Stem Cell Transplantation: Current status

Lalit Kumar
Department of Medical Oncology
IRCH, All India Institute of Medical Sciences, New Delhi 110029
Science without social relevance has very little to recommend it!

Albert Einstein
Stem cell differentiation

- Blastocyst
- Embryonic Stem cells
- Endoderm
  - Liver, GIT, Lungs
    - Mesodermal progenitor cell
    - Hemopoietic progenitor cell
  - Mesoderm
    - Hemopoietic progenitor cell
- Ectoderm
  - Skin, CNS
- BM
# Types of stem cells

<table>
<thead>
<tr>
<th>Differentiation Potential</th>
<th>Number of cell types</th>
<th>Example of stem cell</th>
<th>Cell types resulting from differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Totipotent</td>
<td>All</td>
<td>Zygote blastomere</td>
<td>All cell types</td>
</tr>
<tr>
<td>Pluripotent</td>
<td>All, except cells of embryonic membranes</td>
<td>Cultured human ES cells</td>
<td>Cells from all three germ layers</td>
</tr>
<tr>
<td>Multipotent</td>
<td>Many</td>
<td>Bone marrow</td>
<td>Skeletal, cardiac muscle, liver cells, all blood cells</td>
</tr>
<tr>
<td>Quadripotent</td>
<td>4</td>
<td>Mesenchymal progenitor cell</td>
<td>Cartilage, fat and stromal cells, bone-forming cells</td>
</tr>
<tr>
<td>Tripotent</td>
<td>3</td>
<td>Glial-restricted precursor</td>
<td>2 types of astrocytes, oligodendrocytes</td>
</tr>
<tr>
<td>Bipotent</td>
<td>2</td>
<td>murine fetal liver</td>
<td>B cells, macrophages</td>
</tr>
<tr>
<td>Unipotential</td>
<td>1</td>
<td>Mast cell precursor</td>
<td>Mast cells</td>
</tr>
</tbody>
</table>
Common terms

- Stem cell therapy or cell therapy
- Haemopoietic stem cell transplant (HSCT):
  - Sources
    - Bone marrow transplant (BMT)
    - Peripheral blood stem cell transplant (PB-SCT)
    - Umbilical cord or placental blood SCT
Stem cell therapy
Stem cells found in “mature” organs

Adult Stem Cell Locations

- Brain and Spinal Cord
- Cornea and Retina
- Liver
- Lungs
- Blood Vessels
- Skeletal Muscle
- Pancreas
- Epithelia of Digestive System
- Epithelia of Skin
- Bone Marrow

Illustration by Matt Bohan, 2007
Stem Cell Clinical Trials Worldwide
Clinical Research-AIIMS

- **Cardiac**
  - Myocardial Infarction
  - Dilated Cardiomyopathy

- **Brain** : Stroke

- **Eye**
  - Limbal Stem Cell Deficiency (LSCD)
  - Age Related Macular Degeneration
  - Retinitis Pigmentosa

- **Peripheral Vascular Disease**

- **Liver** : Extra Hepatic Billary Atresia

- **Skin** : Vitiligo

 Bone Marrow- MNC
- Autologous
- No ethical issue
- No tissue rejection
Myocardial Infarction - open trial

- Patients undergoing CABG with an area of non-viable myocardium
- Bone marrow obtained from the same patient processed stem cells injected directly into myocardium at the end of CABG procedure
Intracardiac Stem Cell Injection
Group A
Stem Cell Trials - Cardiac Diseases

Perfusion FDG-PET glucose metabolism

- Reduction in scar volume & Ischemia
- Increase in viable area
- Increase in Ejection fraction

Slide courtesy: Dr S Mohanty

Heart surgery forum, 2008
Stem Cells Used for Therapy

- Bone marrow mononuclear cells
- Mesenchymal stem cells
- Skeletal myoblasts
- Multipotent adult progenitor cells
- Resident cardiovascular progenitor cells
- Endothelial progenitor cells
- Adipose derived stem cells
- Umbilical cord blood
- Blood stem cells

Routes of Injection

- Intravenous
- Intracoronary
- Intramyocardial
- Epicardial
- Intrapericardial

Possible mechanisms of functional improvement

- Paracrine secretions
- Cell fusion
- Vasculogenesis
- Transdifferentiation
- Recruitment of resident stem/progenitors
- Prevention of apoptosis
- Remodelling of matrix
Limbal Stem Cell Transplantation
N=180

VA : FC at 1ft

44 mo FU
VA : 1/60

50 months LSCT FU
VA : 3/60

Stable ocular surface and improved VA

Pre op

4 months post op

Cornea 2011

Courtsey : Dr S Mohanty
Limbal Stem Cell Transplantation (AIIMS)

Chemical Injury

PreOp

10 months Post Op

Chemical Injury

PreOp

5 months Post Op

Stable ocular surface and improved VA

Chemical Injury

Pre op

4 months post op
Liver cirrhosis due to congenital surgical anomalies

- Cause of major concern - Uniformly fatal if untreated. No specific therapy for biliary atresia.

- Liver failure, Ascitis

- Even after surgery, liver continues to worsen
Transplantation of Hair Follicle Progenitor Stem Cells in Vitiligo, N=15

Non-cultured hair follicle outer root sheath cell suspension for transplantation in vitiligo. Br J Dermatology, 2011
Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial.


CONCLUSIONS:

With the methods used, results of this hitherto first randomized controlled trial indicate that intravenous infusion of BMSCs is safe, but there is no beneficial effect of treatment on stroke outcome.
The Promise of Stem Cell Research

- Identify drug targets and test potential therapeutics
- Study cell differentiation
- Understanding prevention & treatment of birth defects

Cultured Pluripotent Stem Cells

- Toxicity Testing
- Tissues/Cells for Transplantation

- Bone marrow for leukemia & chemotherapy
- Nerve cells for Parkinson's & Alzheimer's disease
- Heart muscle cells for heart disease
- Pancreatic islet cells for diabetes
Stem cell therapy: summary

- Autologous BM stem cells: encouraging results in single centre and phase II trials
- Data from randomized trials awaited
- Cultured tissue specific stem cells: in vitro data promising

Issues:
- Dose, tracking of stem cells
- Long term fate
- Large scale production
Haemopoietic stem cell transplantation (HSCT)
Hierarchy of Haemopoiesis
## HSCT: developments

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Work</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>Barnes &amp; Loutit</td>
<td>Anti-leukemic effect of BMT in mouse model</td>
</tr>
<tr>
<td>1959</td>
<td>ED Thomas</td>
<td>1st human transplant using BM</td>
</tr>
<tr>
<td>1962</td>
<td>ED Thomas</td>
<td>First transplant in dogs using irradiation</td>
</tr>
<tr>
<td>1965</td>
<td>Mathe</td>
<td>Long term engraftment of a sibling BM</td>
</tr>
<tr>
<td>1967</td>
<td>J van Bekkum</td>
<td>Developed monkey model</td>
</tr>
<tr>
<td>1968</td>
<td>Van Rood &amp; Dausset</td>
<td>HLA system</td>
</tr>
<tr>
<td>1970</td>
<td>Bortin</td>
<td>Published data on 203 Tx, 3 survivors</td>
</tr>
</tbody>
</table>
First successful transplant was done in 1968 in Minnesota (USA) in a child with SCID from an HLA matched sibling.

E Donnall Thomas was awarded Nobel Prize in Medicine in 1990 for his work on Clinical and Experimental Transplantation.
Haemopoietic stem cell transplant: Types

- Syngeneic
- Allogeneic (HLA matched)
  - Sibling
  - Matched unrelated
- Autologous

Sources:
- Bone marrow
- Peripheral blood
- Umbilical cord
Principle

- HLA matching
- Conditioning
- Stem Cell Infusion
- Post transplant period
- Engraftment

High dose chemotherapy / RT
- To ablate patient’s BM
- Immunosuppression
- Space in BM (Niche)

Space

Immuno-suppression

Tumor cell kill
The MHC: Gene Organization

SET OF GENES ON CHROMOSOME 6 OF MAN

DISCOVERED IN 1958

MAJOR FUNCTION - TO CONTROL GRAFT REJECTION

EXTRAORDINARY POLYMORPHISM
HLA Typing

HLA Inheritance

Mother
A 1 2
B 3 4
C 5 6
DR 7 8

Father
A 9 10
B 11 12
C 13 14
DR 15 16

Child 1
A 1 9
B 3 11
C 5 13
DR 7 15

Child 2
A 1 10
B 3 12
C 5 14
DR 7 16

Child 3
A 2 9
B 4 11
C 6 13
DR 8 15

Child 4
A 2 10
B 4 12
C 6 14
DR 8 16
<table>
<thead>
<tr>
<th>Stage</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>DNA based HLA-A,B,C Full Family, Reverse SSO</td>
</tr>
</tbody>
</table>
| Stage II | DNA based matching Pt & Class I matched sib  
(i) HLA-DRB -low res RLS and/ or SSP |
| Stage III | DNA based matching Pt & Class I matched sib  
(ii) HLA-DRB High Resolution SSP and SSOP  
(iii) HLA-C, HLA-DQA1 and DQB1 SSP |
| Stage IV | Confirmatory typing SSO- Luminex based SBT  
*(Novel alleles)* |
1. Bone Marrow Harvest

- Operating room
- General anesthesia
- Prone position
- Iliac crest
- 1-2 hours
- Done as outpatient
2. Peripheral Blood Stem Cell Collection

Mobilization regimen:
- G-CSF 5-10 mcg/kg/day
- Harvesting on day 5 and 6

Venous access:
- Central catheter
- Peripheral veins
Apheresis Machines
3. Umbilical Cord Blood / Placental Blood

- Rich in Hematopoietic Stem Cells
- Capable of hemopoiesis in HLA matched recipient
- Immune System naive
- Risk and severity of Graft vs Host Disease - Low
- Mainly used in children
Collection of Cord - blood

Collection bag contains 15 ml citrate phosphate dextrose solution as anti-coagulant
Cryopreservation of Stem Cells

-80 °C

-196 °C

- Liquid Nitrogen
- Computerized Freezer Programmed To Cool at 1°C/min
- Store for many years

- Relatively inexpensive
- Rapid, No rate control required
- Highly viable cells: >95%
- Maximum of 18 months (9-10 months)
Post Freezing Evaluation of Stem Cells

Viability of Stem Cells - Trypan Blue Dye

CFU-GM Colonies (In methyl cellulose)
Stem cell enumeration by Flowcytometry

CD 45 – Pan T marker

CD 34 – Stem cell marker

Stem Cells-Giemsa Stained (Under 100X; Oil immersion)
# Phenotypic markers of HSCs

<table>
<thead>
<tr>
<th>Human HSCs</th>
<th>Mouse HSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34$^+$</td>
<td>CD34$^{\text{low/-}}$</td>
</tr>
<tr>
<td>CD59$^+$</td>
<td>Sca-1$^+$</td>
</tr>
<tr>
<td>CD90/Thy1$^+$</td>
<td>CD90/Thy1$^{+/\text{low}}$</td>
</tr>
<tr>
<td>CD38$^{\text{low/-}}$</td>
<td>CD38$^+$</td>
</tr>
<tr>
<td>c-kit$^{-/\text{low}}$</td>
<td>c-kit$^{+}$</td>
</tr>
<tr>
<td>Lin$^-$</td>
<td>Lin$^-$</td>
</tr>
</tbody>
</table>
Stem Cell: Dose

- BM: $3 \times 10^8$ / kg nucleated cells
- PBSCT: $5 \times 10^8$ / kg MNCs
- CD-34+: $2.5 \times 10^6$ / kg
- U Cord: $1 \times 10^7$ / Kg
# Conditioning Regimens

<table>
<thead>
<tr>
<th>Conventional</th>
<th>RICT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bu-Cy</td>
<td>FAB</td>
</tr>
<tr>
<td>Busulfan 4 mg/kg/dx4</td>
<td>Busulfan 4 mg/kg/dx2</td>
</tr>
<tr>
<td>Cycloph.60mg/kgx2d</td>
<td>Fludarabine 25mg/m2x4d</td>
</tr>
<tr>
<td></td>
<td>ATG 20-30 mg/kgx4d</td>
</tr>
<tr>
<td>Cyclo-TBI</td>
<td>Fludara-TBI</td>
</tr>
<tr>
<td>Cycloph.60mg/kgx2d</td>
<td>Fludarabine 25mg/m2x4d</td>
</tr>
<tr>
<td>TBI 10-12 Gys</td>
<td>TBI 2 Gys</td>
</tr>
<tr>
<td>Cyclo-ATG</td>
<td>FAB</td>
</tr>
<tr>
<td>Cycloph.60mg/kgx2d</td>
<td>Busulfan 4 mg/kg/dx2</td>
</tr>
<tr>
<td>ATG 20-40 mg/kgx4d</td>
<td>Fludarabine 25mg/m2x4d</td>
</tr>
<tr>
<td></td>
<td>Alemtuzumab (Compalh)</td>
</tr>
</tbody>
</table>
Central Line
Stem cell infusion

- BM ABO mismatch, deplete RBCs
- PBSC RBC depletion not needed

Autologous

- Remove stem cells from deep freezer, thaw to 37 degree C
- Infusion Intravenous
# Prophylaxis

## Infections

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>Antiviral</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Antifungal</td>
</tr>
</tbody>
</table>

## GVHD

<table>
<thead>
<tr>
<th>Drug</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporin</td>
<td>Day -1 onwards</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Day +1, +3, +6, +11</td>
</tr>
</tbody>
</table>
Engraftment post transplant

- Platelets

Days post transplant: 0, 5, 10

Platelets range: 100 - 1,000,000

Graph showing changes in platelet levels over time post transplant.
SAA: Bone Marrow Biopsy (SKJ)

1. Pre-Transplant (10X)
   2001-22780

2. Post transplant (day +35)
   2001-26168

3. Early Relapse (10X)
   02-20324

4. After stem cells (10X)
   03-2804
Post transplant Infections

Post- transplant Infections

Staphylococcal Infection

Pyoderma gangrenosum Infection

PULMONARY ASPERGILLOSIS

Herpes Zoster - Post transplant

Hepatosplenic
Nodular lesions, Halo sign

Air crescent sign (day 7)

Cavity with fungal wall
CMV Pneumonia

26 M, case of Refractory ALL Post allo BMT, day +44
Graft versus host disease
Graft vs Host Disease

Acute GVHD

Chronic GVHD: Oral Cavity

Acute GVHD

Chronic GVHD: Skin
Chimerism
**Chimerism:**
**Sex chromosome as a marker**

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex mismatched Tx</td>
<td>44</td>
</tr>
<tr>
<td>Evaluated</td>
<td>33</td>
</tr>
<tr>
<td>Complete</td>
<td>24 (relapse-6)</td>
</tr>
<tr>
<td>Mixed</td>
<td>07 (relapse-6)</td>
</tr>
<tr>
<td>• complete</td>
<td>01 (SAA)</td>
</tr>
<tr>
<td>Failed</td>
<td>2</td>
</tr>
</tbody>
</table>
GENESCAN READOUT ABI 377

COMPLETE CHIMERISM

REJECTION
Results
Transplant activity worldwide 1980-2009

Autologous

Allogeneic

Transplants

0 5,000 10,000 15,000 20,000 25,000 30,000 35,000

'80 '81 '82 '83 '84 '85 '86 '87 '88 '89 '90 '91 '92 '93 '94 '95 '96 '97 '98 '99 '00 '01 '02 '03 '04 '05 '06 '07 '08 '09
Indications for hematopoietic stem cell transplant

- **Multiple Myeloma**
- **NHL**
- **AML**
- **HD**
- **ALL**
- **MDS/MPD**
- **Aplastic Anemia**
- **CML**
- **Other Leuk**
- **Other Cancer**
- **Non-Malignant Disease**

- **Allogeneic (Total N=6,672)**
- **Autologous (Total N=10,302)**
Survival after Allogeneic Transplants for SAA, 2001-2011

By Donor Type and Age
## Beta Thalassemia

<table>
<thead>
<tr>
<th>Class</th>
<th>2 Yr OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>84-93%</td>
</tr>
<tr>
<td>II</td>
<td>70-84%</td>
</tr>
<tr>
<td>III</td>
<td>60%</td>
</tr>
</tbody>
</table>

Splenomegaly, fibrosis, poor Iron chelation
Probability of survival after HLA-matched sibling donor transplant for AML, by disease status, 1998-2008

Probability of Survival, %

Years

Early (N=6,898)
Intermediate (N=1,805)
Advanced (N=3,011)
Survival after HLA-identical Sibling Donor Transplants for CML, 2001-2011

- CP, 2001-2011 (N=2,560)
- AP, 2001-2011 (N=378)

P < 0.005
Transplant at I RCH-AIIMS

Sept 1990 to Sept 2014

N= 1018

Auto transplant = 801

Allo transplant = 217

(including 9 cord blood)
Allogeneic SCT: Indications

- AML
- CML
- ALL
- NHL
- HD
- CLL
Indications of autotransplant

N = 704 Adults

- MM
- HL
- NHL
- AML
- Solid tumors
Transplant indications in children

N = 143

TRM
Auto – 7%
Allo – 10.6%
Presentation
Myeloma: CR as Predictor of Survival

Number of allogeneic transplants, by disease, registered with CIBMTR 1998-2008
Trends in Transplants by Transplant Type and Recipient Age*
Unrelated Donor Stem Cell Sources by Recipient Age
Challenges in Stem cell Transplantation in India
Indian stem cell transplant registry

N = 4888

Years

No. of transplants


1 3 2 1 0 1 4 5 7 18 41 55 85 145 149 191 155 253 330 381 455 496 629 767

N = 4888
SCT: Indications (n=4888)

- **MM**: 19%
- **AML**: 18%
- **Hemoglobinopathy**: 15%
- **AA**: 10%
- **CML**: 9%
- **ALL**: 6%
- **Hodgkin Lymphoma**: 6%
- **Mature T.B.NK Cell lymphoma**: 6%
- **Solid Tumor**: 3%
- **MDS**: 3%
- **Others**: 2%
- **Congenital BM Failure**: 2%
- **Primary immune deficiencies**: 1%
- **MDS**: 3%
- **Solid Tumor**: 3%
- **Others**: 2%
- **Congenital BM Failure**: 2%
- **Primary immune deficiencies**: 1%

**INDIAN STEM CELL TRANSPLANT REGISTRY**
ALLO (N= 2969) AUTO (N=1919)

**ALLO (N= 2969)**
- Congenital BM Failure: 3%
- Others: 2%
- Primary immune deficiencies: 1%
- Mature T.B.NK Cell lymphoma: 1%
- Hemoglobinopathy: 25%
- AML: 23%
- AA: 17%
- CML: 14%
- MDS: 4%

**AUTO (N=1919)**
- Others: 3%
- Lymphoid malignancy: 1%
- Hemoglobinopathy: 25%
- AML: 11%
- AML: 6%
- Hodgkin's Lymphoma: 16%
- Mature T.B.NK Cell lymphoma: 14%
- Solid tumor: 6%
- MDS: 4%
- Congenital BM Failure: 3%
- Others: 2%
Bone Marrow Donors Worldwide

44 countries

Australian Bone Marrow Donor Registry
1,82,367

German Registry of Bone Marrow Donors (DKMS)
4,666,893

Marrow Donor Registry (India)
10,650

France Greffe de Moelle
2,01,777

Brazil Registry
2,855,244

National Marrow Donor Program (USA)
80,00,000

Anthony Nolan Research Center (UK)
4,57,520

BMDW

64 Registries
September 2012
20,000,000 Donors
2 crores
Linguistic Distribution of MDRI voluntary donors (N=10,650)
Challenges in SCT in India...

- No of centres: Small
- Personnel
  - Physicians, nurses,
  - Technical staff
- Primary Treatment, Case selection
- Cost related issues
Cost of stem cell transplant

- Probability of finding a match in family: 30-40%

- Importing stem cells from:
  - NMDP (USA): ₹ 1.5 - 2 m
  - MUD transplantation in USA: ₹ 30 - 40 m
  - MUD transplantation in India: ₹ 3 - 4 m
Stem Cell Transplantation is a team effort
Team

- Clinical Team (Residents, Nursing, Technicians)
- Cryo-preservation lab, Lab oncology
- Blood bank, Haematology
- HLA, Chimerism lab, Genetics
- Microbiology, Pathology
- Anaesthesiology (IRCH)
- Dental surgery
- Psychiatry
Roxy Paine, Conjoined, 2007. Hand-wrought stainless steel, 40 × 45 ft. This sculpture of two conjoined trees was exhibited at Madison Square Park in New York in 2007. It was fabricated from 7,000 metal pipes and rod elements of 30 different diameters.
Thank you
Medicine is the art of Knowledge and Wisdom