Autologous Marrow Derived Stem Cells Transplantation in Spinal Cord Injury Patients

Dr. Ravindra Pratap Singh
Consultant- Transfusion Medicine
Department of Transfusion Medicine
Aditya Birla Memorial Hospital, Pune

MD – Transfusion Medicine (PGIMER, Chandigarh)
Fellow in Transfusion Science (UK); Fellow in Intensive Care Medicine (Hyderabad)
Regenerative Medicine

- **Regenerative medicine** is a branch of translational research in tissue engineering and molecular biology which deals with the "process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal function".

- **Translational research** aims to "translate" findings in basic research into medical and nursing practice with meaningful health outcomes. i.e. research implements a “bench-to-bedside”,
Regenerative Medicine

• The term "regenerative medicine" was first found in a 1992 article on hospital administration by Leland Kaiser.

• The widespread use of the term regenerative medicine is attributed to William Haseltine (founder of Human Genome Sciences), 2003.
  – This field holds the promise of engineering damaged tissues and organs via stimulating the body's own repair mechanisms to functionally heal previously irreparable tissues or organs.
  – Regenerative medicine also includes the possibility of growing tissues and organs in the laboratory and safely implanting them when the body cannot heal itself.
  – If a regenerated organ's cells would be derived from the patient's own tissue or cells, this would potentially solve the problem of the shortage of organs available for donation, and the problem of organ transplant rejection.

• Applications of Regenerative Medicine Through:
  – Stem cells
  – PRP
INTRODUCTION

• Stem Cells – Biological cells that can divide and differentiate into diverse specialized cell types, and can self-renew

• In mammals, there are three broad types of stem cells:
  – **Embryonic stem cells** – obtained from inner cell mass of blastocysts (7-10 days post fertilization).
  – **Fetal stem cells** – obtained from aborted fetuses.
  – **Adult stem cells** – Found in various tissues of human body, e.g. BM, brain, fat, dental pulp etc.
INTRODUCTION

- Stem Cells are obtained by
  - Bone Marrow
  - Umbilical cord blood
  - Adipose tissue
  - Peripheral Blood
  - Dental Pulp
  - Brain etc.

- Stem Cell Therapy - Potential to dramatically change treatment of human disease

- Common example of successful stem cell therapy is bone marrow transplantation in patients of leukaemia
6.0 Categorization of research on stem cells

According to the source of stem cells and nature of experiments, the research on human stem cells is categorized into following three areas:

- Permissible research areas
- Restricted research areas
- Prohibited research areas

6.1 Permissible areas of research

6.1.6 Clinical trials with cells processed as per National GTP / GMP guidelines (minimally manipulated or manipulated with alteration in functionality or genetic characteristics) may be carried out with prior approval of IC-SCRT/ IEC/DCGI (for marketable product) as applicable.

All clinical trials on stem cells shall be registered with NAC-SCRT through IC-SCRT.

13.0 Use of stem cells for therapeutic purposes

13.1 As of date, there is no approved indication for stem cell therapy as a part of routine medical practice, other than bone marrow transplantation (BMT). Accordingly all stem cell therapy other than BMT (for accepted indications) shall be treated as experimental.
### IC-IEC for Clinical Trials

<table>
<thead>
<tr>
<th>Institutional &amp; Ethical Committee (IC-IEC) Members</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Hemang Vasavad (MCh –Neurosurgery)</td>
<td>Chairperson</td>
</tr>
<tr>
<td>Mr. Bhupendra Sachde</td>
<td>Secretary General</td>
</tr>
<tr>
<td>Dr. Bhaumik Bayani (MCh –Plastic Surgery)</td>
<td>Scientific Person</td>
</tr>
<tr>
<td>Dr. Bhavesh Sachde (MS-Ortho)</td>
<td>Scientific Person (Other Institute)</td>
</tr>
<tr>
<td>Mr. Kishor Kotak</td>
<td>Social Worker</td>
</tr>
<tr>
<td>Mr. Udyan Devmurari</td>
<td>Legal Advisor</td>
</tr>
<tr>
<td>Mrs. Heena Majithiya</td>
<td>Lady Social Worker</td>
</tr>
<tr>
<td>Mr. Maulik Kotak</td>
<td>Media Preson</td>
</tr>
</tbody>
</table>
POTENTIAL USAGE OF STEM CELLS

- Stroke
- Traumatic brain injury
- Learning defects
- Alzheimer's disease
- Parkinson's disease
- Missing teeth
- Wound healing
- Bone marrow transplantation (currently established)
- Spinal cord injury
- Osteoarthritis
- Rheumatoid arthritis
- Baldness
- Blindness
- Deafness
- Amyotrophic lateral sclerosis
- Myocardial infarction
- Muscular dystrophy
- Diabetes
- Multiple sites: Cancers
- Crohn's disease
Autologous Marrow Derived Stem Cells Transplantation in Spinal Cord Injury Patients
INTRODUCTION

• Approximately 12,000 new cases of spinal cord injury (SCI) occur per annum in the US, with about 300,000 patients living with neurological consequences [1].

• The annual incidence of SCI is 12.1 – 57.8 cases per million. Although >80% of world's population lives in > 100 developing countries, little information is available regarding epidemiology of SCI in these countries. [2]

• Post-injury medical interventions are aimed at treatment of complications such as autonomic dysreflexia, pain, and urinary tract infections.

• Regenerative approaches using growth factors and various cell therapies are particularly appealing with early clinical reports of improvement using autologous bone marrow cells [3-5].

References:
BACKGROUND

• Transplanted bone marrow stem cells (BMSC) have been found to improve neurologic disease in central nervous system (CNS) injury models by generating neural cells or myelin-producing cells.

• The results in treated patients and animal models suggest that bone marrow derived stem cells could potentially be used as a therapy for spinal cord injury (SCI) patients.

• In our study, autologous bone marrow derived stem cells (BMSC) were transplanted along with granulocyte-colony stimulating factor (G-CSF) administration through clinical trial in 16 spinal cord injury patients (study group 8; control group 8) from September 2011 to December 2012.
METHODS

• Study Group:
  – Eight patients with acute and chronic complete Spinal Cord Injury (SCI) with American Spinal Injury Association (ASIA) Impairment Scale (AIS) and Frankel Impairment Scale Grade A were included in this study.
  – These group received Stem Cells Therapy & G – CSF as per Clinical Trial Protocol.

• Control Group:
  – Eight patients as control group with acute and chronic complete Spinal Cord Injury (SCI) with American Spinal Injury Association (ASIA) Impairment Scale (AIS) and Frankel Impairment Scale grade A were also included in this study.
  – These patients were managed by conventional medical/surgical decompression treatment and none of them received Stem Cells or G-CSF.
Patient Selection Criteria

- Transplantation protocols were approved by institutional review board and all procedures were performed after obtaining written informed consent.
- The inclusion and exclusion criteria of the study are summarized below:
  - **Inclusion criteria**
    - Traumatic spinal cord injury.
    - Age between 16 and 65 years
    - Spinal cord injury with single lesion of ASIA* Impairment Scale (AIS)/Frankel grade A.
  - **Exclusion criteria**
    - Women who are pregnant or lactating
    - Penetrating trauma
    - Fever (above 39°C)
    - Ventilator assistance
    - Anatomical transaction of the cord visualized by MRI
    - Serious pre-existing medical diseases

*ASIA – American Spinal Injury Association*
AIS & FRANKEL SCALES

AIS SCALE:

A = Complete: No motor or sensory function is preserved in the sacral segments S4-S5

B = Incomplete: Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5

C = Incomplete: Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3

D = Incomplete: Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more

E = Normal: motor and sensory functions are normal

FRANKEL SCALE:

A = complete paralysis

B = sensory function below the injury level only

C = incomplete motor function below injury

D = fair to good motor function below injury level

E = normal function
MATERIAL & METHODS

• The BMSC were collected through posterior superior iliac spine by multiple aspiration with bone marrow aspiration needle (16 gauge) and average 150 ml (range from 100 ml to 175 ml) were harvested and collected in CPDA1 anticoagulated blood bag with maintaining 1:7 anticoagulation to bone marrow ratio.

• The entire procedure was done in operating room under aseptic condition. The collected bone marrow stem cells were processed on Sepax, Biosafe, USA and final 20 ml BMSC collected in close system and rest were discarded in waste bag.

• Final Processed BMSC was transplanted by injection into the surrounding area of the spinal cord injury site through open spinal surgery.

• In the control group, all patients (n = 8) were treated only with conventional decompression and fusion surgery without BMSC transplantation.
**MATERIAL & METHODS**

A *Post Tx G-CSF (Filgrastim) injection schedule:*

After surgery, a total of five cycles (daily for the first 5 days of each month over 5 months) of G-CSF is injected subcutaneously at the dose of 10 ug/kg body weight.
### Table 1: Patient demographic summary

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study Group (n=8)</th>
<th>Control Group (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age in Years</td>
<td>32.3 (19 – 58)</td>
<td>35.15 (21 – 44)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Injury site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Thoraco-lumber</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
RESULTS

• The patients underwent preoperative and follow-up neurological assessment using the American Spinal Injury Association Impairment Scale (AIS), physiological monitoring.

• The mean follow-up period was 6 months after transplantation. Furthermore, the BMC transplantation and G-CSF administration were not associated with any serious adverse clinical events increasing morbidities.

• All study group patients were completed the protocols successfully.
# Table 2: Patient's Neurological assessment Scales (Before, After 1 Month & 6 Months)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>8</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before Transplant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathic Pain</td>
<td>Not Present</td>
<td>Not Present</td>
</tr>
<tr>
<td>Frankel Neurological Scale and AIS (Grade A-E)</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td><strong>At 1 Month of Transplant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathic Pain</td>
<td>1- Present (Pain Assessment Scale 5/10)</td>
<td>1- Present (Pain Assessment Scale 2/10)</td>
</tr>
<tr>
<td></td>
<td>7 Cases – Grade B</td>
<td>8 Cases – Grade A</td>
</tr>
<tr>
<td></td>
<td>1 Cases - Grade – A</td>
<td></td>
</tr>
<tr>
<td>Frankel Neurological Scale and AIS (Grade A-E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>At 6 Months of Transplant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathic Pain</td>
<td>Not Present</td>
<td>Not Present</td>
</tr>
<tr>
<td>Frankel Neurological Scale and AIS (Grade A-E)</td>
<td>5 Cases - Grade C</td>
<td>1 Cases - Grade B</td>
</tr>
<tr>
<td></td>
<td>2 Cases - Grade – B</td>
<td>7 Cases - Grade A</td>
</tr>
<tr>
<td></td>
<td>1 Cases - Grade – A</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

• Our study suggests that for functional recovery of damaged spinal cord:
  – Human BMSC can be transplanted into spinal cord at contusion or injury site.
  – G-CSF also given after transplant and on monthly basis for 5 months for 5 days at the dose of 10 ug/kg body weight with close monitoring of patient to observe any side effects related to G-CSF.

• All patients managed successfully without any complications except mild fever and body ache which were treated with paracetamol antipyretic and assurance.
Therapeutic strategy of BMC transplantation and G/M-CSF

• Several Clinical Trials explored the hypothesis of SCT may enhance recovery of neurological function.
  – However, significant recovery by only SCT rarely archived and raised concern about treatment protocols. [1-2]

• Recently reported that hematopoietic cytokines (G-CSF, GM – CSF, Erythropoietin) had NEUROPROTECTIVE EFFECTS and improve neurological functions after CNS injury. [3-7]

• On evidence based, our study was planned to treat SCI patients with combined approach of BMSC and G-CSF.

References:
Probable Mechanism of Action of G/M-CSF:

**Direct Pathway:** stimulates and mobilizes the bone marrow stem cells.

**Indirect Pathway:** G/M-CSF can have intrinsic spinal cord repair mechanisms, including:

- Neuroprotection from apoptosis
- Endogenous stem cell activation
- Inhibition of glial scar formation
- Microglial cell activation.

Reference: SEUNG HWAN YOON, YU SHIK SHIM, YONG HOON PARK, JONG KWON CHUNG, JUNG HYUN NAM, MYUNG OK KIM, HYUNG CHUN PARK, SO RA PARK, BYOUNG-HYUN MIN, EUN YOUNG KIM, BYUNG HYUNE CHOI, HYEONSEON PARK, YOON HA. Complete Spinal Cord Injury Treatment Using Autologous Bone Marrow Cell Transplantation and Bone Marrow Stimulation with Granulocyte Macrophage-Colony Stimulating Factor: Phase I/II Clinical Trial. STEMCELLS 2007;25:2066–2073
DISCUSSION

• In our study group, good neurological recovery observed at 1 month and 6 months of follow up with satisfactory sensory and motor power improvement in 7 patients along with psychological improvement as well.

• One patient does not responded, probably due to major spinal cords tracts damage which were beyond regeneration of transplanted marrow cells.

• The control group were not shown any improvement as compared to study group.

• Neuropathic Pain: In study group (Pain Assessment Scale 5/10) & in control group (Pain Assessment Scale 2/10), at one month of follow up one patient each complained pain at lumber region and lower limb.
  – The pain intensity minimized on regular follow up and none of both patients had pain at 6 months of follow up.
  – Probable explanation of neuropathic pain is development of “ABBRENT AXONAL REGENRATION” at injured and transplanted spinal cord site.
DISCUSSION

OTHER POTENTIAL SOURCES OF STEM CELLS FOR SCI

1. **Olfactory ensheathing cells:**
   - Administration of olfactory ensheathing cells across transected spinal cord in several models has resulted in axonal regeneration and restoration of conduction velocity

2. **Schwann cells:**
   - Schwann cells possessed ability to regenerate sensory axons from the dorsal root ganglia and propriospinal axons adjacent to the injury site

3. **Adipose – derived stem and progenitor cells:**
   - Mesenchymal stem cells derived from adipose tissue have been extensively described in the literature, including significant support for the ability of these progenitors to differentiate into many neural cell types

4. **Cord/placenta derived cells:**
   - Umbilical cord and Wharton’s jelly derived MSC offer unique therapeutic characteristics in comparison to bone marrow MSC

5. **Fetal/ES derived neural progenitor:**
   - Fetal-derived neurons have been shown to survive, differentiate and integrate into the host spinal cord after injury.
CONCLUSION

- We used autologous hematopoietic progenitor stem cells, which were rich in mesenchymal stem cells population along with other hematopoietic stem cells subpopulation.

- As these stem cells injected directly at spinal cord injury site through open surgery which assured higher stem cells availability through plasticity and helping in myelination and repair of damaged neurons and tracts, which was added by G – CSF injections through neuronal and microglial cells activations and gliosis inhibition.

- Autologous BMSC also avoid immunologic rejection and graft-versus-host disease (GVHD), which are frequently caused by allografts as well as no association with carcinogenesis, which sometimes occurs with embryogenic stem cell therapy.

- As our study population was comparatively small and short, a long term and large scale multicenter clinical study is required to determine its precise therapeutic effect.
THANK YOU