BHI
THERAPEUTIC SCIENCES

Research Studies Conducted by Blue Horizon International

Umbilical Cord Blood Derived Stem Cell Therapy

Introduction

Stem cells have the potential to treat a wide range of diseases. Adult stem cell therapy could be used to: replace neurons damaged by spinal cord injury, stroke, Alzheimer's disease, Parkinson's disease or other neurological problems; produce insulin that could treat people with diabetes and heart muscle cells that could repair damage after a heart attack; or replace virtually any tissue or organ that is injured or diseased.

Hundreds of allogeneic stem cell (stem cells from another person) therapies are underway in humans for a wide-range of medical conditions. However, there is not a lot of published research that provide definitive proof that stem cell therapies are safe. Our pilot research studies, conducted in collaboration with local partners in Slovakia and China, are directed to evaluate the safety of human umbilical cord blood derived stem cells applications for regenerative medicine use. We conduct our studies in compliance with FDA regulations.

Umbilical Cord Blood Stem Cells Safety and Quality

We have taken every precaution to ensure the safety and viability of Umbilical Cord Blood Derived Mononuclear Cells (UCBMCs).

Umbilical cord bloods were collected from primiparous pregnant women receiving Caesarean section in accordance with the sterile procedure guidelines in the hospital. The pregnant donor women passed medical examinations before they donate UC. They tested for communicable diseases such as HBV, HCV, HIV and Syphilis. After collection, each cord blood sample was tested for communicable diseases such as HBV, HCV, HIV and Syphilis. The isolated mononuclear cells were cultured to test for bacteria and fungus, endotoxins, and to insure viability.

Research subjects received injection of 5 ml of human umbilical cord blood mononuclear cells (~ 4×10^8 mononuclear cells) intravenously or/and via lumbar puncture. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5).

The below overview shows our completed and ongoing research studies approved by Institutional Review Board (IRB).

Research Studies Results

- **Treatment effect and safety of intravenous therapy with human umbilical cord blood derived mononuclear cells in subjects with chronic inflammation**

In the United States we completed safety study of human cord blood stem cells application in the therapy of symptoms related to chronic inflammation. More than 100 research subjects were included in the study. Results are showing that human cord blood stem cells were safe and effective in the improvement of symptoms related to chronic inflammation. Research subjects did not develop serious adverse reactions. Moreover, 3 and 6 months after stem cell therapy, subjects displayed a significant increase in energy level as well as a significant decrease of their pain level.

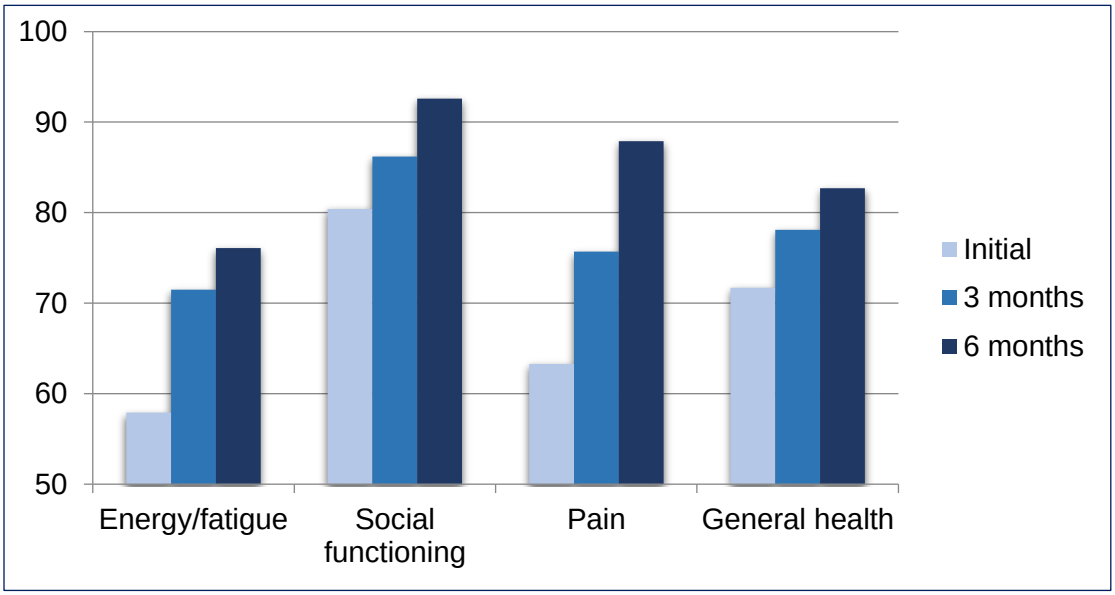
Our study of blood test markers of 28 research subjects with musculoskeletal conditions demonstrates that there are no significant changes before and after stem cell therapy, further demonstrating the safety of stem cell therapy. According to the table below, patients with improvements and no changes in blood work markers (groups 1 and 2) were 76.8 %, which is a statistically significant difference from groups 3 and 4 (t-test, P = 0,004) (Table 1).

Table 1: Four categories of blood test results interpretation

1	Improvement, %	42.9±9,5*
2	No Change, %	35.7±9,2
3	Improvement and Deterioration, %	14.3±6,7
4	Deterioration, %	7.1±4,5

The SF-36 is a self-reported survey of patient health and an indicator of overall health status. The calculated scores from the subjects' SF-36 questionnaires three and six months after stem cell therapy display a significant increase in energy level from 57.9 to 76.1 as well as a significant decrease of their pain level from 63.3 to 87.9 (note: higher pain level indicator implies less physical pain, and vice versa). As shown on Figure 1, stem cell therapy results in a significant improvement in subjects' physical health indicators.

Figure 1. Calculated indications of subjects' general health status collected from initial and follow-up SF-36 questionnaires.



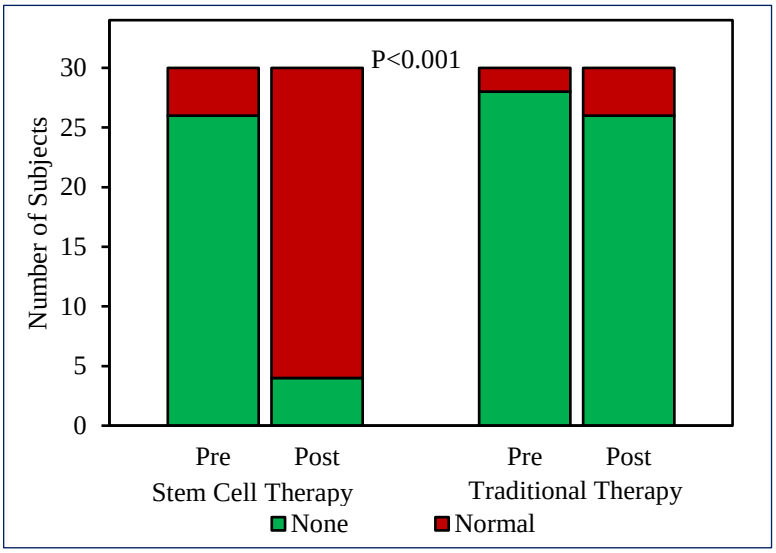
- **<u>Umbilical Cord Blood Mononuclear Cell Therapy for Spinal Cord Injury – A Retrospective Cohort Study</u>**

Stem cells from various sources hold promise to enhance functional recovery after Spinal Cord Injury (SCI). Thirty patients with spinal cord injury were randomly selected from seventy treated with human umbilical cord blood mononuclear cells at the Wuhan Hongqiao Brain Hospital between March 2009 and March 2012. Another thirty patients with SCI, who received only traditional therapy and no stem cell therapy, were included as the control group.

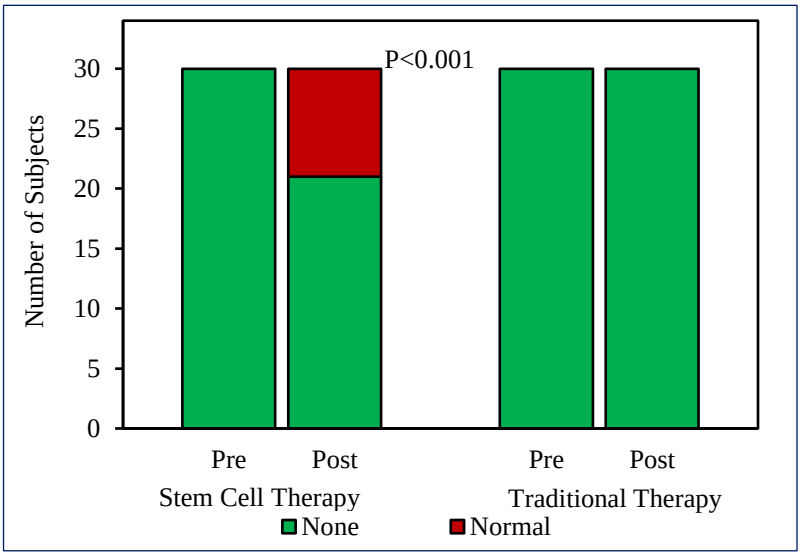
Research subjects showed significant improvement in pain and temperature sensation, lower limb muscle strength, bladder and gastrointestinal function (Fig. 2).

Figure 2.

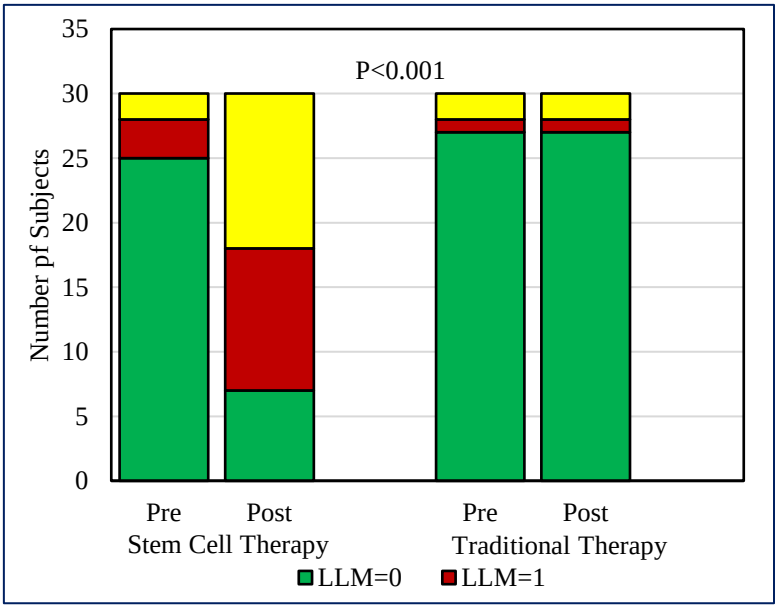
A. Changes in pain sensation



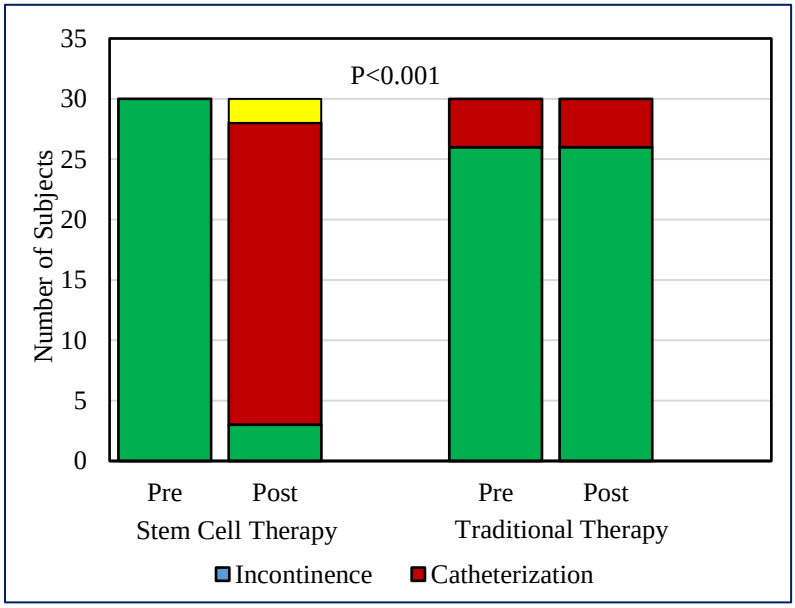
B. Changes in temperature sensation



C. Changes in lower limb muscle (LLM) strength



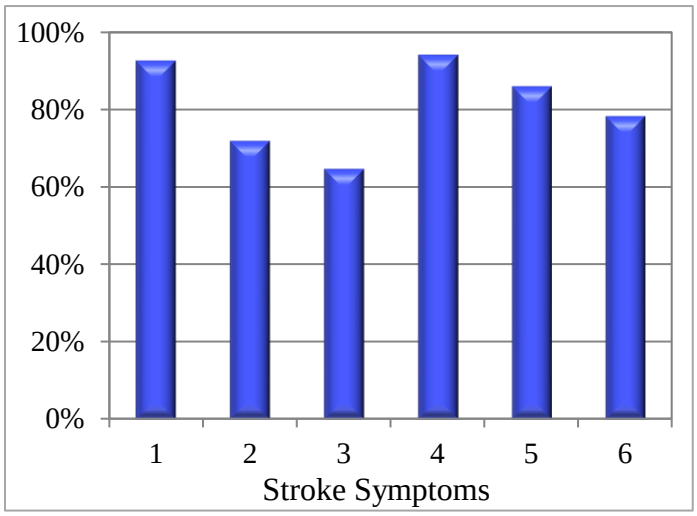
D. Changes in bladder function



- **Umbilical Cord Blood Mononuclear Cell Therapy for Stroke – A Retrospective Cohort Study**

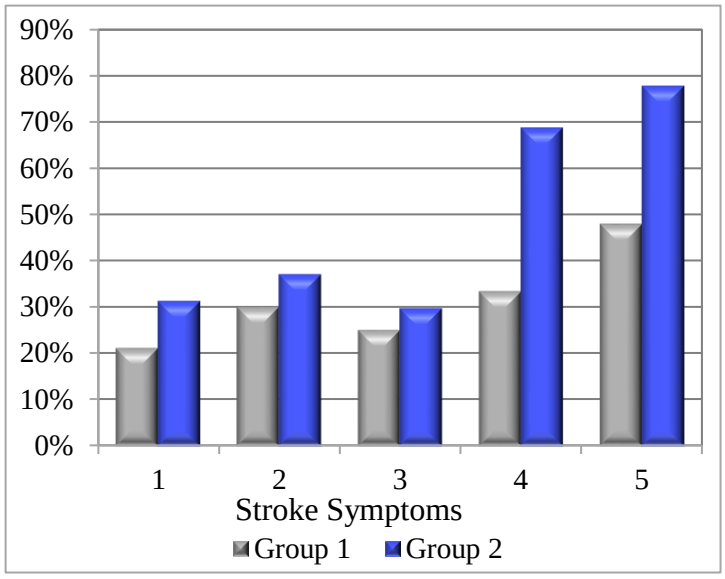
Stroke is a leading cause of adult disability worldwide and the second highest cause of death in the world. Approximately 87% of adult strokes are ischemic in etiology. To date, there are no clinically effective pharmacotherapies that can promote or facilitate cellular functional recovery after an ischemic stroke. Human umbilical cord blood mononuclear cell (hUCMNC) therapy is a promising treatment for ischemic stroke. Ninety-seven subjects with ischemic and hemorrhagic stroke, treated with hUCMNCs in the Wuhan Hongqiao Brain Hospital Co., Ltd. (Wuhan, Hubei) between March 2009 and March 2012, were included in the retrospective cohort study.

Analysis of post-stroke symptoms before and after treatment showed improvement of upper and lower extremity mobility and muscle strength, and neurological function (ability to speak, urinary and bowel function) (Figure 1).



1. Upper extremity mobility
2. Lower extremity mobility
3. Neurological function (ability to speak)
4. Upper extremity muscle strength
5. Lower extremity muscle strength
6. Neurological function (urinary and bowel function)

We analyzed if intrathecal plus intravenous (IT+IV) administration of stem cells in two or three therapy sessions leads to a better treatment outcome of subjects with stroke compared to one IT+IV and two IV or three IV administrations. Application of IT+IV administration in two and three therapy sessions improved all analyzed post-stroke symptoms. Moreover, these subjects showed significant improvement in upper and lower extremity muscle strength comparing to a group of subjects who received one IT+IV plus two IV or three IV administrations (Figure 2).



1. Upper extremity mobility
2. Lower extremity mobility
3. Neurological function (ability to speak)
4. Upper extremity muscle strength
5. Lower extremity muscle strength

Application of hUCMNCs was effective in the therapy of subjects with stroke. For better results, it is our recommendation for subjects with ischemic and hemorrhagic stroke to have at least two IT+IV administrations of hUCMNCs. To obtain statistical significance regarding the improvement of post-stroke symptoms after hUCMNC therapy and to relate the efficiency of stem cell transplantation to age, gender, time or cause of injury, further prospective studies and inclusion of control group are warranted.

- **Long-term Safety Study of Umbilical Cord Blood Mononuclear Cell Therapy**

  Long-term follow-up is important to show the safety of stem cell therapy, but there are not a lot of published research monitoring long-term effect of umbilical cord blood stem cell therapy.

  Our long-term safety study of 30 research subjects, who received umbilical cord blood mononuclear cell therapy in the period 2010-2013, is underway. Administration routes included intravenous (IV) infusion and intrathecal (IT) injection. Short-term follow-up results indicate, that subjects did not develop adverse effects, further demonstrating the safety of therapy. With the application of adverse event/serious adverse event form we are monitoring long-term adverse effects that are possibly related to stem cell therapy. Preliminary results indicate that 17 research subjects did not develop related to therapy adverse effects/medical conditions 4-7 years after stem cell administration, and both IV and IT administration routes were safe.