Enumeration of HSCs

Stem cell evaluation methods:
Why we need a reliable method

André Tichelli
At the end of this presentation you should know

- What the clinician needs from the processing lab
- What the processing lab should know about the HSCT and engraftment
- Which are the methods used for stem cell evaluation and how and when to use them
• Optimal proliferation capacity in vivo
  ▸ Engraftment guaranteed
  ▸ Short engraftment time

• No undesirable cells or products
  ▸ Red blood cells
  ▸ Lymphocytes
  ▸ Malignant cells?

• Safe for the recipient

• Safe for the donor
Graft failure remains an important contributor to morbidity and mortality after HSCT

Survival of patients with aplastic anemia treated with HSCT

Cumulative incidence of sepsis in patients with/out engraftment failure

Piccin A. et al. BMT. 2010; 45: 1008-1013
Kikuchi M et al. Transplant Infectious Disease. 2015; 17: 56-65
Consequences of delayed engraftment on clinical outcome

Transplant related mortality according to delayed engraftment (measured by cell dose) in cord blood transplantation

Late engraftment is associated with higher treatment related mortality (infection; bleeding)

Wagner J et al. Blood. 2002;100:1611-1618
In vivo engraftment is the prove of a good proliferation capacity of the transplant

Need of in vitro surrogate marker: How good is the stem cell unit?

- Volume
- Total nucleated cell (TNC)
- CD34+
- CFU

Characteristics of an ideal surrogate marker for engraftment

- Reliable prediction of engraftment and time to engraftment
- Available at time of decision making
  - Depends on the collected material
- Robust and reproductive method

Proliferation capacity of the stem cell product is not the unique relevant factor predicting engraftment
Factors other than proliferation capacity of the cell product favoring graft rejection

- HLA disparity between donor and recipient
- Multiple transfusions before HSCT
  - Allosensitization of the recipient
- Type of conditioning
  - Reduced intensity conditioning
- T-cell depletion of the graft
- Infections
  - Mainly viral infections (CMV, HHV-6, parvovirus)
- The use of drugs
  - Inducing myelosuppression
- Severe GvHD

Overall survival after unrelated umbilical cord blood transplantation according to HLA-disparity

Wagner J et al. Blood. 2002;100:1611-1618
What type of material are used for HSCT

- Bone marrow (BM)
- Mobilized peripheral blood stem cells (PBSC)
- Cord blood (CB)
## Characteristics of the product depending on transplant sources

<table>
<thead>
<tr>
<th></th>
<th>Bone marrow</th>
<th>Peripheral blood</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>Anesthesia/operating room</td>
<td>Mobilization/Apheresis</td>
<td>During delivery</td>
</tr>
<tr>
<td></td>
<td>Cannot repeated on the next day</td>
<td>Can be repeated on next day</td>
<td>No chance to get more material at a later time in life</td>
</tr>
<tr>
<td><strong>When measurement needed</strong></td>
<td>Immediate need during harvesting BM</td>
<td>Results available at the end of the day</td>
<td>Needed at the end of collection</td>
</tr>
<tr>
<td><strong>Kind of decision making</strong></td>
<td>When to stop harvesting in the operating room</td>
<td>Whether to continue harvesting next day</td>
<td>Economical reasons Proceed and store only units with relevant proliferation capacity</td>
</tr>
<tr>
<td><strong>Marker for immediate decision</strong></td>
<td>TNC (Volume)</td>
<td>CD34</td>
<td>Volume TNC</td>
</tr>
<tr>
<td><strong>Marker for HSCT</strong></td>
<td>TNC CD34</td>
<td>CD34</td>
<td>TNC CD34</td>
</tr>
</tbody>
</table>
Influence of the type of product on what to measure

<table>
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<th>Bone marrow</th>
<th>Peripheral blood</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>10-20 ml/kg BW</td>
<td>End volume: 150-400ml</td>
<td>80-200 ml</td>
</tr>
<tr>
<td></td>
<td>Total volume 1.0-1.7 L</td>
<td>Processing 10-20 L blood volume</td>
<td></td>
</tr>
<tr>
<td><strong>Particularity</strong></td>
<td>Large volume RBC</td>
<td>Lymphocytes</td>
<td>Volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Small cell number</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RBC</td>
</tr>
<tr>
<td><strong>Possible</strong></td>
<td>Volume reduction</td>
<td>Cell selection</td>
<td>Volume reduction</td>
</tr>
<tr>
<td><strong>manipulations</strong></td>
<td>RBC depletion</td>
<td></td>
<td>Cryopreservation</td>
</tr>
<tr>
<td></td>
<td>Cell selection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cryopreservation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In case of</strong></td>
<td>HSCT</td>
<td>HSCT remobilization</td>
<td>HSCT</td>
</tr>
<tr>
<td><strong>insufficient cell</strong></td>
<td>Peripheral blood stem cell</td>
<td></td>
<td>double CB untilis</td>
</tr>
<tr>
<td><strong>number</strong></td>
<td>collection</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Remark</strong></td>
<td>Less GVHD</td>
<td>More GVHD</td>
<td>HSCT over HLA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>barrier</td>
</tr>
</tbody>
</table>
How do clinicians choose the source of stem cells?

- Malignant disorders with a donor
- Aplastic anemia and other non-malignant diseases
- No matched sibling donor or matched unrelated donor ± urgency to transplant
- Preferences

- PBSC
  - More convenient; possible graft-versus-leukemia effect

- Bone marrow
  - Better survival; no GVHD;
  - no graft versus leukemia effect needed

- Alternative donor:
  - Cord blood or haploidentical PBSC

- Donor
- Center
Engraftment after PBSC versus BM transplantation

- With PBSC transplantation
  - Median time to neutrophil engraftment 5 days shorter
  - Median time to platelet engraftment 7 days shorter

Higher CD34+ dose is not necessarily related with better outcomes

- Study on correlation between the composition of PBSC grafts and hematological recovery, GVHD, relapse and relapse-free survival
- CD3+ cell dose was not associated with outcome
- Analysis according to total CD34+ counts (more or less 8.3 x 10^6/kg)

<table>
<thead>
<tr>
<th>CD34+ cell dose</th>
<th>&lt; 8.3x10^6/kg n=53</th>
<th>&gt; 8.3x10^6/kg n=47</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic GVHD</td>
<td>69%</td>
<td>87%</td>
<td>0.05</td>
</tr>
<tr>
<td>Extensive cGVHD</td>
<td>46%</td>
<td>74%</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Causes of deaths
- Relapse: 5 vs 7, NS
- Infection: 4 vs 1, NS
- aGVHD: 9 vs 4, NS
- cGVHD: 3 vs 11, 0.01
Higher CD34+ dose is not necessarily related with better outcomes

* Including patients evaluable for cGVHD

**Multivariate analysis**

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>Confidence Intervall</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ cell dose</td>
<td>2.10</td>
<td>1.2-3.68</td>
<td>0.009</td>
</tr>
<tr>
<td>Relapse-free survival*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34+ cell dose</td>
<td>2.51</td>
<td>1.11-5.70</td>
<td>0.028</td>
</tr>
<tr>
<td>Disease status</td>
<td>2.47</td>
<td>1.02-5.97</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Mohty M et al. Leukemia.2003;17:869-875
### Effect of TNC and CD34+ cell dose on engraftment after allogeneic HSCT

**Single center study**
- 544 patients (227 sibling donor; 317 unrelated donor)
- 292 myeloablative conditioning; 252 RIC
- 121 bone marrow; 423 peripheral blood stem cells

<table>
<thead>
<tr>
<th></th>
<th>Bone marrow</th>
<th>Peripheral blood SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC x10⁸/kg (range)</td>
<td>3.2 (0.8-47)</td>
<td>11.6 (2.0-63.8)</td>
</tr>
<tr>
<td>CD34+ x 10⁶/kg (range)</td>
<td>3.9 (0.3-26.6)</td>
<td>8.1 (1.6-56.4)</td>
</tr>
</tbody>
</table>
Effect of CD34+ cell dose on overall survival in recipients of PBPC

Lower overall survival for
- CD34+ dose ≥ 11 x10^6/kg
- CD34+ dose < 2.5 x10^6/kg
Effect of high CD34+ cell dose on relapse in recipients of allogeneic PBPC

Possible causes for increased risk of relapse

- Not due to GVHD
- Graft rich in regulatory T cells, down-regulating the antileukemic effect?
- Double unit cord blood (CB) transplantation is extendedly use for adult patients
  - Because of the low cell dose per body weight
  - To enhance engraftment

**Optimal CD34+ cell dose of PBSC for autologous and allogeneic HSCH: Guidelines form the ASBMT**

<table>
<thead>
<tr>
<th>Question</th>
<th>Recommendation</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target goals of CD34+ cell doses for PBSC</td>
<td>Minimal dose ≥ 2.0 x10^6/kg</td>
<td>Successful engraftment has occurred with 0.75 x10^6/kg CD34+</td>
</tr>
<tr>
<td></td>
<td>Optimal dose 4-5 x10^6/kg</td>
<td>Higher than minimal doses reduce infection rates and non-relapse mortality</td>
</tr>
<tr>
<td></td>
<td>Too high CD34+ dose? (&gt; 8.0 / 11.0 x10^6/kg)</td>
<td>In allogeneic HSCT increased risk of cGVHD Relapse (?)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No data in autologous HSCT Risk of increased tumor cell infusion?</td>
</tr>
</tbody>
</table>
Proliferation capacity of the graft

- Good indicators for engraftment and time to engraftment
- Low TNC/CD34+ cell number is associated with increased risk for delayed or non-engraftment
- Too high number of CD34+ cell counts may be deleterious in allogeneic HSCT
- No data about too high CD34 counts in autologous HSCT
  - However it could have an influence on postinfusion reaction?
Do we need preleukapheresis CD34 counts for decision making?

- CD34+ cell counts are the best predictors for the yield of harvest CD34+ cells per kilogram
- Preleukapheresis numbers of CD34+ cells >40x10^5/ml in peripheral blood, a single leukapheresis results in an graft containing > 2.5 CD34+ x10^6/kg
- Total mononuclear cell counts or platelet counts of PB are not good predictors

Schwella N et al. JCO. 1996.14:1114-1121
Number of CD34+ cells in peripheral blood as predictor of the CD34+ yield in autologous HSCT

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt; 1.5 x10^6/kg CD34+</th>
<th>≥ 1.5 x10^6/kg CD34+</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>12</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Number of apheresis</td>
<td>1.92 ± 0.5</td>
<td>1.36 ± 0.68</td>
<td>0.01</td>
</tr>
<tr>
<td>CD34+ in PB (cells/ul)</td>
<td>7.76 ± 4.77</td>
<td>121.07 ± 133.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD34+ in graft with ≤3 collections (cells x10^6/kg)</td>
<td>0.71 ± 0.36</td>
<td>7.54 ± 7.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Autologous HSCT performed</td>
<td>0</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

≥ 15 CD34+ cells/ul is the best predictor to begin the apheresis product
- Optimal threshold level to achieve at least 1.5 x10^6/kg CD34 cells in the graft with ≤3 collection
When to begin monitoring peripheral blood CD34+ cell counts?

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<th>Remark</th>
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<tr>
<td>When to begin monitoring peripheral blood CD34+ cell counts?</td>
<td>Mobilization with G-CSF alone</td>
<td>Begin the fourth day of G-CSF</td>
</tr>
<tr>
<td></td>
<td>Growth factors and chemotherapy</td>
<td>Beginning generally 8-10 d after chemotherapy or WBC &gt; 1.0 x10^9/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More unpredictable</td>
</tr>
<tr>
<td></td>
<td>Growth factors and plerixafor</td>
<td>The morning before and after plerixafor administration (days 4 and 5 of G-CSF therapy)</td>
</tr>
</tbody>
</table>

**General remark**  
*Monitoring is recommended in any case*
## When to begin monitoring peripheral blood CD34+ cell counts?

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<tr>
<td>When to begin monitoring peripheral blood CD34+ cell counts?</td>
<td>Mobilization with G-CSF alone</td>
<td>Begin day +4 or +5 after G-CSF initiation</td>
</tr>
<tr>
<td></td>
<td>Growth factors and chemotherapy</td>
<td>When peripheral CD34+ count &gt; 20/ul</td>
</tr>
<tr>
<td></td>
<td>Growth factors and plerixafor</td>
<td>The following morning after plerixafor adminstration</td>
</tr>
</tbody>
</table>
Monitoring of CD34+ in peripheral blood during mobilization

- Very good correlation with CD34+ concentration in PB and yield of the harvest
- Monitoring helps in decision making when to start harvesting mobilized PBSC (particularly for autologous HSCT)
- Reduces the number of harvesting and processing in the lab
  - Ideal is to collect a sufficient number of cells in one apheresis
  - Recognizes the poor mobilizers
- In allogeneic HSCT usually not needed for decision when do start
  - May discover the rare poor mobilizers
- Optimal (and not the minimal) collection of CD34 cell number will make later the transplant procedure easier
Correlation between infused CD34 cell counts and engraftment

- No linear correlation between time to reach platelet counts >20 x10⁹/l and infused CD34+ cell counts
- A cell dose > 2.5 x10⁶ CD34+ / kg seems a safe cell dose (autologous HSCT)
Days to neutrophil and platelet recovery according to CD34+ cell infusion

### Days to neutrophil recovery

<table>
<thead>
<tr>
<th>CD34+/kg (x10^6)</th>
<th>Median time (days)</th>
<th>95% (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5-2.5</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>2.5-5.0</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>5.0-7.5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>7.5-10</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>10.5-12.5</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>&gt;12.5</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

### Days to platelet recovery

<table>
<thead>
<tr>
<th>CD34+/kg (x10^6)</th>
<th>Median time (days)</th>
<th>95% (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5-2.5</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>2.5-5.0</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>5.0-7.5</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>7.5-10</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>10.5-12.5</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>&gt;12.5</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>
Correlation CD34+ in PBSC and engraftment

- There is no linear correlation between cell dose and duration of engraftment
- There is no clear cut-off below which no engraftment will occur
- With a CD34+ cell dose below $2.5 \times 10^6$/kg a prolonged time to engraftment can be expected
- Platelet recovery is more critical and dependent on a high CD34+ cell dose than neutrophil recovery
- A minimal time for recovery will always be needed and cannot be overcome with higher CD34+ cell dose
Volume and TNC of incoming umbilical cord blood units

Relationship between volume and TNC

Distribution of volume of incoming cord blood units

![Histogram of volume distribution](image1)

![Scatter plot of volume vs. TNC](image2)
Erythroid precursor cells can be incorrectly represented as viable TNC resulting in an overestimation

TNC better inter-laboratory reproducibility

TNC is a reliable parameter for selection of CBU containing adequate number of CD34+ cells
Do short-term cultures (CFU) still have a place in the processing lab?

**Background**
- Theoretically the only functional test for hematopoietic precursor cells
- Can precursor cell growth in vitro?

**Problems**
- Needs 14 days to get a result
- Not well standardized method
- Counting the colonies subjective
- Few routine labs have the knowledge
Possible applications for use of short term CFU in routine processing lab

- In case of borderline harvest
- When there has been problems during the stem cell manipulation
- For cord blood units before HSCT
- In case of evaluation of a new processing method
Any graft manipulation leads to a loss of hematopoietic stem cells

- Volume reduction
- RBC depletion
- Cryopreservation
- Thawing
- T-cell or B-cell depletion
- CD34 enrichment

- Great variations in CD34 processing efficiency
- In cord blood units any loss is of relevance
- CD34 enrichment
  - High CD34+ concentration (75-95%)
  - but loss of CD34+ counts
  - Post-manipulation yield 40-90%

*After each graft manipulation, CD34+ counting is mandatory*
T cell depletion methods and CD34 recovery

(A) Efficacy of T cell depletion

(B) CD34 recovery

Influence of TNC and MNC according to processing
Things are changing
Reduced intensity conditioning (RIC)
- Initial state of mixed chimerism in majority during the first 6 months
  - Defined as the coexistence of hematopoietic cells from host and donor origin

- Achievement of full donor T-cell chimerism
  - Strongly correlated with decreased risk of disease progression or relapse

- Graft rejection
  - In about 10-20%
  - Is usually non-fatal

CD34+ cell dose correlated with
- Degree of donor myeloid chimerism
- Not with donor T-cell chimerism

Graft rejection after HSCT

Cell composition and namely TNC/CD34+ cell content play a relevant role in the outcome of HSCT.

The stem cell method to use depends on the source of stem cell and the type of decision making.

Monitoring of CD34+ cell dose on peripheral blood and measurement in the transplant unit are important for planning and processing stem cell units.

Engraftment pattern is different in patients transplanted with RIC.

Therefore a reliable method for stem cell measurement is mandatory.
Thank you very much for your attention