# Research protocol

## Project summary

Spinal muscular atrophy (SMA) is the most common genetic cause of death for children below two years old. Previous studies by us and others have suggested that the fibrinolysis system is involved in nerve degeneration and regeneration and in respiratory failure. In the present study, we investigated the clinical effects of plasminogen, the key substrate of the fibrinolysis system, in SMA. The plasminogen was periodically used in SMA patients, and the treatment effects were observed. The clinical study was an open-label, one arm, and non-randomized study. The plasminogen were administrated to the SMA patients by intravenous injection for up to 72 weeks. The changes of motor function measured by CHOP INTEND scoring system, respiratory function and safety were investigated during plasminogen treatment. It is expected that the patient's motor function and respiratory function will be improved after plasminogen treatment.

## General information

**Protocol title:** A Study of Plasminogen in Subjects With spinal muscular atrophy (SMA)

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## Rationale & background information

Spinal muscular atrophy (SMA) is the most common genetic cause of childhood mortality, affecting 1:6000-1:10 000 live births, with a carrier frequency of 1 in 54[1]. The majority of SMA cases are caused by low levels of the survival motor neuron (SMN) protein caused by mutations in the *SMN* gene on chromosome 5q, termed 5q SMA. A small proportion (4%) seem not to be linked to chromosome 5, which are termed non-5q SMA and are often linked to *IGHMBP2* gene deficiency [1, 2]. Histologically, SMA is characterized by the degeneration of the alpha motor neurons of the spinal cord anterior horn cells [1], loss of myelinated fibers, myelin breakdown, and axonal degeneration in peripheral sensory as well as motor nerves of type I and II patients [3, 4]. These patients usually die of respiratory failure, with significant fibrin deposition in the lung as a clinical feature.

Currently, there are three FDA-approved drugs to treat 5q SMA by increasing SMN protein levels: nusinersen, zolgensma and risdiplam. Although these drugs are among the most expensive drugs in the world (for instance $2.15 million for one injection of zolgensma), the clinical outcome of their use is not ideal [5]. Additionally, there are currently no drugs available to treat non-5q SMA patients. Therefore, there is an urgent need to develop novel and more effective therapeutic alternatives to treat this devastating disease.

The plasminogen activator (PA) system is a general proteolytic system in which the active protease plasmin is formed from its precursor plasminogen by either of 2 physiological PAs: tissue-type PA (tPA) or urokinase-type PA (uPA). Both tPA and uPA can be inhibited by plasminogen activator inhibitor-1 (PAI-1), and excessive plasmin can be inhibited by α2-antiplasmin [6]. It is well known that plasmin is important in degrading the main components of the extracellular matrix, including fibrin, and that fibrin deposition is a key pathological feature of nerve injury and some respiratory disorders [6, 7, 8]. In addition, some studies have shown that the PA system is closely related to pathological processes of nerve degeneration and regeneration after injury, such as remyelination and neuritogenesis [9, 10]. Further, we have shown that added plasminogen accumulates in the injured area and promotes the repair of sciatic nerve injury and dysfunction in diabetic mice [11, 12]. These studies suggest that the PA system may play important roles in nerve degeneration and in regeneration after injury.

One characteristic symptom of SMA patients, especially type I, is that due to respiratory muscle weakness, these patients suffer from gradually worsening breathing difficulties and respiratory failure, and almost all die from this before the age of two [13]. The PA system is closely involved in acute respiratory failure. Administration of plasminogen has been reported to be an effective way to lower the occurrence of acute respiratory distress syndrome (ARDS) and even to save the lives of premature infants [14, 15]. Recently, although some reports have shown that tPA seems ineffective or even detrimental in treating COVID-19 patients with respiratory failure [16, 17], atomization inhalation of plasminogen seems to be an efficient and efficacious method to relieve ARDS and lung injury in COVID-19 patients [18]. These studies suggest that plasminogen may be efficacious for respiratory dysfunctions caused by various disorders.

**References (of literature cited in preceding sections)**


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**Study goals and objectives**

1. To explore the efficacy of plasminogen in the treatment of SMA including the effects on motor function and respiratory function

2. To explore the safety of plasminogen in the treatment of SMA including side effect events such as bleeding, hypersensitivity Reactions and infection.

**Study design**

Study Type: Observational

Actual Enrollment: 20 participants

Observational Model: Other

Time Perspective: Prospective

Official Title: A Study of Plasminogen in Subjects With spinal muscular atrophy (SMA)

Actual Study Start Date : September 05, 2018

Estimated Primary Completion Date: May 2023

Estimated Study Completion Date :October 2023

Inclusion Criteria:

1. Written informed consent/assent obtained prior to any assessment performed

2. Symptomatic SMA diagnosis based on gene mutation analysis with bi-allelic SMN1 mutations (deletion or point mutations) and any copy of SMN2 gene.

3. Naïve to treatment or have discontinued an approved drug/therapy

4. Up-to date on recommended childhood vaccinations and RSV prophylaxis with palivizumab (also known as Synagis), per local standard of care

Key Exclusion Criteria:

1. Previous use of OAV101 or any AAV9 gene therapy

2. Participant with history of aspiration pneumonia or signs of aspiration (eg, coughing or sputtering of food) within 4 weeks prior to Screening

3. Participant dependent on gastrostomy feeding tube for 100% of nutritional intake.

4. Anti-AAV9 antibody titer > 1:50 as determined by ligand binding immunoassay at the time of screening

5. Inability to take corticosteroids

6. Concomitant use of immunosuppressive therapy, plasmapheresis, immunomodulators such as adalimumab, or immunosuppressive therapy within 3 months prior to gene replacement therapy (eg, cyclosporine, tacrolimus, methotrexate, rituximab cyclophosphamide, IV immunoglobulin)

7. Hepatic dysfunction (i.e. AST, ALT, bilirubin, GGT or GLDH, ≥ ULN; CTCAE ≥ 1) at Screening (with the exception of isolated AST elevation: in the absence of

other liver laboratory abnormalities, isolated AST elevation is not considered exclusionary)

8. Previously treated with nusinersen (Spinraza®) or Zolgensma (AVXS-101) within 4 months prior to Screening

9. Previously treated with risdiplam (EvrysdiTM) within 15 days prior to Screening (washout period of at least 5 half-lives before Screening)

## Methodology

**Brief name：** Plasminogen

**Provided：** The clinical doctors or nursing staff with more than 5 years of clinical work experience administrated the intervention.

**The method：** The intervention was administrated by an individual face to face.

**Place：** Based on the condition of patients, the intervention was performed at the home of patients or at Beijing Chang'an Chinese and Western Integrated Medicine Hospital.

**Regimen：** The clinical study was an open-label, one arm, and non-randomized study.

**Treatment duration：** 72 weeks

**Method of administration：** Intravenous injection, at dose of 50-200 mg each time, 1 time per 1-3 days, two weeks as one treatment course, 2 week intervals between courses

**Measurement of outcomes：**

Primary Outcome Measure

The motor function according to the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) was assessed at [ at baseline, 2w、6w、10w、22w、46w]

Secondary Outcome Measures

1. Respiratory function measured by [the value of blood oxygen saturation without Oxygen inhalation] at [baseline 2w, 6w, 10w, 22w, 46w]

2. Anthropometric nutritional status measured by [the proportion of high body weight, body fat, growth parameters] at [baseline, 10w, 22w, 46w]

3. Adverse events measured by [blood routine test, blood biochemistry, coagulation function, hemolysis function, urine routine test, 12 lead ECG, physical examination, vital signs, etc] at [baseline, 22w, 46w]

## Safety considerations

**WARNINGS AND PRECAUTIONS**

**Bleeding**: plasminogen administration may lead to bleeding at lesionsites or worsen active bleeding. Discontinueplasminogen if seriousbleeding occurs. Monitor patients during and for 4 hours after infusionwhen administering plasminogen to patients with bleeding diathesesand patients taking anticoagulants, antiplatelet drugs, and other agentswhich may interfere with normal coagulation.

**Transmission of Infectious Agents**: plasmiogen is made from human blood and therefore carries a risk of transmitting infectious agents, e.g.,viruses, the variant Creutzfeldt-Jakob disease (vCJD) agent, andtheoretically, the Creutzfeldt-Jakob Disease (CJD) agent.

**Hypersensitivity Reactions**: Hypersensitivity reactions, includinganaphylaxis, may occur with plasminogen. If symptoms occur,discontinue RYPLAZIM and administer appropriate treatment.

## Follow-up

All subjects entered the 90 day safety follow-up period after completing the last blood collection and safety inspection of the administration. Since the first administration, they came to the hospital for a safety inspection when they were out of the group or early out of the group at 30 and 90 days, and plasminogen inhibitors and infectious markers were carried out during the screening period and when they were out of the group or early out of the group at 90 days. At the same time, the subjects were interviewed for compliance by telephone/in hospital within 60 days to collect whether there have been adverse events and concomitant medication since the last visit.

**Data management and statistical analysis**

This study will use EDC system to collect and manage data. The data management process should comply with the Good Clinical Practice (GCP) and the corresponding regulatory requirements for data management, comply with the standard operating procedures (SOP) of the data management department, and ensure the authenticity, accuracy, integrity, reliability and traceability of clinical trial data (EDC system will record all audit trails). The details of data management will be provided in the data management plan, and the responsibilities of researchers, supervisors and data managers will be clarified to ensure the quality level of each stage of data management.

In this study, the Data Manager Associate (DMA) writes the data management plan as a guiding document for the entire data management process. All processes of data management should be operated according to the data management plan, which should be updated according to the specific progress of the project.

The biostatisticians and major researchers shall formulate a statistical analysis plan according to the research scheme, and improve the statistical analysis plan document before data locking. The frequency and percentage of the classified indicators in the completion of the study, demography and baseline characteristics were calculated, and the numerical indicators were analyzed descriptively.

(1) Efficacy analysis

The measurement data adopts the sample number, mean, standard deviation, median, Q1, Q3, minimum value, maximum value, error bar chart, broken line chart, etc; Counting data included the number of cases, constituent ratio, rate, 95% CI, straight bar chart, percentage constituent chart, etc.

(2) Security analysis

The method of statistical description is mainly used to describe the number and incidence of adverse events/reactions, serious adverse events/reactions, adverse events/reactions leading to drug withdrawal, drug suspension, and withdrawal from the study. According to MedDRA, the medical code was graded to describe the number and incidence of adverse events/reactions, serious adverse events/reactions, adverse events/reactions leading to drug withdrawal, drug suspension, and withdrawal from the

study under each system organ classification (SOC) and preferred term (PT). The severity of various adverse events/reactions was evaluated according to NCI-CTCAE (Version 5.0), and the number and incidence of adverse events/reactions in each system were described according to the severity. For laboratory, vital signs, ECG, physical examination and other safety data, the baseline data, post treatment data and post treatment change data will be summarized.

### Quality assurance

Before the start of the clinical trial, the researchers should receive the training of the trial plan, so that the researchers can fully understand and understand the clinical trial plan and the specific connotation of each indicator. The quality control personnel shall check the basic conditions of the clinical trial to ensure that the clinical trial conditions can meet the requirements of the scheme. During the trial, the investigator shall carefully carry out the clinical operation and other work according to the requirements of GCP, agency SOP and the trial plan, and make records truthfully, timely, completely and normatively. The quality control personnel shall check the quality of the test process and the corresponding original records. After the test, the research unit shall sort out the corresponding project documents, which shall be checked by the quality control personnel and archived. The quality assurance department of the clinical research unit shall carry out quality control for the feasibility of the tests carried out. When non conformities are found, the researcher and the person in charge of the unit shall be notified in time to make corrections, and the corrections shall be tracked.

### Expected outcomes of the study

The success of this study will provide new treatment methods for SMA patients, reduce treatment costs, bring good news to patients, and reduce the social burden.

## Dissemination of results and publication policy

The purpose, content and results of this clinical trial as well as all future information must be strictly confidential. The copyright of all materials and results (including test

data and its derivative data) belongs to the sponsor.

**Duration of the project**

Actual Study Start Date:September 05, 2018

Estimated Study Completion Date: October 2023

**Problems anticipated**

Since SMA is a rare disease, it may be difficult to recruit patients. Therefore, we should do a lot of publicity in the early stage to collect patients as much as possible.

**Ethics**

The patients are minors or infants, so the ethical review is more strict. All personnel responsible for recruitment shall fully introduce the drug situation, benefits and risks during the trial to the patient's guardian. In addition, the patient shall be given adequate financial compensation to obtain informed consent as much as possible.

**Informed consent forms**

Subjects must give informed consent to participate in the trial before receiving treatment in order to protect their legitimate rights and interests. The investigator has the responsibility to completely and comprehensively introduce the purpose of this study, research methods, drug effects, reasonable expected benefits, possible toxic and side effects and possible risks to the subject or its designated agent. The investigator should let the subject know their rights, risks and benefits to be borne, and should promptly inform the subject of any new information about the drug for the trial. The subjects should be informed that this clinical trial is based on the principle of voluntary participation, and they can withdraw from the trial unconditionally at any time during the trial, and they will not be punished for withdrawing from the trial. The subjects shall be informed that the research party and sponsor have the right to read, save and statistically process the test data of the subjects according to the provisions of relevant laws and regulations. The version, preparation date or modification date of

the informed consent shall be indicated. Only subjects who fully understand the risks and benefits of this clinical trial, as well as potential adverse events, and sign their names and dates on the informed consent form can participate in this clinical trial. If the test protocol has been modified to a certain extent during the trial, the informed consent form needs to be modified accordingly according to the modified content, and the informed consent resigned by the subject after being approved by the Ethics Committee.