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Brian Mehling, MD
Orthopedic Surgeon
CMO, Blue Horizon International

STEM CELL THERAPY IN REGENERATIVE MEDICINE
MSCs in clinical trials

Stem cell research, or regenerative medicine research, holds enormous promise for the future advancement of medical care. A tremendous growth has been observed in last decade in stem cell research especially Mesenchymal Stem Cells (MSCs), with a ratio of 100% increase.
Emerging Applications of Stem Cell and Regenerative Medicine to Sports Injuries

Cell-based therapies and regenerative medicine offer safe and potentially efficacious treatment for sports-related musculoskeletal injuries.

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with head trauma. Although initially believed to affect only boxers, the at-risk population has expanded to encompass a much wider demographic, including football players, hockey players, wrestlers, and military veterans.

There are more than 772 adult stem cell clinical trials approved by the US Food and Drug Administration that are currently being conducted with ongoing recruitment, and many of these involve investigations of sports-related injuries.
Blue Horizon International (BHI), LLC is a healthcare consulting company with a unique concept that combines treatment, research, and philanthropy.

BHI provides the highest quality care and service available in surgical and medical treatments globally, the most advanced technologies currently available in areas such as stem cell therapy, regenerative medicine, immunocancer therapy, and also provides resources for those who are medically underserved.

Our research studies designed to measure the safety and efficacy of intravenous, intra-articular, and intrathecal stem cell treatments. Our comprehensive stem cell protocols employ well-targeted combinations of autologous bone marrow stem cells, autologous adipose stem cells, and allogeneic human umbilical cord mesenchymal stem cells that treat various conditions such as spinal cord injury, traumatic brain injury, and chronic inflammation.
Where we are

Current locations: Wuhan/China, Malacky/Slovakia and Berlin/Germany.

We recently completed pre-FDA, Phase I trials in the United States under a private IRB; Phase II trials in Slovakia; and are preparing for more pre-clinical trials in the USA, Israel, Germany, Hong Kong and Belize.

In collaboration with our local partner in Wuhan, China – cancer patients are being treated in a Phase III trial at the Department of Stem Cells in the Wuhan Hongqiao Brain Hospital using dendritic and killer T-cell therapies. We are about to extend that cancer treatment to our Slovakian facility. Our company is effectively a conduit between potential patients and some of the most prestigious scientific pioneers of cell therapy in the world.
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- **Annie Campbell** – Marketing and Public Relations
Wuhan
- One of the oldest cities in China
- Economic Center of Central China
- 10 million inhabitants

Wuhan University
- 50,000 students
- 5,000 faculty members
- Exchange Program with the University of Trier, Germany

School of Basic Medical Science
- Top 10 Medical School in China
Leader in Spinal Cord Injury Stem Cell Therapy

Collaboration with leading scientific institutions
- China: Wuhan University
- World: Harvard University

Basic clinical research facilities
- Focus on using stem cells to treat untreatable neuronal disease.
- Achievements: more than 600 patients treated to date;
- 75% got clear improvement.
- Sources

- Umbilical cord blood + tissue
  ✓ Rich source of hematopoietic stem and progenitor cells as well as non-hematopoietic stem cells such as: endothelial cells, mesenchymal stem cells (MSCs) and unrestricted somatic stem cells (USSC).
  ✓ Cells from UCB can be used directly after isolation without expansion.
  ✓ The human leukocyte antigen type (HLA) does not need a closely-matched-donor for non-hematopoietic purposes (growth factor production, immune modulation), because these cells are less likely to induce immunological reactions.

- Placenta
  ✓ The human placenta has far more stem cells than there are in umbilical cord blood and they can be easily, safely extracted.

- Origin: Donors / China
Technology (2)

Autologous Stem Cell Therapies

- Sources
  - Adipose tissue
  - Bone marrow

- Preparation: Expansion

- Treatment Modalities
  - Intravenous infusion
  - Intrathecal injection
  - CT-guided injection
  - Local injection
Malacky Hospital is a private hospital located in the western part of Slovakia. It offers high-quality, comprehensive, and customized healthcare solutions to organizations and individuals.

Blue Horizon International Slovakia is licensed by the Ministry of Health of the Slovak Republic to provide adipose stem cell therapies for orthopedic joint applications - knees, hips, shoulders, and ankles. Procedures utilize cutting-edge technology and adult stem cells only. Stem cell therapy is primarily intended for patients with a diagnosis of joint osteoarthritis, whether being a gonarthrosis or coxarthrosis of first and second degree.

BHI Therapeutic Sciences Slovakia offers a novel method of arthritis therapy using a patient’s own stromal vascular fraction (SVF) cells including mesenchymal stem cells.
Stem Cell Therapy Process (adipose tissue derived MSCs)

Removal of adipose tissue under local anesthesia

Isolation of stromal vascular fraction with MSCs

Preparation ready for administration

Lipoaspirate after extraction

Administration of preparation into the hip joint
Stem cells administration modalities for the therapy of sport injuries

**Musculoskeletal disorders**

- One intra-articular injections of adipose tissue derived MSCs
- One IV infusion and one intra-articular injection of umbilical cord blood derived MSCs

**Therapy duration:**
- one week

**Neurological Disorders (TBI, CTE)**

- Three intravenous infusions of umbilical cord blood derived MSCs
- Three intrathecal injections of umbilical cord blood derived MSCs

**AND**

**Therapy duration:**
- three weeks
Clinical Experience – TBI

Patients Treated To Date – 50

Stem Cell Source – Human Umbilical Cord blood stem cells

Treatment Modality – IV + Intrathecal

Application Frequency – Three times in three weeks

Remarks - Significant improvement
21-year-old male

Pre Therapy
- Traumatic brain injury level II
- T11-12 vertebral fracture, spinal cord injury
- Lower extremity muscle strength level: 0
- Sensory loss
- Urinary incontinence

Three Weeks Post Regenerative Stem Cell Therapy
- Bilateral lower extremity muscle strength level: 3
- Tactile and proprioceptive sensation restored
- Urinary and stool incontinence partly improved
- Walks on crutches or with assistance
42-year-old male

Pre Therapy
Traumatic brain injury level II, coma, sensory loss, partial loss of consciousness, speech, language, memory, mobility and recognition of other people, head pressure, edema.

Three Weeks Post Regenerative Stem Cell Therapy
Improvements in speech and understanding, walking and sensorimotor coordination, reduced inflammation/edema, reduced head pressure, and reduced abnormal laughing and crying.
Our study of blood test markers of 28 patients with Musculoskeletal conditions demonstrates that there are no significant changes before and after stem cell treatment. At the same time, to characterize the results of the blood tests, the data were distributed into four groups. 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>
<thead>
<tr>
<th></th>
<th>Improvements, %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42.9±9.5*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>No Changes, %</td>
<td>35.7±9.2</td>
</tr>
<tr>
<td>3</td>
<td>Improvements and Deteriorations, %</td>
<td>14.3±6.7</td>
</tr>
<tr>
<td>4</td>
<td>Deteriorations, %</td>
<td>7.1±4.5</td>
</tr>
</tbody>
</table>

*Mean±Std. Error
Musculoskeletal conditions – Efficacy of human umbilical cord blood MSC therapy

The SF-36 is a patient-reported survey of patient health and an indicator of overall health status. The calculated scores from the patients’ SF-36 questionnaires 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).
In 2015, 73 patients underwent the therapy with their own stromal vascular fraction cells (a total of 127 applications). Sixty-four patients have results from follow-up evaluation. Male to Female ratio is 37:27. Average age is 58.5 years. Affected areas include knees (37 patients), hips (25 patients), hips and knees (2 patients).
Results from six month follow-up evaluation of 48 patients

Pain - Six months after application
- 1 (2.1%) No pain relief
- 37 (77.1%) Pain relief
- 10 (20.8%) No answer provided

Mobility - Six months after application
- 1 (2.1%) No improvement
- 32 (66.7%) Improved mobility
- 15 (31.3%) No answer provided

Application of painkiller medications six months after therapy

33 Patients were taking medications before therapy.
13 Patients completely stopped taking medication six months after therapy.
Initial MRI examination of hip (62-year-old woman) showed stage II hip coxarthrosis and bilateral reduction of cartilage volume.

Five months after the application of stem cells, MRI examination showed increase in cartilage volume.

Pre Therapy

Post Therapy
Initial MRI examination of knee (26-year-old man) showed significant reduction of cartilage on medial femoral condyle and adjacent bone edema.

Six months after application of stem cells, MRI examination showed increase in cartilage volume.

MRI Results (2)
Initial MRI examination of left hip (52-year-old man) showed coxartrosis gr. III; the marked edema of the femoral head with the transition to femoral neck.

Six months after application of stem cells, MRI examination showed significant regression of edema in bone, decrease in intra-articular fluid volume, discrete cartilage growth.
Initial MRI examination of right knee (57-year-old man) showed chondropathies FT media compartment gr. IV., in the lateral compartment gr. III., significant bone edema in lateral condyle and tibial bone cyst. Six months after application of stem cells, MRI examination showed small changes in the cartilage of both compartments, significant regression of bone edema, lateral condyle of the tibia.

Pre Therapy

Post Therapy
Initial MRI examination of hip (64-year-old man) showed right coxartrosis gr. III-IV, with bone edema of the head and the acetabulum with chondropaty gr. IV., degenerative bone cysts. Six months after application of stem cells, MRI examination showed partial regression of bone edema of the humeral head.
Initial MRI examination of hip (65-year-old woman) showed right coxartrosis gr. III., chondropathies gr. III-IV, mild edema and bone overgrowth of intra-articular fluid; left chondropathies gr. II-III, coxartrosis gr. II.

Six months after application of stem cells, MRI examination showed slight bilateral improvement in both hip joints, partial regression of edema and synovial fluid of right cartilage and bone.

Pre Therapy

Post Therapy
Initial MRI examination of left knee (56-year-old woman) showed femoropatellar chondropathies gr. IV.,
Arthrosis gr. III. FP.
Six months after application of stem cells, MRI examination showed slight increase in cartilage on medial facets
and partial regression of edema on the lateral facet.

MRI Results (7)
Our surgical balm is derived from conditioned medium (CM). While growing, stem cells release in culture medium biologically active substances and structures, such as cytokines, growth factors, enzymes. Stem cell-derived CM has a promising prospect for regenerative medicine. Our CM contains key cytokines and growth factors responsible for skin regeneration and healing. Our surgical balm has been designed to help to reduce any scar that is present on your skin in a healthy and safe way.

Research study title is “Safety study of post-surgical balm application for scars reduction”. The primary purpose of this study is to evaluate the safety of surgical balm application, and secondly, to capture efficacy of surgical balm in reduction of scar appearance.
### Chemokine assay results

<table>
<thead>
<tr>
<th>CYTOKINE</th>
<th>UNIT</th>
<th>CONTROL AVERAGE ± STD.ERROR</th>
<th>CM AVERAGE ± STD.ERROR</th>
<th>STATISTICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL21</td>
<td>pg/mL</td>
<td>30 ± 12</td>
<td>485.5 ± 5.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCL13</td>
<td>pg/mL</td>
<td>18 ± 0</td>
<td>171.5 ± 3.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCL27</td>
<td>pg/mL</td>
<td>15 ± 3</td>
<td>603.5 ± 8.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCLX5</td>
<td>pg/mL</td>
<td>208.5 ± 9.5</td>
<td>7458.5 ± 133.5</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL11</td>
<td>pg/mL</td>
<td>11.5 ± 2.5</td>
<td>101 ± 4</td>
<td>YES, P = &lt;0.003</td>
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<tr>
<td>CCL24</td>
<td>pg/mL</td>
<td>14.5 ± 9.5</td>
<td>332.5 ± 7.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCL26</td>
<td>pg/mL</td>
<td>8.5 ± 3.5</td>
<td>61.5 ± 3.5</td>
<td>YES, P = 0.009</td>
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<tr>
<td>CCL19</td>
<td>pg/mL</td>
<td>25 ± 3</td>
<td>73 ± 9</td>
<td>YES, P = 0.037</td>
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<tr>
<td>CX3CL1</td>
<td>pg/mL</td>
<td>36.5 ± 6.5</td>
<td>749 ± 17</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CXCL6</td>
<td>pg/mL</td>
<td>36.5 ± 1.5</td>
<td>232 ± 9</td>
<td>YES, P = 0.002</td>
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<tr>
<td>GM-CSF</td>
<td>pg/mL</td>
<td>26.5 ± 2.5</td>
<td>48 ± 3</td>
<td>YES, P = 0.031</td>
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<tr>
<td>CXCL1</td>
<td>pg/mL</td>
<td>13 ± 1</td>
<td>85 ± 3</td>
<td>YES, P = 0.002</td>
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<tr>
<td>CXCL2</td>
<td>pg/mL</td>
<td>21.5 ± 7.5</td>
<td>503.5 ± 11.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>IFN-gamma</td>
<td>pg/mL</td>
<td>19.5 ± 2.5</td>
<td>50 ± 2</td>
<td>YES, P = 0.011</td>
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<tr>
<td>IL-1b</td>
<td>pg/mL</td>
<td>8.5 ± 3.5</td>
<td>78 ± 5</td>
<td>YES, P = 0.008</td>
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<tr>
<td>IL-2</td>
<td>pg/mL</td>
<td>2.5 ± 0.5</td>
<td>15 ± 1</td>
<td>YES, P = 0.008</td>
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<tr>
<td>IL-4</td>
<td>pg/mL</td>
<td>4.5 ± 0.5</td>
<td>222 ± 3</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>IL-6</td>
<td>pg/mL</td>
<td>25.5 ± 3.5</td>
<td>45 ± 3</td>
<td>NO*, P = 0.052</td>
</tr>
<tr>
<td>IL-8</td>
<td>pg/mL</td>
<td>10.5 ± 2.5</td>
<td>60.5 ± 2.5</td>
<td>YES, P = 0.005</td>
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<tr>
<td>IL-10</td>
<td>pg/mL</td>
<td>36 ± 16</td>
<td>180.5 ± 4.5</td>
<td>YES, P = 0.013</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CYTOKINE</th>
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<th>STATISTICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-16</td>
<td>pg/mL</td>
<td>26.5 ± 1.5</td>
<td>333.5 ± 8.5</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CXCL10</td>
<td>pg/mL</td>
<td>11 ± 1</td>
<td>170 ± 1</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CXCL11</td>
<td>pg/mL</td>
<td>15.5 ± 0.5</td>
<td>184 ± 4</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL2</td>
<td>pg/mL</td>
<td>19 ± 0</td>
<td>211.5 ± 6.5</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL8</td>
<td>pg/mL</td>
<td>1.5 ± 1.5</td>
<td>39 ± 3</td>
<td>YES, P = 0.008</td>
</tr>
<tr>
<td>CCL7</td>
<td>pg/mL</td>
<td>2.5 ± 0.5</td>
<td>15 ± 3</td>
<td>NO*, P = 0.054</td>
</tr>
<tr>
<td>CCL13</td>
<td>pg/mL</td>
<td>0.5 ± 0.5</td>
<td>14.5 ± 0.5</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCL22</td>
<td>pg/mL</td>
<td>44.5 ± 3.5</td>
<td>533 ± 9</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>MIF</td>
<td>pg/mL</td>
<td>13.5 ± 1.5</td>
<td>167 ± 6</td>
<td>YES, P = 0.002</td>
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<tr>
<td>CXCL9</td>
<td>pg/mL</td>
<td>11 ± 1</td>
<td>151 ± 3</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL3</td>
<td>pg/mL</td>
<td>7 ± 1</td>
<td>33.5 ± 1.5</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL15</td>
<td>pg/mL</td>
<td>1.5 ± 0.5</td>
<td>11 ± 1</td>
<td>YES, P = 0.014</td>
</tr>
<tr>
<td>CCL20</td>
<td>pg/mL</td>
<td>3 ± 0</td>
<td>12 ± 2</td>
<td>YES, P = 0.046</td>
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<tr>
<td>CCL19</td>
<td>pg/mL</td>
<td>18 ± 3</td>
<td>154 ± 4</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL23</td>
<td>pg/mL</td>
<td>2 ± 2</td>
<td>37.5 ± 3.5</td>
<td>YES, P = 0.013</td>
</tr>
<tr>
<td>CXCL16</td>
<td>pg/mL</td>
<td>34 ± 4</td>
<td>1205 ± 25</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>pg/mL</td>
<td>59 ± 3</td>
<td>1861 ± 14</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>CCL17</td>
<td>pg/mL</td>
<td>10.5 ± 1.5</td>
<td>124 ± 4</td>
<td>YES, P = &lt;0.001</td>
</tr>
<tr>
<td>CCL25</td>
<td>pg/mL</td>
<td>80.5 ± 11.5</td>
<td>1911 ± 29</td>
<td>YES, P = &lt;0.001</td>
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<tr>
<td>TNF-alpha</td>
<td>pg/mL</td>
<td>8.5 ± 3.5</td>
<td>52 ± 8</td>
<td>YES, P = 0.038</td>
</tr>
</tbody>
</table>

*) The calculated P is not much higher than P=0.05
Bio-Plex Pro Human Chemokine 40-plex Panel is an all-in-one premixed kit for the detection and quantification of 40 chemokines. In order to characterize chemokines in CM, the assays were done using two separate experiments, with each sample tested three times within each experiment. The control media is without conditioning.

In our study of CM, immunoassay tests showed that all analyzed chemokines gave signals in the assays in positive control parallel wells. Thus all chemokines were functional and accurate to the indicated levels. Statistically significant difference was revealed between control group and CM.

Stem cell derived CM therapy is a rapidly advancing field that promises to have a substantial impact on the treatment of different diseases/conditions. Therefore, gaining a more complete characterization of growth factors and cytokines in the stem cell-derived CM is crucial.
"Stem cell treatments are without a doubt the future of medicine..."
Dr. Brian Mehling. PR Newswire: Biotechnology, November 2013

THANK YOU!