MEGA JOURNAL OF CASE REPORTS

Research Article

Compiled Date: November 18, 2023

Plasminogen Shows Rapid Efficacy in Treating Patients with Type I Spinal Muscular Atrophy (SMA)

Ting Wang[1,2#], Dongmei Zhang[3#], Chunying Guo[1,2#], Xiaojing Ge[1,2], Yuanyuan Wu[1,2] and Jinan Li[1,2*]

[1]Department of Applied Research, Talengen Institute of Life Sciences, Shenzhen, P.R. China

[2]Department of Applied Research, Ruijian Xingze Biomedical Co. Ltd, Dongguan, P.R. China

[3] Beijing Chang'an Chinese and Western Integrated Medicine Hospital, Beijing, P.R. China

[#]These authors contributed equally to this work.

[*]**Corresponding author**: Jinan Li, Department of Applied Research, Talengen Institute of Life Sciences, Room C602G, 289 Digital Peninsula, Shunfeng Industrial Park, No.2 Red Willow Road, Futian District, Shenzhen, P.R. China, E-mail: jnl@talengen-pharma.com

Abstract

Background: 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.

Methods: The plasminogen was periodically used in 9 type I and 1 non-5q (*IGHMBP2* gene deficiency) SMA patients, and motor function and respiratory function were observed.

Results: For all nine 5-42-month-old type I SMA patients, within 0.2-2 months of treatment, the motor function scores, measured by the CHOP INTEND scoring system, increased 6.89±4.83 points. After 0.2-4 months of treatment, 3 of the 9 type I SMA patients achieved the motor milestone of sitting unassisted for more than 10 seconds. In addition, the respiratory function of these patients showed quick and significant improvements. The only non-5q SMA patient also showed clinical improvements after plasminogen treatment. In all 10 patients, during the treatment period of up to 24 months, plasminogen exhibited an excellent safety profile, with no adverse events.

Conclusion: This is the first clinical study showing that plasminogen may be a promising drug candidate to improve respiratory and motor functions and even the survival of type I SMA patients.

Trial Registration: trial registration number ISRCTN21188633

Keywords: Plasminogen; PIC; Type I SMA; Motor function; Respiratory function

Introduction

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) **[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 [6-8].** 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. Hence, inspired by our previous studies, we hypothesized that plasminogen supplementation may be efficacious in improving the motor and respiratory functions of SMA patients. In the current study, with approval from the hospital ethics committee, we explored the therapeutic effects of plasminogen manufactured under GMP-compliant conditions on survival, motor functions, and respiratory functions in type I and non-5q SMA patients. We also investigated the levels of routine coagulation/thrombosis markers closely related to the PA system in SMA patients and found that α2-Plasmin Inhibitor-plasmin Complex (PIC) levels were significantly higher than its normal concentration range.

The results suggest that using plasminogen seems to be an efficacious, efficient and relatively simple way to improve the survival and the motor and respiratory conditions of type I and non-5q SMA patients.

Methods

Patient involvement and plasminogen administration

The clinical study was an open-label, one arm, and non-randomized study with trial registration number ISRCTN21188633 (Registration date: 08/01/2023). The 10 study subjects were diagnosed with type I SMA with SMN gene mutation or non-5q SMA with mutation in the gene encoding immunoglobulin-binding protein 2 (*IGHMBP2*), according to genetic tests and clinical symptoms. The profiles of these 10 patients are given in **Table 1.** The clinical study was a joint clinical study between the Talengen Institute of Life Sciences and Beijing Chang'an Chinese and Western Integrated Medicine Hospital. The Ethics Committee of Beijing Chang'an Chinese and Western Integrated Medicine Hospital approved the study, which was performed according to the Helsinki Declaration. Signed informed consent was obtained before treatment for all subjects included in the study, and informed consent was obtained from a parent or guardian as minors are involved in the study. When the minor patients' photographs were used for the publication, the informed consent of the parents was obtained.

Table 1: CHOP INTEND scores of 9 type I SMA infants at baseline and after plasminogen treatment.

Patient ID	Baseline	2 months postdosing	6 months postdosing	7-24 months postdosing
1	12	15	25	22-24*
2	4	5	/	/
3	31	40	/	/
4	1	7	/	/
5	0	8	/	/
6	18	20	/	/
7	25	40	/	/
8	11	24	/	/
9	8	13	/	/

*The treatment was interrupted at months 6, 7, 9, 10, and 20. At month 7, the patient was treated with stem cell therapy, which resulted in a rapid deterioration of his disease condition. /: Indicates that the information was not studied or recorded.

Freeze-dried plasminogen (5 or 50 mg per vial, about 98% purity of lys-plasminogen with trace levels of glu-plasminogen), purified from human plasma fraction III at GMP-compliant facilities, and was provided by the Talengen Institute of Life Sciences. Plasminogen was dissolved in sterile water to produce 5 mg/mL solutions for use in the study. The treatment profiles are shown in **Table 2.**

Table 2: Milestones of motor function of 9 type I SMA infants at baseline and after plasminogen treatment.

Patient ID	Baseline	After treatment
1	Unable to sit unassisted; no head control; unable to roll over	Able to sit unassisted for 30 s; able to keep head upright for 2 min and turn head left and right after treatment for 4 months
2	Able to keep head upright for up to 3 s in the supine position	Able to keep head upright for up to 30 s in the supine position after treatment for 3 days
3	Unable to sit unassisted steadily or roll over; degeneration of head control	Able to sit unassisted for 10 s after treatment for 9 days
4	Unable to move arms in the supine position; unable to move feet	Able to move arms from inside to outside in the supine position and able to bend knees and stand upright for 2 min after 10 days' treatment
5	Able to bend knees and stand upright for 2 s at most	Able to bend knees and stand upright for 2 min after 11 days' treatment
6	Unable to move legs independently	Able to push legs slightly, and move freely to the middle after 4 days' treatment
7	Unable to raise head (occasionally 2-3 s) and turn head when held vertically	Able to keep head up upright >20s after 12 days' treatment
8	Unable to sit unassisted	Able to sit unassisted >10s after 10 days of treatment
9	Unable to raise the arms or legs	Able to raise the legs assisted after 8 days of treatment and autonomously raise the arms after10 days' treatment

Routine coagulation/thrombosis marker examination

Plasma markers of coagulation/thrombosis, including Prothrombin Time (PT), Prothrombin Time Ratio (PTR), international Normalized Ratio (INR), Prothrombin Time activity (PT%), Activated Partial Thromboplastin Time (APTT), Thrombin Time (TT), Fibrinogen (FIB), Fibrin(ogen) Degradation Product (FDP), D-dimer, Thrombomodulin (TM), Thrombin-Antithrombin Complex (TAT), α2-Plasmin Inhibitor-Plasmin Complex (PIC), and tissue-type Plasminogen Activator-Plasminogen Activator Inhibitor complex (t-PAIC), were examined from the plasma of patients 6, 7, 8, and 9 at the Second Affiliated Hospital of Xi'an Jiaotong University or the Third Hospital of Xi'an during plasminogen treatment. PT, PTR, INR, PT%, APTT and TT were determined by the coagulation method with a coagulation detector (Sysmex, CS5100, Japan) using kits from Siemens. D-dimer was determined by an enzyme-linked immunofluorescence assay run in an automatic immunofluorescence enzyme labeling instrument (Biomerieux, VIDAS, France) with matching reagent. FIB, FDP, TAT, TM, PIC and tPAI-C were determined by the chemiluminescence method using the Sysmex HISCL5000 chemiluminescence immunoassay instrument (Sysmex, Japan) with supporting reagents.

Oxygen saturation examination

Oxygen saturation was monitored by a pulse oximeter (Prince-100F, Heal Force, China) at the indicated time points.

Motor function assessment

Trained clinical evaluators assessed the patients' motor function according to the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), as indicated. CHOP INTEND is a motor function scale specifically designed for type I SMA **[19].** Other noticeable behavior changes, not listed in the tables, were also monitored and noted for potential future reference.

Statistical analysis

The CHOP INTEND scores are presented as mean ± SD. Comparisons before and after plasminogen treatment were made using the two-tailed paired t test. The comparison of PIC data between the normal value and SMA patients' detection results used the one-sample t test. P value $<$ 0.05 was considered to be statistically significant.

Results

Plasminogen improves the clinical symptoms of type I SMA patients

It has been suggested that the PA system plays an important role in pathological processes in both the nervous system and the respiratory system **[6,9,14].** After positive results in previous SMA studies on mice **[20,21],** we investigated the clinical efficacy of plasminogen in 9 type I and 1 non-5q (*IGHMBP2* gene deficiency) SMA patients. Although the patient number was limited, the overall efficacy of plasminogen was encouraging. Quick and efficacious improvements in motor function, respiratory function and even the expected lifespan were observed in these patients. As shown in **Figure 1A and B and Table 1**, CHOP INTEND score significantly (P=0.003) increased from 12.22±10.67 at baseline (before plasminogen treatment) to 19.11±13.27 after plasminogen treatment, an increase of 6.89±4.83 points, after 0.2 to 2 months of treatment in cases 1-9.

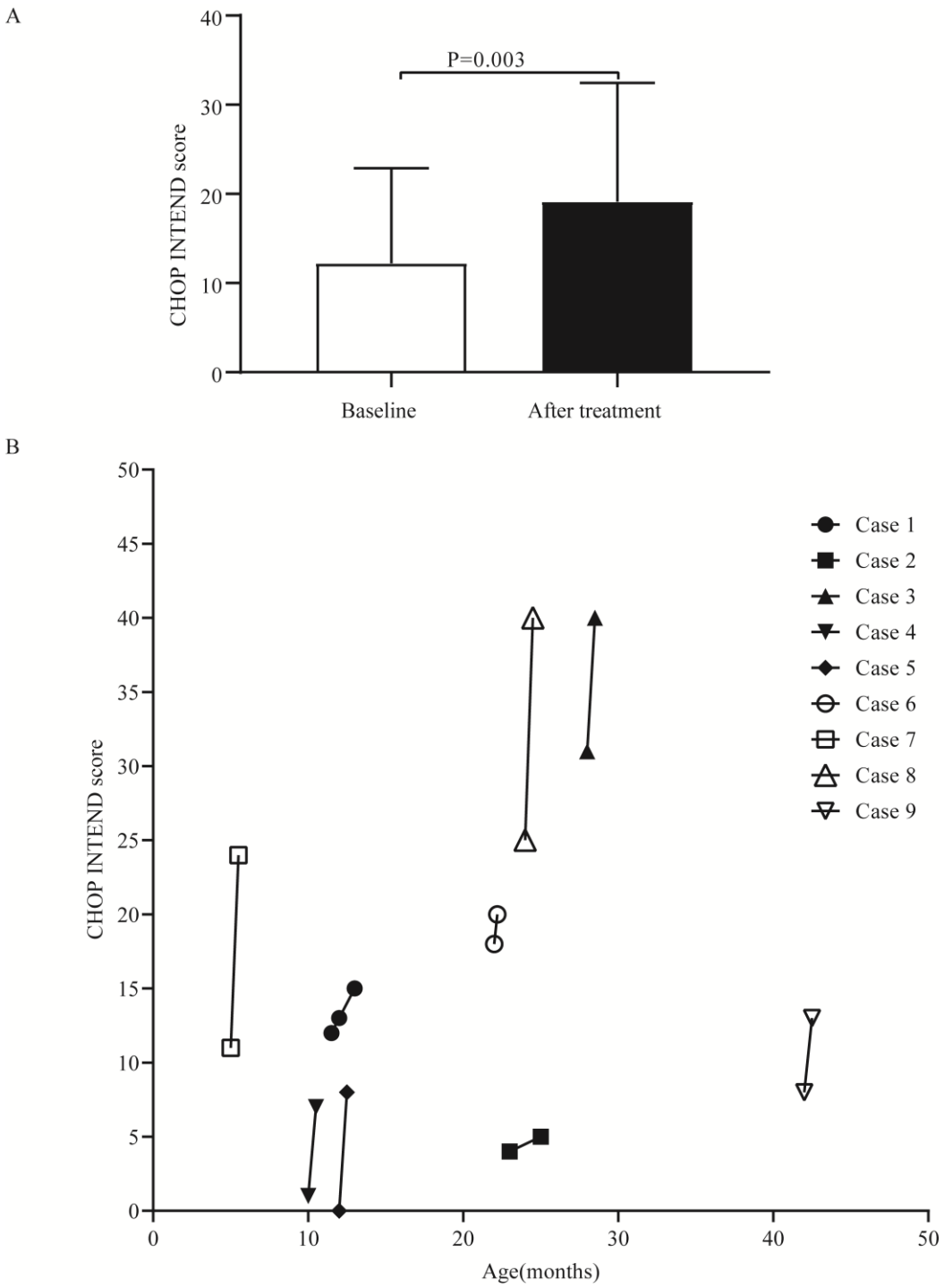

Figure 1: Plasminogen treatment increases the CHOP INTEND scores in type I SMA patients. A. CHOP INTEND scores of 9 type I SMA patients at baseline and after plasminogen treatment for 0.2-2 months; B. CHOP INTEND scores of the 9 types I SMA patients after 0.2-2 months of treatment with plasminogen in the current study.

On the improvement in motor milestones, 3 of 9 patients were able to sit unassisted for at least 10 seconds, and 4 of 9 patients achieved significant improvement in head control after plasminogen treatment for 0.2 to 4 months **(Table 2S).**

Table S2: Treatment profiles of plasminogen for SMA patients.

Patient ID	Type of SMA	Plasminogen treatment
1	I	Intravenous injections, sometimes together with atomization inhalation Atomization inhalation: 2-3 times per day, 10 mg each time Intravenous injection: 1 time every 1-4 days, and gradually increasing from 50 mg to 200 mg One to two weeks as one treatment course, for a total of 18 courses; 2-3-week intervals between courses
2	I	Atomization inhalation: 2-4 times per day, 5-40 mg each time, for two weeks, in the first course Intravenous injections: 1 time every 2 days, 50 mg each time, for a total of 3 days, in the second course. Four-week intervals between two courses
3	I	Intravenous injections: 1 time every 2 days, 50 mg each time Two weeks for one treatment course, for a total of 1 course
4	I	Intravenous injections, sometimes together with atomization inhalation Atomization inhalation: 2 times per day, 5 mg each time Intravenous injection: 1 time every 2 days, 50 mg each time Two weeks for one treatment course, for a total of 1 course
5	I	Intravenous injections: 1 time every 2 days,10-75 mg each time Two weeks as one treatment course, for a total of 1 course
6	I	Atomization inhalation: 1 time every 1-2 days, 10 mg each time, for a total of 1 week
7	I	Atomization inhalation: 20 mg on first day Intravenous injections: 50 mg per day from the second day on, for a total of 13 days
8	I	Intravenous injections: 50 mg per day, 2 weeks for one treatment course, for a total of 1 course
9	I	Intravenous injections: 50 mg per day, 2 weeks for one treatment course, for a total of 1 course
10	non-5q	Intravenous injections, sometimes together with atomization inhalation Atomization inhalation: 2-3 times per day, 10 mg each time Intravenous injection: 1 time every 1-4 days, and gradually increasing from 50 mg to 250 mg 1-2 weeks for one treatment course, for a total of 7 courses; 2-3-week intervals between courses but a four-month interval between the fourth and fifth courses

Rapid and significant improvements in respiratory functions were also observed in type I SMA patients after plasminogen treatment. The blood oxygen saturation of patient 1 was usually 87%, though it went up to a maximum of 92% before the treatment was started. Just 2 days after nebulized inhalation of plasminogen, the

blood oxygen saturation had increased to 97% and stayed around that level thereafter. The blood oxygen saturation of patient 6 was 92% at most before the treatment, increased to 99% by the second day after nebulized inhalation of plasminogen and stayed at approximately 98-99% at later treatments **(Table 3).** The overall gastrointestinal condition and sleep quality also seemed generally improved in these patients **(Table S3).**

Table 3: Characteristics of the respiratory function of 9 type I SMA infants at baseline and after plasminogen treatment.

Patient ID	Baseline	After treatment
1	Oxygen mask needed Oxygen saturation: 87-92%	No ventilation support needed (at 35 months old) Oxygen saturation: 97% the second day after treatment and always around 97% thereafter
2	Noninvasive ventilation	Oxygen saturation: 97-99%
3	/	/
4	Suction 20-30 times per day	50% reduction in the times of suction after treatment for 1 day
5	Suction 3 times per day	Suction 1 times per day after treatment for 5 days
6	Oxygen saturation ≤ 92%	Oxygen saturation: 99% at the second day after treatment and maintained at approximately 98-99%
7	Abdominal breathing; the abdomen fluctuates greatly during sleep	Able to breathe stably, decreased by 20% in amplitude of abdominal breathing after 12 days' treatment
8	Weak breathing, abdominal breathing	Able to breathe stably after 2 days' treatment
9	Auxiliary ventilation required for approximately 8 hours at night.	Auxiliary ventilation reduced to 6 h after 2 days treatment and to 4 h after 3 days' treatment.

/: Indicates that the information was not studied or recorded.

Table S3: Gastrointestinal and sleep conditions of 9 type I SMA infants at baseline and after plasminogen treatment.

	Gastrointestinal condition		Sleep quality	
Patient ID	Baseline	After treatment	Baseline	After treatment
1	Slight deterioration of swallowing function	Feeding orally, choking frequency reduced, Speaking	Easy to wake up	Able to enter deep sleep after 1 week's treatment
2	Gastrostomy feeding	/	Normal	Normal
3	Slight deterioration	/	Poor sleep and	Sleeping better, waking up

	of swallowing function		occasional crying	without crying
4	Gastrostomy feeding	Reduction in feeding time	/	/
5	Gastrostomy feeding	/	/	/
6	Swallowing function normal	Reduced feeding time from 20-25 min to 10 min	Waking up twice, occasionally crying at night	Waking up only 1 time, sleeping time prolonged
7	/	/	Waking up three times, irritable and crying at night	Sleeping time prolonged to 2 hours, able to sleep autonomously after 1 day's treatment
8	/	/	Easy to wake up and cry at night	Prolonged sleeping time to 2 hours after treatment for 1 day
9	Deterioration of swallowing function	Reduced choking frequency after 3 days' treatment	Turn over 5-6 times during the night	Fewer times rolling over at night

/: Indicates that the information was not studied or recorded.

Plasminogen improved the life expectancy and growth of one type I SMA patient

Historical studies have demonstrated that the average lifespan of type I SMA patients is 10 months **[22].** Patient 1's doctors gave him a prognosis for survival of a maximum of 1.5 years of age. After plasminogen treatment for 24 months, he was still alive and in generally good health, having the ability to speak, to swallow without support, and to breathe without ventilation or oxygen help (35 months old, **Figure 2**).

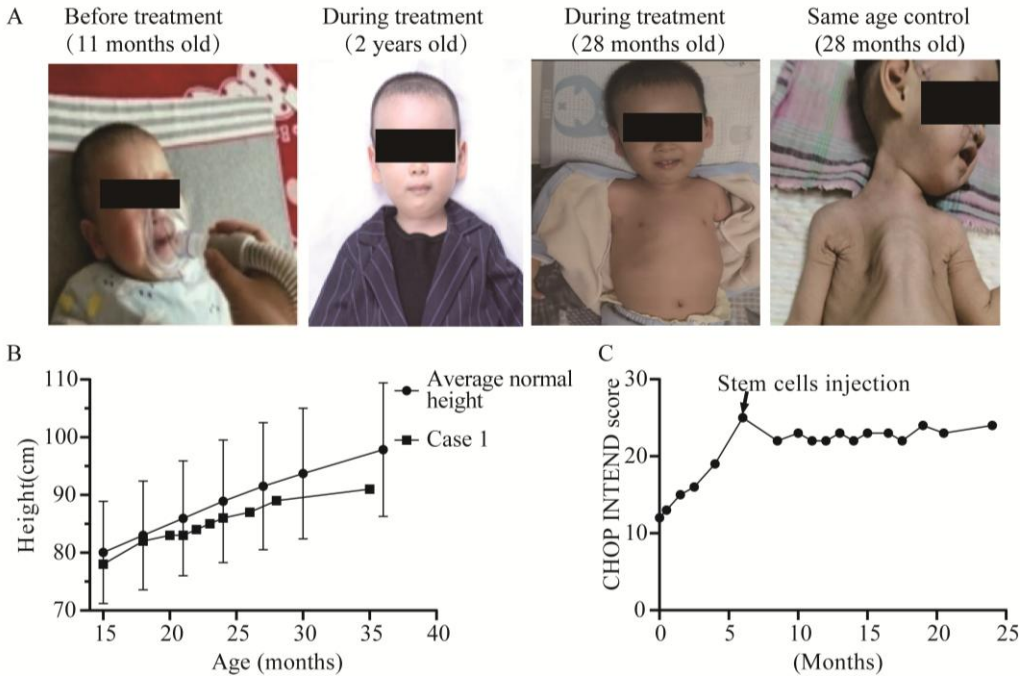

Figure 2: Plasminogen improved the survival, growth and motor functions of a type I SMA patient (patient 1). A: Photographs of patient 1 at the ages of 11 months (before plasminogen treatment, first from left), two years (during plasminogen treatment, second from left), and 28 months (during plasminogen treatment, third from left); age control (28 months old without plasminogen treatment, fourth from left); B: the height development curve of patient 1 (squares) and average normal male infants in China (dots) between 15 and 35 months old; C: CHOP INTEND scores of patient 1 at different time points from baseline through the plasminogen treatment.

In addition, his CHOP INTHEND score increased steadily, going up by 13 points after 6 months of treatment. At month 7 of treatment, his parents decided that he should receive an unknown stem cell therapy. This stem cell treatment seemed to be detrimental to the patient, as his condition quickly worsened in the days after treatment, including fever, insomnia, irritability and crying. His CHOP INTEND score quickly dropped down to 18 points and the ability to sit unassisted and head control was lost. The parents returned to the hospital and requested that the plasminogen treatment be resumed as soon as possible. The restart of plasminogen treatment quickly stabilized the general physical condition of the boy, and his CHOP INTEND score returned to 22 after month 8 of treatment and stayed at 22-24 in the subsequent treatments until the end of the observation at 35 months of age **(Table 1 and Figure 2C).** The long-term treatment period gave us a unique opportunity to follow his body development. His chest shape did not demonstrate a typical 'bell shape' at 28 months of age **(Figure 2A**, third from left), as commonly observed in type I SMA patients (**Figure 2A**, fourth from left) **[23].** Although decreased height/length is noted among type I SMA infants [24], his body development curve showed that his height was within the normal range of the average non-SMA male infants of the same age (Figure 2B).

Plasma PIC level increased in type I SMA patients

Thrombosis and fibrin formation parameters were also measured in the plasma of 4 type I SMA patients (patients 6-9) during their plasminogen treatment. These data showed that the plasma PIC levels were 1.36, 2.49, 1.71 and 0.8 μg/mL for patients 6, 7, 8, and 9, respectively, and that these levels were either significantly

elevated or right above the highest normal limit (< 0.8 μg/mL) **(Table 4).** As PIC is often used to reflect the clinical risk of thrombosis, the results of PIC suggest that SMA patients may be at a higher risk of thrombosis.

Table 4: The results of plasma examination of routine coagulation/thrombosis indices in patients 6, 7, 8, and 9 during plasminogen treatment.

Patient ID		**6**	**7**	**8**	**9#**
PT (s)	Detection value	8.31	10.2	10.4	**8.3↓**
	Normal range	9.8-12.1			
PTR	Detection value	2.35	0.92	0.94	**0.75↓**
	Normal range	0.82-1.15			
INR	Detection value	0.82	0.91	0.93	**0.74↓**
	Normal range	0.9-1.3			
PT% (%)	Detection value	102.1	117.3	112.8	**178.3↑**
	Normal range	70-130			
APTT (s)	Detection value	27.0	30.9	28.0	25.6
	Normal range	22.7-31.8			
TT (s)	Detection value	14.1	20.2	17.1	16.5
	Normal range	14-21			
FIB (mg/dL)	Detection value	240	194	240	301
	Normal range	180-350			
FDP (μg/mL)	Detection value	3.10	1.47	4.12	1.18
	Normal range	0-5			
D-dimer (ng/mL)	Detection value	537	450	550	480
	Normal range	0-1000			
TM (TU/mL)	Detection value	8.31	13.1	9.52	6.75
	Normal range	3.8-13.3			
TAT (ng/mL)	Detection value	2.35	3.15	1.21	3.89
	Normal range	＜4.0			
PIC(μg/mL)	Detection value	**1.36↑**	**2.49↑**	**1.71↑**	**0.8**
	Normal range	**＜0.8**			
tPAI-C (ng/mL)	Detection value	2.56	2.13	1.32	1.05
	Normal range	＜17			

#: Indicates that the plasma sample of case 9 was repeatedly frozen and thawed before examination, which may have affected the accuracy of the results.

Plasminogen improved motor and respiratory functions in one non-5q SMA patient

Considering that essentially no effective drugs are available to treat non-5q SMA patients, one 37-month-old female non-5q SMA patient with severe respiratory failure was also enrolled. She was diagnosed at age 18

months with SMA with respiratory distress type 1 (SMARD1) caused by mutations in the gene encoding *IGHMBP2*, linked to chromosome 11, by a gene sequencing examination **[25].**

During the first course of plasminogen treatment, when the parameters of ventilation remained unchanged, the patient's spontaneous breathing increased by approximately 20-30% (approximately 2-3 spontaneous breaths every 10 breaths), and the blood oxygen saturation increased from above 95% with oxygen inhalation to 98-99% without oxygen inhalation. After four courses of treatment, the patient was completely free of the ventilator and resumed spontaneous breathing **(Table S4).**

Table S4: Clinical characteristics of the non-5q SMA patient (Case 10) before and after plasminogen treatment.

	Before treatment	**After treatment**
Respiratory function	Respiratory weakness; Needed invasive ventilator support Oxygen saturation: above 95% with oxygen inhalation. Much phlegm	After one course's treatment: spontaneous breathing time increased approximately 20-30%; oxygen saturation: 98-99% without oxygen inhalation; phlegm amount decreased After four treatment courses: breathing more strongly, completely detached from the ventilator and spontaneous breathing resumed
Motor function	Limb weakness, no head control ability; CHOP INTEND score: 2	Limb strength and moving flexibility increased Head control increased CHOP INTEND score increased to 7 after 2 weeks of treatment and stayed at 7-9 thereafter
Facial expression	Stiff facial expressions	More facial expressions
Temperature	≥37.5 °C	Back to normal range (36.7-37 °C) after 12 days of treatment
Sleep	Easy to wake up, difficult to fall asleep	Sleep time increased, easy to fall asleep

She was weak in the limbs, could not control her head and had a CHOP INTEND score of 2 before treatment, which improved to 7 after 2 weeks of treatment with plasminogen and stayed at 7-9 in the following 7 courses of treatment. Other meaningful improvements were also observed, including in facial expression, body temperature and sleep **(Table S4).** These results suggest that the administration of plasminogen may improve the general clinical conditions of non-5q SMA patients.

Discussion

SMA is the most common genetic cause of infant mortality in children under two years old. The majority of patients with SMA remain untreated. Plasminogen has been suggested to be involved in pathological processes in the nerve and respiratory systems. In this trial study, we found that the PIC level, a risk marker of clinical thrombosis, was significantly increased in type I SMA patients compared with normal subjects. Administering plasminogen, an important component of the fibrinolysis system, seemed to be efficacious and efficient for the treatment of type I and non-5q SMA patients, such as by improving their survival, developmental growth,

swallowing, speaking, and motor and respiratory functions. Additionally, no overt adverse events were observed during plasminogen treatment. To our knowledge, this is the first clinical study using plasminogen to treat this devastating disease, and these positive study findings are encouraging. Plasmin is formed from the zymogen plasminogen through proteolytic cleavage. Plasmin is extremely fast acting and has a very short half-life (0.02 seconds) **[26],** which makes plasmin almost impossible to be used clinically. Circulating in the blood at a steady concentration as high as 0.2 mg/ml, plasminogen is mostly inert, has a long half-life of 0.8-2.2 days and does not have any active functional roles **[6].** However, we have shown that although plasminogen levels are significantly increased in the wounded areas of diabetic mice, systemic supplementation of plasminogen results in an even further local increase and faster wound healing, suggesting that plasminogen itself has active functional roles in wound healing **[12].** To our knowledge, a few earlier reports have shown that plasminogen can cross the altered Blood–Brain Barrier (BBB) in neurological disorders **[27].** Interestingly, despite its large molecular weight of 90 kD, we have shown that systemically supplemented plasminogen also seems to pass the BBB rapidly and that the active plasmin in the brain increases both in mice with different neurological diseases and even in healthy mice (manuscripts in submission). In SMA, there is a supraphysiological loss of the anterior horn motor neurons in the spinal cord, which results in progressive muscular paralysis with skeletal muscular atrophy **[28].** Since pathological research reports on SMA are rare, to learn more, we collected research materials on Amyotrophic Lateral Sclerosis (ALS), another motor neuron disease caused by upper and lower motor neuron damage that has similar clinical manifestations as SMA **[29].** Examinations of postmortem biopsies from patients who died from ALS have shown that abnormal fibrin deposition in the spinal cord is also one of its pathological features of ALS **[30].** It has been reported that abnormal fibrin deposition can promote neuron degeneration and death **[7]** and inhibit neuron regeneration **[31]** in some central nervous system tissue injury processes. Our previous studies have shown that plasminogen supplementation alone promotes fibrin degradation in thrombolysis and in an injured liver, kidney, or sciatic nerve **[11].** It is reasonable to infer that plasminogen may also improve nerve injury in the spinal cord by promoting fibrin degradation and thus improving motor function in SMA patients. The present study has shown that SMA patients may have a risk of thrombosis, as shown by the PIC data, and that plasminogen administration can improve some SMA clinical symptoms, such as motor and respiratory functions.

Fibrin is also the major component of the hyaline membrane, a barrier that forms and blocks gas exchange and inhibits the action of pulmonary surfactant, which is essential for proper pulmonary expansion, leading to acute respiratory distress and tissue hypoxia, particularly in premature infants with Respiratory Distress Syndrome (RDS) **[15]**. Our results have shown that plasminogen supplementation efficiently and substantially improves oxygen saturation and respiration function in type I and non-5q SMA patients. Of interest, in the 1970s, two double-blind randomized clinical studies indicated that plasminogen improves the clinical condition and decreases the death rate of newborns with RDS caused by hyaline membrane disease **[15,32].** Furthermore, our study of COVID-19 has shown that atomization inhalation of plasminogen is efficient and efficacious in relieving ARDS in COVID-19 patients **[18].** Therefore, we speculated that plasminogen may contribute to improving respiratory functions by promoting fibrin degradation in the lungs of SMA children. Currently, nusinersen, zolgensma and risdiplam, the only three FDA-approved drugs to treat SMA, all modify SMN gene expression, leading to increased expression of the full-length protein and subsequently helping neuron survival. Although 90% of the protein product of the mutated SMN gene is the truncated type with a short half-life,

probably through certain DNA repair mechanisms **[33],** approximately 10% is normal, full-length SMN **[34].** Most type 1 SMA patients have two copies of the SMN2 gene, most type 2 SMA patients have three copies of the SMN2 gene, and most type 3 SMA patients have four copies of the SMN2 gene **[34].** Thus, the more SMN2 genes, the more truncated SMN protein is formed, and also the more full-length SMN protein is formed, so the disease severity is reduced. This is the reason why, by increasing the expression of the SMN2 gene through these treatments, the clinical severity of SMA is reduced. In our earlier animal studies, we also observed that plasminogen upregulates the expression of the transcription factor NF-kB and increases the levels of SMN gene transcription and protein expression (most of them should be full-length proteins since the truncated proteins degrade quickly in the cell) (manuscript in submission). Thus, one simple mechanism to increase full-length SMN protein expression could be through the upregulation of transcription factors, especially NF-kB, in which plasmin is known to play a role **[20,21].** More work is needed to confirm these findings and to elucidate the underlying molecular mechanism. Such work is being conducted at our institute. The finding that plasminogen also positively treats non-5q SMA patients suggests that plasminogen plays a general therapeutic role in different types of SMA, which brings more hope for those patients who are currently not treatable by existing drugs. Examining the inclusion criteria of the clinical trials of the three FDA-approved drugs **[35-37],** most of our patients would not have been eligible for inclusion in the trials due to their severe disease conditions, as shown by their baseline CHOP INTEND scores **(Table S1).** When we plotted the changes in CHOP INTEND score of these patients after treatment with plasminogen, together with the published data from the clinical trials of the approved drugs, it seems likely that plasminogen takes effect with a faster increase in scores and with broader age and severity ranges for its effectiveness **(Figure 1B).** However, we must emphasize that this observation is preliminary. A more comprehensive and better-designed clinical trial is needed to draw firm conclusions.

Table S1: Baseline characteristics of type I and non-5q SMA patients.

Patient ID	Sex	Age at first dose	SMA type	CHOP INTEND score	Brief medical treatment history
1	Male	11.5 months	I	12	Mesenchymal stem cells, antiviral drugs, ambroxol hydrochloride and clenbuterol hydrochloride oral solution
2	Female	23 months	I	4	Nusinersen treatment x4, fructose sodium diphosphate, terbutaline atomization, ipratropium bromide
3	Female	28 months	I	31	Vitamin A&D dropping pills
4	Male	10 months	I	1	Acetylcysteine solution, budesonide suspension, amoxicillin
5	Female	12 months	I	0	None
6	Male	22 months	I	18	None
7	Female	24 months	I	25	None
8	Female	5 months	I	11	Vitamin A&D, vitamin D3, vitamin B12

9	Male	42 months	I	8	Nusinersen treatment x4
10	Female	37 months	Non5q	2	Cefdinir dispersible tablets, shupushen, anesthetic

This is the first clinical study using plasminogen to treat type I and non-5q SMA patients. The findings of significant clinical improvements within days or even hours of plasminogen supplementation suggest that plasminogen is an effective method of treatment. Improvements in overall survival, body development, and motor and respiratory functions in type I and non-5q SMA patients were seen. Currently, an official clinical trial is being developed and submitted for approval to use plasminogen to treat SMA patients. In the meantime, under current ethical approvals, more preclinical and clinical studies are being conducted at our institute to fully understand the molecular mechanisms and the clinical efficacy of plasminogen in SMA.

Acknowledgments

We wish to thank Mr. Chuan Song and Mr. Mengnan Li for fruitful discussions, and acknowledge all other staff involved in the study from but not limited to the institutions of the authors for their assistance, and all the people who helped during the manuscript preparation.

Conflict of Interest

J.L. founded Talengen Institute of Life Sciences. The institute has applied for patents on the use of plasminogen and is developing natural and recombinant plasminogen as a drug candidate to treat SMA and other neurological diseases. Other authors have declared no competing interests.

Funding Sources

No funding support in this article.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval

The Ethics Committee of Beijing Chang'an Chinese and Western Integrated Medicine Hospital approved the study, which was performed according to the Helsinki Declaration. Signed informed consent was obtained before treatment for all subjects included in the study, and informed consent was obtained from a parent or guardian as minors are involved in the study.

Author contributions

JL and TW conceived and supervised the study; JL, DZ and TW designed the experiments; DZ and XG performed the experiments; JL TW, DZ, CG, XG and YW analyzed the data; JL, TW and YW wrote the manuscript; and TW, CG and JL revised the manuscript.

References

1. Sugarman EA, Nagan N, Zhu H, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72 400 specimens. Eur J HumGenet. 2012;20(1):27-32.
2. Peeters K, Chamova T, Jordanova A. Clinical and genetic diversity of SMN1-negative proximal spinal muscular atrophies. Brain. 2014;137(11):2879-96.
3. Yonekawa T, Komaki H, Saito Y, et al. Peripheral nerve abnormalities in pediatric patients with spinal muscular atrophy. Brain Dev. 2013;35(2):165-71.
4. Chien YY, Nonaka I. Peripheral nerve involvement in Werdnig– Hoffmann disease. Brain Dev. 1989;11:221-9.
5. Connock M, Andronis L, Auguste P, Dussart C, Armoiry X. Will the US$5 million onasemnogene abeparvosec treatment for spinal muscular atrophy represent 'value for money' for the NHS? A rapid inquiry into suggestions that it may be cost-effective. Expert Opin Biol Ther. 2020;20(7):823-7.
6. Aisina RB, Mukhametova LI. Structure and function of plasminogen/plasmin system. Bioorg Khim. 2014;40(6):642-57.
7. Cortes-Canteli M, Mattei L, Richards AT, et al. Fibrin deposited in the Alzheimer\"s disease brain promotes neuronal degeneration. Neurobiol Aging, 2015;36(2):608-17.
8. Van Breemen VL, Neustein HB, Bruns PD. Pulmonary Hyaline Membranes Studied with the Electron Microscope. Am J Pathol. 1956;33(4):769.
9. Seeds NW, Akison L, Minor K. Role of plasminogen activator in spinal cord remodeling after spinal cord injury. Respir Physiol Neurobiol. 2009;169(2):141-9.
10. Seeds NW, Williams BL, Bickford PC. Tissue plasminogen activator induction in Purkinje neurons after cerebellar motor learning. Science. 1995;270:1992-4.
11. Li JN. Method for prevention or treating diabetic mellitus nerve injury and related diseases. US20180369345.
12. Shen Y, Guo Y, Mikus P, Sulniute R, Wilczynska M, Ny T, et al. Plasminogen is a key proinflammatory regulator that accelerates the healing of acute and diabetic wounds. Blood. 2012;119(24):5879-87.
13. Pharmacoeconomic Review Report: Nusinersen (Spinraza): (Biogen Canada Inc.): Indication: Treatment of patients with 5q SMA [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2018.
14. Ambrus CM, Weintraub DH, Choi TS, Eisenberg B, Ambrus JLJRCiCP, Pharmacology. Plasminogen in the prevention of hyaline membrane disease. Res Commun Chem Pathol Pharmacol. 1973;6(1):341-4.
15. Ambrus CM, Choi TS, Cunnanan E, et al. Prevention of hyaline membrane disease with plasminogen: A cooperative study. JAMA. 1977;237(17):1837-41.
16. So M, Steiger DJ, Takahashi M, Egorova NN, Kuno T. The characteristics and outcomes of critically Ill patients with COVID-19 who received systemic thrombolysis for presumed pulmonary embolism: an observational study. J Thromb Thrombolysis. 2021:52(4):1061-7.

17. Mazilu L, Katsiki N, Nikolouzakis TK, Aslanidis MI, Lazopoulos G, Kouretas D, et al. Thrombosis and Haemostasis challenges in COVID-19 - Therapeutic perspectives of heparin and tissue-type plasminogen activator and potential toxicological reactions-a mini review. Food Chem Toxicol. 2021;148:111974.
18. Wu Y, Wang T, Guo C, Zhang D, Ge X, Huang Z, et al. Plasminogen improves lung lesions and hypoxemia in patients with COVID-19. QJM. 2020;113(8):539-45.
19. Glanzman AM, Mazzone E, Main M, Pelliccioni M, Wood J, Swoboda KJ, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. Neuromuscul Disord. 2010;20(3):155-61.
20. Li JN. A method and drug for treating spinal muscular atrophy. US17/924,620.
21. Li JN. A method and drug for treating spinal muscular atrophy. WIPO Patent WO/2021/228086A1.
22. Ramdas S, Servais L. New treatments in spinal muscular atrophy: an overview of currently available data. Expert Opin Pharmacother. 2020;21(3):307-15.
23. Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. 2000 Feb 24 [updated 2020 Dec 3]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2021.
24. De Amicis R, Baranello G, Foppiani A, et al. Growth patterns in children with spinal muscular atrophy. Orphanet J Rare Dis. 2021;16(1):375.
25. Luan X, Huang X, Liu X, Zhou H, Chen S, Cao L. Infantile spinal muscular atrophy with respiratory distress type I presenting without respiratory involvement: Novel mutations and review of the literature. Brain Dev. 2016;38(7):685-9.
26. N Kaur, PK Sinha, G Sahni. Site-specific PEGylation of micro-plasmin for improved thrombolytic therapy through engineering enhanced resistance against serpin mediated inhibition. PLoS ONE. 14(5): e0217234.
27. Mezzapesa A, Orset C, Plawinski L, et al. Plasminogen in cerebrospinal fluid originates from circulating blood. J Neuroinflammation. 2014:11:154.
28. Anne M, Duchen LW. Sensory system involvement in infantile spinal muscular atrophy. J Neurol Sci. 1975;26(3):349-59.
29. LM Murray, K Talbot, TH Gillingwater. Review: Neuromuscular synaptic vulnerability in motor neurone disease: amyotrophic lateral sclerosis and spinal muscular atrophy. Neuropathol Appl Neurobiol. 2010;36(2):133-56.
30. Garbuzova-Davis S, Hernandez-Ontiveros DG, Rodrigues MCO, et al. Impaired blood–brain/spinal cord barrier in ALS patients. Brain Res. 2012;1469:114-28.
31. Schachtrup C, Paul L, et al. Fibrinogen inhibits neurite outgrowth via β3 integrin-mediated phosphorylation of the EGF receptor. Proc Natl Acad Sci U S A. 2007;104(28):11814-9.
32. Ambrus CM, Choi TS, Weintraub DH, et al. Studies on the prevention of respiratory distress syndrome of infants due to hyaline membrane disease with plasminogen. Semin Thromb Hemost. 1975;2(1):42-51.
33. Singh RN, Singh NN. Mechanism of Splicing Regulation of Spinal Muscular Atrophy Genes. Adv Neurobiol. 2018;20:31-61.

34. Verhaart IEC, Robertson A, Wilson IJ, et al. Prevalence, incidence and carrier frequency of 5q–linked spinal muscular atrophy–a literature review. Orphanet J Rare Dis. 2017;12(1):124.
35. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1723-32.
36. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al. Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1713-22.
37. Darras BT, Masson R, Mazurkiewicz-Bełdzińska M, Rose K, Xiong H, Zanoteli E, et al. Risdiplam-Treated Infants with Type 1 Spinal Muscular Atrophy versus Historical Controls. N Engl J Med. 2021;385(5):427-35.

Citation of this Article

Wang T, Zhang D, Guo C, Ge X, Wu Y and Jinan Li J. Plasminogen Shows Rapid Efficacy in Treating Patients with Type I Spinal Muscular Atrophy (SMA). Mega J Case Rep. 2023;6(11):2001-2018.

Copyright