Harvest and manipulation of the harvest

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Repopulating Potential of Hematopoietic Stem Cells - Animal Models

- Donor Bone Marrow Isolation
- Purification
- Lethal Irradiation
- Transplantation
- Recipient Secondary Transplantation
Hierarchical pathway of hemopoiesis by colony assay and phenotypic markers

- SCID Repop cells
- Cobble Stone Area forming cells
- CFU-B
- CFU-Blast
- LTC-IC
- Pre-CFC
- CFU-GEMM
- BFU-Mk
- CFU-GM
- BFU-E
- CFU-T
- HPP-CFC
- Pre-CFC
- CD34 HLA-DR CD38 CD45RA CD45R0 CD90 CD117 CD33
- SELF-RENEWAL
- S PHASE

CD34 Neg

CD34
HLA-DR
CD38
CD45RA
CD45R0
CD90
CD117
CD33
Figure 2. A schematic overview of the effects of filgrastim. Filgrastim shortens the marrow maturation time and leads to early exit of the developing neutrophils from the marrow into the blood. By increasing blood counts and the total neutrophil supply filgrastim can enhance the tissue neutrophil response. Through these effects, filgrastim may accelerate clearance of infections.
COLLECTION OF PBPC BY LEUKAPHERESIS

- Whole blood into machine
- PBPC collected in bags
- RBC + plasma returned to patient

Centrifugal separation
- collect MNCs out
- return RBC + plasma out
- Whole blood in

Patient
PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

REFERENCE PARAMETERS:

- **CD34+ CELLS**
  - > 20 uL (PBSC)
  - > 2-5 x 10^6/kg
- **CFU-GM**
  - (> 6-8 x 10^4/kg)

Ist Ematologia, Cremona
Hematopoietic Progenitor Cells
### Table 4.1: The “Gold Standard” priming procedure and timing of PBSC harvest

<table>
<thead>
<tr>
<th>Day 0:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth factor administration 1–2 times per day s.c. or</td>
</tr>
<tr>
<td>*Cy dose 1–4 g/m² + rhG-CSF 1 time per day s.c.</td>
</tr>
<tr>
<td>Day 3 or *10 / 4 or *11:</td>
</tr>
<tr>
<td>Measure blood CD34 level and proceed to apheresis when CD34+ cells &gt; 10–20/μL</td>
</tr>
<tr>
<td>Day 4 or *11–7 or *14:</td>
</tr>
<tr>
<td>Harvest a minimum of CD34+ cells/kg pt weight depending on centre experience</td>
</tr>
</tbody>
</table>

(G-CSF 5-10 ug)
Clinical impact of hematopoietic progenitor quantification (mainly CD34+ cells)

- REAL TIME ANALYSIS (less than 1 hour)
- SELECTION OF PATIENTS WHO MOBILIZE A SUFFICIENT NUMBER OF PROGENITORS AFTER A MOBILIZATION REGIMEN (> 20 μl) (SUITABLE FOR COLLECTION BY LEUKOAPHERESIS)
- TECHNIQUE OF CHOICE FOR THE CLINICAL MANAGEMENT OF PBSC COLLECTIONS (enables the optimal timing of the apheresis sessions and the number of procedures ensuring the harvest of at least 2-5 X 10^6/Kg CD34+ cells)

Hematology Section, Cremona, Italy
Clinical impact of hematopoietic progenitor quantification. II

- Accuracy and reliability in predicting three-lineage short and long-term engraftment following hematopoietic stem cell transplantation (good correlation with the clonogenic attitude of the graft)
- First step in quality assessment of hematopoietic stem cell grafts

Cremona - Italy
BM vs PBSC
PMN: speed of engraftment

ANC per µL

Days from autografting

-7 0 7 14 21 28

BMT
CPC
CD34+ CELL ABSOLUTE COUNTING:
Technical aspects

1. RBC LYSIS, WASHING, FIXATION METHOD
2. DETECTION OF VIABLE CELLS
3. USE OF NEGATIVE AND POSITIVE CONTROLS
4. MULTIPARAMETRIC ANALYSIS: 2-3-4 COLOR ANALYSIS (CD45, CD34+ SUBSETS, 7-AAD)
5. SINGLE OR DUAL PLATFORM (hematology analyzer for absolute leukocyte count)
6. TYPE OF SAMPLE ANALYZED (BM, CB, APHERESIS, fresh, thawed, age of the sample)
7. ASSESSMENT OF THE PERFORMANCE OF THE FLOW CYTOMETER
8. INTERNAL AND EXTERNAL QUALITY CONTROL
ISHAGE/ISCT protocol for CD34+ cells enumeration and apoptotic/dead cells exclusion: single platform Boolean gating strategy-lyse no wash

SHORT TERM ENGRAFTMENT

- The first day in which PMN > 500 uL after BMT. This value should be confirmed 3 times in 3 consecutive days

- Platelet > 20,000/uL. This value should be confirmed 3 times in 3 consecutive days
LONG-TERM ENGRAFTMENT
AFTER BMT (3-6 months)

Incomplete recovery: is considered for platelet count < 50,000/uL, and/or neutrophil count < 1,000/uL

Secondary graft failure is considered to have occurred if, on three consecutive days after full engraftment was documented, granulocytes have decreased to less than 500/uL, and/or platelets to < 20,000/uL.
### Table 3.1: Variables (risk factors) for harvest and engraftment of PBSC

<table>
<thead>
<tr>
<th>Harvest outcome (CD34+ cells)</th>
<th>Engraftment time (Tree lineage recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Age</td>
</tr>
<tr>
<td>Sex</td>
<td>Sex</td>
</tr>
<tr>
<td>Disease type (diagnosis)</td>
<td>Disease type (diagnosis)</td>
</tr>
<tr>
<td>Disease stage</td>
<td>Disease stage</td>
</tr>
<tr>
<td>Haematopoiesis impaired (y/n)</td>
<td>Growth factor levels</td>
</tr>
<tr>
<td>Therapy response (y/n)</td>
<td>Graft CD34+ cell number</td>
</tr>
<tr>
<td>Type of therapy</td>
<td>CD34 subsets (growth factor, chemokine</td>
</tr>
<tr>
<td>Priming regimen w. CT (y/n)</td>
<td>receptors and adherence molecules)</td>
</tr>
<tr>
<td>Blood CD34+ cell level</td>
<td></td>
</tr>
<tr>
<td>Apheresis strategy</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: A proposed algorithm for procurement of autologous grafts

Candidate for high dose therapy

Previous CT < 2 lines
- rhG-CSF 5 µg/kg s.c. x 1–2 day

Previous CT > 2 lines or RT
- High dose Cy + rhG-CSF 10 µg/kg s.c. x 1 day

Blood level of CD34+ <10/µL: Insufficient mobilisation

Blood level CD34+ cells >10–20/µL: Trigger apheresis
- >2 x 10^6 CD34+/kg harvested 1–2 days: Sufficient graft

Insufficient mobilisation: Marrow harvest, Experimental protocols EBMT: Other CT/Growth factors
- >2 x 10^6 CD34+/kg harvested 1–2 days: Sufficient graft
**Proposed definitions**  
*GITMO ad-hoc expert panel*

A patient with MM or lymphoma and candidate for ASCT is a:

| ‘Proven’ poor mobiliser | If he/she received adequate mobilisation (G-CSF dose ≥ 10 µg/kg if used alone or ≥ 5 µg/kg after chemotherapy) and he/she shows: peak CD34\(^+\) circulating cell count < 20/µL on days 4–6 after start of mobilisation with G-CSF alone or up to 18–20 days after chemotherapy and G-CSF OR < 2.5 \(\times\) 10\(^6\) harvested CD34\(^+\) cells/kg per planned SCT by ≤ 3 aphereses |
| ‘Predicted’ poor mobiliser | If he/she fulfils ≥ 1 major criterion or ≥ 2 minor criteria |
| **Major criteria** | • Failed previous mobilisation attempt  
| | • Prior extensive radiotherapy to marrow-bearing tissue  
| | • Full courses of previous therapy including melphalan, fludarabine or other therapies potentially affecting stem cell mobilisation |
| **Minor criteria** | • Advanced phase disease, i.e. ≥ 2 prior cytotoxic lines  
| | • Refractory disease  
| | • Extensive BM involvement at mobilisation  
| | • BM cellularity < 30% at mobilisation  
| | • Age > 65 years |

Olivieri et al., BMT, in press
Optimal Transplant Cell Dose (CD34+/kg)

- Probability of platelet recovery correlated with the number of CD34+ cells transplanted\(^1\)

- In a retrospective study, lack of full platelet recovery (>150 x 10^9/L) was associated with lower CD34+ cell doses\(^2\)

Patients (%) achieving ≥ 6 million CD34+ cells/kg by apheresis day (ITT population)

HR = 2.54, p < 0.0001

Kaplan-Meier estimate of proportion of patients reaching ≥ 6 x 10^6 CD34+ cells/kg

G-CSF + plerixafor (n = 148)

G-CSF + placebo (n = 154)

Figures checked with reference - all OK except for data point 3 in control arm (originally said 49.0 - replaced it with 48.9 as specified in the reference)

dominic.elliston; 4-2-2011
Transplanted CD34+ Cell Dose is Associated with Long-Term Platelet Count Following Autologous Haematopoietic Stem Cell Transplant in Patients with Non-Hodgkin’s Lymphoma and Multiple Myeloma

Patrick J Stiff, MD\textsuperscript{1}, Ivana Micallef, MD\textsuperscript{2}, Auayporn P Nademanee, MD\textsuperscript{3}, Edward A Stadtmauer, MD\textsuperscript{4}, Richard Thomas Maziarz, MD\textsuperscript{5}, Brian J Bolwell, MD\textsuperscript{6}, Gary Calandra, MD\textsuperscript{7}, Gary Bridger, PhD\textsuperscript{7}, John F DiPersio, MD, PhD\textsuperscript{8}, on behalf of 3101 and 3102 Investigators

\textsuperscript{1}Loyola University, Chicago, IL, \textsuperscript{2}Mayo Clinic, Rochester, MN, \textsuperscript{3}City of Hope, Duarte, CA, \textsuperscript{4}University of Pennsylvania, Philadelphia, PA, \textsuperscript{5}Oregon Health & Science University, Oregon, Portland, \textsuperscript{6}Cleveland Clinic, Cleveland, OH, \textsuperscript{7}Genzyme Corporation, Cambridge, MA, and \textsuperscript{8}Washington University, St. Louis, MO
**NHL: Percentage of Patients Reaching ≥ 150 x 10^9/L Platelets by Cell Dose**

<table>
<thead>
<tr>
<th>Transplant Cell Dose (CD34+ cells/kg)</th>
<th>2 – 4 x 10^6 N = 76</th>
<th>4 – 6 x 10^6 N = 75</th>
<th>6 x 10^6 N = 66</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 days</td>
<td>28 (48.3%)</td>
<td>42 (66.7%)</td>
<td>45 (81.8%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>6 months</td>
<td>31 (56.4%)</td>
<td>36 (66.7%)</td>
<td>42 (76.4%)</td>
<td>.026</td>
</tr>
<tr>
<td>12 months</td>
<td>18 (56.3%)</td>
<td>21 (80.8%)</td>
<td>24 (82.8%)</td>
<td>.020</td>
</tr>
</tbody>
</table>

For all transplanted cell dose categories, the proportions of patients are based on the number of patients with available data at each time point.

*P*-value for testing linear trend using Mantel-Haenszel Chi-square test.

### ECOSM DATABASE: DISEASES TREATED

<table>
<thead>
<tr>
<th></th>
<th>NHL</th>
<th>MM</th>
<th>HD</th>
<th>OTHERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients N.740</td>
<td>292</td>
<td>256</td>
<td>63</td>
<td>29</td>
</tr>
<tr>
<td>%</td>
<td>46%</td>
<td>40%</td>
<td>10%</td>
<td>4%</td>
</tr>
</tbody>
</table>

**Pie Chart**

- **NHL**: 46%
- **MM**: 40%
- **HD**: 10%
- **OTHERS**: 4%
**CD34+ CELLS POST-MOBILIZATION WITH PLERIXAFOR**

<table>
<thead>
<tr>
<th></th>
<th>NO MOBIL or CD34 ≤ 2 x 10^6/Kg</th>
<th>CD34 ≥ 2 x 10^6/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients N.</td>
<td>160</td>
<td>480</td>
</tr>
<tr>
<td>%</td>
<td>25%</td>
<td>75%</td>
</tr>
</tbody>
</table>

**PATIENTS MOBILIZED WITH PLERIXAFOR**

Plerixafor for chemomobilization in 12% of cases

- NOMOBIL or CD34 ≤ 2 x 10^6/Kg
- CD34 ≥ 2 x 10^6/Kg
Quality Assessment of Hematopoietic Stem Cell Graft Committee

Chairman: Lanza F (Cremona-Italy)
Secretary: Fruehauf S. (Germany)

Quality Assessment of Autografting: A prospective registration study

http://www.ebmt.org/7Directory/committees/qahscg.htm
Engraftment and blood cell recovery are not the only clinical end points in autografting

- Primary end points should evaluate efficacy, i.e. health economic considerations, including antibiotic administration, transfusion of blood components and time in hospital.

- Secondary end points should evaluate toxicity, in accordance with f.x. Common Toxicity Criteria (CTC), including mucositis, enteritis and haematological toxicity.

- Tertiary end points should evaluate safety, i.e. the risk of regimen related death or disease progression within the first 3 months following graft reinfusion.
Engraftment and blood cell recovery are not the only clinical end points in autografting

8.1. Proposed graded clinical end points in quality assessment

<table>
<thead>
<tr>
<th>Objective</th>
<th>End point</th>
<th>Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary: Efficacy</strong></td>
<td>Days on antibiotics, transfusion of blood components, days in hospital</td>
<td><strong>Favourable:</strong> = 7 days on antibiotics and no transfusions</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Intermediate:</strong> = 7 days on antibiotics and transfusions OR &gt; 7 days on antibiotics and no transfusions</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Unfavourable:</strong> &gt; 7 days on antibiotics and transfusions</td>
</tr>
<tr>
<td><strong>Secondary: Toxicity</strong></td>
<td>Days to ANC &gt;0.5 x 10^6/L and Platelets &gt;20 x 10^6/L</td>
<td><strong>Favourable:</strong> ANC and platelets recovery before 14 days</td>
</tr>
<tr>
<td></td>
<td>Other organ toxicity if appropriate</td>
<td><strong>Unfavourable:</strong> ANC or platelets recovery after 14 days</td>
</tr>
<tr>
<td><strong>Tertiary: Safety</strong></td>
<td>Death or disease recurrence</td>
<td><strong>Favourable:</strong> Alive and without disease progression after 12 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Unfavourable:</strong> Death or disease progression before 12 months</td>
</tr>
</tbody>
</table>
Impact of CD34+ Cell Dose on Patient Outcomes: Conclusions

• The minimal preferred CD34+ cell dose for transplantation is $\geq 2 \times 10^6$ cells/kg

• Published literature suggests that higher cell doses (> $5 \times 10^6$ cells/kg) improve engraftment and patient survival, and impact economic outcomes:
  
  – Correlated with faster neutrophil and platelet recovery\(^1\)-\(^3\)
  
  – Were associated with longer disease-free survival, and overall survival compared to lower transplant doses\(^4\),\(^5\)
  
  – Were associated with significantly shorter hospitalisations and decreased number of blood transfusions in NHL patients\(^6\)

EWGCCA-EUROGRAFT CD34 subsetting trial: 4 sendouts (37 centres)

Intra-site variation < 5% (CD90: 43-46%; CD133: 46-57%); Intersite variation (CD90: 37%- 28% CV; CD133: 39%- 31% CV)

<table>
<thead>
<tr>
<th>Staining</th>
<th>MEDIAN</th>
<th>10th percentile</th>
<th>90th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstained</td>
<td>2.1</td>
<td>0.61</td>
<td>7.15</td>
</tr>
<tr>
<td>CD90</td>
<td>42.1</td>
<td>20.6</td>
<td>53</td>
</tr>
<tr>
<td>CD133</td>
<td>61.2</td>
<td>22.8</td>
<td>75.5</td>
</tr>
</tbody>
</table>
High percentage of CD34-positive cells in autologous AML peripheral blood stem cell products reflects inadequate *in vivo* purging and low chemotherapeutic toxicity in a subgroup of patients with poor clinical outcome.

N Feller, GI Schuurhuis, MA van der Pol, G Westra, GWD Weijers, A van Stoijn, PC Huijgens and GI Ossenkoppele

The stem cell mobilizing capacity of patients with acute myeloid leukemia in complete remission correlates with relapse risk: results of the EORTC-GIMEMA AML-10 trial

S Keating¹, S Suciu², T de Witte¹, R Zittoun³, F Mandelli⁴, A Belhabri⁵, S Amadori⁶, W Fibbe⁷, E Gallo⁸, G Fillet⁹, B Varet¹⁰, G Meloni⁴, A Hagemeijer¹¹, P Fazi¹², G Solbu² and R Willemze⁷ on behalf of the EORTC and GIMEMA Leukemia Groups

**CD34 % > 0.8%**

**CD 34 > 7 x10e6/Kg in a single apheresis**

**CD34 in PB > 200 cmm**

**SUPERMOBILIZER: CD34 dose & RELAPSE RISK**

25% of AL pts are supermobilizers