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

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Local Organizer: C. Peters
May 8-10, 2014
Venue: Vienna, Austria

I, Christian CHABANNON, the undersigned, speaker in the ESH-EBMT Training Course, cited above, hereby disclose the following financial or commercial affiliations:

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<tr>
<td>SANOFI S.A.</td>
<td>X</td>
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<td></td>
</tr>
</tbody>
</table>

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In addition, I certify that I will prepare my own slides and that the content of my presentation will in no way be controlled by the corporate sponsors of this congress.

Dated and Signed April 23, 2014
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 observed 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 ➔ Cell Therapy Products (CTPs)
      2. Intended to alter the functional properties of collected cells = cell manufacturing ➔ ATMPs
   2. Storage
   3. Distribution
# Donor identification

<table>
<thead>
<tr>
<th>Donor – Recipient Relation</th>
<th>HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Matched</td>
</tr>
<tr>
<td>Related</td>
<td>PBSC BM</td>
</tr>
<tr>
<td>Unrelated</td>
<td>PBSC BM</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 Human resource</td>
<td>Apheresis unit</td>
</tr>
<tr>
<td><strong>Safety and comfort</strong></td>
<td>General anaesthesia Post-operative pain</td>
<td>Mobilization treatment</td>
</tr>
<tr>
<td><strong>Cell product</strong></td>
<td>Low content in HSC Low content in lymphocytes RBC (ABO incompatibility)</td>
<td>High content in HSC High content in lymphocytes</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>bone marrow</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>peripheral blood</td>
<td>150-400 ml</td>
<td>8x10^6/kg</td>
<td>250x10^6/kg</td>
<td>2-5x10^6 CD34+ /kg</td>
</tr>
<tr>
<td>umbilical cord blood</td>
<td>80-160 ml</td>
<td>0.2x10^6/kg</td>
<td>0.5-2x10^5/kg</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
Allogeneic HSCT Activity in Europe 2011

Stem cell source and donor type

- CB; 831; 6%
- BM; 3133; 22%
- PBSC; 10503; 72%

- HLA identical sibling/twin; 5711; 39%
- Unrelated; 7799; 54%
- Other relative; 957; 7%

Bone Marrow Transplantation, J R Passweg, et al. 2013. 48, 1151-1167
Stem cell sources for allogeneic HSCT (US, 1988-2013)
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>Ancestim</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 6×10^6 or more CD34+/kg (DiPersio JF et al, Blood 2009)

Kaplan-Meier estimate of proportion of non hodgkin’s lymphoma patients reaching 5×10^6 or more CD34+/kg (DiPersio JF et al, JCO 2009)
REVIEW

Autologous haematopoietic stem cell mobilisation in multiple myeloma and lymphoma patients: a position statement from the European Group for Blood and Marrow Transplantation

M Mohty1,2,7, K Hübel1,2,7, N Kröger3,27, M Aljurf4,27, J Apperley5,27, GW Basak6,27, A Bazarchi7,27, K Douglas8,27, I Gabriel5,27, L Garderet1,2,7, C Geraldes9,27, O Jaksic1,2,7, MW Kattan11,27, Z Koristek12,2,7, F Lanza13,2,7, RM Lemoli14,2,7, L Mendeleeva15,2,7, G Mikala16,2,7, N Mikhailova17,2,7, A Nagler18,2,7, HC Schouten19,2,7, D Selleslag20,2,7, S Suciu21,2,7, A Sureda22,2,7, N Worel23,2,7, P Wuchter24,2,7, C Chabannon25,2,7 and RF Duarte26,2,7

Autologous haematopoietic SCT with PBSCs is regularly used to restore BM function in patients with multiple myeloma or lymphoma after myeloablative chemotherapy. Twenty-eight experts from the European Group for Blood and Marrow Transplantation developed a position statement on the best approaches to mobilising PBSCs and on possibilities of optimising graft yields in patients who mobilise poorly. Choosing the appropriate mobilisation regimen, based on patients’ disease stage and condition, and optimising the apheresis protocol can improve mobilisation outcomes. Several factors may influence mobilisation outcomes, including older age, a more advanced disease stage, the type of prior chemotherapy (e.g., fludarabine or melphalan), prior irradiation or a higher number of prior treatment lines. The most robust predictive factor for poor PBSC collection is the CD34+ cell count in PB before apheresis. Determination of the CD34+ cell count in PB before apheresis helps to identify patients at risk of poor PBSC collection and allows pre-emptive intervention to rescue mobilisation in these patients. Such a proactive approach might help to overcome deficiencies in stem cell mobilisation and offers a rationale for the use of novel mobilisation agents.

Bone Marrow Transplantation advance online publication, 31 March 2014; doi:10.1038/bmt.2014.39
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>129</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>48</td>
<td>21-78</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74</td>
<td>44-130</td>
</tr>
<tr>
<td>CD34 / µL</td>
<td>63</td>
<td>6.7-237.6</td>
</tr>
</tbody>
</table>

![CD34/µL Distribution](chart.png)
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></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>57</td>
<td>65.9 ± 6.0</td>
<td>0.0449</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

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

### TABLE I. Specific Gravities of Blood Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.027</td>
</tr>
<tr>
<td>Platelets</td>
<td>1.04</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.06</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.05–1.07</td>
</tr>
<tr>
<td>Myelocytes/promyelocytes</td>
<td>1.07</td>
</tr>
<tr>
<td>Blasts</td>
<td>1.07–1.08</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>1.08</td>
</tr>
<tr>
<td>Mature granulocytes</td>
<td>1.09</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>1.095</td>
</tr>
</tbody>
</table>

CD34+ progenitor 1.074

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)

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

Table 2. Characteristics of bone marrow and PBSC collections

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow collection (N = 2726)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of anesthesia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>General</td>
<td>2619</td>
<td>96</td>
</tr>
<tr>
<td>Spinal</td>
<td>74</td>
<td>3</td>
</tr>
<tr>
<td>Epidural</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>Local</td>
<td>1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Volume of marrow collected, mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 500</td>
<td>325</td>
<td>12</td>
</tr>
<tr>
<td>500-1000</td>
<td>954</td>
<td>35</td>
</tr>
<tr>
<td>1000-1500</td>
<td>1185</td>
<td>43</td>
</tr>
<tr>
<td>&gt; 1500</td>
<td>261</td>
<td>10</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Median, range</td>
<td>1040</td>
<td>102-2313</td>
</tr>
<tr>
<td>Volume of marrow collected per donor weight, mL/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 10</td>
<td>882</td>
<td>32</td>
</tr>
<tr>
<td>10-15</td>
<td>905</td>
<td>33</td>
</tr>
<tr>
<td>15-20</td>
<td>826</td>
<td>30</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>112</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>Median, range</td>
<td>12.7</td>
<td>1.3-28.7</td>
</tr>
</tbody>
</table>

Pulsipher et al, Blood, 20& »
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
  – 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 ± 0.6 (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 ($10^6$)</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 ($10^6$)</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><strong>Donneur (greffon)</strong></td>
<td><strong>Receveur</strong></td>
<td><strong>Donneur (greffon)</strong></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>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AB</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 (10⁹)</th>
<th>CD34+ cells (10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HES</td>
<td>447</td>
<td>76.82 ± 9.10¹</td>
<td>81.46 ± 12.50</td>
</tr>
<tr>
<td>TB</td>
<td>181</td>
<td>60.72 ± 13.55²</td>
<td>81.99 ± 17.68</td>
</tr>
<tr>
<td>Sepax</td>
<td>213</td>
<td>80.26 ± 7.73²³</td>
<td>86.08 ± 11.63³⁴</td>
</tr>
</tbody>
</table>

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.

Lapierre V et al, 2007
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

• functional 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 origin 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 3. Microbial contamination of HPC grafts (BM and PBPCs): clinical relevance*

<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>Schwell 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>Schwell 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

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

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
REVIEW

Hematopoietic SCT with cryopreserved grafts: adverse reactions after transplantation and cryoprotectant removal before infusion

Z Shu¹, S Heimfeld² and D Gao¹

Transplantation of hematopoietic stem cells (HSCs) has been successfully developed as a part of treatment protocols for a large number of clinical indications, and cryopreservation of both autologous and allogeneic sources of HSC grafts is increasingly being used to facilitate logistical challenges in coordinating the collection, processing, preparation, quality control testing and release of the final HSC product with delivery to the patient. Direct infusion of cryopreserved cell products into patients has been associated with the development of adverse reactions, ranging from relatively mild symptoms to much more serious, life-threatening complications, including allergic/gastrointestinal/cardiovascular/neurological complications, renal/hepatic dysfunctions, and so on. In many cases, the cryoprotective agent (CPA) used—which is typically dimethyl sulfoxide (DMSO)—is believed to be the main causal agent of these adverse reactions and thus many studies recommend depletion of DMSO before cell infusion. In this paper, we will briefly review the history of HSC cryopreservation, the side effects reported after transplantation, along with advances in strategies for reducing the adverse reactions, including methods and devices for removal of DMSO. Strategies to minimize adverse effects include medication before and after transplantation, optimizing the infusion procedure, reducing the DMSO concentration or using alternative CPAs for cryopreservation and removing DMSO before infusion. For DMSO removal, besides the traditional and widely applied method of centrifugation, new approaches have been explored in the past decade, such as filtration by spinning membrane, stepwise dilution-centrifugation using rotating syringe, diffusion-based DMSO extraction in microfluidic channels, dialysis and dilution-filtration through hollow-fiber dialyzers and some instruments (CytoMate, Sepax S-100, Cobe 2991, microfluidic channels, dilution-filtration system, etc.) as well. However, challenges still remain: development of the optimal (fast, safe, simple, automated, controllable, effective and low cost) methods and devices for CPA removal with minimum cell loss and damage remains an unfilled need.

Bone Marrow Transplantation (2014) 49, 469–476; doi:10.1038/bmt.2013.152; published online 30 September 2013

Keywords: Hematopoietic stem cells; cellular therapy; dimethyl sulfoxide; side effects; removal of DMSO
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 Drezet5, Chloé Huynh1, Aurélie Autret4, Anne-Marie Stoppa5, Reda Bouabdallah5, Diane Coso5, Carine Malenfant1, Claude Lemarié1,2,6 and Christian Chabannon1,2,3,6

Case-match analysis using 4 matching factors with distinct 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

- HSC collection
  - donor evaluation
  - storage
  - equipment
  - facilities
  - staff
- collected product qualification
- HSC processing
  - traceability
  - labelling
  - transportation
- 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 arogen-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 endpoint 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 use of unstratified Cox regression models and in an analysis using the landmark start date for the time to the first treatment with a second-generation treatment with a median follow-up of 29.5 months.

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.P.P.); Sharp Healthcare, San Diego, CA (C.H.R.); New York University Medical Center, New York (A.C.F.); Taussig Cancer Institute, Cleveland Clinic, Cleveland (R.D.); Dendreon Corporation, Seattle (R.B.S., Y.Y., 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 reconstitutions

Colleen Delaney, Shelly Heimfeld, Carolyn Brashem-Stein, Howard Voorhis, Ronald L. May, Irwin 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 Kebrhaei, M.D., Simrit Parmar, M.D., Uday Popat, M.D., Chitra Hosing, M.D., Richard Champlin, M.D., Catherine Bollard, M.D., Jeffrey J. Mollod, M.D., Roy S. 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.N. Cooper, M.D., Ph.D., Laura Worth, M.D., Muzaffar H. Qazilbash, M.D., Martin Korb, 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

BACKGROUND

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 for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study


Summary

Background: Severe graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic transplantation with hematopoietic stem cells. Mesenchymal stem cells modulate immune responses in vivo and in vitro. We aimed to assess whether mesenchymal stem cells could ameliorate GVHD after hematopoietic stem cell transplantation.

Methods: Patients with severe acute, resistant, or severe acute GVHD received mesenchymal stem cell (MSC) transplantation on an outpatient basis every 3 weeks in three courses during the first 3 months after transplantation (n = 30). Patients with a complete response and nine showed improved clinical response.

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 cells/kg. 27 patients received one dose, 22 received two doses, and 6 received three or more doses of cells obtained from HLA-identical siblings (n = 1), 10 patients received a complete response and nine showed improved response.

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


Preparation and infusion of MSCs

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

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

Lynn M. Ball, Maria E. Bernardo, Hone J. Roedel, Maarten J. D. Van Tol, Benedetta Corneli, Neel Jan Zoguang, Maria Antonia Avanzini, Antonella Confetti, Alice Bertani, Giovanni Gigliotti, Cornelia M. Job, van der Zee, Marco Zucca, Katarina Lo Bianco, Francesco Fiorenza, Rudolph Maarten Egerer, Willem E. Flibbe, Arjan C. Lankseter, and Franco Locati.

Summary

Mesenchymal stem cell (MSC) infusions have been reported to be effective in patients with steroid-refractory, acute graft-versus-host disease (sGVHD) but comprehensive data on pediatric patients are limited. We retrospectively analysed a cohort of 37 children (aged 3 months-17 years) treated with MSCs for steroid-refractory grade III-IV sGVHD. All patients but three received multiple MSC infusions. Complete response (CR) was observed in 24 children (65%), while 13 children had either partial (n = 8) or no response (n = 8). Cumulative incidence of transplantation-related mortality (TRM) in patients who died or did not achieve CR was 17% and 69%, respectively (P = 0.001). After a median follow-up of 2.9 years, overall survival (OS) was 87% in patients who did or did not achieve CR, respectively (P = 0.001). The median time from starting steroids for graft-versus-host disease (GVHD) treatment to first MSC infusion was 13.9 days (range 5-85). Children treated between 5 and 12 days after steroid initiation showed a trend for better OS (56%) and lower TRM (17%) as compared with patients receiving MSCs 13-85 days after steroids (25% and 53%, respectively, P = 0.02 and 0.06, respectively). Multiple MSC infusions are safe and effective for children with steroid-refractory sGVHD, especially when employed early in the disease course.

Keywords: steroid-refractory acute graft-versus-host disease, mesenchymal stem 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,1,6 Bruce L. Levine,1,6 David L. Porter,1,3 Sharyn Katz,4 Stephan A. Grupp,1,6 Adam Bagg,1,3 Carl H. June,1,6

Tumor immunotherapy with T lymphocytes, which can recognize and destroy malignant cells, has been limited by the ability to isolate and expand T cell populations 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, which are generated in vitro in the absence of cytokines, engraft and expand in the blood and bone marrow in patients receiving high-dose chemotherapy. In three patients treated with advanced chronic lymphocytic leukemia, CAR T cells expanded ≥1000-fold in vivo, trafficked to bone marrow, and continued to rise for at least 6 months. Evidence for the target included B cell aplasia and hypergammaglobulinemia. On average, each infused CAR was estimated to eradicate at least 1000 CLL cells. Furthermore, a CD19-specific immune response was detected in the blood and bone marrow, accompanied by complete remission, in two of three patients. These results suggest potential for the development of CAR T cell therapy in the treatment of selected malignancies.

**CANCER**

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

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We report on 16 patients with relapsed or refractory B cell acute lymphoblastic leukemia (B-ALL) who were treated with autologous T cells expressing the 19-28z chimeric antigen receptor (CAR) specific to the CD19 antigen. The overall response rate was 90%, 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 (ATMP) 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 centres 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 centres 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?

**U.S. Regulation of Stem Cells as Medical Products**

Douglas R. Sipp and Leigh Turner

A recent decision by a U.S. District Court judge could have profound implications for the increasing number of U.S. clinics that advertise putative "stem cell treatments" for a wide range of clinical, rejuvenation, and aesthetic applications. In *United States v. Regenerative Sciences LLC et al.*, the court upheld the authority of the Food and Drug Administration (FDA) to require the premarketing approval of human stem cell-derived products that meet any of several broad criteria (1). The court concluded that grafted cells to patients solely on an intrastate basis constitutes interstate commerce. The court followed precedent in ruling that any drug containing a component from out of state triggers the interstate commerce clause, which is crucial, as the FDA only has authority over commercial activities that involve the crossing of state lines. The defendants had contended this point, and it seems likely that FDA authority to regulate stem cell-based interventions might extend to these activities as well.

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