Role of Transfusion Medicine Consultant in Peripheral Blood Stem Cell Transplant Program

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Blood banks:
Not just a blood dispensing facility

Collaborating with clinicians
Contents

• Stem cells
• Indications
• Pre-procedure workup
• Procedure
• Cryopreservation
• Evaluation and Quality control
• Thawing and Infusion
• Clinical aspects: GVHD and GVL, engraftment
• Transfusion support
• HPC processing: positive / negative selection
• Unrelated donor registry
• Pediatric patients
• Bone marrow harvest: decline
• Our data
What are stem cells?

HSC ~ HPC

Hematopoietic Stem Cells

Hematopoietic stem cells can generate all lymphoid and myeloid lineages.
### Source of Stem cells

<table>
<thead>
<tr>
<th></th>
<th>Marrow</th>
<th>Mobilized Peripheral Blood</th>
<th>Umbilical Cord Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cell content</td>
<td>Adequate</td>
<td>Good</td>
<td>Low</td>
</tr>
<tr>
<td>Progenitor cell content</td>
<td>Adequate</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>T-cell content</td>
<td>Low</td>
<td>High</td>
<td>Very low</td>
</tr>
<tr>
<td>Risk of tumor contamination</td>
<td>High</td>
<td>Low</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>
## Classification: HSCT

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autologous</strong></td>
<td>Patients own HSCT collected and stored before patient's treatment with irr/chemo/both</td>
</tr>
<tr>
<td><strong>Allogenic</strong></td>
<td>HSCT infusion from another human into patient following treatment with irr/chemo/both</td>
</tr>
<tr>
<td><strong>Reduced Intensity/Nonmyeloablative</strong></td>
<td>Like allogeneic transplant, the stem cells are from a healthy person (the donor), but the chemotherapy given is less intensive</td>
</tr>
<tr>
<td><strong>Syngenic</strong></td>
<td>Transplant using an identical donor</td>
</tr>
</tbody>
</table>

**HSCT**

- Hematopoietic Stem Cell Transplantation
Indications: Autologous HSCT

- Hodgkin disease
- Multiple myeloma
- Non-Hodgkin lymphoma
- Neuroblastoma
- Acute myeloid leukemia
- Germ cell tumors
- Autoimmune disorders (systemic lupus erythematosus [SLE], systemic sclerosis)
- Amyloidosis
Indications: Allogeneic HSCT

- Acute myeloid leukemia
- Acute lymphoblastic leukemia
- Chronic myeloid leukemia
- Aplastic anemia
- Myelodysplastic syndromes
- Chronic lymphocytic leukemia
- Myeloproliferative disorders
- Multiple myeloma
- Non-Hodgkin lymphoma
- Hodgkin disease
- Pure red-cell aplasia
- Paroxysmal nocturnal hemoglobinuria

- Fanconi anemia
- Thalassemia major
- Sickle cell anemia
- Severe combined immunodeficiency (SCID)
- Wiskott-Aldrich syndrome
- Hemophagocytic lymphohistiocytosis
- Inborn errors of metabolism
- Epidermolysis bullosa
- Severe congenital neutropenia
- Shwachman-Diamond syndrome
- Diamond-Blackfan anemia
- Leukocyte adhesion deficiency

TMC data
Pre-procedure assessment
(within 30 days)

- HLA typing
- Complete Blood Count
- Anti HIV 1& 2
- Anti HCV
- HBsAg
- VDRL
- PS for Malarial Parasite
- Blood group
- Antibody titers
- Direct Coombs test
- Indirect Coombs test

Additional tests in patients
- CMV IgG/IgM
- Toxoplasma IgG/IgM
- Serum Electrolytes
- Serum Uric acid
- Serum Calcium
- Serum Magnesium
- LDH
- Blood Sugar – F/PP
- Liver function tests
- Renal function tests
- Prothrombin time / PTTK
Consent form for Peripheral Blood Stem Cell Collection

TATA MEMORIAL HOSPITAL
DEPARTMENT OF TRANSFUSION MEDICINE

INFORMED CONSENT

Date: _____________

I, ________________________ (full name of patient) TMH Case No. ___________ undergoing peripheral blood stem cell collection for myself have been explained by the physician from the Department of Transfusion Medicine in the language I understand about the procedure of peripheral blood stem cell harvesting. I have been explained that donor morbidity from this procedure is very small and is usually limited to procedure related discomfort, vaso vagal attacks, reversible calcium deficiency and pain at the venepuncture sites. I have been informed that I might have to undergo multiple procedures on the cell separator in order to collect the required quantity of stem cells. I have been explained that during harvesting I might have to be transfused with platelets and in rare situations I may have to be given blood from some other donor of my blood group. Understanding all these facts fully in my consciousness, I willingly give my consent for the procedure of peripheral blood stem cell harvest.

________________________________________
Signature of patient

________________________________________
Signature of witness

________________________________________
Signature of parent (in case of minor)

________________________________________
Name of witness
HLA compatibility between donor and patient

**Sibling Search**
- Best first approach
- Match found for about 30% of patients

**Extended Family Search**
- An HLA expert decides if this is of benefit
- May involve parents and other close relatives

**UNRELATED DONOR SEARCH**
- Search Australia and worldwide for one of the millions of registered donors

**Diagram**
- **A. 8 of 8 Match / 10 of 10 Match**
  - Patient
  - Donor
  - Match for DRB1 and DQ

- **B. 7 of 8 Match / 9 of 10 Match**
  - Patient
  - Donor
  - Match for DRB1 and DQ
HSC mobilization

- Mobilization, and its reciprocal process – homing – are regulated by a complex network of molecules on the surface of stem cells and stromal cells, and enzymes and cytokines released from granulocytes and osteoclasts.
Clinical Mobilization regimens

• Chemotherapy

• Combined growth factor and chemotherapy

• Hematopoietic Growth Factor (GCSF, GMCSF)
  – Dose is 5-20 µg/kg/day SC (Neupogen/Plerixafor)
  – Perform apheresis 4 days after the first dose.
  – Side effects (bone pain, headache, muscle ache, fatigue, sweating)
Poor mobilizers

- Failure to achieve a minimum level of
  - 5-20 CD34+ cells/µL in peripheral blood after completion of mobilisation regimen
  - 1-2X10^6 CD34+ cells/µL during a single apheresis procedure
  - 5X10^6 CD34+ cells/µL in all collections

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**Mozobil (plerixafor injection) Administration**

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF (10 micrograms/kg)</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Mozobil (0.24 mg/kg)</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Apheresis</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>

- Administer G-CSF (10 micrograms/kg) each morning for 4 days prior to first evening dose of Mozobil and each morning of apheresis
- Administer Mozobil (0.24 mg/kg) approximately 11 hours prior to initiation of each apheresis
Venous access

• The eligible donor must have prominent veins on both the arms.

• Autologous: Specially designed large-bore, double-lumen catheters are used.

• Most serious adverse events during apheresis are related to the use of venous catheters. This includes thrombosis, infection, bleeding or pneumothorax
Procedure
# Cell Separators

**Cobe spectra**

**Comtec**

**Amicus**

**Spectra Optia**

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**Table 33-3. Instrument Settings for Peripheral Blood Progenitor Cell Collections**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>RBC Content (%)</th>
<th>Flow Rate (mL/min)</th>
<th>Anticoagulant to Whole Blood Ratio</th>
<th>Cycle Volume (mL)</th>
<th>Number of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectra</td>
<td>1 to 3</td>
<td>60 to 150</td>
<td>1:12 to 1:15</td>
<td>Continuous</td>
<td>N/A</td>
</tr>
<tr>
<td>Amicus</td>
<td>6 to 8</td>
<td>40 to 75</td>
<td>1:12 to 1:15</td>
<td>1000 to 1400</td>
<td>7 to 14</td>
</tr>
<tr>
<td>COM.TEC</td>
<td>6 to 8</td>
<td>40 to 60</td>
<td>1:10 to 1:14</td>
<td>300 to 500</td>
<td>25 to 40</td>
</tr>
</tbody>
</table>

*Rossi’s Principles of Transfusion Medicine, 4th ed.*
Apheresis is generally continued till the following doses (per Kg body weight of the patient) are collected.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimal Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+ve cells / Kg</td>
<td>$&gt;2 \times 10^6$</td>
</tr>
<tr>
<td>MNCs / kg</td>
<td>$4-8 \times 10^8$</td>
</tr>
<tr>
<td>CFU-GM/Kg</td>
<td>$&gt;2\times10^5$</td>
</tr>
</tbody>
</table>
The optimal time for collecting PBSC is when a peripheral blood sample contains at least $20 \times 10^3$ circulating CD34+ cells/ml.
Cryopreservation

- It is a process where cells are preserved for longer periods by cooling to low sub-zero temperatures.

- Cryoprotectant like DMSO (8.7%) in equal volumes as the product is added to achieve a final concentration of 4.3%.

- DMSO prevents **ice crystal formation**, prevents formation of **toxic solute** concentrations that can result from cell dehydration and **stabilizes** the cell membrane in order to prevent damage during thawing.
Cryopreservation

Perform gently:
Exothermic reaction

Approx 100 ml can fit in one cassette
Freezing

• Controlled rate freezing
  – 1-2°C/min to -30 °C, then 2-10 °C/min to -90 °C

• Uncontrolled rate freezing
  – -70 to -80 °C

• Mechanical freezer (<=-80 °C) or in liquid nitrogen freezer (-196 °C)

• All freezers and liquid nitrogen tanks containing HPC should be regularly monitored and have alarm systems
Thawing

• Product is thawed rapidly by immersing in a 37°C waterbath just before infusion. Rapid thawing may lead to bag breakage.

• Thawing and washing may be done in laboratory to reduce DMSO toxicity
Quality Control of PBSC Product

- **TNC**: Automated cell counter (KX-21, Sysmex, Transasia)
- **MNC %**: by Microscopy
- **Viability**: Trypan Blue dye exclusion test (fresh product and before infusion)
- **Sterility** testing (Bactec/eBDS): Done after collection, after addition of DMSO and after thawing
- **CD34/CD3** enumeration: by BD FACS Calibur
• Trypan blue exclusion test to determine cellular viability. Viable cells do not take up the stain

• Performed just before infusion of the product from the segment preserved during cryopreservation
• May be infused rapidly if patients condition permits at 5-20 ml/min with a BT set. (in line filter: 170-210 µ)

• Special instructions to NOT use LR filters.

• Look out for symptoms: nausea, vomiting, hypertension, hypotension, flushing, chest tightness, cramps, bradycardia.

• If DMSO >0.8 to 1 g/kg, cardiac dysfunction and fatal dysrhythmias may occur, do multiple bag infusions over 2 days.
Engraftment

- The number of days after infusion of the graft until a defined threshold of circulating neutrophils or platelets is reached, typically the first of 3 days with PMN \( > 500/\mu L \) and platelet \( > 20,000/\mu L \), untransfused.

- Shorter engraftment times are associated with fewer complications.
BMT-GVHD

• Acute GVHD presents from a few days to 100 days after transplantation

• Skin, GIT and liver are most commonly involved

• Risk is higher with unrelated and mismatched rather than with HLA matched transplants

• Maximising the GVL effect while minimizing the GVHD is a major clinical challenge
The main effectors for both graft-versus-leukemia (GVL) effect and GVHD are T lymphocytes.

These two processes share many similar pathways, it has not been easy to separate GVL from GVHD.

Because the clinically used pan immunosuppressive therapy for GVHD prevention also results in decreased GVL effect, the success of allogeneic hematopoietic cell transplantation relies on a small and unpredictable therapeutic window at the present time.
Transfusion support

• Irradiated

• Leukoreduced

• Bacterial detection
Stem cells do not express ABO antigens
### ABO incompatible HSCT

#### Table: Transfusion Support Recommendations for ABO-Incompatible HPC Transplantation

<table>
<thead>
<tr>
<th>Recipient Blood group</th>
<th>Donor Blood group</th>
<th>O</th>
<th>A</th>
<th>B</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>Compatible</td>
<td>Major</td>
<td>Major</td>
<td>Major</td>
</tr>
<tr>
<td>A</td>
<td>Minor</td>
<td>Compatible</td>
<td>Bidirectional</td>
<td>Major</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Minor</td>
<td>Bidirectional</td>
<td>Compatible</td>
<td>Major</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>Minor</td>
<td>Minor</td>
<td>Minor</td>
<td>Compatible</td>
<td></td>
</tr>
</tbody>
</table>

#### Table: Transfusion Support Recommendations for ABO-Incompatible HPC Transplantation (continued)

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All Products</td>
<td>RBCs</td>
<td>Platelets</td>
</tr>
<tr>
<td>O</td>
<td>A</td>
<td>Recipient</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>O</td>
<td>B</td>
<td>Recipient</td>
<td>O</td>
<td>B</td>
</tr>
<tr>
<td>O</td>
<td>AB</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
</tr>
<tr>
<td>A</td>
<td>AB</td>
<td>Recipient</td>
<td>A</td>
<td>AB</td>
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<tr>
<td>B</td>
<td>AB</td>
<td>Recipient</td>
<td>B</td>
<td>AB</td>
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<tr>
<td>A</td>
<td>O</td>
<td>Recipient</td>
<td>O</td>
<td>A</td>
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<tr>
<td>B</td>
<td>O</td>
<td>Recipient</td>
<td>O</td>
<td>B</td>
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<td>AB</td>
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<td>A</td>
<td>B</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
</tr>
</tbody>
</table>

NA indicates not applicable.

1. Time period from diagnosis to transplantation.
2. Time period from transplantation to RBC engraftment.
3. Engraftment established, as indicated by direct antiglobulin testing being negative, along with 2 consecutive independent samples with the forward and reverse typing showing donor ABO status.
ABOi SCT impact on outcome

Fig 6. Impact of ABO-compatibility on survival among nonmyeloablative HCT recipients. No statistically significant differences were observed among the three cohorts ($P = 0.17$).

Wang Z et al BJH 2010
HPC processing:
Positive/Negative selection

Isolex300i to isolate stem cells

CliniMACS
Special considerations in pediatrics

• Larger volume needs to be processed.

• Venous access can be challenging. Central or femoral lines preferred in <10 yrs age group

• Pediatric donors are minors and screening for medical history is done through parents

• C/I in donor: hemodynamic instability, anemia, active infection, ACE inhibitors

• Hypocalcemia incidence more in pediatric donors
75 stem cell donor registries from 53 countries
53 cord blood registries from 36 countries
Bone marrow harvest: on the decline

Operating room needed
General /local /both anesthesia
More staff personnel needed to
Preparing syringes, perform collections and filter marrow
Overnight hospital stay for donor
Donor: back pain, absence form work
More engraftment times
More transfusion support
Increased infection and hospital stay
Overall costs high
Immunologic recovery inferior
Potentially more tumour cell contamination of product
Data (Tata Hospital)
June 2008-August 2015

- No. of Hematopoietic Stem Cell Transplantation (HSCT) : 467
Type of HSCT

- Autologous (n=251) - 53.8%
- Allogenic (n=216) - 46.2%
Distribution of ABO incompatible HSCT

- ABO compatible: 56.3%
- ABO incompatible: 43.7%
  - Major: 3.6%
  - Minor: 27.7%
  - Bidirectional: 28.7%
In HSCT patients, there is more requirement of SDP than PRBC.
Conclusion

*Disease relapse and Graft-versus-host disease* remain the two major causes of mortality with unsatisfactory progress.

*Transplant-related mortality* has decreased due to:

- improved *supportive care*, including better strategies to prevent severe *infections*
- incorporation of *reduced-intensity conditioning protocols*.

*Donor availability* has dramatically increased thanks to the international collaboration and unrelated volunteer donor registries.
"None of us is as smart as all of us."

-Ken Blanchard