Hematopoietic Stem Cell Transplantation – General Aspects
Donor Identification & Screening
Stem Cell Mobilization
Stem Cell Collection

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WE WANT STEM CELLS!
Why do we want Stem Cells

- To hasten the correction of chemotherapy-induced aplasia in the autologous setting
- To install hematopoietic chimerism in the allogeneic setting
  - Essential for anti-tumour alloreactive effects
  - Essential to correct functional defects observes in inherited or acquired non-malignant disorders
How do we identify Stem Cells?

• Grossly!
• CD34⁺ cells
  – PBSC / Apheresis products
  – [Cord Blood Units]
• Total nucleated cells
  – Bone marrow
  – Cord Blood Units
Two main steps in the production of an hematopoietic cell graft

1. Cell procurement
   1. Donor identification
   2. Donor screening / donor recruitment
   3. Donor mobilization
   4. Donor collection
   5. Donor follow-up

2. Cell processing / Cell manufacturing
   1. More or less extensive cell engineering
      1. Minimally-manipulated cell products $\Rightarrow$ Cell Therapy Products (CTPs)
      2. Intended to alter the functional properties of collected cells = cell manufacturing $\Rightarrow$ ATMPs
   2. Storage
   3. Distribution
## Donor identification

<table>
<thead>
<tr>
<th>Donor –Recipient Relation</th>
<th>Matched</th>
<th>Mismatched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related</td>
<td>PBSC BM</td>
<td>PBSC BM</td>
</tr>
<tr>
<td>Unrelated</td>
<td>PBSC BM</td>
<td>CBU</td>
</tr>
</tbody>
</table>
Donor identification

• Historically, donors have been perceived as a « rare resource »
  – geno-identical (siblings)
  – MUD
  – even CBU
• However, recruiting haplo-identical donors change the paradigm
  – having a choice between several potential donors becomes the rule rather than the exception
  – introduce additional criteria in the algorithm to select a donor
Donor identification

• Requires a close cooperation between
  – Donor centre /
  – Transplant centre / transplant team
  – HLA / immuno-genetics laboratory (EFI accredited)
  – Registries

• Anticipation is key for success!
  – Average delay for identification of an HLA-matched related donor (sibling): 27 days
  – Average delay for identification of an HLA-matched unrelated donor (MUD): 64 days
• More than 23,217,895 millions unrelated donors registered worldwide

• More than 610,791 Cord Blood Units
Donor screening / donor recruitment

• Clinical & Biological screening
  – medical questionnaire
  – physical examination
  – biological testing with limited validity

• Looking for health conditions and risk factors
  – that may cause a risk to the donor
  – that may cause a risk to the recipient
Donor screening / donor recruitment

• Testing for donor / recipient ABO incompatibility

• Increasing age of related donors
  – screening for occult malignant blood diseases or immune disorders

• Donors with positive or incomplete testing or behavioural issues
Donor care

• Growing pressure to separately organize patient and donor care, under the supervision of different medical teams
• provides a more appropriate setting for the expression of donor free-will
## Comparisons and choice of collection procedures

<table>
<thead>
<tr>
<th></th>
<th>Bone marrow collection</th>
<th>Peripheral blood stem collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Logistical considerations</strong></td>
<td>Access to OR</td>
<td>Apheresis unit</td>
</tr>
<tr>
<td></td>
<td>Human resource</td>
<td></td>
</tr>
<tr>
<td><strong>Safety and comfort</strong></td>
<td>General anaesthesia</td>
<td>Mobilization treatment</td>
</tr>
<tr>
<td></td>
<td>Post-operative pain</td>
<td></td>
</tr>
<tr>
<td><strong>Cell product</strong></td>
<td>Low content in HSC</td>
<td>High content in HSC</td>
</tr>
<tr>
<td></td>
<td>Low content in lymphocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RBC (ABO incompatibility)</td>
<td></td>
</tr>
</tbody>
</table>
# Stem cell sources

<table>
<thead>
<tr>
<th></th>
<th>volume collected</th>
<th>med. CD34+ content</th>
<th>med. CD3+ content</th>
<th>target cell-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bone marrow</strong></td>
<td>10-20 ml/kg</td>
<td>2-3x10^6/kg</td>
<td>25x10^6/kg</td>
<td>2x10^8 TNC/kg</td>
</tr>
<tr>
<td><strong>peripheral blood</strong></td>
<td>150-400 ml</td>
<td>8x10^6/kg</td>
<td>250x10^6/kg</td>
<td>5-10x10^6 CD34+/kg</td>
</tr>
<tr>
<td><strong>umbilical cord blood</strong></td>
<td>80-160 ml</td>
<td>0.2x10^6/kg</td>
<td></td>
<td>&gt; 3x10^7 TNC/kg</td>
</tr>
</tbody>
</table>
Peripheral blood stem cell donation by apheresis is nowadays the most commonly used stem cell source for allogeneic HSCT

- High progenitor and T-cell content
- Accelerated cell recovery following myeloablative and reduced intensity conditioning regimen
Stem cell sources for allogeneic HSCT (US, 1988-2010)
Peripheral blood HSC collection

- HSC collection from peripheral blood results from the combination of
  - effective mobilisation protocols
  - efficient apheresis techniques development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Active molecule</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim</td>
<td>Granulocyte colony-stimulating factor</td>
<td>Produced via recombinant DNA technology; most widely used</td>
</tr>
<tr>
<td>Sargramostim</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
<td>Rarely used today</td>
</tr>
<tr>
<td>Pegfilgrastim</td>
<td>Pegylated filgrastim</td>
<td>Course can be completed with a single dose</td>
</tr>
<tr>
<td>Lenograstim</td>
<td>Glycosylated filgrastim</td>
<td>Widely used in Europe</td>
</tr>
<tr>
<td>Anestim</td>
<td>Recombinant human stem cell factor</td>
<td>Rarely used today</td>
</tr>
<tr>
<td>Plerixafor</td>
<td>Partial agonist CXCR-4 and CXCR-7</td>
<td>Used for mobilization failures</td>
</tr>
</tbody>
</table>

Gertz MA, 2010
“Stem cell mobilization”: plerixafor use

• Only for autologous collection / transplantation
  – not marketed for allogeneic collection / transplantation

• For adult patients:
  – with lymphoid malignancies
  – “… who mobilize poorly … “
  – in combination with rhG-CSF

• Cost
  – cost / efficiency
Kinetics of CD34+ collection in randomized trials of G-CSF vs G-CSF + plerixafor

Kaplan-Meier estimate of proportion of **multiple myeloma patients** reaching 6x10^6 or more CD34+/kg (DiPersio JF et al, Blood 2009)

HR = 2.54, p < 0.001

Kaplan-Meier estimate of proportion of **non Hodgkin’s lymphoma patients** reaching 5x10^6 or more CD34+/kg (DiPersio JF et al, JCO 2009)

HR = 3.64
95% CI, 2.39 to 5.45
P < .0001
Peripheral blood HSC collection

• The choice of a mobilization regimen
  – Depends on the context (induction chemotherapy, salvage regimen, degree of myelo-suppression induced by therapeutic agents)
  – Must comply with marketing authorizations and regulatory aspects
    • rhG-CSF not allowed in patients < 18 years old
    • plerixafor allowed only for autologous collection in patients with multiple myeloma or with lymphoma, and in association with rhG-CSF)
Peripheral blood HSC collection

• The choice of a mobilization regimen
  – Affects the quality of stem and progenitor (CD34+) cells mobilization and collection, but also affects the stem cell product for its contents in other cell populations
    • immune effectors with potential consequences for the risk of GVHD in the allogeneic setting
    • neutrophil precursors with potential consequences on graft processing and freeze/thaw in the autologous setting)
Peripheral blood HSC collection

• Inter-individual variability in response to mobilization treatments
  – Clinical factors
    • age, sex, ethnic origin, weight ....
  – Genetic factors:
    • Benboubker L et al, Br J Haematol, 2001, 113, 247
    • Bogunia-Kubik K et al, Bone Marrow Transpl, 2009,
    • Martin-Antonio et al, Haematologica, 2011, 96, 102

• Donor collection and recipient transplantation are usually synchronized
  – “uncomfortable” situation in case of poor mobilization
### Donor characteristics and mobilization

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>129</td>
<td>96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Men &lt;60</th>
<th>Men ≥60</th>
<th>Women &lt;60</th>
<th>Women ≥60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>48</td>
<td>21-78</td>
<td>21-78</td>
<td>21-78</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>74</td>
<td>44-130</td>
<td>44-130</td>
<td>44-130</td>
</tr>
<tr>
<td><strong>CD34 / µL</strong></td>
<td>63</td>
<td>6.7-237.6</td>
<td>6.7-237.6</td>
<td>6.7-237.6</td>
</tr>
</tbody>
</table>

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The scatter plot shows the distribution of CD34/µL levels across different groups. The x-axis represents different age groups, and the y-axis shows CD34/µL values.
ANOVA analyses of SNP influence on CD34+ cell mobilization

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Event</th>
<th>CD34+ cells /µL</th>
<th>p (Anova)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL12 (SDF-1)</td>
<td>AA</td>
<td>9</td>
<td>50.3 ± 11.4</td>
<td>0.5591</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>68</td>
<td>65.8 ± 5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>126</td>
<td>63.3 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>AA</td>
<td>15</td>
<td>54.3 ± 5.6</td>
<td>0.6188</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>98</td>
<td>62.0 ± 3.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>96</td>
<td>64.9 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>CSF3 (G-CSF)</td>
<td>CC</td>
<td>160</td>
<td>66.1 ± 3.3</td>
<td>0.1855</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>41</td>
<td>53.4 ± 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>4</td>
<td>69.6 ± 15.9</td>
<td></td>
</tr>
<tr>
<td>CSF3R (G-CSF Receptor)</td>
<td>CC</td>
<td>74</td>
<td>64.3 ± 5.0</td>
<td>0.6883</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>98</td>
<td>63.9 ± 3.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>36</td>
<td>57.7 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>VCAM-1</td>
<td>CC</td>
<td>12</td>
<td>34.8 ± 6.4</td>
<td>0.0449</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>57</td>
<td>65.9 ± 6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>138</td>
<td>63.8 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>VLA4</td>
<td>AA</td>
<td>56</td>
<td>62.1 ± 5.8</td>
<td>0.0698</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>107</td>
<td>66.3 ± 3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>43</td>
<td>53.5 ± 5.4</td>
<td></td>
</tr>
</tbody>
</table>
Peripheral blood HSC collection

- cytapheresis is based on the specific gravity of cell types
  - Table 1: Specific Gravities of Blood Components
    | Component                        | Gravity |
    |----------------------------------|---------|
    | Plasma                           | 1.027   |
    | Platelets                        | 1.04    |
    | Monocytes                        | 1.06    |
    | Lymphocytes                      | 1.05-1.07|
    | Myelocytes/promyelocytes         | 1.07    |
    | Blasts                           | 1.07-1.08|
    | Metamyelocytes                   | 1.08    |
    | Mature granulocytes              | 1.09    |
    | Erythrocytes                     | 1.095   |
  - CD34+ progenitor: 1.074

- improvements in collection techniques
  - circulating CD34 monitoring strategies
  - large volume leukapheresis (at least 3 blood vol.)
  - new continuous flow separators (Spectra Optia® & )

Okafor C et al, 2010
Different types of cell processors are marketed

SPECTRA OPTIA (Terumo BCT)

AMICUS CS3000 (Fresenius Kabi)
Circulating CD34+ cell numbers are predictive of collected CD34+ cell numbers

  
  Predicted CD34+ dose = (Peripheral CD34 count x Benchmark Collection Efficiency for cell separator being used x Volume of blood to be processed) / (Patient's weight in kg x Metric conversion factor)


\[
\text{CD34}^+ \text{ cells predicted to be collected per liter} = \frac{\text{(peripheral blood CD34}^+ \text{ cells/L) } \times \text{(30\%)}\dagger}{\text{processed blood}}
\]

\[
\text{body weight in kg}
\]
Adverse events after peripheral blood donation

- most frequent AE are related to
  - G-CSF : myalgias
  - ACD infused during apheresis : hypocalcemia
  - hematologic changes
    - hyperleukocytosis
      - leukostasis not reported
    - thrombocytopenia
      - hemorrhage not reported
  - venous access
    - central line complications
- rare but serious AE
  - spontaneous splenic rupture after G-CSF treatment
  - incidence 1/5,000 – 1/10,000

Table 2. Symptoms reported by National Marrow Donor Program (NMDP) peripheral blood stem cell (PBSC) donors, excluding reports of bone pain.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>All Donors (N = 1080)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myalgia</td>
<td>54%</td>
</tr>
<tr>
<td>Headache</td>
<td>52%</td>
</tr>
<tr>
<td>Malaise</td>
<td>49%</td>
</tr>
<tr>
<td>Insomnia</td>
<td>28%</td>
</tr>
<tr>
<td>Nausea</td>
<td>15%</td>
</tr>
<tr>
<td>Sweats</td>
<td>14%</td>
</tr>
<tr>
<td>Other flu-like Symptoms</td>
<td>12%</td>
</tr>
<tr>
<td>Anorexia</td>
<td>11%</td>
</tr>
<tr>
<td>Fever</td>
<td>6%</td>
</tr>
<tr>
<td>Chills</td>
<td>6%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2%</td>
</tr>
</tbody>
</table>

Horowitz MM et Confer DL, 2005
Bone marrow collection

• harvest under general or regional anesthesia
  – around 10-20ml/kg by multiple aspirations
  – usually from the posterior superior iliac crests

• procedure limited by
  – contraindication to general anesthesia
  – operating room availability

• development of new techniques
  – less invasive devices, without general anesthesia
  – Development was abandoned for the Marrow Miner (Hospira)
Adverse events after bone marrow donation

Pulsipher et al, Blood, 20&

<table>
<thead>
<tr>
<th>Table 2. Characteristics of bone marrow and PBSC collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Bone marrow collection (N = 2726)</td>
</tr>
<tr>
<td>Type of anesthesia</td>
</tr>
<tr>
<td>General</td>
</tr>
<tr>
<td>Spinal</td>
</tr>
<tr>
<td>Epidural</td>
</tr>
<tr>
<td>Local</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Volume of marrow collected, mL</td>
</tr>
<tr>
<td>≤ 500</td>
</tr>
<tr>
<td>500-1000</td>
</tr>
<tr>
<td>1000-1500</td>
</tr>
<tr>
<td>&gt; 1500</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Median, range</td>
</tr>
<tr>
<td>Volume of marrow collected per donor weight, mL/kg</td>
</tr>
<tr>
<td>≤ 10</td>
</tr>
<tr>
<td>10-15</td>
</tr>
<tr>
<td>15-20</td>
</tr>
<tr>
<td>&gt; 20</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Median, range</td>
</tr>
</tbody>
</table>

A

B

C

BM Donors

BM Donors

BM Donors

Fatigue  Insomnia  Site Reaction  Dizziness  Anorexia  Nausea
Donor follow-up

• Biological
  – Check normalization of haematological parameters within short delay after donation

• Clinical

• Psychological
  – Pay special attention to unfit or fragile donors
  – Pay special attention to children < 18 years old, who are asked to donate (family pressure, cultural pressure …)
  – Pay special attention to children, even > 18 years old, donating for their parents in the haplotype-mismatch setting
Long-term safety of rhG-CSF use in normal donors?

- Reports on biological alterations in leucocytes of normal donors post donation

- Long-term survey
Umbilical cord blood collection

- collection at term (> 37 weeks)
  - umbilical vein puncture with placenta *in utero*, after
    - vaginal delivery
    - cesarean section
  - collection by gravity using a bag system containing ACD

- since cell dose is the main limitation of it is important to optimize
  - donor selection
  - collection method
Hematopoietic Stem Cell Transplantation – General Aspects
Basic aspects of cell processing and delivery

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Two major steps in the production of an hematopoietic cell graft

1. Cell procurement
   1. Donor identification
   2. Donor screening / donor recruitment
   3. Donor mobilization
   4. Donor collection
   5. Donor follow-up

2. Cell processing
   1. More or less extensive cell engineering
      1. Minimally-manipulated cell products → Cell Therapy Products (CTPs)
      2. Intended to alter the functional properties of collected cells = cell manufacturing → ATMPs
   2. Storage
   3. Distribution
More or less extensive processing

- Minimally manipulated cell products
  - Regulated by competent authorities at a national level
    - Cell or tissue establishment license

- Largely produced and delivered by hospital or blood bank based (« academic ») facilities that work for clinical programmes in their immediate vicinity
More or less extensive processing

- More than minimally manipulated cell products or substantially manipulated cell products
  - Regulated since 2007 by EMA as « Advanced Therapy Medicinal Products » (Regulation EC No 1394/2007 of the European Parliament)
  - Marketing Authorization
- Regulation designed to foster the development of the industry in the field of somatic cell therapy, tissue engineering, gene therapy and combined products, and to facilitate access of patients to this new class of medicines
Minimally manipulated cell products

• The vast majority of autologous and allogeneic HSC grafts falls in this category

• Definition:
  – centrifugation
  – cryopreservation
  – immune-selection of various populations contained in the collected cell product
  – deserythrocytation
  – ….
Bone marrow processing

- erythrocytes depletion and/or volume reduction
  - mandatory in case of major ABO mismatch to prevent severe hemolytic reactions
  - necessary for low-weight recipients

- use of continuous flow cell-separator such as Cobe Spectra allows for
  - residual erythrocyte volume < 0.2 ml/kg
  - final volume < 10/ml/kg
  - CD34+ recovery around 80-90%

<table>
<thead>
<tr>
<th>Variable</th>
<th>Initial BM</th>
<th>After processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>1099 ± 385 (390-2450)</td>
<td>135.9 ± 42 (57-300)</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>27.9 ± 3.9 (16-43)</td>
<td>2.9 ± 0.9 (0-9)</td>
</tr>
<tr>
<td>RBCs (mL)</td>
<td>309.9 ± 117.7 (107.3-647.2)</td>
<td>4.0 ± 1.8 (0-10.99)</td>
</tr>
<tr>
<td>RBCs (mL)/kg of recipient body weight</td>
<td>7.6 ± 4.7 (1.5-25.5)</td>
<td>0.1 ± 0.09 (0-0.687)</td>
</tr>
<tr>
<td>RBCs depletion (%)</td>
<td></td>
<td>98.6 ± 0.8 (95.1-100)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell subpopulation</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNCs (10^6)</td>
<td>33.66 ± 12.2 (10.3-76.3)</td>
</tr>
<tr>
<td>Granulocytes (10^5)</td>
<td>48.98 ± 27.0 (7.5-124.7)</td>
</tr>
<tr>
<td>CD3+ cells (10^6)</td>
<td>82.02 ± 17.9 (25.4-146.5)</td>
</tr>
<tr>
<td>CD34+ cells (10^5)</td>
<td>82.2 ± 21.1 (26.7-159.8)</td>
</tr>
<tr>
<td>CFU-GM (10^5)</td>
<td>93.9 ± 55.3 (20.5-707.7)</td>
</tr>
</tbody>
</table>

Larghero J et al, 2006
Bone marrow processing

<table>
<thead>
<tr>
<th>Identité ABO</th>
<th>Incompatibilité ABO mineure</th>
<th>Incompatibilité ABO majeure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donneur (greffon)</td>
<td>Receveur</td>
<td>Donneur (greffon)</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td>O</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>O</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>O</td>
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<td>B</td>
<td>AB</td>
<td>A</td>
</tr>
<tr>
<td>AB</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>
Umbilical cord blood processing

• Volume reduction before cryopreservation is a common procedure
  – minimize storage space
  – reduce DMSO volume (allow reinfusion without washing)

• Cell loss is unavoidable

Table 3. TNC and total CD34+ cell recoveries, mean ± SD.

<table>
<thead>
<tr>
<th>Technique</th>
<th>No.</th>
<th>TNC $(10^9)$</th>
<th>CD34+ cells $(10^6)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HES</td>
<td>447</td>
<td>76.82 ± 9.10$^1$</td>
<td>81.46 ± 12.50</td>
</tr>
<tr>
<td>TB</td>
<td>181</td>
<td>60.72 ± 13.55</td>
<td>81.99 ± 17.68</td>
</tr>
<tr>
<td>Sepax</td>
<td>213</td>
<td>80.26 ± 7.73$^{2,3}$</td>
<td>86.08 ± 11.63$^4$</td>
</tr>
</tbody>
</table>

Lapierre V et al, 2007

1, HES vs. TB, $P < 0.0001$; 2, Sepax vs. HES, $P < 0.0001$; 3, Sepax vs. TB, $P < 0.0001$; 4, Sepax vs. HES $P < 0.0001$ and Sepax vs. TB, $P = 0.008$. 
HSC cryopreservation

• should minimize cell injury during the freeze–thaw process
  – intracellular ice crystal damages

• critical process for UCB
  – controlled-rate freezing with cooling rate around 1-2°C/min
  – 5-10% of cryoprotectant (DMSO)
HSC storage

- Storage in liquid nitrogen at a temperature of
  - -156°C (vapor phase)
  - -196°C (liquid phase)

- Container should be temperature-monitored 24h/24h to avoid transient warming during storage.

- No use-by date
  - BM autologous SCT after 21 years of cryopreservation
  - Recovery of functional UCB progenitor after 15 years
    - Broxmeyer HE et al, 2003
• loss of integrity may occur
  – during shipment (1/3)
  – at the time of thaw (2/3)

• incidence of bag breakage is 1-5%

• occurrence of breaks depends upon
  – bag material and manufacturer
  – attention paid to handling
Umbilical cord blood thawing

- cryopreserved UCB grafts should be thawed at the laboratory
  - trained staff
  - quality control before infusion
- UCB are either
  - diluted after thawing
  - washed after thawing
- thawed HSC products are stable
  - during 4-6h
  - at +18-24°C

Regan DM et al, 2010
Quality control of HSC grafts

• numeration of viable CD34+ and lymphocytes sub-population
  – single-platform flow cytometry techniques
  – standardized methodologies using commercially available kits including viability dye

• functionnal assays: colony-forming units
  – difficult to interpret due to the subjective nature of the readout
  – important inter-laboratory variability
  – but unique predictor of graft potency!

Dauber K et al, 2011
Quality control of HSC grafts

• microbial contamination
  – incidence depends on the origine of the cell product
    • 1-5% for PB
    • 5-10% for BM
    • 10-15% for UCB
  – mostly due to coagulase-negative *Staphylococcus sp.*
  – cryopreservation usually do not eradicate bacteria

• in case of culture-positive products
  – results not available at the time of infusion for fresh products
  – infections attributable to the culture positive product extremely rare
  – antibiotic prophylaxis should be discussed depending on the type of contaminant

<table>
<thead>
<tr>
<th>Study</th>
<th>Total harvest</th>
<th>Contaminated harvests</th>
<th>Potentially pathogenic</th>
<th>Iatrogenic sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Webb et al.¹</td>
<td>2,632</td>
<td>85 (3.2)</td>
<td>5 (0.18)</td>
<td>2 (0.07)</td>
</tr>
<tr>
<td>Schewla et al.²</td>
<td>290</td>
<td>13 (4.4)</td>
<td>1 (0.34)</td>
<td>0</td>
</tr>
<tr>
<td>Prince et al.⁴</td>
<td>1,662</td>
<td>54 (3.2)</td>
<td>5 (0.3)</td>
<td>0</td>
</tr>
<tr>
<td>Attarian et al.⁵</td>
<td>1,263</td>
<td>3 (0.2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jestice et al.⁶</td>
<td>128</td>
<td>23 (17.9)</td>
<td>2 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Nasser et al.⁷</td>
<td>1,660</td>
<td>11 (0.6)</td>
<td>2 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Espinosa et al.⁸</td>
<td>1,040</td>
<td>3 (0.2)</td>
<td>0</td>
<td>NA†</td>
</tr>
<tr>
<td>Padley et al.⁹</td>
<td>893</td>
<td>22 (2.4)</td>
<td>1 (0.1)</td>
<td>0</td>
</tr>
<tr>
<td>Schewla et al.¹⁰</td>
<td>239</td>
<td>63 (26.3)</td>
<td>13 (5.4)</td>
<td>0</td>
</tr>
<tr>
<td>Rowley et al.¹¹</td>
<td>100</td>
<td>22 (22)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lazarus et al.¹²</td>
<td>194</td>
<td>13 (6.7)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Cohen et al.¹³</td>
<td>227</td>
<td>16 (7.0)</td>
<td>2 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>10,888</td>
<td>332 (3.0)</td>
<td>30 (0.27)</td>
<td>3 (0.027)</td>
</tr>
</tbody>
</table>

* Data are reported as number (%).
† All contaminants discarded.

Kamble R et al, 2005
Key steps in HSC grafts procurement and processing

• steps that are not completely under control
  – quality of the collected product (BM, PB)

• steps that should be under control
  – temperature stability during storage and shipment
  – efficiency of erythrocytes depletion and/or volume reduction (UCB, BM)
  – efficiency of the thawing/dilution/washing procedure
  – consistency of controlled-rate freezing
HSC grafts distribution and infusion

• verification of release criteria
  – storage/transportation temperature
  – cell dose and if applicable cell recovery and viability
  – product conformity / ABO compatibility
  – labelling (ISBT128 conformity)

• distribution to transplant units
  – check patient/donor identity and labelling
  – infuse immediately after reception
  – pre-medication as per institutional guidelines
HSC products shipment

- lack of precise standards (JACIE +4-24°C)
  - based upon published data, storage and transportation
    - of fresh HSC products at +4-8°C
    - of thawed HSC product at +20-24°C
  - PB is more sensitive to temperature than BM

- use of cooling elements should be restricted to medical coolers that will not freeze the product

- temperature monitoring (data logger) is mandatory

Antonenas V et al, 2006
Adverse events associated with HSC infusion

- AE after infusion of fresh HSC grafts are
  - sporadic and moderate to mild
  - mostly related to temperature and volume

- AE after infusion of thawed HSC grafts are
  - more common (20-60%)
  - sometimes life-threatening
    - cardiovascular
    - neurological

- suspected causative agents
  - lysis products of cellular contaminants (granulocytes)
  - idiosyncratic reactions to residual DMSO or dextran-40

---

Fig. 1. Prefreeze HPC graft content in granulocytes according to the severity of AEs.

Calmels B et al, 2007
AEs following HSCT infusion

Acute life-threatening cardiovascular toxicity with umbilical cord blood infusion: The role of dextran

LETTER TO THE EDITOR

Ischemic stroke associated with the infusion of DMSO-cryopreserved auto-PBSCs

Severe vasospastic angina with hemodynamic compromise related to the infusion of dimethyl sulfoxide (DMSO)-cryopreserved autologous peripheral blood stem cells

Original Article

Neurotoxicity upon infusion of dimethylsulfoxide-cryopreserved peripheral blood stem cells in patients with pre-existing cerebral disease and without pre-existing cerebral disease

Temporary vision loss because of dimethyl sulfoxide in autologous stem cell transplantation

Neurological events associated with the infusion of cryopreserved bone marrow and/or peripheral blood progenitor cells

Adverse reactions during transfusion of thawed haematopoietic progenitor cells from apheresis are closely related to the number of granulocyte cells in the leukapheresis product
Thawing at the bedside vs thawing in the cell processing facility?

Automated washing of autologous hematopoietic stem cell grafts after thawing does not impair engraftment

Boris Calmels1,2,3,*, Alexandre Drezet3,*, Chloé Huynh1, Aurélie Autret4, Anne-Marie Stoppa5, Reda Bouabdallah5, Diane Coso5, Carine Malenfant1, Claude Lemarié1,2, and Christian Chabannon1,2,3,6

Table 1: Patients, autografts and clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Unwashed (n=65)</th>
<th>Washed (n=130)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>30/35</td>
<td>52/78</td>
<td>0.41</td>
</tr>
<tr>
<td>Age (median [range])</td>
<td>55.3 [17-71]</td>
<td>56.7 [23-71]</td>
<td>0.74</td>
</tr>
<tr>
<td>Diagnosis / High dose chemotherapy (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cell disorders / Melphalan</td>
<td>48 [73.8]</td>
<td>97 [74.6]</td>
<td>0.15</td>
</tr>
<tr>
<td>Lymphoma / BEAM</td>
<td>8 [12.3]</td>
<td>23 [17.7]</td>
<td></td>
</tr>
<tr>
<td>Acute leukemia / BU-MEL</td>
<td>5 [7.7]</td>
<td>2 [1.5]</td>
<td></td>
</tr>
<tr>
<td>Solid tumors / CY-MEL</td>
<td>4 [6.2]</td>
<td>8 [6.2]</td>
<td></td>
</tr>
<tr>
<td>Number of CD34+ cells cryopreserved (10^6/kg)</td>
<td>3.7</td>
<td>3.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Patients according to CD34+ cell dose (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[2; 3.5]</td>
<td>27</td>
<td>44</td>
<td>0.29</td>
</tr>
<tr>
<td>[3.5; 5]</td>
<td>38</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Days to neutrophils &gt; 0.5 G/L (median ± sd [range])</td>
<td>12.4 ± 1.4 [10-15]</td>
<td>12.5 ± 1.6 [8-17]</td>
<td>0.67</td>
</tr>
</tbody>
</table>

- case-match analysis using 4 matching factors with distincts relative weights (diagnosis 200, CD34 cell dose 10, age 2 and sex 1)
- washing does not impair hematopoietic engraftment as compared to bedside thawing
« Good Manufacturing Practices » for HSC processing

• good manufacturing practices (GMPs) should ensure
  – product safety
  – product characterization

• establishment of a consistent and scalable manufacturing process
  – process does not introduce external contaminants
    • closed systems
    • biological safety cabinet (BSC) : class 100 (ISO 5) environment
    • environment surrounding the BSC : class 10,000 (ISO 7) clean room
    • personnel trained in aseptic processing to “protect” the product
  – specifications for key parameters of the therapeutic product must be defined
    • cell count, viability, recovery...
  – qualification of each components used in the manufacturing process
    • biological (HSA...), chemical (DMSO, mAb...)
Quality Management in Collection & Processing Facilities

- Mandatory to obtain cell or tissue establishment licenses from national competent authorities

- Voluntary certification or accreditation processes
  - ISO 9001
  - ISO 15189
  - JACIE: Joint Accreditation Committee for ISCT & EBMT

- Need for cell collection and cell processing facilities to access patient data and transplant outcome.
HSC procurement and processing

- Storage
- Equipment
- Facilities
- Staff
- Traceability
- Labelling
- Transportation

Donor evaluation

HSC collection

- Collected product qualification

HSC processing

- Product transportation

HSC graft distribution

Manufacturing validation
ATMPs & HSC Transplants?

- Substantially manipulated products
  - ex-vivo expanded / activated defined cell populations
  - dendritic cells (tumor vaccines), natural killer cells, T cells
  - genetically modified cells
  - gene therapy of SCID and hemoglobinopathies, genetically-modified T cells (CAR)

- Most if not all of these products are currently delivered in the context of clinical trials
  - Phase I / Phase II trials
  - Small numbers of patients
  - Few established orphan indications
Examples of the first ATMPs that were granted a marketing authorization

<table>
<thead>
<tr>
<th>Commercial denomination (Manufacturer)</th>
<th>Marketing Authorization obtained on</th>
<th>Nature</th>
<th>Clinical use</th>
<th>Indication</th>
<th>Characteristics of candidate patient population</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChondroCelect® (Tigenix NV)</td>
<td>05/10/2009</td>
<td>Autologous chondrocytes</td>
<td>Regenerative medicine (orthopédie)</td>
<td>Repair of focal and symptomatic cartilage lesions in the knee</td>
<td>Large</td>
</tr>
<tr>
<td>Maci® (Genzyme Europe B.V.)</td>
<td>27/06/2013</td>
<td>Autologous chondrocytes on a collagen matrix</td>
<td>Regenerative medicine (orthopédie)</td>
<td>Knee repair</td>
<td>Large</td>
</tr>
<tr>
<td>Provenge® (Dendreon Corporation)</td>
<td>06/09/2013</td>
<td>Autologous dendritic cells activated with GM-CSF-PAP (Sipuleucel-T)</td>
<td>Oncology</td>
<td>Anti-tumour vaccine for patients with a drogen-independent prostate cancer</td>
<td>Large</td>
</tr>
</tbody>
</table>
Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer

Philip W. Kantoff, M.D., Celestia S. Higano, M.D., Neal D. Shore, M.D., E. Roy Berger, M.D., Eric J. Small, M.D., David F. Penson, M.D., Charles H. Redfern, M.D., Anna C. Ferrari, M.D., Robert Dreicer, M.D., Robert B. Sims, M.D., Yi Xu, Ph.D., Mark W. Frohlich, M.D., and Paul F. Schellhammer, M.D., for the IMPACT Study Investigators*

ABSTRACT

BACKGROUND
Sipuleucel-T, an autologous active cellular immunotherapy, has shown evidence of efficacy in reducing the risk of death among men with metastatic castration-resistant prostate cancer.

METHODS
In this double-blind, placebo-controlled, multicenter phase 3 trial, we randomly assigned 512 patients in a 2:1 ratio to receive either sipuleucel-T (341 patients) or placebo (171 patients) administered intravenously every 2 weeks, for a total of three infusions. The primary end point was overall survival, analyzed by means of a stratified Cox regression model adjusted for baseline levels of serum prostate-specific antigen (PSA) and lactate dehydrogenase.

RESULTS
In the sipuleucel-T group, there was a relative reduction of 22% in the risk of death as compared with the placebo group (hazard ratio, 0.78; 95% confidence interval [CI], 0.61 to 0.98; P=0.03). This reduction represented a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group). The 36-month survival probability was 31.7% in the sipuleucel-T group versus 23.0% in the placebo group. The treatment effect was also observed with the first and second doses of therapy.

From the Dana–Farber Cancer Institute, Harvard Medical School, Boston (P.W.K.); School of Medicine, University of Washington, Seattle Cancer Care Alliance, Seattle (C.S.H.); Carolina Urologic Research Center, Myrtle Beach, SC (N.D.S.); School of Medicine, SUNY at Stony Brook, Stony Brook, NY (E.R.B.); Urologic Oncology Program, University of California San Francisco, San Francisco (E.J.S.); Vanderbilt University Medical Center, Nashville (D.F.P.); Sharp Healthcare, San Diego, CA (C.H.R.); New York University Clinical Cancer Center, New York University Langone Medical Center, New York (A.C.F.); Taussig Cancer Institute, Cleveland Clinic, Cleveland (R.D.); Dendreon Corporation, Seattle (R.B.S., Y.X., M.W.F.); and Eastern Virginia Medical School, Norfolk (P.F.S.). Address reprint requests to Dr. Kantoff at the Lank Center for Genitourinary Oncology, Dana–Farber Cancer Institute, Harvard Medical School, 44 Binney St., Dana 1230, Boston, MA 02115, or at philip_kantoff@dfci.harvard.edu.
Cord Blood Expansion

TECHNICAL REPORTS

Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid recor

Colleen Delaney, Shelly Heimfeld, Carolyn Brashem-Stein, Howard Voorhis, Ronald L. MaIrwin D. Bernstein

Original Article

Cord-Blood Engraftment with Ex Vivo Mesenchymal-Cell Coculture

Marcos de Lima, M.D., Ian McNiece, Ph.D., Simon N. Robinson, Ph.D., Mark Munsell, M.S., Mary Eapen, M.D., Mary Horowitz, M.D., Amin Alousi, M.D., Rima Saliba, Ph.D., John D. McMannis, Ph.D., Indreshpal Kaur, Ph.D., Partow Kebrniai, M.D., Simrit Parmar, M.D., Uday Popat, M.D., Chitra Hosing, M.D., Richard Champlin, M.D., Catherine Bollard, M.D., Jeffrey J. Molldrem, M.D., Roy B. Jones, M.D., Ph.D., Yago Nieto, M.D., Ph.D., Borje S. Andersson, M.D., Nina Shah, M.D., Betul Oran, M.D., Laurence J. Cooper, M.D., Ph.D., Laura Worth, M.D., Muzaffar H. Qazilbash, M.D., Martin Korbling, M.D., Gabriela Rondon, M.D., Stefan Ciurea, M.D., Doyle Bosque, R.N., Ila Maewal, Pharm.D., Paul J. Simmons, Ph.D., and Elizabeth J. Shpall, M.D.

ABSTRACT

Poor engraftment due to low cell doses restricts the usefulness of umbilical-cord-blood transplantation. We hypothesized that engraftment would be improved by transplanting cord blood that was expanded ex vivo with mesenchymal stromal cells.
Mesenchymal stem cells (MSCs) are being studied for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study

Katarina Leffler1, Francesco Feresin2, Lynn Laiti3, Franco Locati4, Helene Reecher1, Ian Lewis1, Eibardt Lenoir5, Bente Sundberg5, Mario di Bartolomeo6, Rita Kermberger6, Claude Delaunay7, Antonino Avanzini8, Mario Ricci9, William Htibbs3, Olga Nikitina3, On behalf of the European Group for Blood and Marrow Transplantation.

Summary

Mesenchymal stem cells (MSCs) are a potential treatment for steroid-resistant, severe, acute graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation. MSCs can modulate immune responses in vitro and vivo and we aimed to assess whether mesenchymal stem cells could ameliorate GVHD after hematopoietic stem cell transplantation.

Methods

Patients with steroid-resistant, severe acute GVHD were treated with mesenchymal stem cells derived from the European Group for Blood and Marrow Transplantation’s donor pool and eight experimental patients, in a multicenter, phase II, open-label study. We recorded response, transplantation-related deaths, and adverse events for up to 60 months follow-up from infusion of the cells.

Findings

Between October 2001 and January 2007, 55 patients were treated. The median dose of bone-marrow derived mesenchymal stem cells was 1.4 x 10^6 (min-max range 0.2 x 10^6-1.8 x 10^6) cells per kg body weight. 27 patients received one dose, 23 received two doses, and six three to five doses of cells obtained from HLA-matched donors (n=5), haploidentical donors (n=1), and third-party HLA-matched donors (n=9). 30 patients had a complete response and nine showed improvement. No patients had side-effects during or immediately after infusions of the mesenchymal stem cells. Response rate was not related to donor HLA-match. Three patients had recurrent graft-versus-host disease and one developed de-novo acute myeloid leukemia of recipient origin. Complete responders had lower transplantation-related mortality 1 year after infusion than did patients with partial or no response (11% (95% CI 36% to 14% vs 12% (95% CI 23% to 5%)) and higher overall survival 2 years after hematopoietic stem cell transplantation (86% (95% CI 80% to 94% vs 80% (95% CI 76% to 84%)).

Interpretation

Infusion of mesenchymal stem cells expanded in vitro, irrespective of the donor, might be an effective therapy for patients with steroid-resistant, acute GVHD.

Funding


Preparation and infusion of MSCs

Clinical-grade MSCs were generated under Good Manufacturing Practice conditions according to a protocol approved by the Dutch Central Committee on Research involving Human Subjects (CCMO). Mononuclear cell islands were isolated from 50-100% of MSC donor BM, and separated by density gradient centrifugation as previously described (Ball et al, 2007; Leffler et al, 2007).

Multiple infusions of mesenchymal stromal cells induce sustained remission in children with steroid-refractory, grade III-IV acute graft-versus-host disease

Lynne M. Ball1, Maria E. Bernardino2, Helene Reecher1, Marian J. D. van Tol3, Beatrice Cordell1, Ijsa Jan Zounganga1, Maria Antonia Avanzini8, Antonella Confetti3, Alice Bertani1, Giovanni Giorgioni3, Cornelia M. Joel van der Zijden1, Marco Zuccheri1, Katarina Leffler1, Francesco Feresin2, Rudolph Maarten Eigeler4, Willem E. Fibbe5, Arjan C. Lankster6 and Franco Locati4

1Department of Paediatrics, Stem Cell Transplantation Unit, Leiden University Medical Centre, Leiden, The Netherlands, 2Department of Paediatric Haematology/Oncology, University Hospital Maastricht, Maastricht, The Netherlands, 3Department of Paediatric Haematology/Oncology, University Hospital Leiden, Leiden, The Netherlands, 4Department of Paediatric Tissue Engineering, Italian Tissue Engineering and Blood Transplantation, Udine, Italy, 5Department of Paediatric Haematology/Oncology, University Hospital Maastricht, Maastricht, The Netherlands, 6Department of Paediatric Tissue Engineering, Italian Tissue Engineering and Blood Transplantation, Udine, Italy, 7Department of Paediatric Tissue Engineering, Italian Tissue Engineering and Blood Transplantation, Udine, Italy, 8Department of Paediatric Tissue Engineering, Italian Tissue Engineering and Blood Transplantation, Udine, Italy, 9Department of Paediatric Tissue Engineering, Italian Tissue Engineering and Blood Transplantation, Udine, Italy.

Summary

Mesenchymal stromal cell (MSC) infusions have been reported to be effective in patients with steroid-refractory, acute graft-versus-host disease (aGVHD). However, recent studies have shown that MSCs can induce sustained remission in children with grade III-IV aGVHD, indicating a potential therapeutic approach for these patients.

Keywords: steroid-refractory acute graft-versus-host disease, mesenchymal stromal cells, transplantation-related mortality, hematopoietic stem cell transplantation in children.
Engineered immune cells

RESEARCH ARTICLE

LEUKEMIA

T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia

Michael Kalos,1,2* Bruce L. Levine,1,2# David L. Porter,1,3 Sharyn Katz,4 Stephan A. Grupp,5,6 Adam Bagg,1,3 Carl H. June1,3*

Tumor immunotherapy with T lymphocytes, which can recognize and destroy malignant cells, has been limited by the ability to isolate and expand T cells restricted to tumor-associated antigens. Chimeric antigen receptors (CARs) composed of antibody binding domains connected to domains that activate T cells could overcome tolerance by allowing T cells to respond to cell surface antigens; however, to date, lymphocytes engineered to express CARs have demonstrated minimal in vivo expansion and antitumor effects in clinical trials. We report that CAR T cells contain a costimulatory domain from CD137 and the T cell receptor (TCR) αβ chain have potent non-specific after infusion, in three of three patients treated with advanced chronic lymphocytic leukemia (CLL) cells expanded >100-fold in vivo, trafficked to bone marrow, and continued to high levels for at least 6 months. Evidence of target tumor cells included B cell aplasia and hypogammaglobulinemia. On average, each infused CAR-activated to eradicate at least 1000 CLL cells. Furthermore, a CD19-specific immune reagent in the blood and bone marrow, accompanied by complete remission in two of three patients of these cells persisted as memory CAR T cells and retained anti-CD19 effector potential of this major histocompatibility complex-independent approach for the effector cell 9.


RESEARCH ARTICLE

CANCER

Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia

Marco L. Davila,1,2,4 Isabelle Riviere,1,2,4 Xiuyan Wang,4 Shirley Bartido,4 Jae Park,4 Kevin Curran,5 Stephen S. Chung,5 Jolanta Stefanksi,5 Oriana Borquez-Ojeda,5 Malgorzata Olzewska,4 Jinrong Qu,1 Teresa Wasilewska,3 Qing He,4 Mitsu Fink,3 Himay Shinglot,3 Maher Youssif,3 Mark Satter,3 Yongzeng Wang,4 James Hosey,4 Hilda Quintanilla,4 Elizabeth Halton,4 Yvette Bernal,4 Diana C. G. Bouhassira,4 Maria E. Arcila,6 Mithat Gonen,6 Gall J. Roboz,2 Peter Maslak,6 Dan Douer,1 Mark C. Frattini,9 Sergio Girak,1,2 Michel Sadelain,2,3,6 Reiner Brentjens1,3,9

We report on 16 patients with relapsed or refractory B cell acute lymphoblastic leukemia (B-ALL) that we treated with autologous T cells expressing the 19-28z chimeric antigen receptor (CAR) specific to the CD19 antigen. The overall response rate was 86%, which allowed us to transition most of these patients to a standard-of-care allogeneic hematopoietic stem cell transplant (allo-SCT). This therapy was as effective in high-risk patients with Philadelphia chromosome-positive (Ph+) disease as in those with relapsed disease after previous allo-SCT. Through systematic analysis of clinical data and serum cytokine levels over the first 21 days after T cell infusion, we have defined diagnostic criteria for severe cytokine release syndrome (sCRS), with the goal of better identifying the subset of patients who will likely require therapeutic intervention with corticosteroids or interleukin-6 receptor blockade to curb the CRS. Additionally, we found that serum C-reactive protein, a readily available laboratory study, can serve as a reliable indicator for the severity of the CRS. Together, our data provide strong support for conducting a multicenter phase 2 study to further evaluate 19-28z CAR T cells in B-ALL and a roadmap for patient management at centers now contemplating the use of CAR T cell therapy.

Ongoing dispute on the contribution of academic facilities in the production and delivery of ATMPs

- The 2007 regulation has been the topic of a recent public consultation

- Different viewpoints for
  - EMA and competent authorities
  - Professionals from academic facilities

- Hospital exemption
  - “ATMPs which are prepared on a non routine basis according to specific quality standards and used within the same member state under the exclusive responsibility of a medical practitioner, in order to comply with a medical prescription for a custom-made product for an individual patient, should be excluded from the scope of this regulation.”
PUBLIC CONSULTATION PAPER

ON THE REGULATION ON ADVANCED THERAPY MEDICINAL PRODUCTS

Deadline for Public Consultation: 31 March 2013

REGULATION (EC) No. 1394/2007 ON ADVANCED THERAPY MEDICINAL PRODUCTS

SUMMARY OF THE RESPONSES TO THE PUBLIC CONSULTATION
ATMPs & clinical research?

- DLI = minimally manipulated cells
  - (collected), processed and released by a cell or tissue establishment

- Substitution of a defined number of well-characterized immuno-selected and ex-vivo activated NK cells
  - falls within the definition of ATMPs?
  - should obtain MA?
  - can no longer be (collected), processed and released by a cell or tissue establishment, but should be bought from a biotechnology / pharmaceutical company?
Engineered Natural Killer cells (1)

Donor apheresis
CD3 depletion then CD56 selection
activation with IL-2 (7 days @ 37°C)
CD3- CD56+ activated NK cells
Regulation of advanced therapy medicinal products in Europe and the role of academia

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Abstract
Background aims. Advanced therapy medicinal products (ATMPs) are gene therapy, somatic cell therapy or tissue-engineered products regulated under (EC) No. 1394/2007 to ensure their free movement within the European Union while guaranteeing the highest level of health protection for patients. Academic good manufacturing practice (GMP) centers are major contributors in the development of ATMPs and this study assessed the impact of regulations on them. Methods. European academic and non-industrial facilities (n = 747) were contacted, and a representative sample of 50 replied to a detailed questionnaire. Experienced centers were further selected in every Member State (MS) for semi-structured interviews. Indicators of ATMP production and development success were statistically assessed, and opinions about directive implementation were documented. Results. Facilities experienced in manufacturing cell therapy transplant products are the most successful in developing ATMPs. New centers lacking this background struggle to enter the field, and there remains a shortage of facilities in academia participating in translational research. This is compounded by heterogeneous implementation of the regulations across MS. Conclusions. GMP facilities successfully developing ATMPs are present in all MS. However, the implementation of regulations is heterogeneous between MS, with substantial differences in the definition of ATMPs and in the approved manufacturing environment. The cost of GMP compliance is underestimated by research funding bodies. This is detrimental to development of new ATMPs and commercialization of any that are successful in early clinical trials. Academic GMP practitioners should strengthen their political visibility and contribute to the development of functional and effective European Union legislation in this field.

Key Words: advanced therapy medicinal products, European Union, good manufacturing practice, manufacturing, regulation
Outside of Europe?
Acknowledgements

• Centre de Thérapie Cellulaire
  – Didier Bechlian
  – Maelys Berthomieu
  – Guillaume Bouchet
  – Farida Braham
  – Boris Calmels
  – Jérôme Couquiaud
  – Valérie de Cesare
  – Charlotte Durousseau
  – Julie Gaudé
  – Angéla Granata
  – Cathy Giustinelli
  – Anne-Marie Imbert
  – Claude Lemarié
  – Pierre Lignée
  – Benbella Mahi
  – Carine Malenfant
  – Boudra Makni
  – Sarah Ouffaï
  – Patricia Parc
  – Sylvie Portelli
  – Bénédicte Puddu
  – Lionel Regimbaud
  – Isabelle Sielleur
  – Sandrine Sow
  – Olivfier Vicari

• Unité de Transplantation et de Thérapie Cellulaire (U2T). Département d’Onco-Hématologie
  – Didier Blaise
  – Laurence Caymaris
  – Roberto Crocchiolo
  – Lucas Castagna
  – Raynier Devillier
  – Jean El-Cheikh
  – Catherine Faucher
  – Samia Harbi
  – Bilal Mohty
  – Sabine Fürst

Marseille Schtroumph Tribe
EBMT CELL PROCESSING COMMITTEE