data, or statistical analysis data. These differences have no effect on the completeness of the data and the interpretation of the results.

In this study, different calculation models (regression models) and computer programs will be used to analyze and summarize the data. Because different models round or retain the date in different ways (for example: mean, standard deviation) in some tables may be slightly different from the data in other tables, separately calculated data, or statistical analysis data. These differences have no effect on the completeness of the data and the interpretation of the results.

The general observation, urinalysis parameters and ophthalmologic examination results will be listed in table for analysis.

Pathology and bone marrow examination results (if any) will be described in detail.

1. **Study Protocol, Amendments and Deviations**

The study protocol and any study amendment (if any) shall be validated in writing with the signature of the study director and the personnel of the Quality Assurance Department; The representative of the sponsor shall sign on the signature page or send an email to approve the study protocol; And protocol amendment (if necessary) shall require the signature of the representative of the sponsor or an email for confirmation. All deviations from the study protocol shall be recorded, and the possible effect on the study shall be analyzed in writing by study director, and corresponding correction measures shall be taken if necessary.

# Final Report

* 1. **Main Contents to be Recorded in the Report**

Including but not limited to the following:

* Study name, study number and study objective
* Names, addresses and contact information of test facility and the sponsor
* Study start and end dates, experiment start and end dates
* Code, batch number, content and other characteristics of the test article; formulation analysis results of the test article
* Species, strain, number, age, sex, body weight range, source, animal certificate number, issuing unit, receipt data, and housing conditions of laboratory animals
* Type, source and batch number of animal feed, bedding and drinking water
* Routes, dosages, methods, frequency and duration of administration of test article and reference item.
* Justification for dose design of test article
* Determination frequency and assay method of various indicators
* Names and responsibilities of study director and key study personnel
* Statistical methods used for data analysis
* Study results, discussion/conclusion
* Abnormalities that deviate from the study protocol
* Storage location of original data and specimens
  1. **Writing Process**

The draft report will be prepared based on comprehensive study results and submitted to QA for review. After finalization, it will be signed by the study director and QA, and then delivered to the Sponsor.

1. **Storage of Relevant Data**
   1. **Archiving Time and Storage Period**

(1) The study director should ensure that all study data are transferred to the archives for archiving within 2 weeks after the completion of the study.

(2) If this study is used for registration application, the archive storage period should be at least five years after drug marketing; if this study is not used for registration application, the archive storage period should be at least five years after the date of the study report approval.

(3) The wet specimens and other biological specimens generated under the above application conditions should be stored for a period that does not affect the quality of their evaluation, and if the period exceeds the storage period specified in (2), the storage period in (2) shall prevail.

* 1. **Archived Data**

Including but not limited to the following:

* Letter of appointment for study director
* Study protocol and protocol amendments (if any)
* Various written documents or reports related to the study
* Study raw data (including electronic data)
* Specimens: formalin-fixed specimens, paraffin blocks, pathological sections, etc.
* Final report and relevant data
* Animal room temperature and humidity report, etc.
* Retention samples of test articles
* Other materials
  1. **Storage Location and Storage Condition**

Storage place: Archives Management Department, Building 1, CTI Biotechnology (Suzhou) Co., Ltd.;

Storage conditions: Conventional;

Contact person: Li Wu;

Contact Telephone: 0512-36801688.

1. **Study-related Main SOPs**

|  |  |
| --- | --- |
| Grouping of Laboratory Animal | Q/CTI MR-BTC-MAN-001 |
| Laboratory Animal Cage Space Animal Number | Q/CTI MR-BTC-IAC-004 |
| General Principles for Health Observation of Laboratory Animals | Q/CTI MR-BTC-MAN-017 |
| Body Weight Measurement of Laboratory Animals | Q/CTI WI-BTC-MAN-020 |
| Numbering and Identification of Laboratory Animals | Q/CTI MR-BTC-MAN-095 |
| Study Management Process | Q/CTI MR-BTC-GEN-030 |
| Preparation and Change of Study Protocol | Q/CTI WI-BTC-GEN-032 |
| Preparation and Revision of Final Report | Q/CTI WI-BTC-GEN-034 |
| Handling of Deviations | Q/CTI WI-BTC-GEN-037 |
| Raw Data and Storage | Q/CTI MR-BTC-GEN-039 |
| General Principles for the Management of Nonclinical Study Archives of Drugs | Q/CTI MR-BTC-GEN-055 |
| General Principles for Blood Collection of Laboratory Animals | Q/CTI MR-BTC-MAN-028 |
| Rodents Animal Husbandry | Q/CTI MR-BTC-ANI-003 |
| Dosing Procedures in Rodents | Q/CTI MR-BTC-MAN-013 |
|  |  |

1. **Key References**

None.