Clinical Study Protocol

A pilot study for the safety and expression of dystrophin in skeletal muscle after SPOT-03 administration in Duchenne Muscular Dystrophy (DMD) patients

NCT Number:	**NCT07188012**
Project Number：	**SPOT-MG02**
Protocol Number：	**FM-T3-SH**
Institution：	**Shanghai Children's Medical Center**
Principal Investigator：	**Wang Jiwen**
Co-sponsor：	**Shanghai Siponuoyin Biotechnology Co., Ltd**
CRO：	**Beijing Leadingpharm Medicine Technology Development Co., Ltd**
Version：	**V3.0**
Date：	**Dec 18, 2025**

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Investigator's Statement

I the undersigned, have reviewed this protocol (protocol no: FM-T3-SH, Version: v3.0, Date: Dec 18, 2025) and I agree to conduct this protocol in accordance with International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP) and the Declaration of Helsinki. This protocol will be carried out upon the approval from the Ethics Committee (EC) or Institutional Review Board (IRB).

I understand that the protocol may not be modified without written approval of the sponsor and that all changes to the protocol must be submitted to the applicable regulatory authorities and the EC/IRB prior to implementation.

Principal Investigator:

_____

Signature *Date (mm/dd/yyyy)*

Name:

_____

Please Print

Institution:

_____

Collaborator's Statement

I the undersigned, have reviewed this protocol (protocol no: FM-T3-SH, Version: v3.0, Date: Dec 18, 2025) and I agree to conduct this protocol in accordance with International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP) and the Declaration of Helsinki. I will fulfill the responsibilities of the sponsor including initiation, monitoring, and funding of this trial. I agree to conduct this clinical study in accordance with the design and provisions in the protocol.

Signature of Responsible Party:

_____

Signature *Date (mm/dd/yyyy)*

Name:

_____

Please Print

Collaborator:

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Abbreviation List

Abbreviations	Full Name
AE	Adverse Event
AR	Adverse Reaction
BMI	Body Mass Index
CBC	Complete Blood Count
CN	Copy Number
CRF	Case Report Form
CRO	Contract Research Organization
CRU	Clinical Research Unit
CTCAE	Common Terminology Criteria for Adverse Events
D/d	Day
DMD	Duchenne Muscular Dystrophy
EC	Ethics Committee
ECG	12-Lead Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
EMG	Electromyography
EOS	End of Study
EVs	Extracellular Vesicles
FAS	Full Analysis Set
FIH	First-in-Human
h	Hour
IC	Informed Consent
IIT	Investigator-Initiated Clinical Trial
IRB	Institutional Review Board
ISRs	Injection Site Reactions
IV	Intravenous
GCP	Good Clinical Practice
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
NMPA	National Medical Products Administration
NSAA	North Star Ambulatory Assessment
PET	Pulmonary Function Test
PK	Pharmacokinetics
PD	Pharmacodynamics

Abbreviations	Full Name
PP	Per-Protocol Set
qPCR	Quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SAS	Safety Analysis Set
SAP	Statistical Analysis Plan
TRAEs	Treatment Related Adverse Reactions
W/w	Week

Synopsis

Title:	A pilot study for the safety and expression of dystrophin in skeletal muscle after SPOT-03 administration in Duchenne Muscular Dystrophy (DMD) patients
Project Number:	SPOT-MG02
Protocol Number:	FM-T3-SH
Collaborator:	Shanghai Siponuoyin Biotechnology Co., Ltd
Clinical Trial Phase:	Investigator-initiated clinical trial (IIT)
Investigational Drug:	SPOT-03 injection
Indication:	Duchenne Muscular Dystrophy（DMD）
Study Population	Ambulatory boys with DMD, aged 2 to less than 8 years
Sample Size	6~9 subjects; Group I: 3 subjects, Group II: 3~6 subjects
Duration of Study	The screening period is 0-30 days, Group I: with 4 weeks of treatment period and a follow-up period of 6 months; Group II: with 4 months of treatment period and a follow-up period of 12 months.
Objectives	**Primary objective:** To evaluate the safety and tolerability of SPOT-03 administered by intravenous infusion (IV) to DMD patients. Secondary objectives: ● To evaluate the changes of dystrophin nucleic acid in blood and muscles of DMD patients after IV infusion of SPOT-03. ● To evaluate the changes in dystrophin expression and engraftment in muscles of DMD patients after IV infusion of SPOT-03. ● To evaluate the changes of anti-dystrophin antibodies and cytokines in serum of DMD patients after IV infusion of SPOT-

	03. ● To evaluate the changes in fat tissue mass and lean tissue mass in patients with DMD after intravenous infusion of SPOT-03.
Endpoints	**Primary endpoints:** ● The primary endpoints are the collection and quantification of adverse events (event description, onset time and response, severity assessment, and causal relationship with the study product). Including various clinical examination results: physical examination, vital signs, ECG, changes in clinical laboratory test results (blood routine, blood biochemistry, urine routine). Safety is evaluated through the reported medical history and symptom observations. **Secondary endpoints:** **Group I:** ● Changes in dystrophin nucleic acid concentration in blood at different timepoints after the first dose administration (see Table 5 for Group I schedule), as well as before and after the 2nd, 4th, 6th, and 8th doses and during follow-up. And the changes in dystrophin nucleic acid concentration in muscles at D29 (after completion of the last dose), measured by qPCR. ● Changes in the expression and engraftment level of dystrophin in muscle biopsies at baseline and D29 (after the completion of the last dose), measured by western blot and/or immunofluorescence. ● Changes in anti-dystrophin antibodies and cytokines in the serum at baseline and before the 2nd, 4th, 6th, and 8th administration and during follow-up (see Table 5 for Group I schedule). **Group II:** ● Changes in dystrophin nucleic acid concentrations in blood at different timepoints after the first and last dose administration (see Table 5 for group II schedule), as well as before and after the 4th, 8th, 12th, 16th, 20th, 24th, and 28th doses, measured by qPCR. ● Changes in dystrophin nucleic acid concentrations in the muscle

	at baseline, during administration, and after the last dose administration, measured by qPCR. ● Changes in dystrophin expression levels in the muscle at baseline, during administration, and after the last administration, measured by western blot and/or immunofluorescence. ● Changes from baseline in cytokine levels before administration of the 4th, 8th, 12th, 16th, 20th, 24th, 28th, 32nd doses and during follow-up period (see Table 5 for group II schedule), measured by ELISA assay. ● Changes from baseline in serum anti-dystrophin antibody levels before administration of the 4th, 8th, 12th, 16th, 20th, 24th, 28th, 32nd doses and during follow-up period (see Table 5 for group II schedule). ● Changes from baseline in adipose tissue mass and lean tissue mass at different follow-up time points after administration (see Table 5 for Group II schedule).
Study Description	This is a FIH, open-label, single-arm, exploratory clinical study of SPOT-03 injection in patients with DMD. The primary objective is to evaluate the safety and tolerability of SPOT-03 injection. The secondary objectives are to investigate changes in dystrophin nucleic acid concentration, dystrophin protein expression and engraftment, cytokine and anti-dystrophin antibody concentrations, and adipose tissue mass and lean tissue mass in subjects after administration. The study is divided into two dose groups: Group I: 4.0E+11 copies/kg , administered 8 times Group II: 4.0E+11 copies/kg, administered 32 times A total of 6 to 9 DMD patients aged 2 to less than 8 years will be enrolled in this study according to the inclusion criteria. Complete data from at least 3 subjects will be obtained for each dose group. All subjects will begin oral tacrolimus (0.05-0.2 mg/kg/d, adjustable according to actual clinical conditions) 3 days before the initial administration of SPOT-03 (D-3) and will continue administration for

	approximately 1 month after completion. In Group I, the first dose of SPOT-03 will be administered by intravenous infusion on D1, followed by twice a week administration (once every 4 days) for a total of 8 doses. In Group II, the first dose of SPOT-03 will be administered by intravenous infusion on D1, followed by twice a week administration (once every 4 days) for a total of 32 doses. After all subjects in the previous dose group have completed administration, the next dose group may proceed after the investigators and sponsors have discussed and determined that there are no serious adverse reactions related to the drug. Safety tests and evaluations will be conducted for the patients during each administration and during follow-up. Muscle biopsy (see Table 4 for specific time points) will be performed in the biceps brachii during the Screening Period + Baseline Period (before the first administration), during administration (Group II), and after the last administration of SPOT-03 on D29 (Group I) / after the 32nd dose (Group II). Dystrophin protein expression will be detected by western blot. Fiber strength and the percentage of dystrophin-positive fibers will be detected by immunohistochemistry (IHC). Changes in dystrophin nucleic acid before and after SPOT-03 administration will be measured by qPCR. MRI and electromyography (EMG) will be performed before the first administration of SPOT-03 and at 12 weeks after the last administration of SPOT-03 is completed (Group I: W16, Group II: W30).
Investigational Drug	**SPOT-03：** Colorless and odorless clear liquid **Active Pharmaceutical Ingredients:** Dystrophin nucleic acid loaded EV **Pharmaceutical excipients：** CryoStorCS10 (containing 10% dimethyl sulfoxide (DMSO)), phospate-buffered saline (PBS), human serum albumin (HSA), (pharmaceutical grade) **Strength：** 8.0E+11 copies/mL **Storage：** -60℃ ~ -90℃

Route of Administration	Intravenous infusion: twice a week (once every 4 days). Each subject in group I will receive a total of 8 doses, and each subject in group II will receive a total of 32 doses. SPOT-03 should be transported on dry ice and stored at -60 ° C to -90 ° C for an expected period of 3 months. The SPOT-03 product is frozen in 3 mL vials and thawed at room temperature before use. The thawed product is a colorless, odorless, clear solution. Thawed SPOT-03 should be used as soon as possible unless otherwise stored at 4° C for no more than 4 hours. After thawing, SPOT-03 should be diluted with normal saline to different concentrations for clinical use. Use immediately after dilution. The opened and diluted solution should not be left at room temperature for more than 4 hours. The required dose will be calculated based on the body weight of the subjects measured at baseline and the dose group to which they are assigned. The appropriate site for intravenous infusion will be selected based on the patient's individual venous condition. The infusion time is approximately 60 to 120 minutes. If the patient experiences mild allergic reactions such as dizziness, chills, rash, or extravasation during the infusion, administration should be immediately suspended. After recovery, the investigator will determine whether to continue administration and may appropriately reduce the infusion rate (as determined by the investigator). The infusion time may be extended accordingly (as determined by the investigator). After administration is completed, the actual dosage and the start and end times of administration will be recorded. Photograph of the infusion site will be taken before and after each infusion, and the response at the administration site and any infusion reactions will be closely observed.
Concomitant Medication	The newly generated dystrophin will be recognized by the immune system of the subjects as an exogenous protein, triggering an immune response. Any immune response to dystrophin can affect its engraftment and potentially induce further muscle damage, reversing

	any benefits that patients should have received. Therefore, the subjects should begin to orally take 0.05-0.2 mg/kg (adjusted according to the actual clinical situation) tacrolimus daily at D-3 (before the initial administration of SPOT-03) until the last dose of SPOT-03 has been administered.
Inclusion Criteria	**Only those who meet all the following criteria can be enrolled:** 1) According to fthe requirements of the region/country and/or IRB/IEC, the patient and/or legal guardian have signed a written informed consent form and are aware of all relevant study content. 2) Boys aged ≥ 2 years to < 8 years and capable of walking independently for at least 10 meters. 3) Confirmed diagnosis of DMD through multiplex ligation-dependent probe amplification (MLPA) and whole-exome sequencing. 4) Tolerance for muscle biopsy under anesthesia with no absolute contraindications to the procedure. 5) Heart, liver, lung, and kidney functions are sufficient: a) The left ventricular ejection fraction (LVEF) should be ≥ 50%; b) Forced vital capacity (FVC) ＞ 50% of the expected value, and do not require nighttime ventilation; c) Patient's glomerular filtration rate (GFR) > 30 mL/min/1.73 m^2
Exclusion Criteria	**Those who meet any one of the following criteria must be excluded:** 1) Complications other than DMD that may cause muscle weakness and/or motor dysfunction. 2) There are severe intellectual disabilities (such as severe autism, severe cognitive impairment, and severe behavioral disorders) that, according to the investigator's judgment, can affect the study. 3) Hospitalization for respiratory failure within 8 weeks prior to screening. 4) Asthma or underlying lung diseases that are poorly controlled, such

	as bronchitis, bronchiectasis, emphysema, or recurrent infectious pneumonia that investigator believes may affect respiratory function. 5) Severe uncontrolled heart failure (NYHA III-IV), including any of the following conditions： a) Intravenous administration of diuretics or positive inotropic drugs is required within 8 weeks prior to screening. b) Hospitalization due to worsening heart failure or arrhythmia within 8 weeks prior to screening. 6) Abnormal laboratory values considered clinically significant: a) GGT > 3 × upper limit of normal b) Bilirubin ≥ 3.0 mg/dL c) Creatinine ≥ 1.8 mg/dL d) Hemoglobin < 8 or > 18 g/dL e) White blood cell count > 18,500/μL 7) There are arrhythmias that require antiarrhythmic treatment. 8) Subjects who are undergoing immunosuppressive therapy. 9) Has used other gene therapy, investigational drugs, or any treatment aimed at increasing dystrophin expression. 10) Subjects with a history of major surgeries within 12 weeks prior to the initial infusion or planning to undergo major surgeries (such as scoliosis surgery) during this study. 11) Subjects who are allergic to investigational products or local anesthetic drugs or have a history of severe allergies or genetic allergic reactions. 12) Within 6 months prior to the initial infusion, the subjects are exposed to another investigational drug or are participating in an intervention clinical trial. 13) Subjects with positive of hepatitis B core antibody or hepatitis C antibody or HIV antibody during screening. 14) Investigator believes that the presence of any other serious diseases, medical conditions, or chronic drug treatment needs can pose unnecessary risks to gene transfer.

Withdrawal Criteria	1) Considering the safety, the investigator believes that withdrawing from the study is in the best interest of the subjects. 2) Subjects with poor compliance cannot insist on completing the trial as planned or have other circumstances that may affect the judgment of the research results. 3) The subject voluntarily requested to withdraw from the trial and withdraw the informed consent.
End of Study	• Group I: The study will end after enrolled subjects complete all administrations and follow-up at week 28. • Group II: The study will end after enrolled subjects complete all administrations and follow-up at week 70.
Dystrophin Protein Expression	1) Evaluation indicators a) Dystrophin protein levels will be determined by western blot of muscle biopsy samples. b) Fiber strength and the percentage of dystrophin-positive fibers will be detected by immunohistochemistry (IHC). 2) Muscle biopsy sampling a) Surgical biopsy: In the operating room, the biceps brachii or other appropriate sites will be selected, and the sampling location will be confirmed. The site will be disinfected with iodophor. After local anesthesia, a muscle tissue sample approximately 0.5 cm × 1 cm × 0.5 cm in size will be extracted. The extracted sample will be sent to the analysis/pathology laboratory for biopsy testing. Photographs will be taken to record the sampling site before and after each muscle biopsy. b) Puncture biopsy: In the operating room, the quadriceps femoris or other appropriate sites will be selected, and the sampling location will be confirmed. The site will be disinfected with iodophor. After local anesthesia, a 16G biopsy needle will be used to perform muscle biopsy sampling. The extracted sample will be sent to the analysis laboratory for testing. Photographs will be taken to record the sampling site before and after each

muscle biopsy.

3) Sampling time points

Group I:

a) One muscle biopsy (one sample in total, one site) will be taken on Screening Period and Baseline Period

b) One puncture sample (one sample in total, one site) will be taken on the day of the last SPOT-03 administration (dose 8)

c) One muscle biopsy (one sample in total, one site) will be performed within 3 days after administration.

Group II;

a) One muscle biopsy (one sample in total, one site) will be taken on Screening Period and Baseline Period

b) One puncture sample (one sample in total, one site) will be taken during SPOT-03 administration, with the time of collection determined by the investigator

c) One puncture sample (one sample in total, one site) will be taken on the day of the last SPOT-03 administration (dose 32)

d) One muscle biopsy (one sample in total, one site) will be performed within 3 days after administration.

In the study, it is advisable to perform a quadriceps muscle puncture biopsy and sampling on the same day as the last SPOT-03 administration, and then conduct a biceps brachii surgical muscle biopsy after the last administration. If a subject is unable to complete muscle tissue collection from the above-mentioned sampling sites due to medical or anatomical reasons, the investigator may determine alternative sampling points based on the muscle condition of the subjects and perform the muscle biopsy accordingly.

4) Sample testing method

a) Western blot: Dystrophin protein concentration changes will be evaluated at baseline, during administration (Group II only), and after the last administration is completed;

b) Immunohistochemistry (IHC) / Immunofluorescence (IF): Muscle fiber strength and the percentage of dystrophin-positive

	fibers will be evaluated at baseline and after the last administration.
Safety Assessment	• Vital signs (pulse, respiratory rate, blood pressure, temperature) and physical examination will be performed at each visit. • Safety assessments including laboratory tests (hematology, chemistry, urinalysis) and 12-lead ECG will be performed on some visits (see Table 5 for details). • ISRs (redness, swelling, heat, pain, and itching) will be observed within 30 minutes each post-treatment. According to the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0), all adverse events that occur throughout the study period will be evaluated and graded. The investigator will collect a description of the events, time of onset and resolution, assessment of severity and causal relationship to SPOT-03.
Pharmacokinetics Assessment	The changes of dystrophin nucleic acid in blood and muscle biopsy of subjects will be measured by qPCR. **Group I:** **Blood sample collection for PK:** • Intensive blood collection will be conducted before the dose 1 and dose 8 administration and at the following time points after the 1st and 8th administration: 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h, as discussed by the investigator and collaborators. Note: PK blood sample collection before the first administration may be conducted during the Screening Period / Baseline Period if no more than 7 days elapse between baseline and the first administration. • PK blood samples will also be collected before dose 2, dose 4, and dose 6 administrations at 0h+10 min after each of these doses is completed. • Late-stage PK blood collection may be adjusted based on previously obtained PK data and safety data. • PK blood samples will also be collected on W8, W12, and W16

follow-up periods. If dystrophin nucleic acid cannot be detected at W8 follow-up, blood sample collection for this test at subsequent follow-ups may not be necessary.

Muscle tissue testing:

The changes in dystrophin nucleic acid concentration in muscle tissue will be evaluated at baseline and after the last administration (D29).

Group II:

Blood sample collection for PK:

- Intensive blood collection will be conducted before dose 1 administration, within 30 minutes before dose 32 administration, and at the following time points after dose 1 and dose 32 administration: 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h.
- PK blood collection will also be conducted within 30 minutes before dose 4, dose 8, dose 12, dose 16, dose 20, dose 24, dose 28 administration and at 0h+10min and 24h±1h after each of these doses.
- If a subject withdraws early for any reason during the administration period, intensive blood collection will be performed within 30 minutes before the last administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after the last administration.
- Late-stage PK blood collection may be adjusted based on previously obtained PK data and safety data after discussion by the investigators and collaborators.
- PK blood samples will also be collected at W22, W26, W30, W34, W38, W42, W46, W50, W54, W58, W62, W66, W70 follow-up periods. If dystrophin nucleic acid cannot be detected at W22 or at any subsequent follow-up, it may not be necessary to collect blood samples for this test at subsequent follow-ups.

Muscle tissue testing:

The changes in dystrophin nucleic acid concentration in muscle tissue will be evaluated at baseline, during the administration period, and

	after the last administration.
Pharmacodynamic Assessment	• Muscle biopsies will be performed at the Screening + Baseline Period, during administration (one sample in total, one site; for Group II only), and after the last dose to detect the expression of dystrophin in the muscle. • Quantity of dystrophin is measured by western blot of biopsied muscle tissue. Levels are described as change from baseline, in order to remove any background signal in the assay. Levels are expressed as a percent of control (i.e., as a percent of the level of normal, wild-type dystrophin present in muscle tissue of healthy individuals without DMD or Becker muscular dystrophy). • MRI and electromyogram examinations will be performed at the Screening + Baseline Period and at W12 following the last administration to assess the health of muscles and the nerve cells that control them. • Only Group II will undergo DEXA testing. DEXA examinations will be conducted during the Screening Period / Baseline Period before administration, at dose 7 (±7 days), dose 15 (±7 days), dose 23 (±7 days), dose 31 (±7 days), W20 follow-up period (±7 days), W22 follow-up period (±7 days), and at W26, W30, W34, W38, W42, W46, W50, W54, W58, W62, W66, and W70 follow-up periods to analyze fat tissue mass and lean tissue mass. The number of examinations may be adjusted as deemed necessary by the investigator.
Immunogenicity	Evaluation indicators include biomarker detection (changes in concentrations of TNF-α, INF-γ, IL-2, IL-6, and IL-10) and changes in the concentration level of anti-dystrophin antibody measured by ELISA assay. **Biomarker detection:** **Group I:** • Blood samples for biomarker detection test will be collected during the Screening Period / Baseline Period / before the first

	administration (no additional collection is required before the first administration if no more than 7 days from baseline to the first administration), before dose 2, dose 4, dose 6, dose 8 administrations, and during the W8, W12 and W16 follow-up periods. • If biomarker levels return to normal or are undetectable at W8 follow-up, blood samples may not be collected for the test at subsequent follow-ups. **Group II:** • Blood samples for biomarker detection will be collected during the Screening Period / Baseline Period/ before the first administration (no additional collection before the first administration if no more than 7 days from baseline to the first administration), before dose 4, dose 8, dose 12, dose 16, dose 20, dose 24, dose 28, dose 32 administration. • Blood samples for biomarker detection will also be collected during the follow-up periods of W22, W26, W30, W34, W38, and W42. • If biomarker levels return to normal or are undetectable at W22 or at any subsequent follow-up, blood samples may not be collected for the test at subsequent follow-ups. **Anti-dystrophin antibody test:** **Group I:** • Blood samples for anti-dystrophin antibody test will be collected during the Screening Period / Baseline Period / before the first administration (no additional collection before the first administration if no more than 7 days from baseline to the first administration), before dose 2, dose 4, dose 6, dose 8 administrations, and during the W8, W12 and W16 follow-up periods. • If the anti-dystrophin antibody level is undetectable in the follow-up sample at W8, blood samples may not be collected for this test at subsequent follow-ups.

	Group II: • Blood samples for anti-dystrophin antibody test will be collected during the Screening Period / Baseline Period During the screening period/baseline period/before the first administration (if no more than 7 days from baseline to dose 1 administration, no additional collection is required before dose1 administration), before dose2, dose 4, dose 6, dose 8, dose 12, dose 16, dose 20, dose 24, dose 28, dose 32 administration. • Blood samples for anti-dystrophin antibody test will also be collected during the follow-up periods of W22, W26, W30, W34, W38, and W42. • If anti-dystrophin antibody levels cannot be detected at W22 or at any subsequent follow-up, blood samples may not be collected for the test at subsequent follow-ups.
Statistical Analysis of Population	1) Full analysis set (FAS): FAS will be used to report medication compliance and to summarize demographic characteristics (age, gender, race, height and weight) and background characteristics (medical history, concomitant medications, and physical examination, etc.) for all randomized subjects by treatment group. 2) Safety analysis set (SAS): All subjects who have been randomized into cohorts, have used the investigational drug, and have safety evaluation data post-treatment constitute the safety population of this study. The safety population will be used for safety analysis.
Statistical Method	Due to the small sample size and open-label design of this study, only descriptive statistics will be conducted for all endpoints, including the number of subjects, mean and standard deviation, minimum and maximum values of continuous variables, and the number and percentage of categorical variables.

1. Background

Duchenne muscular dystrophy (DMD) is the most common X-linked recessive male hereditary disease and the most common fatal monogenic disease. The incidence rate of DMD in male newborns is about 1/3500. Approximately 400-500 cases of DMD are born in China every year, with a total of approximately 70.000 confirmed cases, making it one of the countries with the highest number of DMD patients in the world. There is no obvious geographical distribution characteristic of DMD patients in China [1].

1.1. Dystrophin gene and its mutation types

DMD is caused by mutations in the gene encoding the dystrophin protein. This gene is located in the Xp21.2 region of chromosome, with a total length of 2.3 Mb and 79 exons. The mRNA sequence encoded by this gene has a total length of 11 Kb. Dystrophin gene is the largest known human gene [2][3][4]. The normal dystrophin protein size generated by the translation of this gene in muscle cells is 427 kDa, and it is expressed in muscle cells throughout the body, including the heart, skeletal muscles, smooth muscles, retina, and brain [5].

The mutation types in the dystrophin gene are mainly exon deletions/duplications, while mutations in large segments (≥ 1 exon) with deletions or duplications account for about 79% (of which deletions account for about 68% and duplications account for about 11%). The most common hotspots of deletions and duplications are located in exons 45-55 and 3-9 of the gene [2][4]. Other types of small mutations (<1 exon) account for approximately 21% (of which point mutations account for about 11%, small deletions account for about 5%, small insertions account for about 2%, and splice site mutations account for about 3%) [4][5]. These point mutations are distributed throughout the entire DMD gene and there are no obvious hotspots. DMD patients are unable to synthesize normal dystrophin in their bodies due to mutations in the dystrophin gene.

The phenotype of DMD mainly depends on whether mutations in the dystrophin gene have disrupted the open reading frame. Out-frame mutations lead to transcription and translation disorders of the dystrophin gene, resulting in a more severe DMD phenotype. In-frame mutations, while retaining an open reading frame, can transcribe and translate into truncated, partially functional dystrophin proteins, resulting in milder phenotypes, such as Becker muscular dystrophy (BMD) [2].

1.2. Clinical therapy for DMD patients

Based on the guidelines of the Neurology Branch of the Chinese Medical Association [7], the therapy of DMD patients mainly involves Multi-Disciplinary Treatment (MDT)

symptomatic treatment, usually starting in boys aged 4 or above. MDT mainly includes drug treatment, rehabilitation treatment, treatment of respiratory complications, treatment of heart disease, surgical orthodontic treatment, and other treatments etc [1]. Although advances in multidisciplinary nursing and disease management strategies for DMD have slowed down disease progression, the disease is still incurable [6][8][9][10].

At present, the drug treatment for DMD patients mainly is glucocorticoids, such as prednisone (0.75mg/kg/d), which can improve the strength and lung function of DMD patients, reduce the need for spinal surgery, and delay the occurrence of cardiomyopathy. But hormone therapy may cause many side effects, including obesity, Cushing's face, hirsutism, osteoporosis, and so on. To reduce side effects, the dose can be reduced by one-third if necessary [7].

In recent years, in addition to hormone therapy, with the increasing maturity of core technologies such as gene delivery vector technology and gene editing technology, gene therapy has also been gradually developing and heating up. Due to its potential to cure rare diseases in one go, it has long received global attention and is known as the "dawn of rare disease treatment".

1.3. Gene therapy for DMD

The DMD gene therapy strategy is designed for different types of gene mutations aimed at restoring dystrophin expression in muscle tissue. It mainly includes readthrough therapy [11], exon skipping therapy [12], vector mediated gene replacement therapy [13], clustered regularly interspaced short palindromic repeat，CRISPR/ CRISPR-associated nuclease 9，(Cas9）gene editing therapy [14].

The above four therapies are suitable for DMD patients with different mutation types, and each has its own advantages and disadvantages, see Table 1.

Table 1 Summary of gene therapy strategies for DMD [15]

Therapy strategy	Mechanism of action	Applicable to patients	Advantage	Disadvantage	R&D progress
Readthrough	Prevent signal recognition of termination codon and induce read-through.	Nonsense mutation	Good safety and tolerability	Applicable to few patients and limited clinical benefits	Some drugs approved by EMA
Exon skipping therapy	Antisense oligonucleotides mask mRNA splicing signals and achieve exon skipping.	Exon deletion	Good safety and tolerability	Limited clinical benefits, frequent administration requirement and low myocardial	Some drugs have been approved by FDA, and some clinical trials are ongoing.

				transduction efficiency	
Vector mediated dystrophin gene replacement therapy	AAV loaded micro-dystrophin	AAV antibody negative	Restore stable high expression of dystrophin	High demand for AAV, time-consuming manufacturing, high cost, and susceptibility to immune reactions	Accompanied by questioning and controversy, the gene therapy drug-Elevidys, jointly developed by Sarepta & Roche, has been approved for market by FDA.
CRISPR/Cas9 gene editing	AAV loaded CRISPR/Cas9 precise repair gene mutation	AAV antibody negative	one-time treatment	High demand for AAV, time-consuming manufacturing, high cost, susceptibility to immune reactions, and off-target risk	The most studies are in the animal experimental stage, and one clinical trial has been terminated

EMA：European Medicines Agency，FDA：U.S. Food and Drug Administration，AAV：Adeno-associated virus，CRISPR：Clustered regularly interspaced short palindromic repeat，Cas9：CRISPR-associated nuclease 9

1.4. Approved drugs for DMD and their scope of application

In recent years, the approved drugs for DMD treatment are mainly steroid and gene therapy products. See Table 2.

Table 2 Approved drugs for the treatment of DMD [16][17]

Drug classification	Drug name	R&D company	Approval time	Indication
Exon skipping therapy（ASO）	Casimersen	Sarepta	2021	DMD patients with exon 45 gene mutation
	Viltolarsen	Nippon Shinyaku Co Ltd	2020	DMD patients with exon 53 gene mutation
	Golodirsen	Sarepta	2019	DMD patients with exon 53 gene mutation
	Eteplirsen	Sarepta	2016	DMD patients with exon 51 gene mutation
Corticosteroid	Deflazacort	PTCT	2017/2019	DMD patients aged 2 years and above
	Vamorolone	Catalyst Pharms	2023	DMD patients aged 2 years and above
Readthrough	Ataluren	PTCT	2014	Ambulatory DMD patients with nonsense mutation
AAV vector mediated dystrophin gene replacement therapy	Elevidys	Sarepta	2023	Used to treat DMD outpatient patients aged 4-5 with confirmed mutations

1.4.1. Steroid drugs

In 2017, the corticosteroid drug Deflazacort was approved by the US Food and Drug

Administration (FDA) for the treatment of DMD patients. On October 26, 2023, the FDA approved the marketing application of Vamorolone oral suspension 40 mg/mL for the treatment of DMD patients aged 2 years and above. Vamorolone is a first-in-class steroid drug, which differs from normal steroid drugs in that it can selectively activate certain signaling pathways of steroids, resulting in better safety [16].

1.4.2. Gene therapy for DMD

In 2014, EMA approved the drug Ataluren (trade name: Translarna) to enter the European market. This drug can reduce the sensitivity of ribosomes to early termination codons, allowing mRNA to continue translation without stopping at the termination codon during translation (i.e. "readthrough"). It can be used to treat gene nonsense mutations, with approximately 10% of DMD patients [17].

The four exon skipping therapies approved by the FDA for the treatment of DMD are Eteplarirsen, Golodirsen, Viltolasen, and Casimersen. These drugs can only be used for DMD patients with specific gene mutations: Eteplarirsen (Exonys 51), approved for marketing on September 19, 2016, is used to treat approximately 13% of DMD patients with exon 51 mutations [19]. Golodirsen (Vyondys 53), approved for sale on December 12, 2019, and Viltolarsen, approved for sale in Japan and the United States in March and August 2020, respectively, are used to treat approximately 8% of DMD patients with exon 53 mutations [20][21]. Casimersen (Amondys 45), approved for marketing on February 25, 2021, is used to treat approximately 8% of DMD patients with exon 45 mutations [22]. These four drugs are all ASO that modify the splicing of dystrophin mRNA to translate into truncated, partially functional dystrophin, transforming more severe DMD phenotype into lighter BMD phenotype [23][24]. These types of drugs have a low affinity for proteins, so they will be quickly cleared in the body after systemic injection. The biggest challenge for this type of drug currently is the difficulty of entering the cell, therefore, it is necessary to administer larger drug doses or increase the frequency of administration to improve bioavailability. All four drugs require regular intravenous injection [25]. More importantly, these products are conditionally marketed and have not yet completed the required confirmatory clinical studies, so their clinical benefits are not fully understood.

On June 22, 2023, the FDA accelerated the approval of SRP-9001 (trade name: Elevidys) of Sarepta Therapeutics for the treatment of DMD patients aged 4-5. SRP-9001 (AAV loading micro-dystrophin) has also become the world's first one-time gene therapy for DMD. This drug uses the AAVrh74 vector. The gene loading capacity of this type of vector is limited, and it

cannot load entire dystrophin gene. Therefore, the main functional regions of dystrophin gene can only be selected for Elevidys design based on existing studies. After administration of Elevidys, the final product generated in the body, the micro-dystrophin (138 kDa), only contains the selected domain of the normal wild-type dystrophin (427 kDa). The micro-dystrophin lacks the domain that can bind to neuronal nitric oxide synthase and α- syntrophic factors, which of them can protect muscle cells by synergistically regulating blood flow. Therefore, this gene therapy method can only alleviate the symptoms of DMD patients. Although in theory, the function of the micro-dystrophin may be similar to that of normal dystrophin, but it is a novel protein that does not exist in nature. It is different from both the normal wild-type and the endogenous shortened form of dystrophin in BMD patients, as well as the internally truncated dystrophin produced through exon skipping drug therapy expression. The relationship between micro-dystrophin and various shortened forms of dystrophin is still unclear, and whether its actual performance is close enough to that of natural dystrophin remains questionable. In addition, there is no clear answer to how high the expression level of micro-dystrophin can benefit patients clinically. Meanwhile, due to the lack of epidemiological data on this protein, it is not clear how it affects the pathophysiology of DMD patients [26].

The use of AAVrh74 vector also limits the clinically treatable population, requiring patients with anti-AAVrh74 antibody titers<1:400 to be selected. The existing clinical studies of Elevidys have shown that after large-scale administration, the titer of anti-saferrh74 antibody in patients significantly increases. Therefore, even if the patient's initial dose is insufficient or there is no therapeutic efficacy after treatment, they cannot accept additional doses of the same drug for re-administration. And due to the possibility of immune cross reactivity with other AAV subtypes, DMD patients who have not benefited from Elevidys treatment may not be able to accept gene therapy based on other serotype AAV vectors in the future [26].

In addition, multiple experts have raised doubts about the structure, animal study, and clinical trial results of Sarepta's micro-dystrophin. They questioned whether there is a significant correlation between the micro-dystrophin level and muscle performance in DMD patients. Although the FDA has approved the product for market through accelerated approval, it requires further post market study to be conducted [26].

At present, there are no approved DMD gene therapy in China. From the above description, although gene therapy brings hope for the clinical treatment of DMD patients, the gene therapy products currently on the market have different shortcomings. Existing clinical studies have shown that they cannot meet the clinical treatment needs of DMD patients.

Therefore, R&D personnel still need to make efforts, as well as a large amount of funding to develop more promising and effective gene therapy products for the clinical treatment of DMD patients.

1.5. Global ongoing study on DMD treatment drugs

According to the Clinicaltrials.gov [18], there are a total of 381 clinical trials for the treatment of DMD (2023/8/29). The clinical trial information of new drugs that are currently ongoing or in recruitment status is detailed in Table 3. Whether from the approved DMD drugs or the current number of ongoing new drugs, the industry's enthusiasm for DMD drug development is gradually increasing.

Table 3 Global information of the ongoing new drugs for DMD

Drug name	R&D company	Treatment strategies	Phase	ID
CAP-1002	Capricor Inc	Cell therapy	III	NCT05126758
CRD-TMH-001	Cure Rare Disease	Gene editing	I	NCT05514249
PF-06939926	Pfizer	Gene replacement therapy	III	NCT04281485
SGT-001	Solid Biosciences		I/II	NCT03368742
GNT 0004	Genethon-Sarepta		III	/
RGX-202	REGENXBIO		II	NCT05693142
AOC 1044	Avidity Biosciences	Exon skipping therapy	II	NCT05670730
WVE-N531	Wave Life Sciences		II	NCT04906460
DYNE-251	Dyne Therapeutics		II	NCT05524883
SQY51	Sqy Therapeutics		II	NCT05753462
Brogidirsen	NS Pharma		II	NCT05996003
ENTR-601-44	Entrada Therapeutics		II	/
PGN-EDO51	PepGen		II	NCT06079736
Renadirsen	Daiichi Sankyo		II	NCT04433234
Vesleteplirsen	Sarepta Therapeutics		II	NCT04004065
TAS-205	Taiho Pharmaceutical Co., Ltd.	Chemical drug	III	NCT04587908
EDG-5506	Edgewise		III	NCT05540860
Givinostat	Italfarmaco Group		NDA	NCT01761292
Edasalonexent	Catabasis		III	NCT03703882
Ezutromid	Summit Therapeutics		II	NCT02858362
Pamrevlumab	FibroGen	Monoclonal antibody	III（Failed）	NCT04632940

2. SPOT-03 Introduction

2.1. SPOT-03 design

SPOT-03 is a gene therapy product for DMD, using the patented technology of Spot Biosystems - Cellular nanoporation (CNP), which can rearrange cell membrane molecules and improve membrane permeability through high-intensity electric field. Thereby, constructing a channel for introducing exogenous molecules into cells [27]) for transfecting

human mesenchymal stem cells (MSCs) and introducing target molecules and molecular targeted peptides (which can be co-expressed with transmembrane proteins of EVs). Compared with the traditional transfection technology, this technology can significantly improve the transfection efficiency, promote the cells to load about 14kb of full-length dystrophin nucleic acid molecules encoding dystrophin with high flux, and simultaneously generate a large number of EVs loaded with target dystrophin. After transfection, collect the supernatant containing the target product, purify and concentrate it, add phosphate buffer, cell cryopreservation solution CS10, and human serum albumin to prepare SPOT-03 injection.

2.2. Mechanism of Action of SPOT-03

SPOT-03 is an EVs loaded full-length dystrophin nucleic acid molecule, and the EVs membrane surface expresses a molecular targeted peptide, which can be used as a gene therapy product for DMD. In order to improve the targeting ability of SPOT-03 into cells, the molecular targeting peptide was co-transfected with the transmembrane protein of EVs, thus introducing the molecular targeting peptide onto the EVs membrane. After intravenous injection of SPOT-03 into DMD patients, the targeting ability of SPOT-03, endowed by molecular targeting peptides on the EVs membrane, can guide SPOT-03 into the patient's muscle cells, allowing dystrophin deficient receptor cells to utilize intracellular ribosomes and amino acids to translate and secrete endogenous full-length intact dystrophin. Simultaneously, the DNA cargo traffics to the nucleus, where it is transcribed into mRNA, followed by cytoplasmic translation, providing sustained dystrophin expression. The generated dystrophin can tightly bind with various proteins in the inner, transmembrane, and outer regions of the cell membrane, such as sarcoglycan and dystroglycan, and are interrelated to form a whole inside and outside the cell membrane. It maintains material exchange and connection inside and outside the cell membrane, protects the integrity and stability of the cell membrane structure, and thus protects muscles from damage and/or promotes muscle repair and regeneration.

3. Risk and Benefit Assessment

3.1 Risk Assessment

The main components of SPOT-03 are EVs with molecular targeted peptides secreted by MSCs and full-length dystrophin nucleic acid molecules loaded on them. After administration, SPOT-03 can target muscle cells and generate dystrophin. EVs can be secreted by all cells in the human body, with low immunogenicity and good biocompatibility.

It is hard to induce the production of antibodies and to cause immunogenicity in the body. EVs of SPOT-03, integrating corresponding molecular targeted peptides on the lipid layer membrane, possess strong targeting ability, which can directly deliver loaded dystrophin nucleic acid to target cells. In addition, the dystrophin nucleic acid sequence loaded on SPOT-03 will carry or get transcribed into the same mRNA molecular sequence encoding dystrophin in the human body.

According to the mechanism of action of SPOT-03, endogenous full-length intact dystrophin can be generated and secreted in dystrophin deficient receptor. Because there are not the intact dystrophin proteins in DMD patients, it may be recognized by the immune system as an exogenous protein in the patient's body, leading to a more severe immune response and potentially inducing further muscle damage, reversing any benefits that the patient should have received. There have been reports in other clinical studies of gene therapy for DMD [27]. According to reports, independent clinical studies of three different AAV products have shown a total of approximately 200 patients, of which 5 experienced severe immune reactions. Symptom onset occurred 3 to 6 weeks after administration: all five patients had severe weakness of the proximal and distal limb muscles that led to loss of ambulation, as well as weakness of the bulbar and respiratory muscles, which led to receipt of transient ventilatory support in three of the patients (two with noninvasive ventilation and one with endotracheal intubation). In addition, all patients had symptoms of myositis, and three patients also experienced symptoms of myocarditis. Therefore, various immunomodulatory treatments were used among the trials, including pulse dose glucocorticoids, intravenous immunoglobulin, plasmapheresis, and tacrolimus. The above clinical symptoms were resolved within 3 months after treatment. To prevent the occurrence of immune response, this study orally administered tacrolimus daily starting from D-3 before treatment of the study drug, and during the drug administration period, continuous medication is required, and the dosage should be adjusted based on the results of blood drug concentration monitoring. After the last administration, continue taking the medication for approximately one month. The researcher may adjust the medication period according to the subject's condition. Since these two drugs are immunosuppressants, it is necessary to closely monitor the adverse reactions of medication to prevent the occurrence of infections. The detailed adverse reactions of this drug can be found in section 8.3.

In addition, SPOT-03 belongs to a hypertonic solution with high osmotic pressure (>600 Osm/L) and before intravenous infusion, normal saline is used to prepare an injection solution

with an osmotic pressure similar to that of blood. Appropriate venous access should be selected for administration, and appropriate vascular access device should be used. During the infusion process, administer slowly, carefully observe the patient's infusion site, evaluate the infusion site reaction, and strictly prevent adverse events such as extravasation and venous infusion complications. If extravasation is observed, stop administration immediately. The preclinical sin- and multi- dose toxicity study of SPOT-03 showed that SPOT-03 had no significant systemic adverse reactions in the animal body. At present, there are no similar products to SPOT-03 on the market, and there are also no corresponding reports of ongoing clinical trials. There are over 400 registrations or related clinical trials based on EVs, most of which focus on the diagnosis, treatment, or prognostic strategies for various cancers, cardiovascular diseases, and neurodegenerative diseases. So far, these clinical trials have reported that EVs based therapies have no significant toxicity or adverse effects, indicating the safety and feasibility of the technology.

In summary, from the perspective of product design, production, and mechanism of action, the most likely adverse reactions caused by SPOT-03 infusion in DMD patients are infusion reaction and immune reaction, which may pose safety risks in the clinical application of this product. However, the toxicity studies of SPOT-03 and other relevant reports of gene therapy indicate that the safety risks of SPOT-03 in clinical application are controllable.

3.2 Benefit Assessment

SPOT-03 is the latest gene therapy product for DMD. After administration to DMD patients, the molecular targeting peptide on the EVs membrane can guide SPOT-03 to enter the patient's muscle cells, allowing dystrophin deficient receptor cells to utilize intracellular ribosomes and amino acids to translate and secrete endogenous full-length intact dystrophin. Dystrophin is a cytoskeletal protein that has an anti-mechanical stretching effect and can prevent damage to muscle cells during contraction. The generated dystrophin can tightly bind with various proteins in the inner, transmembrane, and outer regions of the cell membrane, such as sarcoglycan and dystroglycan, and are interrelated to form a whole inside and outside the cell membrane. It maintains material exchange and connection inside and outside the cell membrane, protects the integrity and stability of the cell membrane structure, and thus protects muscles from damage and/or promotes muscle repair and regeneration. Therefore, SPOT-03 to DMD patients can theoretically alleviate the clinical symptoms and improve the quality of life of DMD patients.

Since SPOT-03 is completely different from existing gene therapy products on the market, it

is the first to achieve in vivo targeted delivery of full-length dystrophin nucleic acid molecules, which can generate full-length and complete dystrophin in the body. Therefore, SPOT-03 based on dystrophin nucleic acid loaded on EVs can provide a new and effective protein replacement therapy for DMD patients, making clinical cure of DMD possible.

4. Study Objectives and Endpoints

Table 4 The objectives and endpoints of this study

Objectives	**Endpoints**
Primary objective:	**Primary endpoints:**
To evaluate the safety and tolerability of SPOT-03 administered by intravenous infusion (IV) to DMD patients.	Collection and quantification of adverse events (description of event, time of onset and resolution, assessment of severity and causal relationship to the investigational drug), as well as the data of physical examination, vital signs, 12-lead ECG, clinical laboratory test results (hematology, biochemistry, urinalysis) in this study.
Secondary objectives:	**Secondary endpoints:**
To evaluate the changes of dystrophin nucleic acid in serum and muscles of DMD patients after IV infusion of SPOT-03.	**Group I:** Changes of dystrophin nucleic acid concentration in blood before and different timepoints after the initial administration (see Table 5 for schedule) as well as before and after the 2nd, 4th, 6th, and 8th administration and during follow-up. And the changes in dystrophin nucleic acid concentration in muscles at baseline and D29 after the completion of the last dose (if the biopsy time is between 12-24 hours after the last dose) (by qPCR). **Group II:** Changes in dystrophin nucleic acid concentrations in blood at different timepoints after the first and last dose administration (see Table 5 for group II schedule), as well as before and after the 4th, 8th, 12th, 16th, 20th, 24th, and 28th doses, measured by qPCR. And the changes in dystrophin nucleic acid

Objectives	Endpoints
	concentrations in the muscle at baseline, during administration, and after the last dose administration.
To evaluate the changes in dystrophin expression and engraftment in muscles of DMD patients after IV infusion of SPOT-03.	**Group I:** Changes in the expression and engraftment level of dystrophin in muscle biopsies at baseline and D29 (after the completion of the last dose), measured by western blot and/or immunofluorescence. **Group II:** Changes in dystrophin expression levels in the muscle at baseline, during administration, and after the last dose administration, measured by western blot and/or immunofluorescence.
To evaluate the changes in serum anti-dystrophin antibodies and cytokines in DMD patients after IV infusion of SPOT-03.	**Group I:** Changes in anti-dystrophin antibodies and cytokines in the serum at baseline and before the 2nd, 4th, 6th, and 8th administration and during follow-up, measured by ELISA assay. **Group II:** Changes from baseline in anti-dystrophin antibodies and cytokine levels before administration of the 4th, 8th, 12th, 16th, 20th, 24th, 28th, 32nd doses and during follow-up period (see Table 5 for group II schedule), measured by ELISA assay.
To evaluate the changes in fat tissue mass and lean tissue mass in DMD patients after intervenous infusion of SPOT-03.	Changes from baseline in adipose tissue mass and lean tissue mass at different follow-up time points after administration (see Table 5 for Group II schedule).

5. Study Design

5.1. Overall Design

This is a FIH, open-label, single-arm exploratory clinical study of SPOT-03 administered via IV infusion for DMD patients. The primary objective of this study is to evaluate the safety and tolerability of SPOT-03 for DMD patients. And the secondary objectives are to preliminarily investigate the concentration change of dystrophin nucleic acid, the expression and engraftment level changes of dystrophin protein, as well as changes in cytokines and immunogenicity, adipose tissue mass and lean tissue mass in subjects after administration.

The study has two dose groups:

Group I：4.0E+11 copies/kg, administered 8 times

Group II：4.0E+11 copies/kg, administered 32 times

A total of 6 to 9 DMD patients aged 2 to less than 8 years will be enrolled in this study according to the inclusion criteria. Complete data from at least 3 subjects will be obtained for each dose group. All subjects will begin oral tacrolimus (0.05-0.2 mg/kg/d, adjustable according to actual clinical conditions) 3 days before the initial administration of SPOT-03 (D-3) and will continue administration for approximately 1 month after completion.

In Group I, the first dose of SPOT-03 will be administered by intravenous infusion on D1, followed by twice a week administration (once every 4 days) for a total of 8 doses. In Group II, the first dose of SPOT-03 will be administered by intravenous infusion on D1, followed by twice a week administration (once every 4 days) for a total of 32 doses. After all subjects in the previous dose group have completed administration, the next dose group may proceed after the investigators and sponsors have discussed and determined that there are no serious adverse reactions related to the drug.

Safety tests and evaluations will be conducted for the patients during each administration and during follow-up. Muscle biopsy (see Table 4 for specific time points) will be performed in the biceps brachii during the Screening Period + Baseline Period (before the first administration), during administration (Group II), and after the last administration of SPOT-03 on D29 (Group I) / after the 32nd dose (Group II). Dystrophin protein expression will be detected by western blot. Fiber strength and the percentage of dystrophin-positive fibers will be detected by immunohistochemistry (IHC). Changes in dystrophin nucleic acid before and after SPOT-03 administration will be measured by qPCR. MRI and electromyography (EMG) will be performed before the first administration of SPOT-03 and 12 weeks after the last administration of SPOT-03 is completed (Group I: W16, Group II: W30).

5.2. Duration of Study

The screening period is 0-30 days, Group I with 4 weeks of treatment period and a follow-up period of 6 months, and Group II with 18 weeks of treatment period and a follow-up period of 12 months.

5.3. Termination Criteria

1) According to CTCAE v5.0 criteria, during the dose escalation process, more than 1/2 of the subjects have grade 2 or above liver, kidney, cardiac and hematological TRAEs, or more than 1/3 of the subjects have other system AEs of grade 3 or above occur, the trial should be terminated.
2) The collaborator requests to terminate the study due to financial or management reasons, etc.
3) Termination of the trial is requested by IRB/EC.

5.4. Schedule of Events

Table 5 Schedule of Events

Group I

Study Period	**Screening**	**Baseline**	**Treatment Period**								**Follow-up Period**					
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visit Time[a]	D-30~D-8	D-7~D-1	D1	D5	D9	D13	D17	D21	D25	D29	W8	W12	W16	W20	W24	W28
Visit Window (Days)				±2	±2	±2	±2	±2	±2	±2	±7	±7	±7	±7	±7	±7
Informed Consent	X															
Demographics	X															
Medical History[b]	X															
Vital Signs[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECHO[e]	X												X			
12-lead ECG [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PFT[g]	X								X		X	X		X		X
Chest X-Ray	X															
Haematology[h]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Biochemistry[i]	X	X			X		X		X	X	X	X	X	X	X	X
Urinalysis[j]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Complement C3/C4[k]	X						X			X	X	X	X	X	X	X
Hepatitis B & C, HIV[l]	X															
Coagulation[m]	X															X
SPOT-03 Administration			X	X	X	X	X	X	X	X						
EMG	X												X			
MRI[n]	X												X			
PK[o]	X		X	X		X		X		X	X	X	X			
Muscle		X								X						

Study Period	**Screening**	**Baseline**	**Treatment Period**								**Follow-up Period**					
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Visit Time[a]	D-30~D-8	D-7~D-1	D1	D5	D9	D13	D17	D21	D25	D29	W8	W12	W16	W20	W24	W28
Visit Window (Days)				±2	±2	±2	±2	±2	±2	±2	±7	±7	±7	±7	±7	±7
Biopsy[p]																
Photography[q]		X	X	X	X	X	X	X	X	X						
BiomarkerTesting[r]	X		X	X		X		X		X	X	X	X			
Immunogenicity[s]	X		X	X		X		X		X	X	X	X	X	X	X
NSAA[t]	X								X		X	X		X		X
6-minute walk distance, four-step test[u]	X								X		X	X		X		X
Tacrolimus blood concentration monitoring[v]			X													
Administration of tacrolimus		Starting from D-3, take 0.05-0.2mg/kg/d, tacrolimus orally daily until approximately one month after the completion of the last SPOT administration. If there are special circumstances, the duration of administration may be adjusted at the discretion of the investigator														
Adverse Events	Continuous monitoring records from initial administration to last visit															
Concomitant Medications	Continuous recording from signing informed consent to the last follow-up															

ABBREVIATIONS: D = study day; W = study week; ECG = electrocardiogram; ECHO = echocardiogram; PFT = pulmonary function test; EMG = electromyography; MRI = magnetic resonance imaging; EOS = end of study; AE = adverse events. Unscheduled tests may be performed at the discretion of the Investigator if clinically indicated and required for safety.

a) Study date: The first dosing date of the investigational drug is defined as Day 1 (D1). The first dose time of the study drug was defined as t=0. The baseline is the result of the last assessment before the first dose. There was no day 0 in the study; The day before day 1 (D1) is D-1.

b) Obtain the complete medical history of the subject to determine eligibility. If the AE is recorded from the time of administration rather than from the signing of the consent form, any medical events that occurred during the screening period and baseline period prior to the first administration of the study drug will be recorded as medical history. Collect the current medical history of DMD (genetic test results and corresponding treatment information), as well as other treatment histories within 6 months.

c) Vital signs include blood pressure, pulse, respiration, and body temperature. Vital signs will be checked within 1 hour before and after each administration during treatment. In addition, vital sign tests will be conducted 30 minutes (±10 minutes), 1 hour (±10 minutes), 2 hours, and 4 hours (±30 minutes) after the first administration is completed. Note: Blood pressure of the subjects will be monitored daily during tacrolimus administration and recorded.

d) Physical examination includes height, weight, skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, spine and limbs and joints, musculoskeletal system, and nervous system. Check before each cycle of administration. Calculate individual doses based on baseline body weight.

e) Echocardiography tests will be performed at screening and at follow-up at week 16.

f) 12-lead-ECG: Tests are required before administration and 2 hours (±1 hour) after administration for D1, and before administration for other administration cycles. When ECG measurement results are abnormal (including QTcF interval abnormalities such as QTcF>500 ms or an increase of >60 ms relative to baseline) or other abnormalities are clinically significant, two additional ECG measurements are required, with an interval of at least approximately 2 minutes between repeated measurements.

g) Pulmonary function tests include forced vital capacity (FVC), forced expiratory volume in one second (FEV1), the ratio of FEV1 to FVC, and maximal voluntary ventilation (MVV). Tests were conducted during the screening/baseline period, after the 7th administration (+4 days), during the 8-week follow-up period (-14 days), the 12-week follow-up period, the 20-week follow-up period, and the 28-week follow-up period.

h) The blood routine includes: White blood cell count (WBC), lymphocyte count (LY#), monocyte count (MO#), neutrophil count (NE#), eosinophil count (EO#), basophhil count (BA#), lymphocyte percentage (LY%), monocyte percentage (MO%), neutrophil percentage (NE%), eosinophils Percentage (EO%), basophil percentage (BA%), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count (PLT), reticulocyte (RET) (test results within 7 days prior to administration are acceptable at baseline). During the study, the investigator determined whether additional tests were needed based on the subjects' vital signs and symptoms, with safety concerns for the subjects.

i) Blood biochemical tests included: Bilirubin (Total bile, interbile and direct bile), total protein (total Protein, albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine (Cr), Urea, glucose (GLU), serum sodium (Na^+), serum potassium (K^+), serum calcium (Ca^{2+}), serum magnesium (Mg^{2+}) Serum chlorine (CL^-), serum phosphorus (Pi), lactic acid (LAC), lactate dehydrogenase (LDH), serum creatine kinase (CK), serum creatine kinase-MB isoenzyme (CK-MB) and C-reactive protein (CRP), brain natriuretic peptide (BNP, NT-pro BNP), cardiac troponin (Tn I). Calculate eGFR [CKD-EPI] (test results within 7 days prior to administration are acceptable at baseline). During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects.

j) Urine routine tests included: Urine bilirubin (UBG), urine bilirubin (BIL), urine ketone bodies (KET), urine occult blood (BLD), urine protein (PRO), urine nitrite (NIT), urine glucose (GLUU), urine specific gravity (SG), urine PH (pH), red blood cells (RBC), and white blood cells (WBC) (test results within 7 days before administration are acceptable at baseline. (). During the study, investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering their safety.

k) Complement C3 and C4: Complement C3 and C4 tests are conducted, and test results within 7 days before administration are acceptable during the baseline period. During the study period, the investigator determined whether additional tests were needed based on the subjects' vital signs and symptoms, considering the subjects' safety.

l) Pathogen tests include: hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B e antigen, hepatitis B e antibody, hepatitis B core antibody, hepatitis C virus antibody, HIV antibody (test results within 14 days prior to the screening period are acceptable).

m) Coagulation function includes prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT) (test results within 14 days prior to screening may be accepted at the time of screening).

n) Magnetic Resonance Imaging (MRI). Use only conscious sedation during cardiac magnetic resonance imaging. MRI scans of the lower extremities (including thighs and calves) and buttocks of the subjects were performed at the time points specified in the flowchart during the study period, and cardiac MRI examinations were added if the investigator determined if necessary. MRI results within 4 weeks before administration were received during the screening period/baseline period.

o) Blood samples for the PK trial will be collected before the 2nd, 4th, and 6th doses and at 0 hours +10 minutes after the completion of the administration. First time (Note:) PK blood sample collection before the first administration may be conducted during the screening period/baseline period if no more than 7 days from baseline to the first administration, Intensive blood collection (no additional collection required before the first administration) and before the eighth administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after administration, as discussed by the investigator and collaborators The time points for PK blood collection in the later stage can be adjusted based on the PK data and safety data obtained in the earlier stage. PK blood samples will also be collected during the 8-week, 12-week, and 16-week follow-up periods. If Dystrophin nucleic acid cannot be detected at the 8-week follow-up, it may not be necessary to collect blood samples for the test at subsequent follow-ups.

p) Muscle biopsies will be performed during the screening + baseline period and after the last administration (in the study, a quadriceps muscle puncture sample will be performed on the day of the last administration as much as possible, and a biceps brachii muscle tissue sample will be taken during surgery after the last administration. If the subjects are unable to complete the collection of muscle tissue from the above-mentioned sampling sites due to their own reasons, the investigators will determine the sampling points based on the muscle condition of the subjects.

q) Sampling site and injection site photography: Take pictures of the biopsy site before and after each muscle biopsy; Take pictures of the infusion site about 1 hour after each administration to observe the response of the infusion site.

r) Biomarker detection: Also known as cytokines, mainly detects TNF-α, INF-γ, IL-2, IL-6 and IL-10. Blood samples were collected during the screening period/baseline period/before the first administration (no additional collection is required before the first administration if no more than 7 days from baseline to the first administration), before the second, fourth, sixth, and eighth administrations, and during the 8-week, 12-week, and 16-week follow-up periods. If Biomarker levels return to normal or are undetectable at the 8-week follow-up, blood samples may not be collected for the test at subsequent follow-ups.

s) Immunogenicity: Anti-dystrophin antibody test. Blood samples were collected during the screening period/baseline period/before the first administration and before the 2nd, 4th, 6th, and 8th administrations (if no more than 7 days from baseline to the first administration, no additional collection is required before the first administration) and at each subsequent follow-up. If Anti-dystrophin antibody levels cannot be detected in the follow-up samples at week 8, blood samples may not be collected for this test at subsequent follow-ups.

t) NSAA: The North Star Ambulatory Assessment outpatient assessment scale was conducted during the screening/baseline period, after the 7th administration (+4 days), during the 8th week follow-up period (-14 days), during the 12th week follow-up period, during the 20th week follow-up period, and during the 28th week follow-up period to assess the differences in activity ability before and after administration in subjects. The NSAA examination included standing, walking, getting up from a chair, standing on one leg (right leg), standing on one leg (left leg), straddling the box (right leg), straddling the box (left leg), straddling the box (right leg), straddling the box (left leg), sitting up, getting up from the ground, raising the head, standing on the heel, jumping, jumping with the right leg, jumping with the left leg, and running (10m), with video recording.

u) 6-minute walk distance, four-step test: conducted during the screening period/baseline period, after the 7th dose (+4 days), at the 8th week follow-up period (-14 days), at the 12th week follow-up period, at the 20th week follow-up period, and at the 28th week follow-up period;

Tacrolimus blood concentration monitoring: Before administration of the investigational drug on D1, between D1 and W8, blood samples were collected for tacrolimus blood concentration once a week for the first two weeks and once every two weeks for the last six weeks, with the time of collection determined by the investigator. During the study period, the investigator may add tacrolimus blood concentration monitoring and/or unplanned tests based on the symptoms of the subjects or abnormal laboratory tests to ensure the safety of the subjects.

Group II

Visit	**Screening period**	**Baseline period**	**Treatment period (administered once every 4 days for a total of 32 doses)**									**Follow-up period**							**Early withdrawal**
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Visit Time[a]	D-30~D-8	D-7~D-1	dose 1	dose 2	dose 3	dose 4	dose 5	dose 6	dose 7	dose 8	dose9 ～ dose32	W2 2	W2 6	W3 0	W3 4	W3 8	W4 2	W46 ~W70	
Visit Window (day)				±2	±2	±2	±2	±2	±2	±2	±2	±7	±7	±7	±7	±7	±7	±7	±7
Informed consent	X																		
Demography	X																		
Past medical history and allergy history[b]	X																		
Vital signs[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Echocardiography[e]	X													X					X
12-lead ECG [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lung function test[g]	X								X		X	X	X	X	X	X	X	X	X
Chest X-ray examination	X																		
Blood routine[h]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood biochemistry[i]	X	X				X				X	X	X	X	X	X	X	X	X	X
Urine routine[j]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complement C3, C4, C5b-9[k]	X									X	X	X	X	X	X	X	X	X	X
Pathogen examination[l]	X																		
Coagulation test[m]	X										X							X	X

Visit	**Screening period**	**Baseline period**	**Treatment period (administered once every 4 days for a total of 32 doses)**									**Follow-up period**							**Early withdrawal**
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Visit Time[a]	D-30~D-8	D-7~D-1	dose 1	dose 2	dose 3	dose 4	dose 5	dose 6	dose 7	dose 8	dose9 ～ dose32	W2 2	W2 6	W3 0	W3 4	W3 8	W4 2	W46 ~W70	
Visit Window (day)				±2	±2	±2	±2	±2	±2	±2	±2	±7	±7	±7	±7	±7	±7	±7	±7
SPOT-03 administration			X	X	X	X	X	X	X	X	X								
Electromyography	X													X					X
DEXA(Dual-energy X-ray absorptiometry)detection	X									X	X	X	X	X	X	X	X	X	X
MRI (Lower extremities including thighs and calves, buttocks)[n]	X													X					X
PK[o]	X		X			X					X	X	X	X	X	X	X	X	X
Muscle biopsy[p]	X									X	X								X
Take pictures of the sampling site and the injection site[q]	X		X	X	X	X	X	X	X	X	X								
Biomarker detection[r]	X		X			X				X	X	X	X	X	X	X	X	X	X
Immunogenicity[s]	X		X	X		X		X		X	X	X	X	X	X	X	X	X	X
NSAA[t]	X								X		X	X	X		X		X	X	X
6-minute walking distance and four-step test[u]	X								X		X	X	X	X	X	X	X	X	X
Tacrolimus blood concentration monitoring[v]		X																	X
Tacrolimus administration		Starting from D-3, oral tacrolimus is administered daily (0.05-0.2mg/kg/d, with a recommended blood concentration of tacrolimus maintained at 5-																	X

Visit	**Screening period**	**Baseline period**	**Treatment period (administered once every 4 days for a total of 32 doses)**									**Follow-up period**							**Early withdrawal**
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Visit Time[a]	D-30~D-8	D-7~D-1	dose 1	dose 2	dose 3	dose 4	dose 5	dose 6	dose 7	dose 8	dose9 ～ dose32	W22	W26	W30	W34	W38	W42	W46 ~W70	
Visit Window (day)				±2	±2	±2	±2	±2	±2	±2	±2	±7	±7	±7	±7	±7	±7	±7	±7
		10ng/mL, which may be adjusted according to the actual clinical situation). Tacrolimus is administered approximately one month after the last dose administration of SPOT-03, and the duration of administration may be adjusted by the investigator if there are special circumstances																	
Adverse events		Continuous monitoring records were kept from the first administration to the last visit																	
Combination therapy	Continuous recording from signing the informed consent to the last follow-up																		

Notes:
Abbreviation: D= day; W= week; 12-lead-ECG= 12-lead electrocardiogram; ECHO= echocardiogram; PFT= Lung function test; EMG= Electromyography; MRI= Resonance imaging; AE= Adverse events.
The investigator may decide whether to conduct unplanned tests based on clinical indications and for safety reasons.

a) Study date: Baseline is the result of the last assessment before dose 1 administration. The day before dose 1 administration was D-1. In this dose group, dose1 was administered every 4 days for a total of 32 doses. dose1 is the first dose, and each dose is dose1, dose2, dose3... according to the number of doses administered. The last administration week is W18, and the follow-up period is calculated from W18, with W22 being the fourth week after the last dose is completed. W70 refers to the 52nd week after the last administration. From W46 to W70, follow-up visits were conducted every 4 weeks. Safety follow-up was conducted respectively at W46, W50, W54, W58, W62, W66, and W70.
b) The complete medical history of the subjects was obtained to determine eligibility. AE is recorded from the time of administration of the study drug, not from the time of signing the consent form. Any medical events that occurred during the screening period and baseline period prior to the first administration of the study drug will be recorded as medical history. Collect the current medical history (genetic test results and corresponding treatment information) of DMD, as well as other treatment histories within 6 months.
c) Vital signs include blood pressure, pulse, respiration, and body temperature. Vital signs will be checked within 1 hour before and after each administration during treatment. In addition, vital sign tests will be conducted 30 minutes (±10 minutes), 1 hour (±10 minutes), 2 hours (±30 minutes), and 4 hours (±30 minutes) after the first administration is completed. Note: Blood pressure of the subjects will be monitored daily during tacrolimus administration and recorded.
d) Physical examination included height, weight, skin and mucous membranes, lymph nodes, head and neck, chest, abdomen, spine and limbs and joints, musculoskeletal system, and nervous system. Check before each cycle of administration. Calculate individual doses based on baseline body weight.
e) Echocardiography will be performed during screening and at follow-up at week 30.

f) 12-lead-ECG: dose1 should be tested before administration and 2 hours (±1 hour) after administration completion, and before administration for other administration cycles. When ECG measurement results are abnormal (including QTcF interval abnormalities such as QTcF>500 ms or an increase of >60 ms relative to baseline) or other abnormalities are clinically significant, two additional ECG measurements are required, with an interval of at least approximately 2 minutes between repeated measurements.

g) Pulmonary function tests include forced vital capacity (FVC), forced expiratory volume in one second (FEV1), the ratio of FEV1 to FVC, and maximal voluntary ventilation (MVV).. Tests were conducted during the screening/baseline period, dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d), and during the follow-up period at week 22 (-14 days), week 26, week 34, week 42, week 46, week 50, week 54, week 58, week 62, week 66 and week 70.

h) The blood routine includes: White blood cell count (WBC), lymphocyte count (LY#), monocyte count (MO#), neutrophil count (NE#), eosinophil count (EO#), basophhil count (BA#), lymphocyte percentage (LY%), monocyte percentage (MO%), neutrophil percentage (NE%), eosinophils Percentage (EO%), basophil percentage (BA%), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelet count (PLT), reticulocyte (RET) (test results within 7 days before dose1 administration are acceptable at baseline). During the study, the investigator determined whether additional tests were needed based on the subjects' vital signs and symptoms, with safety concerns for the subjects. In order to maintain consistency in the test results, it is recommended that fingertip blood tests be used for routine blood tests.

i) Blood biochemical tests include: Bilirubin (Total bile, interbile and direct bile), total protein (total Protein, albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine (Cr), Urea, glucose (GLU), serum sodium (Na^+), serum potassium (K^+), serum calcium (Ca^{2+}), serum magnesium (Mg^{2+}) Serum chlorine (CL^-), serum phosphorus (Pi), lactic acid (LAC), lactate dehydrogenase (LDH), serum creatine kinase (CK), serum creatine kinase-MB isoenzyme (CK-MB) and C-reactive protein (CRP), brain natriuretic peptide (BNP, NT-pro BNP), cardiac troponin (Tn I). Calculate eGFR [CKD-EPI] (test results within 7 days before dose1 administration are acceptable during the baseline period). During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects.

j) Urine routine tests included: Urine bilirubin (UBG), urine bilirubin (BIL), urine ketone bodies (KET), urine occult blood (BLD), urine protein (PRO), urine nitrite (NIT), urine glucose (GLUU), urine specific gravity (SG), urine PH (pH), red blood cells (RBC), and white blood cells (WBC) (baseline period is acceptable within 7 days before dose1 administration Results of the test). During the study, investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering their safety.

k) Complement C3, C4, C5b-9: Complement C3, C4, and C5b-9 tests should be conducted. During the baseline period, test results within 7 days before dose1 administration can be accepted. During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects.

l) Pathogen tests include: hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B e antigen, hepatitis B e antibody, hepatitis B core antibody, hepatitis C virus antibody, HIV antibody (test results within 14 days prior to the screening period are acceptable).

m) Coagulation function includes prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (APTT) (test results within 14 days prior to screening may be accepted at the time of screening). Coagulation function was tested during the screening period / at the baseline / before the first administration (the results within 14 days before the screening period could be accepted), after dose 32 administration, on day W70, and during the early withdrawal from follow-up.

n) Magnetic Resonance Imaging (MRI). Use only conscious sedation during cardiac magnetic resonance imaging. MRI scans of the lower extremities (including thighs and calves) and buttocks of the subjects were performed at the time points specified in the flowchart during the study period, and cardiac MRI examinations were added if the investigator determined if necessary. MRI results within 4 weeks before dose1 administration during the screening period/baseline period.

o) Blood samples for the PK trial will be collected intensively before dose1 administration, within 30 minutes before dose32 administration, and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after dose1 and dose32 administration. PK blood samples will be collected within 30 minutes before dose4, dose8, dose12, dose16, dose20, dose24, and dose28 administration and at 0h+10min and 24h±1h after administration. If the subject withdraws early for any reason during the administration period, the subject will have an intensive blood collection within 30 minutes before the last administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after the last administration. After discussion by the investigator and collaborators, the time points for PK blood collection in the later stage may be adjusted based on the previously obtained PK data and safety data. PK blood samples will also be collected at the 22nd, 26th, 30th, 34th, 38th, 42nd, 46th, 50th, 54th, 58th, 62nd, 66th and 70th follow-up periods. If Dystrophin nucleic acid cannot be detected at week 22 or at any subsequent follow-up, it may not be necessary to collect blood samples for this test at subsequent follow-ups.

p) A quadriceps muscle puncture sample will be taken during the screening + baseline period, during administration, and a muscle biopsy will be performed after the last administration (in the study, a quadriceps muscle puncture sample will be taken on the day of the last administration, and a biceps brachii muscle tissue sample will be taken during surgery after the last administration as much as possible.) If the subjects are unable to complete the collection of muscle tissue from the above-mentioned sampling sites due to their own reasons, the investigators will determine the sampling points based on the muscle condition of the subjects).

q) Sampling site and injection site photography: Take pictures of the biopsy site before and after each muscle biopsy; Take pictures of the infusion site about 1 hour after each administration to observe the response of the infusion site.

r) Biomarker detection: That is, cytokines, mainly detecting TNF-α, INF-γ, IL-2, IL-6 and IL-10. During the screening period/baseline period/before the first administration (no additional collection is required before dose1 if no more than 7 days from baseline), before dose4, dose8, dose12, dose16, dose20, dose24, dose28, dose32 administration Blood samples were collected during the follow-up periods of week 22, week 26, week 30, week 34, week 38, week 42, week 46, week 50, week 54, week 58, week 62, week 66 and week70. If Biomarker levels return to normal or are undetectable at week 22 or at any subsequent follow-up, blood samples may not be collected for the test at subsequent follow-ups.

s) Immunogenicity: Anti-dystrophin antibody test. During the screening period/baseline period/before the first administration (no additional collection is required before dose1 administration if no more than 7 days from baseline to dose1), before dose2, dose4, dose6, dose8, dose12, dose16, dose20, dose24, dose28, dose32 administration Blood samples were collected during the week 22, week 26, week 30, week 34, week 38, week 42, week 46, week 50, week 54, week 58, week 62, week 66 and week70. If Anti-dystrophin antibody levels cannot be detected at week 22 or in subsequent follow-up samples, blood samples may not be collected for this test at subsequent follow-ups.

t) NSAA: North Star Ambulatory Assessment Outpatient Assessment Scale It was conducted during the screening/baseline period, dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d), the 22-week follow-up period (-14d), the 26-week follow-up period, the 34th follow-up period, and the 42nd follow-up period to assess the differences in activity ability before and after administration. NSAA tests included standing, walking, getting up from a chair, standing on one leg (right leg), standing on one leg (left leg), straddling the box (right leg), straddling the box (left leg), straddling the box (right leg), straddling the box (left leg), sitting up, getting up from the ground, raising the head, standing on the heel, jumping, right leg jumping, left leg jumping, running (10m), and video recording.

u) 6-minute walk distance, four-step test: conducted during the screening period/baseline period, dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d), week 20(+4d), week 22 (+4d), week 26, week 30, week 34, week 38, week 42, week 46, week 50, week 54, week 58, week 62, week 66 and week70;

v) Tacrolimus blood concentration monitoring: Before dose1, dose2, dose4, dose8, dose16, dose24, dose32 administration, tacrolimus blood concentration was measured within 1 month after dose32 administration, the time of collection was determined by the investigator. During the study period, the investigator may add tacrolimus blood concentration monitoring and/or unplanned tests based on the symptoms of the subjects or abnormal laboratory tests to ensure the safety of the subjects;

w) Early withdrawal: When a subject exits, regardless of whether the 32-dose administration is completed or not, the following safety tests should be conducted on the subject: vital signs, physical examination, 12-lead ECG, blood routine, blood biochemistry, urine routine, complement C3, C4, C5b-9, coagulation function and other laboratory tests, cytokine and immunogenicity blood collection; 1) When a subject withdraws early and has not completed the 32-dose administration, in addition to completing the safety check, the tacrolimus blood concentration of the subject should be tested. The investigator should instruct the subject to continue taking tacrolimus for about one month before discontinuing the drug and recommend that the subject return to the hospital or go to another hospital for tacrolimus blood concentration test as the subject wishes. At the same time, with respect for the wishes of the subjects' guardians, PK blood samples of the subjects should be collected as much as possible for muscle biopsy, pulmonary function test, NSAA, 6-minute walk distance, and four-step test results; 2) If a subject withdraws during the follow-up period, the investigator should communicate with the subject's guardian as much as possible and recommend that the subject return to the hospital to complete the safety check for early withdrawal; While respecting the wishes of the subjects' guardians, collect the results of the subjects' pulmonary function test, NSAA, 6-minute walk distance, and four-step test as much as possible; If the guardian of the subject does not wish to return to the hospital for early withdrawal safety follow-up, telephone follow-up should be conducted for the subject as much as possible, and recent abnormal symptoms, concomitant medications, adverse events, etc. should be recorded. If the above laboratory test results and efficacy indicator results are not collected, it should not be regarded as PD.

6. Study Population

6.1 Inclusion criteria

Only those who meet all the following criteria can be enrolled:

1) According to the requirements of the region/country and/or IRB/IEC, the patient and/or legal guardian have signed a written informed consent form and are aware of all relevant study content.
2) Boys aged ≥ 2 years and < 8 years and capable of walking independently for at least 10 meters.
3) Confirmed diagnosis of DMD through multiplex ligation-dependent probe amplification (MLPA) and whole-exome sequencing.
4) Tolerance for muscle biopsy under anesthesia with no absolute contraindications to the procedure
5) Heart, liver, lung, and kidney functions are sufficient:
    a) The left ventricular ejection fraction (LVEF) should be ≥ 50%;
    b) Forced vital capacity (FVC) ＞ 50% of the expected value, and do not require nighttime ventilation;
    c) Patient's glomerular filtration rate (GFR)>30 mL/min/1.73 m^2

6.2 Exclusion Criteria

Those who meet any one of the following criteria must be excluded:

1) Complications other than DMD that may cause muscle weakness and/or motor dysfunction.
2) There are severe intellectual disabilities (such as severe autism, severe cognitive impairment, and severe behavioral disorders) that, according to the investigator's judgment, can affect the study.
3) Hospitalization for respiratory failure within 8 weeks prior to screening.
4) Asthma or underlying lung diseases that are poorly controlled, such as bronchitis, bronchiectasis, emphysema, or recurrent infectious pneumonia that investigator believes may affect respiratory function.
5) Severe uncontrolled heart failure (NYHA III-IV), including any of the following

conditions：

a) Intravenous administration of diuretics or positive inotropic drugs is required within 8 weeks prior to screening.

b) Hospitalization due to worsening heart failure or arrhythmia within 8 weeks prior to screening.

6) Abnormal laboratory values considered clinically significant:

a) GGT > 3 × upper limit of normal

b) Bilirubin ≥ 3.0 mg/dL

c) Creatinine ≥ 1.8 mg/dL

d) Hemoglobin < 8 or > 18 g/dL

e) White blood cell count > 18,500/μL

7) There are arrhythmias that require antiarrhythmic treatment.

8) Subjects who are undergoing immunosuppressive therapy.

9) Has used other gene therapy, investigational drugs, or any treatment aimed at increasing dystrophin expression.

10) Subjects with a history of major surgeries within 12 weeks prior to the initial infusion or planning to undergo major surgeries (such as scoliosis surgery) during this study.

11) Subjects who are allergic to investigational products or local anesthetic drugs or have a history of severe allergies or genetic allergic reactions.

12) Within 6 months prior to the initial infusion, the subjects are exposed to another investigational drug or are participating in an intervention clinical trial.

13) Subjects with positive of hepatitis B core antibody or hepatitis C antibody or HIV antibody during screening.

14) Investigator believes that the presence of any other serious diseases, medical conditions, or chronic drug treatment needs can pose unnecessary risks to gene transfer.

7. Subject Coding and Grouping

Group I: 4.0E+11 copies/kg, administered 8 times

Group II: 4.0E+11 copies/kg, administered 32 times

This study does not have a placebo group, is open-label, and is not blinded. After administration to all subjects in the previous dose group is completed, the next dose group could be administered after the investigators and sponsor discussed and determined that there were no serious adverse reactions related to the drug.

In Group II, if under abnormal circumstances during the administration period, the subjects unable to receive the drug at the visiting points specified in the flowchart, within one week after the latest dose, the investigators will determine whether the subject could continue to receive the drug based on the recovery status. If a subject is not suitable for continued administration due to safety concerns, the subject will withdraw from the study. New subjects enrollment will be discontinued when 3 subjects have completed the entire treatment and full study data have been collected. Because the study was the first clinical study of SPOT-03 injection in humans, with a small number of subjects enrolled and no blinding, the subjects were coded, grouped and dosed only based on the time they participated in screening and enrollment. Once the subjects signed the informed consent form, they were assigned a unique screening number 01,02,03 ----, and so on. After enrollment, subjects were assigned unique enrollment numbers AZ01, AZ02, AZ03----, and so on.

8. Study Procedures

8.1. Treatment and Follow-up

Those subjects who are eligibility will receive an enrollment number and participate in subsequent clinical items. The detailed items that subjects need to participate in at each visit are as follows.

8.1.1. Screening (D-30 ~ -8 Group I and Group II)

1) Sign informed consent form: explain the study process to the subjects or legal guardian and obtain a written informed consent form from the subjects.
2) Assign screening numbers in the order of obtaining written informed consent.
3) Demographics: gender, age, race, height, weight.
4) Disease history：medical history and surgical history within the 6 months prior to screening.
5) Medical history：drug allergy history and medication history within the 6 months prior to screening.
6) Physical examination: height, weight, skin and mucous membranes, lymph nodes,

head and neck, chest, abdomen, spine, limbs and joints, musculoskeletal system, and nervous system.

7) Vital signs: blood pressure, pulse, respiration, and body temperature.
8) ECHO and 12-lead ECG：the ECHO and ECG records will be checked by an investigator to determine if the patient meets the inclusion and exclusion criteria.
9) Pulmonary function tests: including forced vital capacity (FVC), forced expiratory volume in one second (FEV1), ratio of FEV1 to FVC (FEV1/FVC), maximal voluntary ventilation (MVV).
10) Chest X-Ray.
11) Lab tests: the items include Haematology, Biochemistry, Urinalysis, Coagulation, and Hepatitis B & C, HIV examination.
12) Complement C3, C4, C5b-9 (only Group II) tests.
13) Electromyography: One electromyography test will be conducted during the screening period or baseline period, and electromyography results within one month prior to administration will be accepted during screening.
14) MRI (Lower extremities including thighs and calves, buttocks): MRI is performed on the lower extremities and buttocks of the subjects to observe muscle and nerve damage, and cardiac MRI is added if necessary, as determined by the investigators;
15) DEXA: Measure the mass of adipose tissue and lean tissue; (Only Group II)
16) Muscle biopsy sampling: A total of one muscle biopsy will be performed within the screening or baseline time range, with a biopsy sample taken from the biceps brachii and photographs taken of the sampling site before and after the sampling.
17) PK blood sample, Biomarker blood sample collection.
18) Immunogenic blood sample collection.
19) NSAA assessment scale for assessing motor activity ability of subjects The examination included standing, walking, getting up from a chair, standing on one leg (right leg), standing on one leg (left leg), straddling the box (right leg), straddling the box (left leg), straddling the box (right leg), straddling the box (left leg), sitting up, getting up from the ground, looking up, standing on the heel, jumping, right leg jumping, left leg jumping, running (10m), and video recording.
20) 6-minute walk distance, four-step step test.
21) Collect concomitant medication information.

Determine the eligibility of subjects based on the test results during the screening period.

Eligible subjects will participate in the baseline examinations listed below.

8.1.2. Baseline (D-7 ~ -1 Group I and Group II)

1) Vital signs and physical examination
2) 12-lead ECG
3) PFT
4) Lab tests: Haematology, Biochemistry and Urinalysis (if the screening period test is conducted within 7 days before administration, this test may not be performed here)
5) Complement C3, C4, C5b-9 (only for dose II group) tests: If not conducted during the screening period, one should be conducted at baseline
6) Tacrolimus blood concentration tests should be taken before administration
7) Electromyography: If an electromyography is not performed during the screening period, an electromyography should be performed at baseline
8) MRI: If an MRI test is not performed during the screening period, an MRI test should be conducted at baseline. Receive MRI results within 4 weeks prior to administration during the baseline period.
9) DEXA: If not performed during the screening period, adipose tissue mass and lean tissue mass need to be tested during the baseline period. Receive DEXA results within 2 weeks prior to administration during the baseline period (only dose II group).
10) Muscle biopsy sampling: If muscle biopsy sampling is not performed during the screening period, perform it at baseline, in biceps brachii, and take pictures of the sampling site before and after sampling.
11) PK blood samples, Biomarker blood samples should be collected at baseline if not collected during the screening period.
12) Starting from D-3, oral tacrolimus (0.05-0.2mg/kg/d, adjustable according to actual clinical conditions) is taken daily.
13) Collect the blood sample required for immunogenicity testing, and if not collected during the screening period, collect it during the baseline period.
14) Collect information on the combination of medications.
15) Because of the use of tacrolimus, pay attention to abnormal conditions and record them as medical history
16) The NSAA assessment scale, used to assess the motor activity of the subjects.

The examination included standing, walking, getting up from a chair, standing on one leg (right leg), standing on one leg (left leg), straddling the box (right leg), straddling the box (left leg), straddling the box (right leg), straddling the box (left leg), sitting up, getting up from the ground, looking up, standing on the heel, jumping, right leg jumping, left leg jumping, running (10m), and video recording; If not conducted during the screening period, it should be done once during the baseline period.

17) 6-minute walk distance, four-step test: If not conducted during the screening period, it should be conducted once during the baseline period.

8.1.3. Treatment (D1 ~ 29)

Group I: D1 to D29

The subjects participating in the baseline will receive treatments of SPOT-03 via IV on D1.

Before the initial administration on D1

1) Vital signs and physical examination.
2) 12-lead ECG
3) If PK and Biomarker blood samples were not collected at baseline, blood samples required for PK and Biomarker testing should be collected before administration; If immunogenic blood samples were not collected during the baseline period, they should also be collected at this time;
4) For tacrolimus blood concentration monitoring before administration, if not collected during the baseline period, it should be collected before the first administration on D1;
5) The investigator determined whether to conduct pre-treatment for allergic reactions based on the subjects' conditions;
6) Oral tacrolimus daily (0.05-0.2mg/kg/d, adjustable according to actual clinical conditions);
7) Collect information on concomitant medications;
8) Collect information on adverse reactions.

Calculate the dosage of SPOT-03 based on the concentration of SPOT-03, subject weight, and dose cohort participating in the study.

After the initial administration on D1

1) Take photos of the infusion site and observe the infusion response within 4 hours.
2) Vital signs: testing will be conducted 30 min (± 10 min), 1 h (± 10 min), 2 h (± 30 min), and 4 h (± 30 min) after the initial infusion.
3) The blood samples required for PK testing will be collected densely at timepoints of 0h +10min, 1h ± 10min, 2h ± 20min, 8h ± 1h, 24h ± 1h, 48h ± 2h, 72h ± 3h after the initial infusion, respectively. Later PK blood collection was adjusted by the investigator and collaborators based on the previously obtained PK data and safety data.
4) 12-lead ECG needs to be tested 2 hours (± 1 hour) after the end of infusion.
5) Collect adverse events information.

Other treatment cycles

1) Vital signs: vital signs should be tested within 1 hour before and after each administration.
2) Physical examination should be performed prior to each administration.
3) 12-lead ECG should be performed prior to each administration.
4) Pulmonary function test: after the 7th administration (+4 days);
5) Laboratory tests: including blood routine, blood biochemistry and urine routine tests; Blood routine and urine routine tests should be conducted before the 2nd, 3rd, 4th, 5th, 6th, 7th administration and after the 8th administration; Blood biochemical tests are conducted before the 3rd, 5th, 7th administration and after the 8th administration; During the study period, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects;
6) Complement C3, C4: To be examined before the fifth administration and after the eighth administration; During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects;
7) Take pictures of the infusion site; Closely observe the subjects for any infusion response within 4 hours after each administration;
8) The investigator determined whether to conduct pre-treatment for allergic reactions before administration based on the subjects' conditions;
9) Oral administration of tacrolimus daily (0.05-0.2mg/kg/d, adjustable according to actual clinical conditions) for 4 weeks;

10) A quadriceps muscle puncture sample is performed on the day of the last administration, and a biceps brachii muscle tissue sample is taken after surgery. If the subject is unable to complete the collection of muscle tissue from the above-mentioned sampling site due to their own reasons, the investigator will determine the sampling point based on the subject 'muscle condition and take pictures of the sampling site before and after the sampling;
11) PK Blood sample collection: Blood samples were collected before the 2nd, 4th, and 6th administration and at 0h+10min after administration. Intensive blood collection was conducted before the 8th administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, and 72h±3h after administration. The PK blood collection for later administration was adjusted by the investigator and collaborators based on the previously obtained PK data and safety data.
12) Biomarker detection: Blood samples are collected before the 2nd, 4th, 6th, and 8th administrations.
13) Immunogenicity: Blood samples were collected before the 2nd, 4th, 6th, and 8th dosing;
14) During the administration of the investigational drug from D1 to D29, blood samples were collected for tacrolimus concentration once a week for the first two weeks and once for the last two weeks, with the time of collection determined by the investigator;
15) NSAA assessment: After the 7th administration (+4 days);
16) 6-minute walk test, four-step test assessment: After the 7th administration (+4 days);
17) Collect concomitant medication information;
18) Collect information on adverse reactions.

Treatment period

Group II: Administered once every 4 days for a total of 32 doses.

Subjects participating in the baseline examination will receive intravenous infusion of SPOT-03 injection in dose1.

Before the first dose administration

1) Vital signs (within 1 hour before administration) and physical examination;
2) 12-lead ECG examination;

3) If Biomarker and PK blood samples were not collected at baseline, blood samples required for Biomarker and PK testing should be collected before administration; If immunogenic blood samples were not collected during the baseline period, they should also be collected at this time;
4) For tacrolimus blood concentration monitoring before administration, if not collected during the baseline period, it should be collected before the first administration on D1;
5) The investigator determined whether to conduct pre-treatment for allergic reactions based on the subjects' conditions;
6) Take tacrolimus orally daily (0.05-0.2mg/kg/d, which can be adjusted according to the actual clinical situation);
7) Collect information on concomitant medications.

Administration: Calculate the SPOT-03 dosage based on SPOT-03 concentration, subject body weight, and dose groups participating in the study.

After the first dose administration is completed

1) Take pictures of the infusion site and observe the infusion response within 4 hours;
2) Vital sign detection: 30 minutes (±10 minutes), 1 hour (±10 minutes), 2 hours (±30 minutes), and 4 hours (±30 minutes) after the first administration is completed;
3) Blood samples required for PK testing were collected intensively at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, and 72h±3h after the first administration. Later PK blood collection was adjusted by the investigator and collaborators based on the previously obtained PK data and safety data.
4) 12-lead ECG should be tested 2 hours (±1 hour) after administration;
5) Collect concomitant medication information;
6) Collect information on adverse reactions.

Administer other doses

1) Vital signs: During treatment, test for vital signs within 1 hour before and 1 hour after each administration;
2) Physical examination: Check before each administration;
3) 12-lead ECG test: Check before each administration;

4) Pulmonary function tests: dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d);
5) Laboratory tests: including blood routine, blood biochemistry and urine routine tests; Blood routine and urine routine: dose8, dose16, dose24, dose32 after administration, others before administration per dose; Blood biochemistry: dose4, dose12, dose20, dose28 before administration, dose8, dose16, dose24, dose32 after administration; During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects;
6) Complement C3, C4, C5b-9: Examined after dose8, dose16, dose24, dose32 administration; During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects;
7) Take pictures of the infusion site; Closely observe the subjects for any infusion response within 4 hours after each administration;
8) The investigator determined whether to conduct anti-allergic reaction pretreatment before administration based on the subjects' conditions.
9) Oral administration of tacrolimus daily (0.05-0.2mg/kg/d, adjustable according to actual clinical conditions) for about 1 month;
10) A quadriceps muscle puncture sample will be taken during administration, and the exact time will be determined by discussion between the partner and the investigator; A quadriceps muscle puncture sample will be performed on the same day after the last administration, and a biceps brachii muscle tissue sample will be taken during surgery after the last administration. If the subject is unable to complete the collection of muscle tissue from the above-mentioned sampling site due to their own reasons, the investigator will determine the sampling point based on the subject 'muscle condition and take pictures of the sampling site before and after the sampling;
11) PK Blood sample collection: Intensive blood samples were collected within 30 minutes before dose32 administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after administration. PK blood collection was conducted within 30 minutes before dose4, dose8, dose12, dose16, dose20, dose24, and dose28 administration, and at 0h+10min and 24h±1h after administration. If the subject withdraws early for any reason during the administration period, the subject will undergo intensive blood collection within

30 minutes before the last administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after the last administration; After discussion by the investigator and collaborators, the PK blood collection for later administration was adjusted by the investigator and collaborators based on the PK data and safety data obtained earlier.

12) Biomarker testing: dose4, dose8, dose12, dose16, dose20, dose24, dose28, dose32 blood samples collected before administration;
13) Immunogenicity: Blood sample collection before dose2, dose4, dose6, dose8, dose12, dose16, dose20, dose24, dose28, dose32;
14) Tacrolimus concentrations were collected before dose2 administration, dose4 administration, dose8 administration, dose16 administration, dose24 administration, dose32 administration, and the time of collection was determined by the investigator;
15) NSAA assessment: dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d);
16) 6-minute walk test, four-step test assessment: dose7 (+4d), dose15 (+4d), dose23 (+4d), dose31 (+4d);
17) DEXA tests: dose8 (+7d), dose16 (+7d), dose24 (+7d), dose32 (+7d);
18) Collect combined medication information;
19) Collect information on adverse reactions.

8.1.4. Follow-up

Group I:

The subjects will be followed up for various tests and sample collection at weeks 8, 12, 16, 20, 24 and 28 after the initial administration, as follows:

1) Vital signs, physical examination and 12-lead ECG examination were performed at each follow-up;
2) Take tacrolimus orally daily (0.05-0.2mg/kg/d, which can be adjusted according to the actual clinical situation) until approximately the 8th week.
3) Echocardiography: Echocardiography should be followed up at week 16 after the first administration;
4) Pulmonary function tests: Perform pulmonary function tests at the 8-week follow-up period (-14 days), the 12-week follow-up period, the 20-week follow-up period, and the 28-week follow-up period after the first administration;
5) Laboratory tests: Laboratory tests at 8 weeks of follow-up, 12 weeks of follow-

up, 16 weeks of follow-up, 20 weeks of follow-up, 24 weeks of follow-up, 28 weeks of follow-up, including blood routine, blood biochemistry, urine routine; During the study period, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects. Coagulation tests were conducted during the 28-week follow-up period.

6) Complement C3, C4: Laboratory tests at Week 8, Week 12, week 16, Week 20, Week 24, Week 28; During the study period, the investigator determined whether additional tests were needed based on the subjects' vital signs and symptoms, with safety concerns for the subjects.
7) PK Blood sample collection: Blood samples were collected during the 8-week follow-up, 12-week follow-up, and 16-week follow-up. If Dystrophin nucleic acid cannot be detected at the 8-week follow-up, it may not be necessary to collect blood samples for this test at subsequent follow-ups;
8) Biomarker test: Blood samples are collected during the 8-week follow-up period, the 12-week follow-up period, and the 16-week follow-up period. If Biomarker levels return to normal or are undetectable at the 8-week follow-up, blood samples may not be collected for the test at subsequent follow-ups;
9) Immunogenicity test: Blood samples are collected during the 8-week follow-up period, 12-week follow-up period, 16-week follow-up period, 20-week follow-up period, 24-week follow-up period, and 28-week follow-up period. If Anti-dystrophin antibodies cannot be detected in the 8-week blood sample, no further blood sample collection for this test is required during subsequent follow-ups.
10) Blood samples were collected every two weeks for monitoring of tacrolimus concentration from the last administration to the completion of the last administration of tacrolimus at week 8;
11) Electromyography and MRI (both lower extremities and buttocks) tests were performed at the 16-week follow-up, and cardiac MRI was added if the investigator determined it to be necessary;
12) NSAA assessment: Conducted during the 8-week follow-up period (-14 days), the 12-week follow-up period, the 20-week follow-up period, and the 28-week follow-up period, with video recording;
13) 6-minute walk test, four-step test assessment: assessed at 8-week follow-up period (-14d), 12-week follow-up period, 20-week follow-up period, and 28-

week follow-up period;

14) Collect concomitant medication information;

15) Collect information on adverse reactions.

The study ended after the subjects completed the 28-week follow-up. The study will be ended after completing follow-up at W28.

Follow-up

Group II：

The subjects were followed up at 4, 8, 12, 16, 20 and 24 weeks after the completion of the last administration (dose32) for various tests and sample collection as follows:

1) Vital signs, physical examination and 12-lead ECG examination were performed at each follow-up;

2) Oral tacrolimus daily (0.05-0.2mg/kg/d, adjustable according to actual clinical conditions) until approximately 1 month after the completion of the last administration;

3) Echocardiography: Echocardiography should be followed up at week 12 after the last administration;

4) Pulmonary function tests: Pulmonary function tests are conducted during the 4-week follow-up period (-14 days), the 8-week follow-up period, the 16-week follow-up period, and the 24-week follow-up period after the last administration.

5) Laboratory tests: Laboratory tests, including blood routine, blood biochemistry, and urine routine, are conducted at the 4-week, 8-week, 12-week, 16-week, 20-week, and 24-week follow-up periods after the last administration. During the study period, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects. Coagulation tests were conducted during the 24-week follow-up period after the last administration.

6) Complement C3, C4, C5b-9: Examinations should be conducted at the follow-up periods of the 4th week, 8th week, 12th week, 16th week, 20th week, and 24th week after the last administration. During the study, the investigator determined whether additional tests were needed based on the vital signs and symptoms of the subjects, considering the safety of the subjects.

7) PK Blood sample collection: Blood samples were collected at the 4-week, 8-week,

12-week, 16-week, 20-week, and 24-week follow-up periods after the last administration. If Dystrophin nucleic acid cannot be detected at the 4-week follow-up after the last administration, it may not be necessary to collect blood samples for this test at subsequent follow-ups;

8) Biomarker test: Blood samples were collected during the 4-week follow-up, 8-week follow-up, 12-week follow-up, 16-week follow-up, 20-week follow-up, and 24-week follow-up after the last administration. If Biomarker levels return to normal or are undetectable at the 4-week follow-up after the last administration, blood samples may not be collected for the test at subsequent follow-ups;

9) Immunogenicity test: Blood samples were collected at the 4-week, 8-week, 12-week, 16-week, 20-week, and 24-week follow-up periods after the last administration. If Anti-dystrophin antibodies were not detected in the blood sample at the 4-week follow-up period after the last administration, during subsequent follow-ups, No further blood sample collection for the test is required;

10) Blood samples are collected every two weeks from the completion of the last administration until approximately one month after the last administration to monitor tacrolimus concentration in the blood;

11) Electromyography and MRI (lower extremities and buttocks) tests were performed at the 12-week follow-up after the last administration, and cardiac MRI was added if the investigator determined it to be necessary;

12) NSAA assessment: The assessment was conducted during the 4-week follow-up period (-14 days), the 8-week follow-up period, the 16-week follow-up period, and the 24-week follow-up period after the last administration, with video recording;

13) 6-minute walk test, four-step test assessment: Assessed at the 4-week follow-up period (-14d), 8-week follow-up period, 16-week follow-up period, and 24-week follow-up period after the last administration;

14) DEXA tests: Evaluated at 4 weeks, 8 weeks, 12 weeks, 16 weeks, 20 weeks, and 24 weeks after the last administration, with a time window of ±7 days;

15) Collect concomitant medication information;

16) Collect information on adverse reactions.

The study ended after the subjects completed the 24-week follow-up following the end of the last administration.

Note: If no additional time window is added during the follow-up period, visits should be conducted according to the time window specified in the flow chart.

8.1.5 Early withdrawal (only for Group II)

Regardless of whether the subjects have completed the 32-dose administration or not, the following safety checks must be conducted on the subjects when they withdraw early:

1) Vital signs, physical examination and 12-lead ECG examination.

2) Laboratory tests: Blood routine, blood biochemistry, urine routine, coagulation function.

3) Complement C3, C4.C5b-9.

4) Biomarker detection.

5) Immunogenicity test.

6) Collect concomitant medication information;

7) Collect information on adverse reactions.

When a subject withdraws before completing the 32-dose administration, in addition to completing the safety check, the tacrolimus blood concentration of the subject should also be tested. The investigator should instruct the subject to continue taking tacrolimus for about one month before discontinuing the drug, and suggest that the subject return to the hospital or go to another hospital for tacrolimus blood concentration test as the subject wishes. At the same time, with respect for the wishes of the subjects' guardians, PK blood samples of the subjects should be collected as much as possible for muscle biopsy, pulmonary function test, NSAA, 6-minute walk distance, and four-step test results.

When a subject withdraws during the follow-up period, the investigator should communicate with the subject's guardian as much as possible and recommend that the subject return to the hospital to complete the early withdrawal safety check; While respecting the wishes of the subjects' guardians, collect the results of the subjects'

pulmonary function test, NSAA, 6-minute walk distance, and four-step test as much as possible; If the guardian of the subject does not wish to return to the hospital for early withdrawal safety follow-up, telephone follow-up should be conducted for the subject as much as possible, and recent abnormal symptoms, concomitant medications, adverse events, etc. should be recorded.

8.2. Supply of Investigational drugs/treatments

8.2.1. Name, Source and Strength

【Generic Name】Dystrophin Nucleic Acid Injection

【Product code】SPOT-03

【Strength】8.0E+11 copies/mL

【Expiry Date】3 months

【Active Pharmaceutical Ingredients】Dystrophin nucleic acid loaded EV

【Pharmaceutical excipients】CryoStorCS10 frozen storage solution (containing 10% dimethyl sulfoxide (DMSO)), phosphate-buffered saline (PBS), human serum albumin (HSA)

【Storage】-60℃~ -90℃

8.2.2. Preparation and Storage

SPOT-03 should be transported on dry ice and stored at -60 ° C to -90 ° C for an expected period of 3 months. The SPOT-03 product is frozen in 3mL vials and thawed at room temperature before use. The thawed product is a colorless, odorless, clear solution. Thawed SPOT-03 should be used as soon as possible unless otherwise stored at 4 ° C for no more than 4 hours. After thawing, SPOT-03 should be diluted with normal saline to different concentrations for clinical use. Use immediately after activation. The opened and diluted solution should not be left at room temperature for more than 4 hours.

8.2.3. Route of Administration and Dosage Adjustment

Route of Administration

Group I: Intravenous drip, twice a week (once every 4 days), for a total of 8 doses.

Group II: Intravenous drip, twice a week (once every 4 days), for a total of no more than 32 doses.

Each bottle of SPOT-03 should be thawed at room temperature before use, and the thawing

process should be completed within approximately 60 minutes. The thawed SPOT-03 should be used as soon as possible unless there are special circumstances.

Dosage Adjustment

Calculate the required dose based on the body weight of the subjects measured at baseline and the dose group they were scheduled to attend. Select the appropriate site of intravenous infusion based on the patient's individual and venous condition. The infusion time is approximately 60 to 120 minutes. If the patient experiences mild allergic reactions such as dizziness, chills, rash, or extravasation during the infusion, the administration should be immediately suspended. After recovery, the investigator will determine whether to continue the administration and may appropriately reduce the infusion rate (as determined by the investigator). The infusion time may be extended accordingly (as determined by the investigator). After administration is completed, record the actual dosage and the start and end times of administration.

Photograph

Take photos for the infusion site before and after each infusion, and carefully observe the infusion reaction.

8.3. Concomitant medication

The newly generated dystrophin will be recognized by the immune system of the subjects as an exogenous protein, triggering an immune response. Any immune response to Dystrophin can affect its engraftment and potentially induce further muscle damage, reversing any benefits that patients should have received. Therefore, the subjects should begin to orally take 0.05-0.2 mg/kg tacrolimus daily for 4 weeks at D-3 before SPOT-03 administration until about one month after the last administration was completed.

Tacrolimus is a potent immunosuppressant and may cause the following adverse reactions in clinical practice, which should be handled by an experienced clinician.

1) Cardiovascular system symptoms: including hypertension, angina, palpitations, heart failure, etc.
2) Sensory system symptoms: Some patients have tremors, headaches, insomnia, depression, hallucinations, forgetfulness, etc.

3) Digestive system symptoms: including constipation, diarrhea, nausea, vomiting, weight changes, difficulty swallowing, stomach pain, etc.
4) Respiratory symptoms: Some patients have asthma, respiratory failure, lung function damage, pulmonary fibrosis, etc.
5) Skin symptoms: may cause itching, sweating, rash, hirsutism, etc.
6) Symptoms of blood and lymphatic system: leukopenia, anemia, thrombocytopenia, bone marrow suppression, etc. Daily manifestations can include sudden high fever, pale complexion, mental fatigue, limb weakness, memory loss, bleeding, etc.
7) Renal dysfunction: renal failure, tubular necrosis, oliguria, etc. Absence of urine and uremia are rare.
8) Other: blurred vision, photophobia, tinnitus, edema, local pain, urinary and fecal incontinence, thyroid abnormalities, etc.

8.4. Patient Compliance and Withdrawal

8.4.1. Screening Failure

Screening failure is defined as the subject who signed the informed consent form not being eligibility for the study according to the results of various examination during screening period. Subjects who failed the screening should not participate in this trial, and the reasons for the screening failure must be recorded in the original records.

8.4.2. Withdrawal Criteria

1) Considering the safety, the investigator believes that withdrawing from the study is in the best interest of the subjects.
2) Subjects with poor compliance cannot insist on completing the trial as planned or have other circumstances that may affect the judgment of the research results.
3) The subject voluntarily requested to withdraw from the trial and withdraw the informed consent.

8.4.3. Management of Subjects During the Study

Participants in this study should follow the process arrangement of the study. At each visit, participants should be arranged to participate in relevant study items as required. Inform

the subjects and guardians of the specific time and the testing items to be implemented for the next follow-up at the end of each follow-up. At each visit, the study logs of the subjects should be checked.

Participants can withdraw from this study at any time during the trial process. investigators should understand the reasons for subjects withdrawing from the trial and whether any adverse events have occurred. If any adverse events occur, follow-up should be conducted. Participants who withdraw early should complete the corresponding experimental procedures (including physical examinations, vital signs, ECG, and laboratory tests) as much as possible according to the protocol requirements.

Subjects who withdrew from the study due to adverse events or abnormal safety evaluations with clinical significance should be followed up by the investigators until the adverse events are resolved or stabilized until the investigators deems follow-up unnecessary or lost.

The date and reason for early withdrawal from the study must be recorded in the corresponding case report form (CRF). Adverse events, concomitant medications, tracking of abnormal laboratory test results, and the end page of CRF must be completed as much as possible.

8. Study Assessment

9.1 PK Assessment

9.1.1 Assessment Indicators

Application of qPCR method to detect changes in dystrophin nucleic acid concentration in the blood, serum and muscle tissue of subjects. Intensive collection of blood samples after the initial administration for detecting single-dose PK. Subsequent blood collection is used to detect steady-state PK after multiple administration.

PK indicators: Cmax (peak concentration), Tmax (peak time), C trough, ss (steady-state minimum blood drug concentration), Css,max (steady-state maximum blood drug concentration), AUC0-t (area under the curve of the last measurable concentration time point) and t1/2 (elimination phase half-life).

9.1.2 Timepoints of Sampling

Group I: Blood sample collection for the PK trial will be conducted before the 2nd, 4th, and 6th administrations and at 0 hours +10 minutes after the administration is completed. First time (Note:) PK blood sample collection before the first administration may be

conducted during the screening period/baseline period if no more than 7 days from baseline to the first administration, Intensive blood collection (no additional collection required before the first administration) and before the eighth administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after administration, as discussed by the investigator and collaborators Late-stage PK blood collection was adjusted based on the previously obtained PK data and safety data. PK blood samples will also be collected during the 8-week, 12-week, and 16-week follow-up periods. If Dystrophin nucleic acid cannot be detected at the 8-week follow-up, it may not be necessary to collect blood samples for the test at subsequent follow-ups.

Group II: Intensive blood collection will be conducted before dose1 administration, within 30 minutes before dose32 administration, and 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after dose1 and dose32 administration. PK blood collection will be conducted within 30 minutes before dose4, dose8, dose12, dose16, dose20, dose24, and dose28 administration and at 0h+10min and 24h±1h after administration. If the subject withdraws early for any reason during the administration period, the subject will have an intensive blood collection within 30 minutes before the last administration and at 0h+10min, 1h±10min, 2h±20min, 8h±1h, 24h±1h, 48h±2h, 72h±3h after the last administration. After discussion by the investigator and collaborators, the time points for PK blood collection in the later stage may be adjusted based on the previously obtained PK data and safety data. PK blood samples will also be collected at the 22nd, 26th, 30th, 34th, 38th, 42nd, 46th, 50th, 54th, 58th, 62nd, 66th and 70th follow-up periods. If Dystrophin nucleic acid cannot be detected at week 22 or at any subsequent follow-up, it may not be necessary to collect blood samples for this test at subsequent follow-ups.

9.2 Pharmacodynamic Assessment

9.2.1 Dystrophin Expression

1) **Assessment Indicators**

Determination of the amount of Dystrophin protein by Western Blot of muscle biopsy; Fiber strength and the percentage of dystrophin-positive fiber were detected by IHC.

1) **Muscle biopsy sampling**

Select the biceps brachii or other appropriate site, confirm the sampling location, disinfect with iodophor, extract muscle tissue about 0.5cm×1cm×0.5cm after local anesthesia, and send the extracted sample to the pathology laboratory for biopsy. Take

pictures of the sampling site before and after each muscle biopsy.

2) **Sampling time points**

Group I:

- Screening period + one muscle biopsy (one sample in total, one site) during baseline period;
- One puncture sample will be taken on the day of the last administration (D29), and one muscle biopsy (one sample in total, one site) will be performed within 3 days after administration.

Group II :

- Screening period + one muscle biopsy (one sample in total, one site) during baseline period;
- One puncture sample will be taken during administration (one sample in total, one site), with the time of collection determined by the investigator;
- One puncture sample will be taken on the day of the last administration, and a muscle biopsy (one sample in total, one site) will be performed within 3 days after administration.

In the study, it is advisable to perform a quadriceps muscle puncture and sampling on the same day as the last administration, and then conduct a biceps brachii muscle tissue sampling during the surgery after the last administration. If the subjects were unable to complete the collection of muscle tissue from the above-mentioned sampling sites due to their own reasons, the investigator will determine the sampling points based on the muscle condition of the subjects and perform muscle biopsies.

3) **Sample testing method**

The expression of Dystrophin protein in biopsy muscle tissue was detected by the following method:

- By Western blotting, changes in Dystrophin protein concentration were evaluated at baseline, during the administration of the study drug (only for the Group II), and after the last administration is completed;
- Muscle fiber strength and the percentage of dystrophin-positive fibers at baseline and after the last administration were evaluated by immunohistochemical (IHC) assays;

Concentration of Dystrophin nucleic acid in biopsy muscle tissue:

- By qPCR, changes in Dystrophin nucleic acid concentration were evaluated at baseline, during the administration of the study drug (only for the second dose group), and after the last administration was completed.

9.2.2 Electromyography

Electromyography is performed during the screening period + baseline period (once in total) and at week 12 after the completion of the last administration to assess muscle health.

9.2.3 MRI

MRI (both lower extremities including thighs and calves, buttocks) examinations are performed during the screening/baseline period before administration and at week 12 after the completion of the last administration to assess muscle health. Cardiac MRI should be added if necessary, as determined by the investigators.

9.2.4 DEXA

Only the second dose group was tested for DEXA. DEXA tests were performed during the pre-administration screening/baseline period, dose8(+7day), dose16(+7day), dose24(+7day), dose32(+7day), and after the completion of the last administration, and during the 4-week follow-up period, 8-week follow-up period, 12-week follow-up period, 16-week follow-up period, 20-week follow-up period, and 24-week follow-up period after the completion of the last administration to analyze adipose tissue quality and lean tissue quality. The number of tests may be increased if necessary, as determined by the investigator.

9.3 Immunogenicity Assessment

9.3.1 Assessment Indicators

1) Changes of concentration of cytokine including TNF-α, INF-γ, IL-2, IL-6, and IL-10 will be detected.
2) Changes of anti-dystrophin antibody level can be measured.

9.3.2 Timepoints of Sampling

Group I: Blood samples were collected during the screening period/baseline period/before the first administration (no additional collection is required before the first administration if no more than 7 days from baseline to the first administration), before the

second, fourth, sixth, and eighth administrations, and during the 8-week, 12-week, and 16-week follow-up periods. If Biomarker levels return to normal or are undetectable at the 8-week follow-up, blood samples may not be collected for the test at subsequent follow-ups.

Group II: During the screening period/baseline period/before the first administration (no additional collection before dose1 if no more than 7 days from baseline to dose1), before dose4, dose8, dose12, dose16, dose20, dose24, dose28, dose32 administration Blood samples were collected during the follow-up periods of week 22, week 26, week 30, week 34, week 38, and week 42. If Biomarker levels return to normal or are undetectable at week 22 or at any subsequent follow-up, blood samples may not be collected for the test at subsequent follow-ups.

Anti-dystrophin antibody test:

Group I: Blood samples were collected during the screening period/baseline period and before the 2nd, 4th, 6th, and 8th administrations (if no more than 7 days from baseline to the first administration, no additional collection is required before the first administration) and at each subsequent follow-up. If the Anti-dystrophin antibody level is undetectable in the follow-up sample at week 8, blood samples may not be collected for this test at subsequent follow-ups.

Group II: During the screening period/baseline period/before the first administration (if no more than 7 days from baseline to dose1, no additional collection is required before dose1 administration), before dose2, dose4, dose6, dose8, dose12, dose16, dose20, dose24, dose28, dose32 administration Blood samples were collected during the 22-week follow-up, 26-week follow-up, 30-week follow-up, 34-week follow-up, 38-week follow-up and 42-week follow-up. If Anti-dystrophin antibody levels cannot be detected at week 22 or in subsequent follow-up samples, blood samples may not be collected for this test at subsequent follow-ups.

9.3.3 Detection Method

Immunogenicity and cytokine tests will be detected by ELISA method.

9.4 Safety Assessment

9.4.1 Safety Parameters

All AEs and clinically significant laboratory abnormalities will be graded according to Common Terminology Criteria for AEs, Version 5.0 dated 27 November 2017.

Safety parameters include vital signs (including temperature, pulse, respiratory rate, blood

pressure), physical examination (including general condition, mucous membranes, lymph nodes, head and neck, abdomen, musculoskeletal, respiratory rate, cardiovascular, nervous and psychiatric conditions) and laboratory tests (including hematology, urinalysis, blood chemistry) and 12-lead ECG. In case of any abnormality, the corresponding indicators shall be rechecked.

Evaluate the TRAEs occurred during the clinical study, record its clinical manifestation, severity, occurrence time, duration, treatment method and prognosis, and determine its correlation with the investigational drug.

9.4.2 Baseline Signs and Symptoms

The baseline value serves as the starting point for clinical evaluation, and a series of physical examinations or laboratory tests are conducted to confirm the premedication status of the subjects, in order to facilitate comparison between post-treatment and pre-treatment. All subjects are required to undergo baseline measurement, which should be done before randomization. However, if the examination is invasive (such as biopsy), it should be performed after chemical examination as much as possible to reduce harm to the subjects. In addition, the baseline value should be as close as possible to the initial treatment. Before the study begins, conduct a comprehensive physical and laboratory examination of the study subjects, and record relevant data. Inquire and record in detail the lifestyle and past medication history of the study subjects. Based on the collected data, determine the baseline status of each study subject and record it in the CRF.

9.4.3 Laboratory Safety Assessment

According to the study Schedule in Table 5, list the ***Hematology***, ***blood biochemistry, Urinalysis, coagulation, and serum pathogen test*** for each subject. If applicable, it will also be marked as above or below the corresponding normal range. Evaluate changes in laboratory results before and after administration. We will also compare the indicators before and after the study to discover whether the parameters have returned to the pre study level.

1) ***Haematology***: white blood cell count (WBC), neutrophil count (NEUT #), eosinophil count (EO #), basophil count (BASO #), lymphocyte count (LYMPH #), red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT), and hematocrit (HCT) (the test results within 7 days before administration can be

accepted).

2) ***Urinalysis:*** Urinary bilirubin (UBG), urinary bilirubin (BIL), urinary ketone body (KET), urinary occult blood (BLD), urinary protein (PRO), urinary nitrite (NIT), urinary glucose (GLUU), urinary specific gravity (SG), urinary pH (pH), red blood cells (RBC), and white blood cells (WBC).
3) ***Biochemistry:*** bilirubin (total, indirect and direct), total protein, albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine (Cr), blood urea (Urea), blood glucose (GLU), serum sodium (Na+), serum potassium (K^+), serum calcium ($Ca2^+$), serum magnesium (Mg^{2+}), serum chloride (CL^-), lactate dehydrogenase (LDH), serum creatine kinase (CK), serum creatine kinase MB isoenzyme (CKMB) and c-reactive protein (CRP). Calculation of eGFR [CKD-EPI].
4) ***Coagulation:*** prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR).
5) ***Hepatitis B & C and HIV serology tests***: hepatitis B surface antigen (HBs Ag), anti-hepatitis B surface antibodies (anti-HBs Ab) anti-hepatitis B core antibodies (anti-HBc Ab), hepatitis B e antigen (HBe Ag), anti-hepatitis B e antibodies (anti-HBe Ab), hepatitis C antibodies, anti-HIV1 and anti-HIV2 antibodies.

9.4.4 Vital Signs and Physical Examination

Perform vital signs and physical examinations at each visit and record any clinically significant changes. The changes in vital signs from baseline to the EOS will be provided, and the incidence of significant abnormalities in all vital signs will be summarized and listed. The safety assessment of vital signs and physical examinations will only be provided in the list.

Vital signs: temperature, resting pulse, blood pressure, and respiratory rate. Vital signs will be recorded at every visit.

Physical examination: Including general condition, mucous membranes, lymph nodes, head and neck, abdomen, musculoskeletal, respiratory rate, cardiovascular, nervous and psychiatric conditions. Weight and height (Screening visit only) should be recorded and calculated for BMI. BMI=weight (kg) /height^2 (m^2).

9.4.5 12-Lead ECG

A 12-lead ECG will be recorded measuring the RR, PR, QRS, and QT interval durations. Summarize the changes in ECG parameters during the study period relative to baseline values and present abnormal results. All ECG data will also be provided in the subject list.

9.4.6. NSAA Scale

The NSAA (North Star Ambulatory Assessment) rating scale is a validated rating scale that can be used to assess the impact of Duchenne muscular dystrophy on the mobility of patients. The examination included standing, walking, getting up from a chair, standing on one leg (right leg), standing on one leg (left leg), straddling the box (right leg), straddling the box (left leg), straddling the box (right leg), straddling the box (left leg), sitting up, getting up from the ground, looking up, standing on the heel, jumping, right leg jumping, left leg jumping, running (10m).

The subjects completed one NSAA score scale during the screening period/baseline period, and the scores of the subjects' motor activity before administration were recorded. The first dose group was evaluated with the NSAA score after the 7th administration (+4 days), at the 8-week follow-up (-14 days), at the 12-week follow-up, at the 20-week follow-up, and at the 28-week follow-up. The second dose group was evaluated with the NSAA score at dose 7 (+4 days), at dose 15 (+4 days), at dose 23 (+4 days), at dose 31 (+4 days), at the 22-week follow-up (-14 days), at the 26-week follow-up, at the 34-week follow-up, and at the 42-week follow-up., and the scores were recorded again.

Outpatient performance will be based on the NSAA score. The higher the score, the better functional performance. The NSAA results of the subjects will be compared with their previous scores to track the improvement, maintenance, or decline in their motor ability over time.

Scores and Results

Score 0 cannot achieve the goal independently;

Score 1 Change the way the activity is completed, but be able to complete the goal independently without any other assistance;

A score of 2 is normal, with no obvious changes in activity.

For 2-3 years old, the highest score for all 8 items is 16 points;

For 3-4 years old, the maximum score for 13 items is 26 points;

For 4-8years old, the highest score is 34 points.

NSAA Accessment		**Subject ratings**
Standing	Stand barefoot for as long as possible without external support.	
Walk	Take at least 10 steps forward (about 8-10 feet) and keep your heel to toe gait consistent.	
Stand up from the chair	Start sitting with your arms crossed over your chest, then stand up from the chair without letting go of your arms.	
Stand on one leg (right leg)	Stand on your right leg with your arms hanging down for as long as possible.	
Stand on one leg (left leg)	Stand on your left leg with your arms hanging down for as long as possible.	
Step onto the square box (right leg)	Step onto a box step at least 15 cm high with your right foot (or dominant foot).	
Step onto the square box (left leg)	Step onto a box step at least 15 cm high with your left foot (or non-dominant foot) and keep it close to your other foot.	
Step down the box (right leg)	Face forward and walk down the steps with your right foot (or main foot).	
Step down from the box (left leg)	Face forward and come down the steps with your left foot (or non-dominant foot), with the other foot together.	
Sit up	Lie flat on the floor with your arms together, move to a sitting position without turning to the floor or getting up with your hands. Use one hand to reach the highest score.	
Stand up from the ground	Lie flat and get up as soon as possible. Do not roll into a four-point kneeling or prone position (Gower movement).	
Lift your head	Lie flat on the floor with your arms crossed over your chest and your hands under your shoulders. Then, lift your head and touch your chin to your chest	

	while keeping your arms crossed.	
Stand on your heels	With your heels leaning back barefoot, do this three times. For the highest score, both feet must be lifted simultaneously, with the instep noticeably turned outward (the foot lifted towards the tibia).	
Jump	Stand on the floor with your feet together and jump as high as possible without moving forward as much as you can.	
Jump with your right leg	Stand on your right leg and jump on one foot without touching the ground with both feet.	
Jump with your left leg	Stand on your left leg and jump on one foot without touching the ground with both feet.	
Run (10m)	Run as fast as possible for about 32 feet. To get the highest score, both feet must be off the ground while running.	
NSAA total score		

9. Adverse Event Reporting

9.1. Definition of Adverse Event

10.1.1. Definition

Adverse Event：

Serious adverse events: Refer to adverse medical events such as death, life-threatening, permanent or severe disability or loss of function, the need for hospitalization or extended hospital stay, and congenital abnormalities or birth defects that occur in subjects after they receive the investigational drug.

But hospitalization in the following circumstances shall not be regarded as a serious adverse event:

1) Expected or elective treatment for an existing disease that did not worsen after the start of the study;

2) Conventional treatment or testing for DMD is not related to the deterioration of the condition.

3) Common clinical manifestations of the natural course of DMD, such as aggravated readmission due to various causes;

4) Admission due to social reasons or rehabilitation treatment, with no deterioration in the general condition of the subjects;

5) Admitted to the hospital for rehabilitation treatment, the general condition of the

subject did not deteriorate.

If a subject has an SAE related to the investigational drug during the study period, for the safety of the subject, it is recommended to discontinue the study; If a subject experiences an SAE not related to the study drug and recovers or returns to the baseline level within one week of the last administration after treatment measures are taken, the investigator will determine whether the subject can continue to receive the drug based on the subject's recovery status.

10.1.2. Categorization of AEs

All AEs and clinically significant laboratory abnormalities will be graded according to Common Terminology Criteria for AEs, Version 5.0 dated 27 November 2017. For any term that is not specifically listed on the CTCAE scale, intensity will be assigned a grade of 1 through 5 using the CTCAE guidelines Table 6。

Table 6 The CTCAE displays grade 1 through 5 criteria of adverse event

Grades	Criteria
Grade 1	Mild； asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention not indicated
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL△.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

△Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Pay attention to distinguishing the severity and intensity of adverse events. Severe is used to describe intensity and is not necessarily SAE. For example, a headache may manifest as severe in intensity but cannot be included in SAE unless it meets SAE criteria.

10.1.3. Causes of AEs

According to the standards formulated by the Adverse Drug Reaction Monitoring Center of the Ministry of Health, the five-level classification is "Definitely related, Probably related, Possibly related, Unlikely related, Unrelated". The first three levels were judged to be related to the investigational drug. The judgment criteria of the five-level

classification method on AEs are shown in Table 7 below：

(1) Whether there is a reasonable order of time.

(2) Whether it is consistent with the known AR type of the investigational drug.

(3) Whether it can be explained by the volunteers' clinical status, concomitant medication, combined therapy and previous therapy.

(4) If the investigational drug is stopped or the dosage is reduced, whether it is reduced or disappeared.

(5) Whether the same reaction occurs again after repeated exposure to the same drug.

Table 7 Causality or relatedness assessment criteria in AEs and investigational drugs

Analysis results	Indicators				
	1	2	3	4	5
Definitely Related	+	+	-	+	+
Probably Related	+	+	-	+	?
Possibly Related	+	+	±	±	?
Unlikely related	+	-	±	±	?
Unrelated	-	-	+	-	-

The relationship between AEs (including SAEs) and drugs should be determined as far as possible. If it is believed that it is “Definitely related, Probably related and Possibly related”, it should be considered as an adverse reaction caused by investigational drug, and whether it is a SAE should be considered according to the severity. Adverse reactions should be listed and classified according to organ system or adverse event syndrome.

9.2. Adverse Event Recording and Reporting

The investigator should tell the subject who is required to truthfully feedback the changes after the medication but should avoid inductive questions. While observing the curative effect, the investigator should pay close attention to the AEs or unexpected toxic and side effects (including symptoms, signs and laboratory tests), analyze the causality, make judgments, track the observation and record, and count the incidence of adverse events.

For the AEs occurred during the trail, the investigator should record the time, severity, duration, treatment measures, outcome, etc. of the occurrence in the CRF, judge the correlation with the investigational drug, sign and indicate the date. In case of SAE in the trail, the investigator must immediately take measures to protect the safety of the subjects, and report to the department responsible for adverse drug reaction reporting in the study site in a timely manner. Then the adverse drug reaction report form should carefully be

filled in according to their opinions. Finally, continue reporting should follow the site's process.

9.3. Risk Prevention and Handling

When AEs occur, the investigator can decide whether to terminate the trial based on the severity, and the researcher will provide corresponding treatment in a timely manner according to the subject's condition. In case of SAEs, Emergency handling measures must be taken to protect the safety of the subjects. All AEs should be tracked and followed up, with detailed records of the handling process and results, until they are properly resolved or the condition stabilizes. If there are laboratory abnormalities, they should be traced back to normal or baseline levels. The follow-up can be selected based on the severity of AEs, including hospitalization, outpatient, home visits, phone calls, mail, and other forms.

10. Statistical Analysis

10.1. Sample Size

This trial was an IIT trial and no sample size estimation was performed; The number of subjects was determined based on the purpose of the study and relevant guidelines. 6 to 9 DMD patients were planned to be enrolled. Subjects who were not administered the drug after enrollment would be replaced, and the replacement subjects would be assigned a new random number, which was the enrollment number of the replaced subject +10.

10.2. Populations for Analysis

Boys aged 2 to 8 years (excluding 8 years) who can walk independently for at least 10 meters and have been diagnosed with DMD.

The age, gender, and race of the subjects, as well as their height and weight, should be recorded. Information about allergies/drug sensitivity or drug abuse should be obtained. All the information of these subjects should be recorded in descriptive language in the table.

11.2.1 Full Analysis Set

Full analysis set (FAS): FAS will be used to report medication compliance and to summarize demographic characteristics (age, gender, race, height and weight) and background characteristics (medical history, concomitant medications, and physical

examination, etc.) for all randomized subjects by treatment group.

11.2.2 Per-Protocol Set

Per-protocol set (PP)：Per-protocol analysis is a comparison of treatment groups that includes only those patients who completed the treatment originally allocated.

11.2.3 Safety Analysis Set

Safety analysis set (SAS): All subjects who have been randomized into cohorts, have used the investigational drug, and have safety evaluation data post-treatment constitute the safety population of this study. The safety population will be used for safety analysis.

11.2.4 Pharmacokinetic Dataset

The pharmacokinetic dataset (PKS) consists of all enrolled subjects who have received at least one study drug and have post-treatment PK evaluation data, forming the PK analysis set for this study. In the PK analysis set, different pharmacokinetic parameters may include different numbers of subjects based on the actual completion of the study.

10.3. Efficacy Analysis and Statistical Methods

Due to the small sample size and open-label design of this study, only descriptive statistics will be conducted for all endpoints, including the number of subjects, mean and standard deviation, minimum and maximum values of continuous variables, and the number and percentage of categorical variables.

Using descriptive statistical methods, describe the number and proportion of subjects entering each statistical analysis set by treatment group, the number and proportion of participants who completed the trial and withdrew midway, and the reasons for withdrawing midway (and their proportion). Describe demographic and other baseline characteristics by treatment group.

11.3.1 Analysis of Primary Endpoints

The primary endpoint is safety. Using a safety analysis set, summarize the baseline data, post-treatment data, and change from baseline data by follow-up and treatment group based on laboratory tests, vital signs, electrocardiograms, and other safety data. A cross table will be used to describe the changes from baseline to each follow-up after administration

regarding the normality and clinical significance of various examination results used as categorical data.

11.3.2 Analysis of Secondary Endpoints

1) PK Parameter Indicators and Assessment

The main pharmacokinetic parameters include t1/2, AUC, Vss, CL, Cmax, Tmax, etc. Descriptive statistical analysis of pharmacokinetic parameters for different dose groups or queues, calculating the arithmetic mean, standard deviation, coefficient of variation, median, maximum, minimum, and geometric mean of pharmacokinetic parameters for each dose group or queue.

2) PD Assessment

Statistically describe each pharmacodynamic indicator according to its data characteristics, and compare the changes after administration with the baseline characteristics. Analyze pharmacodynamic parameters according to actual needs.

3) Immunogenicity Assessment

Descriptive statistical analysis was conducted to compare the changes in cytokine production and anti-dystrophin antibodies in subjects at different time points after treatment with the baseline period.

10.4. Safety Analysis and Statistical Methods

Using the safety analysis set, AEs, ARs, and SAEs of each treatment group will be summarized and utilized. Compare the incidence rates among different treatment groups using the χ^2-test/Fisher exact probability method. Adverse events and reactions will also be standardized medical codes based on System Organ Class and Preferred Term.

All completed examination items including physical examination, 12-lead ECG, lab tests (Haematology, Urinalysis, Biochemistry, coagulation function, etc.), and descriptive statistics are listed in the form of a cross tabulation of pre- and post- treatment (based on the judgment of clinical doctors). The items of abnormal ECG, physical examination and laboratory test at each visit shall be listed in the form of a list. Descriptive recording of vital sign data, including values at each timepoint and their relative changes relative to baseline.

List all AEs, including subject number, dosage group, AE name, description, start date,

stop date, NCI-CTCAE level, severity of AEs, relationship with study drug, impact on study drug, outcome, etc.

10.5. Mid-term Analysis

Due to the small sample size of this study, which is only an exploratory IIT clinical study, no criteria for mid-term analysis/stop analysis are set. Both parties can obtain study data. If there are SARs during the study, both parties can negotiate and decide on the continued implementation of the study.

10.6. Data Monitoring Committee

Due to the small sample size and short study duration, which is only an exploratory IIT clinical study, no data monitoring committee was established.

11. Data Collection and Management

11.1. CRF/EDC

Due to the small sample size in this study, the data will be entered into a paper CRF. Clinical investigators or data entry personnel (clinical coordinators) designated by the investigators shall promptly and accurately input source data into CRF. The data entry personnel or investigators can modify the data after verifying it, and the modified data needs to be filled in the modification reason on the CRF. The investigators has signature permission for all final data.

11.2. Data Management

1) Investigators should keep all detailed original files of the subjects to ensure that the data is accurate, complete, and timely. The original documents, medical records, etc. should be clear, detailed, and easily identifiable by personnel participating in this clinical trial.
2) The data in CRF can only be modified by investigators or authorized personnel.
3) During the monitoring visit, the inspector can contact the staff of the research center, obtain source documents, and provide an appropriate environment to complete the review of study related documents. The monitor will meet regularly with the investigators during the study to provide feedback on the progress of the study.
4) The inspector will compare the CRF data with hospital records (source files). The

nature and location of all source files will be clarified to ensure understanding of all sources of raw data required for filling out CRF. Supervisors can also contact the study center to review these data sources.

5) After the study is completed, archive the CRF as needed. The experimental data should be retained for 5 years after the end of the study. But if there are requirements in current regulations or agreements with partners, these materials should be kept for a longer period of time. The collaborators will notify the investigators in writing when these materials will no longer need to be preserved.

12. Ethical Considerations

12.1. Ethics Committee/Institutional Review Board

This protocol, written informed consent form, and materials directly related to the subjects must be submitted to the ethics committee for written approval before the study can be officially conducted. Investigators must submit their annual study report to the ethics committee at least annually (if applicable). When the study is terminated and/or completed, the investigators must notify the ethics committee in writing. Investigators must promptly report all changes that have occurred in their study work to the ethics committee (such as revisions to relevant study documents such as protocols and/or informed consent forms), and these changes must not be implemented without approval from the ethics committee, unless they are made to eliminate obvious and direct risks to the subjects. When such situations occur, the ethics committee will be notified.

12.2. Informed Consent

In this study, all subjects are required to voluntarily sign a written informed consent form. Investigators must provide informed consent forms that are easy to understand and approved by the ethics committee to the subjects or their guardians and give them sufficient time to consider this study. Before obtaining a signed written informed consent form from the subject, the subject is not allowed to join the study. During the study, all updated versions of the informed consent form and written information will be provided to the participants. The informed consent form should be kept as an important document for clinical trials for future reference.

12.3. Protocol Amendment

The protocol amendment may affect the legal and ethical status of the study, as well as the statistical evaluation and the possibility of achieving the primary purpose of the study.

The terms of the protocol and its annexes must be strictly observed, except in emergency situations. If amendment is required, it must be provided in writing according to the corresponding SOP of the study site and approved. The protocol amendment will be presented to the investigator and explained. All protocol amendments must be submitted to the EC for review and approval before implementation, as well as to the management department for approval/notification when appropriate. Major amendments to the protocol must obtain the approval of Haining Spot Biotech Co., Ltd., the site's EC and the management department (if required) before implementation.

13. Confidentiality

Only investigators and monitors participating in clinical trials may have access to the personal medical records of the subjects, and they will sign a "Researcher Declaration" or "Confidentiality Commitment" that includes confidential information. Data processing will adopt a "data anonymity" approach, omitting information that can identify the individual identity of the subjects. The medical records of the subjects will be kept in the data archive room of the clinical trial center.

The results of this study may be published in medical journals, but we will keep patient information confidential in accordance with legal requirements, unless personal information of patients is not disclosed due to relevant legal requirements. When necessary, government management departments, hospital ethics committees, and their relevant personnel may access patient information in accordance with regulations.

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