Hematopoietic stem cell mobilization and collection

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Transplant Activity in the U.S.
1980-2010

- Autologous
- Related Donor
- Unrelated Donor

Transplants

'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 '10
Source of Stem Cells: 1990 <-> 2000

EBMT, Blood100:7, 2002
Autologous Stem Cell Sources by Recipient Age
2000-2009

<table>
<thead>
<tr>
<th>Year Range</th>
<th>Age ≤ 20 yrs</th>
<th>Age &gt; 20 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-2004</td>
<td>Bone Marrow (BM)</td>
<td>Bone Marrow (BM)</td>
</tr>
<tr>
<td>2005-2009</td>
<td>Peripheral Blood (PB)</td>
<td>Peripheral Blood (PB)</td>
</tr>
<tr>
<td>2000-2004</td>
<td>BM + PB</td>
<td>BM + PB</td>
</tr>
<tr>
<td>2005-2009</td>
<td>BM + PB</td>
<td>BM + PB</td>
</tr>
</tbody>
</table>
Allogeneic Stem Cell Sources by Recipient Age 2000-2009

Transplants, %

- Bone Marrow (BM)
- Peripheral Blood (PB)
- Cord Blood (CB)

Age ≤ 20 yrs

- 2000-2004
- 2005-2009

Age > 20 yrs

- 2000-2004
- 2005-2009
Bone Marrow Harvest

- Surgical procedure requiring general anaesthesia
- (Chest bone, anterior and) posterior iliac crest
- 2-3 hours of hard labour for 2 physicians
  - (+anaesthesist and nursing staff)
- Important blood loss (>1litre or 15-20 mL/kg)
  - Autologous peroperative transfusion in healthy donors
- Side effects
  - Bone and muscle pain
  - DVT and PE
  - Osteomyelitis
  - Recovery: 1-3 weeks
Apheresis: History in a nut shell

1914: John J Abel, Pharmacology Laboratory, John Hopkins University

- Αφαίρησος or « apheresis » : to take away with force
- Large amounts of plasma can be removed/replaced from dogs
Principle of apheresis: sedimentation of blood

Separation by Specific Gravity

- Plasma
- Packed Red Cells
- Buffy Coat

Platelet ± 1.040
Lymphocyte 1.050 - 1.061
Monocyte 1.065 - 1.06
Granulocyte 1.087 - 1.092
Centrifugation separates cellular components based on their density.

This requires "hard" centrifugation: this means long enough and hard enough for the cells to take an equilibrium position.
Principle of Apheresis
Peripheral Blood Stem Cells

60’s (Goodman et al):
- Hematopoietic stem cells circulate in steady state blood
- Frequency 1% of what is generally observed in bone marrow

80’s:
- First successful peripheral blood stem cell transplants in CML patients (Goldman et al)
- CFU-GM in peripheral blood markedly increase after endotoxin, dextran and chemotherapy (Cline and Gold, and Richman et al)

90’s:
- G-CSF and GM-CSF increase peripheral blood CFU-GM up to 100-fold (Dürrsen et al, Socinski et al)

2003:
- CXCR4 antibody mobilizes CD34+ cells within hours (Dale et al)
Peripheral Blood Stem Cell Harvest

WBC COUNT (/microL)

CD34 COUNT (/microL)

DAY 0  DAY 1  DAY 2  DAY 3  DAY 4  DAY 5  DAY 6  DAY 7  DAY 8

G-CSF

PBSC Collection
Peripheral Blood Stem Cell Harvest

- **WBC COUNT (µL):**
  - Week 1: 10^4
  - Week 2: 10^3
  - Week 3: 10^2

- **CD34 COUNT (µL):**
  - Week 1: 10^5
  - Week 2: 50
  - Week 3: 40

- **G-CSF** injection

- **PBSC Collection**

**CHEMOTHERAPY**
G-CSF:

1. Promotes CD34+ cell proliferation
   - CD34+ cells retained by local adhesion molecules: VCAM-1/VLA-4 and SDF-1α/CXCR4 interactions

2. Protease release from neutrophils (Elastase, Cathepsin G, MMPs)
   - Degradation of CAM-1/VLA-4 and SDF-1α/CXCR4 interactions

3. SDF-1α retention signals degraded, CD34+ cells migrate to bloodstream
Peripheral Blood Stem Cell Harvest
Leucocyte Collections

WHOLE BLOOD IN

Plasma and Platelets out

WHITE BLOOD CELLS OUT

RBC out

WHOLE BLOOD IN
Peripheral Blood Stem Cell Harvest

Safe and Painless Procedure
- Outpatient Basis

Mobilization Related Complications:
- Chemotherapy: neutropenia, infections
- G-CSF:
  - Bone and Muscle Aches, Mild Flu-like Symptoms
  - Local Redness and Erythema, rarely Urticarial Rash
  - Splenomegaly
  - No long term effects known

Harvest Related Complications:
- Blood Pressure
- Ca Depletion Symptoms

Children:
- Central venous line
- Blood priming of the set
Cell dose recommendations

Minimal requirement
- $<1.5 - 2.5 \times 10^6/kg$: delayed neutrophil recovery, and significantly delayed platelet engraftment
- $<1 \times 10^6/kg$: risk of permanent loss of engraftment

Optimal requirement: 
- $>3.5 \times 10^6/kg$: faster engraftment
- $>6 \times 10^6/kg$: lower transfusion requirements – selection bias?

Acceptable dose: $3-5 \times 10^6/kg$
- $2.5 \times 10^6/kg$ may be acceptable
- Collection 1 – $2 \times 10^6/kg$ acceptable if cost/benefit is beneficial
## Allogeneic donor complications

**BM donors (n=166) | PBSC donors (n=164)**

<table>
<thead>
<tr>
<th>Any AE</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>95 (57%)</td>
<td>107 (65%)</td>
<td></td>
</tr>
</tbody>
</table>

### Harvesting procedure-related AEs

<table>
<thead>
<tr>
<th>Any harvest-related</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>91 (55%)</td>
<td>61 (37%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Access pain</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>39 (23%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anemia</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pain back</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (10%)</td>
<td>4 (2%)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Nausea</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (6%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arthralgia</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vomiting</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pain skeletal</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (2%)</td>
<td>10 (6%)</td>
<td></td>
</tr>
</tbody>
</table>

### Filgrastim-related AEs

<table>
<thead>
<tr>
<th>Any filgrastim-related</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 (59%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Musculoskeletal</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>71 (43%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Headache</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 (12%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LDH increased</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alkaline phosphatase increased</th>
<th>BM donors</th>
<th>PBSC donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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BMT, 2003, 32:873-880. Favre et al
Allogeneic donor complications

**Figure a**
Number of donors (%)

- **BM**
- **PBSC**

<table>
<thead>
<tr>
<th>Nights of hospitalization</th>
<th>BM</th>
<th>PBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2 to 7</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>&gt;7</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**Figure b**
Number of donors (%)

- **BM**
- **PBSC**

<table>
<thead>
<tr>
<th>Days of restricted activity</th>
<th>BM</th>
<th>PBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1</td>
<td>9</td>
<td>38</td>
</tr>
<tr>
<td>2 to 14</td>
<td>70</td>
<td>59</td>
</tr>
<tr>
<td>&gt;14</td>
<td>21</td>
<td>2</td>
</tr>
</tbody>
</table>

BMT, 2003, 32:873-880. Favre et al
Allogeneic PBSC: Faster engraftment...

Neutrophil recovery

Thrombocyte recovery

Cornelissen et al, Exp Hem 2002
...but also more GVHD

Cornelissen et al, Exp Hem 2002
Unrelated donors: Bone marrow preferred?

Anasetti et al, ASH 2011 : BMT CTN 0201
- OS PBSC vs BM : 51 vs 46% (p=0.288)
- No difference in relapse, non relapse mortality and acute GVHD
- Engraftment faster in PBSC group
- Less graft failure (2.7 vs 9.1%)

More extensive cGVHD in PBSC group
- 48 vs 32 %
- Cost-effectiveness?
# Graft composition BM vs PBSC

<table>
<thead>
<tr>
<th>Cell yield a</th>
<th>BM</th>
<th>PBSC</th>
<th>PBSC/BM ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC (10^8/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>166</td>
<td>163</td>
<td>3.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–38.6</td>
<td>2.4–32.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ (10^6/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>160</td>
<td>163</td>
<td>2.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median</td>
<td>2.7</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–154.5</td>
<td>1.5–68.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (10^6/kg)</td>
<td></td>
<td></td>
<td>8.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N</td>
<td>134</td>
<td>149</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>35.7</td>
<td>300.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.6–1699</td>
<td>15.6–2123.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK (10^6/kg)</td>
<td></td>
<td></td>
<td>7.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N</td>
<td>119</td>
<td>139</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.6</td>
<td>28.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–154.5</td>
<td>0–665.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% donors with target CD34+ yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 10^6/kg</td>
<td>34</td>
<td>81</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2 10^6/kg</td>
<td>61</td>
<td>98</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*BM, 2003, 32:873-880. Favre et al*
Poor Mobilizer: Definitions

- Failed collection of minimum or target number
- Failed to reach minimal PB CD34+ count to undergo apheresis
- Risk factors for poor or failed mobilization:
  - Treatment related
    - >2 lines previous chemotherapy
    - Melphalan, Fludarabine, Gemcitabine, Bleomycine, Platinum, alkylating agents, lenalidomide
    - Radiation therapy (to the bone marrow)
  - Patient/Donor related
    - Age
    - NHL
    - Diabetes
  - Bone marrow related
    - Bone marrow involvement
    - Thrombocytopenia
Plerixafor as additional mobilizer

S Fruehauf and G Tricot, BBMT 2010
Plerixafor vs Placebo in G-CSF mobilization in NHL

DiPersio J F et al. JCO 2009;27:4767-4773
Plerixafor vs Placebo in G-CSF mobilization in MM

**A**

HR = 2.54, p < 0.001

Percent reaching target

<table>
<thead>
<tr>
<th>Days</th>
<th>Plerixafor + G-CSF</th>
<th>Placebo + G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.173%</td>
<td>0.173%</td>
</tr>
<tr>
<td>2</td>
<td>0.353%</td>
<td>0.353%</td>
</tr>
<tr>
<td>3</td>
<td>0.469%</td>
<td>0.469%</td>
</tr>
<tr>
<td>4</td>
<td>0.559%</td>
<td>0.559%</td>
</tr>
<tr>
<td>5</td>
<td>0.868%</td>
<td>0.868%</td>
</tr>
</tbody>
</table>

**B**

 Bars indicate the number of CD34+ cells/kg x 10^6

<table>
<thead>
<tr>
<th>Day</th>
<th>Plerixafor + G-CSF</th>
<th>Placebo + G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>2.29</td>
<td>1.78</td>
</tr>
<tr>
<td>Day 2</td>
<td>4.02</td>
<td>2.66</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.78</td>
<td>1.16</td>
</tr>
<tr>
<td>Day 4</td>
<td>1.33</td>
<td>0.7</td>
</tr>
</tbody>
</table>
## Plerixafor: side effects

<table>
<thead>
<tr>
<th></th>
<th>Plerixafor (n = 147)</th>
<th>Placebo (n = 151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any related adverse events, n (%)</td>
<td>95 (64.6)</td>
<td>67 (44.4)</td>
</tr>
<tr>
<td><strong>Gastrointestinal disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>27 (18.4)</td>
<td>8 (5.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>24 (16.3)</td>
<td>11 (7.3)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8 (5.4)</td>
<td>4 (2.7)</td>
</tr>
<tr>
<td><strong>General disorders and administration site conditions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>12 (8.2)</td>
<td>5 (3.3)</td>
</tr>
<tr>
<td>Injection site erythema</td>
<td>30 (20.4)</td>
<td>5 (3.3)</td>
</tr>
<tr>
<td><strong>Musculoskeletal and connective tissue disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone pain</td>
<td>14 (9.5)</td>
<td>12 (7.9)</td>
</tr>
<tr>
<td><strong>Nervous system disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>8 (5.4)</td>
<td>13 (8.6)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>11 (7.5)</td>
<td>11 (7.3)</td>
</tr>
</tbody>
</table>

Plerixafor in chemo + G CSF mobilization in NHL and MM

Plerixafor in chemo + G CSF mobilization in NHL and MM

Adverse events

Gastrointestinal disorders
- Nausea: 8 (20.0%)
- Diarrhea: 6 (15.0%)
- Flatulence: 2 (5.0%)
- Hypoesthesia oral: 2 (5.0%)

General disorders and administration site conditions
- Injection site erythema: 5 (12.5%)

Nervous system disorders
- Headache: 4 (10.0%)
- Paresthesia: 4 (10.0%)
- Dizziness: 2 (5.0%)
- Psychiatric disorders
  - Anxiety: 2 (5.0%)
  - Eye disorders: 2 (5.0%)

All patients n (%)
- Gastrointestinal disorders: 13 (32.5%)
  - Nausea: 8 (20.0%)
  - Diarrhea: 6 (15.0%)
  - Flatulence: 2 (5.0%)
  - Hypoesthesia oral: 2 (5.0%)
- General disorders and administration site conditions: 11 (27.5%)
  - Injection site erythema: 5 (12.5%)
- Nervous system disorders: 9 (22.5%)
  - Headache: 4 (10.0%)
  - Paresthesia: 4 (10.0%)
  - Dizziness: 2 (5.0%)
  - Psychiatric disorders
    - Anxiety: 2 (5.0%)
    - Eye disorders: 2 (5.0%)

Plerixafor mobilization

- Peak effect 4-9 h after administration
  - Effect sustained much longer to facilitate timing of apheresis

- Mobilized cell population has different characteristics
  - More growth phase cells
  - More CD34+CD38- cells
  - More B, T lymphocytes, more DC and NK cells
  - Increased expression of VLA-4 and CXCR4
  - Increased expression of genes that promote cell adhesion, cell motility, cell cycle and antiapoptosis

**MAY SUGGEST HIGHER REPOPULATION POTENTIAL AND FASTER IMMUNE RECOVERY**
Mobilization strategies

G CSF alone:
- Nice kinetics make apheresis timing more predictable
- Up to 40% mobilization failures

Chemotherapy + G CSF
- CT part of planned therapy
- Mobilization CT: Cyclophosphamide
- More cells mobilized but similar failure rate than G CSF alone
  - Recent studies suggest better mobilization in traditionally poor mobilizers
  - Eg incorporating mobilization into traditional lymphoma therapy reduces failure: < 3%
- In vivo purging???
  - No impact on CR rate, TTP, EFS, OS
Mobilization strategies

Plerixafor:

- Part of initial mobilization (G-CSF + P)
  - In US: similar to lower cost compared to CT + G-CSF
  - In Belgium: not possible reimbursement-wise
- Preemptive/risk adapted use
- How to identify at risk patients?
- In Belgium:
  - Only after mobilization failure
    - <15 CD34/microL
    - <2x10^6/kg collection
  - 6500 euro/day!
Peripheral blood stem cells are a safe and reliable method for graft collection in both patients and allogeneic donors.

Peripheral blood stem cells have largely replaced bone marrow in adults as a graft source.

- Autologous transplants: faster and more reliable engraftment.
- Allogeneic: similar, but concerns about cGVHD.
PBSC mobilization depends on the amount and kind of chemotherapy administered

- Think about early collection if HSCT is an option
  - But worry about graft contamination
- If mobilization fails, rescue options are at hand
  - Plerixafor
  - What about poorly mobilizing allogeneic donors?