Medical Issues in a Clinical Cellular Therapy Laboratory

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Objectives

I. Major and Minor ABO Incompatibility in HPC transplants
II. Blood components transfusion in the 3 stages of transplant
III. Stem cell infusion reactions
IV. Engraftment failure
Allogeneic Donor Selection

HLA (chrom 6) and ABO (chrom 9) antigens are encoded by different genes, inherited separately.

HLA matching is paramount.
HLA antigens are expressed on pluripotent stem cells,

ABO antigens are not expressed on pluripotent stem cells,
ABO antigens are expressed on endothelial and epithelial tissues.
30-50% of Allogeneic HPCT are ABO incompatible.
Major and Minor ABO Incompatibility in HPCT
ABO incompatible HPCT

It is known that clinically significant hemolysis can occur at:

• Infusion
• Post transplant

Q: Does ABO Incompatibility affect overall survival, non-relapse mortality or GVHD?
ABO-Incompatible HSCT

**Major:** Recipient has ABO Ab directed against donor RBCs

e.g. A donor \( \rightarrow \) O recipient

\[ \begin{array}{c}
\text{A donor} \\
A \\
\alpha A
\end{array} \quad \begin{array}{c}
\text{O recipient} \\
\Rightarrow \Rightarrow \Rightarrow \alpha A
\end{array} \]

**Minor:** Donor has ABO Ab directed against recipient RBCs

e.g. O donor \( \rightarrow \) A recipient

\[ \begin{array}{c}
\text{O donor} \\
\alpha A \\
<
\end{array} \quad \begin{array}{c}
\text{A recipient} \\
A \\
A \\
O
\end{array} \]

**Bi-directional:** Ab going in both directions

e.g. A donor \( \rightarrow \) B recipient

\[ \begin{array}{c}
\text{A donor} \\
A \\
\alpha B \\
<
\end{array} \quad \begin{array}{c}
\text{B recipient} \\
B \\
B \\
B \\
\alpha A
\end{array} \]
ABO INCOMPATIBLE ALLO TRANSPLANT

Pre-transplant Phase I
- Myeloablation (MA)
- Reduced intensity conditioning (RIC)
- Non-MA

Peri-transplant Phase II

Post Engraftment Phase III
- Engraftment: dynamic chimera stable chimera

Transfusion, cellular components
- Gamma irradiated
- Leukoreduced
- CMV neg --> CMV safe

- blood products compatible with both, recipient and donor
Q: Incompatibility?

- RBCs (Graft)
- Plasma (Recipient)
RBCs (Graft)

Plasma (Recipient)

A: Major Incompatibility
Problems with Major Incompatibility
Problems with Major Incompatibility

First stage: Infusion

**Problem:** Incompatible RBC infused with the HPC product may be hemolyzed by native ABO antibody in recipient

**Analysis:**
- RBC content in the HPC product
- Ab Titer in the Recipient

**Solution:**
- RBC depletion of the product with goal of <20-30 mL RBCs or < 0.4 ml/kg in final product
- For very high titers in recipient, consider apheresis
Problems with Major Incompatibility

Second stage: Engraftment

Problem: Recipient ABO antibodies may persist and hemolyze newly formed engrafting incompatible red blood cells. Delayed red blood cell recovery / Pure red cell aplasia.

Analysis: - CBC / Retic
- LDH, Bili, Haptoglobin
- Transfusion burden

Solution:
» Transfuse donor and recipient compatible red cells and plasma until reverse typing consistent with donor type.
» For very high titers in recipient, consider apheresis.
» Rituximab, Bortezomib.
Engraftment Problems with Major Incompatibility

- Delayed red blood cell recovery / Pure red cell aplasia

Donor RBC chimerism was markedly delayed following reduced intensity vs. myeloablative SCT
  (median, 114 versus 40 days; P < .0001)
- strongly correlated with decreasing host anti-donor isohemagglutinin levels.
  (Bolan CD, Blood, 2001)
Prolonged RBC Aplasia post Major ABO mismatched BMT:

18 plasma exchange with donor-type plasma

Q: Incompatibility?

Plasma (Donor)

RBCs (Recipient)
A: Minor Incompatibility

Plasma (Donor)

RBCs (Recipient)
Problems with Minor ABO incompatibility

First stage: Infusion

Problem: Incompatible plasma infused with HPC product, may hemolyze recipient red cells

Analysis: - HPC product volume
- Ab Titer of the Donor

Solution: Plasma reduction, prevents passive transfer of isohemaagglutinins (IH) i.e. prevents acute hemolysis
Problems with Minor ABO incompatibility

Second stage: Engraftment

Problem: Passenger Lymphocyte Syndrome

Viable B lymphocytes in HPC product produce isoagglutinins against residual recipient RBCs, 5-15 days post-transplant

Analysis:
- Positive DAT
- Donor derived isoagglutinins in serum and eluate
- 10-15% of patients with +DAT develop hemolysis

Solution:
» Supportive RBC transfusion
» RBC exchange (rare),
» Rituximab,
» Methotrexate
» (CD34 selection)
Passenger Lymphocyte Syndrome

Hemolysis subsides as residual recipient RBCs are destroyed or replaced by donor or transfused RBCs

Problems with Bi-Directional HPCT transplant
Bi-Directional ABO INCOMPATIBILITY

ABO INCOMPATIBILITY

MAJOR

- Lysis of Infused donor RBCs
  - by Recipient IH

- Keep CTP RBC volume < 0.4ml/kg

- Delayed RBC engraftment
  - Pure RBC Aplasia
    - by Persistent Recipient IH
  - Consider apheresis

- Passenger lymphocyte Syndrome (PLS) = GVHD!
- New IH synthesis
- Lysis of residual Recipient RBC
- Early Provision of Donor compatible RBC Tx
  - Rituximab

minor

- Infusion of plasma IH
- Lysis of Recipient RBC
- CTP Plasma reduction

IH - Isohemagglutinins
Role of Antibody Titers

- Anti-A or Anti-B
- Titer level depends on the individual’s immune system and prior exposure to ABO antigens
- Can be performed on donor (minor) and recipient (major)
- Quantified as $2^x$ (2, 4, 8, ...64,...512,...)
Role of Antibody Titers

Rowley, Bone Marrow Transplantation, 2011
**Laboratory Processing of HPC**

Red cell depletion:

Goal $< 20\text{-}30 \text{ ml} \text{ red cells}$

or $< 0.4 \text{ mL/kg} \text{ recipient weight}$

- **Required for BM!**
- **Umbilical cords** Many products now red cell depleted prior to cryopreservation, if not, cryopreservation lyses red cells and the product can be washed prior to infusion

**Plasma depletion**

(avoid acute hemolysis of recipient RBCs)
Does ABO Incompatibility change the risk of GVHD?

- Higher risk of GVHD after minor ABO-incompatible tx
- A or B antigens expressed on endothelial and epithelial tissue of the host are target for GVH response
ABO incompatibility and survival

Center for International Blood and Marrow Transplantation (CIBMTR) analysis of overall survival in patients receiving ABO matched, minor MM, major MM, or bidirectionally (Bidir) MM hematopoietic allografts for lymphoma Ratanatharathorn et al. 2009 (A) or AML/MDS Luger et al. 2012 (B) MM = mismatch C) Logan et al. Biol Blood Marrow Transplant 2015
Clinical Outcomes of ABO-Incompatible HPC Transplants

**Can Cause:**

- Immediate or delayed hemolysis
- Pure red cell aplasia (PRCA) - Major
- Passenger Lymphocyte Syndrome - Minor

**May Cause:**

- Delayed neutrophil or platelet engraftment*
- Increased risk of GVHD or rejection?
- Decreased Survival?
Transfusion Support for ABO-Incompatible Transplant
Transfusion Support ABO-I Tx

• Consider both donor and recipient type
• Donor and recipient compatible RBCs
• Donor-compatible plasma and platelets are used to avoid isoagglutinins directed against donor RBCs and to avoid delayed red cell engraftment
• Recipient compatible plasma and PLTs to avoid hemolysis
### Table 25-2. Transfusion Support for Patients Undergoing HSCT by Type of ABO Incompatibility and Stage of Transplant

<table>
<thead>
<tr>
<th>Type of Incompatibility</th>
<th>Transplant Stage</th>
<th>ABO Blood Group Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Preparative regimen</td>
<td>Recipient</td>
<td>Donor</td>
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<td>Transplantation</td>
<td>Recipient</td>
<td>Donor</td>
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<tr>
<td>Recipient antibodies detected</td>
<td>Recipient</td>
<td>Donor</td>
</tr>
<tr>
<td>Recipient antibodies no longer detected</td>
<td>Donor</td>
<td>Donor</td>
</tr>
<tr>
<td><strong>Minor incompatibility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparative regimen</td>
<td></td>
<td>Donor</td>
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<tr>
<td>Transplantation</td>
<td></td>
<td>Donor</td>
</tr>
<tr>
<td>Recipient cells circulating</td>
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<td>Donor</td>
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<tr>
<td>Recipient cells no longer circulating</td>
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<td>Donor</td>
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<td><strong>Bidirectional incompatibility</strong></td>
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<tr>
<td>Preparative regimen</td>
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<td>Group O</td>
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<tr>
<td>Transplantation</td>
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<td>Group O</td>
</tr>
<tr>
<td>Recipient antibodies detected/recipient cells circulating</td>
<td></td>
<td>Group O</td>
</tr>
<tr>
<td>Recipient antibodies no longer detected/recipient cells no longer circulating</td>
<td></td>
<td>Donor</td>
</tr>
</tbody>
</table>

*Due to the short shelf life and limited availability of group AB platelets, it may not always be possible to provide fully matched ABO platelet products as recommended in the table. Therefore, blood banks and transfusion services may consider providing ABO-mismatched platelets that have been volume reduced to diminish their plasma content. RBCs = Red Blood Cells.
# Transfusion Support ABO Incompatible HPC Transplants

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>All components</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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<tbody>
<tr>
<td>O</td>
<td>A</td>
<td></td>
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<td>A</td>
<td>AB;B;O</td>
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<td>O</td>
<td>AB</td>
<td>A;B;O</td>
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<td>O</td>
<td></td>
<td>O</td>
<td>A</td>
<td>AB;B;O</td>
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<tr>
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<td>B</td>
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<td>O</td>
<td>AB</td>
<td>A; B; O</td>
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<tr>
<td>A</td>
<td>AB</td>
<td></td>
<td>A</td>
<td>AB</td>
<td>A;B;O</td>
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<tr>
<td>B</td>
<td>O</td>
<td></td>
<td>O</td>
<td>B</td>
<td>AB;A;O</td>
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<tr>
<td>B</td>
<td>A</td>
<td></td>
<td>O</td>
<td>AB</td>
<td>B; A; O</td>
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<tr>
<td>B</td>
<td>AB</td>
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<td>AB</td>
<td>B;A;O</td>
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<tr>
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<td>A;B;O</td>
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<td>A</td>
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<tr>
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<td>B</td>
<td></td>
<td>B</td>
<td>AB</td>
<td>B;A;O</td>
</tr>
</tbody>
</table>
New Proposal - Priority: Protecting engraftment in Phase II

### TABLE 1. RBC, PLT, and plasma transfusion support for patients undergoing ABO-incompatible HSCT

<table>
<thead>
<tr>
<th>ABO incompatibility</th>
<th>Recipient</th>
<th>Donor</th>
<th>All products</th>
<th>RBCs</th>
<th>PLT</th>
<th>Plasma</th>
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<tr>
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<td>First choice</td>
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<td>B</td>
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<td>O</td>
<td>A</td>
<td>AB, A, O</td>
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<tr>
<td></td>
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<td>AB</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
<td>A, B, O</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>AB</td>
<td>Recipient</td>
<td>B</td>
<td>AB</td>
<td>A, B, O</td>
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<tr>
<td></td>
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<td>Recipient</td>
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<td>AB</td>
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<td>Recipient</td>
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<td>AB, B, O</td>
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<td>Recipient</td>
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</tr>
<tr>
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<tr>
<td></td>
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<td>AB</td>
<td>A, B, O</td>
</tr>
<tr>
<td>Major and minor</td>
<td>B</td>
<td>A</td>
<td>Recipient</td>
<td>O</td>
<td>AB</td>
<td>B, A, O</td>
</tr>
</tbody>
</table>

*The transition from Phase I (before HSCT) to Phase II should occur no later than the infusion of the HSC product, but can be defined by institutional guidelines to occur earlier. Day 14 before HSCT is often chosen, like at the initiation of induction chemotherapy.*

†The transition from Phase II to Phase III is marked by complete engraftment as defined by the recipient's peripheral blood typing as donor in both forward and reverse blood group typing. Institutional guidelines may define a delayed transition, like 1 year or later after HSCT; patients with successful engraftment may soon become transfusion independent, while continuing transfusion need may indicate a clinically relevant engraftment issue.

‡In order of preference.

§NA = not applicable.

‖For practical reasons, institutional guidelines often may define the use of donor type PLTs as first choice.

O’Donghaile D et al, Transfusion, 2012
# Provision of Blood Products

<table>
<thead>
<tr>
<th>RBC Blood Type</th>
<th>Donor</th>
<th></th>
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<tbody>
<tr>
<td>Patient</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>A</td>
<td>O</td>
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<td>B</td>
<td>O</td>
<td>O</td>
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<tr>
<td>AB</td>
<td>O</td>
<td>A</td>
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</table>

<table>
<thead>
<tr>
<th>Plasma/PLT 1ST CHOICE</th>
<th>Donor</th>
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</thead>
<tbody>
<tr>
<td>Patient</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
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</tr>
<tr>
<td>A</td>
<td>A</td>
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</tr>
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<td>B</td>
<td>B</td>
<td>AB</td>
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<td>AB</td>
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<table>
<thead>
<tr>
<th>PLT Blood Type 2nd CHOICE</th>
<th>Donor</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>O</td>
<td>A</td>
</tr>
<tr>
<td>O</td>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>A</td>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>B</td>
<td>AB</td>
<td>A</td>
</tr>
<tr>
<td>AB</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>
ABO incompatibility cases:
54 yo, woman with angioimmunoblastic T cell lymphoma Group O with history of multiple pregnancies, Received allogeneic Group A, PBSC from matched unrelated donor (MUD)
Conditioning and transplant delayed. However, donor was not available later. Product collected, shipped and cryopreserved by NYP. PBSC thawed and post-thaw washed

- Anti-A Titer of 4096!
What type of incompatibility is this?

Risk of hemolysis at transplant infusion?
   Unlikely, donor RBCs will be lysed during cryopreservation and red cell stroma will be removed during post-thaw washing

Risk of hemolysis at engraftment?
   Possibly
   Consider plasmapheresis if severe

Risk of pure red cell aplasia (PRCA)?
   20-30%
Follow-up

23 mLs of RBCs infused
Split in 2 bags for infusion
No reaction at time of infusion

No PRCA observed
ABO incompatibility case 2

35 yo male with cutaneous T cell lymphoma

Non-myeloablative allogeneic PBSC transplant (cyclophosphamide/fludarabine)
GVHD prophylaxis: cyclosporine
4x10^6 CD34/kg from MRD
Donor Group O, Recipient Group B

Day +7 patient developed fever, hyperbilirubinemia, LDH elevation and anemia (Hb3.4 g/dL), hemoglobinuria, and acute renal failure
What type of incompatibility is this?

Risk of hemolysis at transplant infusion?

Why does patient have hemolysis at Day 7?

Patient had a positive Direct Coombs with anti B in eluate

Transfer of mature donor lymphocytes in the stem cell product: **Passenger Lymphocyte Syndrome**
Why was the hemolysis so profound in this case of minor incompatibility?

PBSC graft has larger number of B cells than bone marrow
G-CSF mobilization activates B cells
GVHD prophylaxis is cyclosporine
   – mainly inhibits T cell function
Low intensity preparative regimen,
   – such regimens may allow preservation of the host’s erythropoietic capacity for longer times after transplant
Follow up

Cyclosporine discontinued due to deteriorating renal function
Grade II GVHD develops
Hemodialysis initiated

GVHD treated, however patient develops aspergillus pneumonia and dies of pulmonary hemorrhage
ABO incompatibility case 3

Transfusion dependent anemia post Allo MUD HPCT  A to O

- Hemoglobin concentration (mg/dl)
- WBC
- SDP Transfusion
- PLATELET-COUNT
- HEMOGLOBIN
- pRBC Transfusion

Post Transplant Day (PTD)
Myeloperoxidase

α1 Spectrin

α1 Spectrin
# ABO blood type conversion

<table>
<thead>
<tr>
<th>PTD</th>
<th>Forward (RBC) Typing</th>
<th>Reverse (Serum) Typing</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Anti-A</td>
<td>Anti-B</td>
<td>Anti-D</td>
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<td>0</td>
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<tr>
<td>99</td>
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<td>314</td>
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<td>4+</td>
</tr>
<tr>
<td>349</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>

What is appropriate ABO type for Blood Components transfusion?
Stem cell infusion reactions
Stem cell infusion reactions

Reported rates of mild reactions 20-30%

Reported rates of severe reactions <1%

Incidence varies depending on the type of product infused:
• BM, PBSC, or cord
• Autologous versus allogeneic
• Fresh or thawed
• Thawed washed versus bedside thaw
Reactions similar to all blood components

- Bacterial contamination/Sepsis
- TACO/volume overload
- Allergic
- Febrile non-hemolytic
- Hemolytic
- TRALI

Reactions unique to stem cell products

- DMSO reactions
- Granulocyte reactions
Microbial Contamination

Skin flora and environmental organisms are the predominant bacteria, mostly coagulase negative staphylococci

Padley et al conducted a large study, reviewing the microbiology data from 7,233 HPC products -1.6% of products had at least one positive culture result.

The clinical transplant team must be immediately notified, so that a collaborative discussion on the fate of the product can occur.

Factors to consider:
• Pathogenicity of the organism, gram negative rods raise particular concern
• Time to growth and the number of positive cultures
• Stage of the patient’s treatment

If proceeding with the infusion of a culture positive HPC product, prophylactic antibiotic therapy may be administered.
Volume Overload:

Incidence: unknown
may be higher than that seen with red blood cell transfusion given
the large volume of the transplant, which is often greater than a liter.

Many HSC transplant recipients are at a higher risk due to their age or
renal/cardiac comorbidities and often require transfusion support for
thrombocytopenia or anemia during conditioning, so they may already
be in positive fluid balance.

A careful assessment of the patient’s volume status should be
performed prior to infusion of the HPCs.
HPCs should always be infused slowly with close monitoring.

Preventing Volume Overload
Splitting of large volume HPC product with infusion on two consecutive
days and diuresis in between
Volume reduction of the product
Allergic reactions

Manifest in a similar way to allergic transfusion reactions.

Allergen may be a protein in an allogeneic donor’s plasma or a product added during processing, such as dextran or DNAse.

Allergic reactions can often be prevented by premedication with antihistamines, steroids, or a combination of both.
Reactions related to cryopreserved/thawed products

DMSO (Dimethyl sulfoxide)

- Polar, osmotically active cryoprotectant
- <1 g/kg DMSO per 24 hour period
- 100 mL total volume HPC unit containing 10% DMSO concentration of will have 10 g DMSO

DMSO reactions related to histamine release and mast cell degranulation

May also cause hemolysis and negative chronotropic effects on cardiac tissue
DMSO reactions

**Mild**

- Headache
- Nausea
- Shivering
- Garlic or sweet corn taste
- Pain at IV insertion

**Severe**

- Cardiovascular: Heart block, hypotension, hypertension, cardiac arrest
- Neurologic: amnesia, encephalopathy, seizure
- Pulmonary
- Hemolysis
DMSO reactions

**Figure 1** Mean centre incidence of DMSO toxicity by DMSO reduction strategy. Error bars show standard errors.

Windrum P Bone Marrow Transplantation, 2005;36, 601-603.
Adverse reactions due to granulocytes in the product

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

Calmels, Transfusion. 2007;47, 1268-1275
Engraftment not Affected by Adverse Reactions

Fig. 3. Hematopoietic reconstitution of patients with or without AEs. ANC = absolute neutrophil count.
AE Documentation and Reporting

FDA, CAP, AABB and Foundation of Accreditation of Cellular Therapy (FACT) require a process for the detection, reporting, evaluation, and documentation of adverse events related to stem cell infusion.

Severe adverse events should be reported to the laboratory immediately.

Events should be summarized and monitored, through committees such as Transfusion committee or Transplant Quality committees.
Engraftment failure
Causes of engraftment failure

Clinical

• Relapse
• Anti-HLA Ab (DSA)
• Isoagglutinins
• Conditioning regimen
  (non-myeloablative)
• Viruses (HSV6, CMV, parvo)
• Drugs that damage the BM post tx
  (Cotrimoxazole and Ganciclovir)
• Low B12 and folate levels
• Insufficient cell dose (inadequate mobilization)

Laboratory

• Insufficient Cell Dose (miscalculation)
• Damage to cells during collection
• Damage to the cells during storage
• Damage to cells during manipulation or cryopreservation
Engraftment Failure Case

41 yo F with AML who received a related allogeneic PBSC transplant from her 10/10 HLA matched brother.
B positive to B positive.

Product was collected in house on Day -1 @ 15:05 and received into the laboratory minutes later.
The product was stored overnight at 4C.

Product required dilution with Plasmalyte-A due to the high pre WBC count.
The product was infused on Day 0 at 11:45.
Time elapsed between product collection and infusion was approximately 21 hours.
Laboratory approach to engraftment failure

- Review all initial processing and calculations
- Thaw a QC cryovial (cryopreserved sample from the initial product) and perform re-characterization
  - WBC
  - Viability (expected to be lower than HPC)
  - CFUs
- Confirm no reagents were recalled/expired
Review of Characterization:

All calculations were rechecked to confirm correct dosing

- WBC Post-processing: $3.96 \times 10^8$/ml
- Viability CD34 (7-AAD): 98.10%
- Viability (TB): 100%
- CD34 Dose infused/kg: $4.985 \times 10^6$
- CD3 Dose infused/kg: $19.78 \times 10^7$
- RBC content infused: 6.52 ml (0.15ml/kg)
- CFU (Results from QC cryovial): No growth

- Reagent recalls: Plasmalyte (500ml) Baxter lot #: C873505, EXP: 9/30/13-NO RECALL
Why did this patient have engraftment failure?

Lack of engraftment potential for donor’s cells?  
Unlikely

Damage to cells during collection?  
No evidence

Damage to cells during processing?  
No evidence

Patient related reason for engraftment failure?

More common with AML, in the end patient had relapse
She also was treated with ganciclovir during conditioning
Later after the transplant developed CMV infection
"Boost"

This recipient received another transplant on Day 22. The patient received some of the remaining aliquots from the original collection.

The post-thaw/wash characterization results were as follows:

- CD34/kg: $8.6 \times 10^6$/kg
- CD3/kg: $39.98 \times 10^7$/kg
- Trypan blue viability: 86%
- RBC/kg: 0.08 ml/kg.
Donor was patient’s brother, only unusual PMH from brother was mild eosinophilia. He came in for a BM biopsy, at which time we set up CFUs. CFU had adequate growth; BM biopsy was normal.

2nd Matched Related (MRD) Transplant

Brother recollected PBSC on Day 50. 10x10^6 CD34 were infused. CFU showed adequate growth on this collection. Patient engrafted.
Clinical

- Relapse
  - HLA Ab (DSA) or Isoagglutinins
  - Conditioning regimen (non-myeloablative)

- Viruses (HSV6, CMV, parvo)
  - Use of drugs that damage the BM

- Post tx (Cotrimoxazole and Ganciclovir)
  - Low B12 and folate levels
  - Insufficient cell dose (inadequate mobilization)

Laboratory

- Insufficient Cell Dose (miscalculation)
- Damage to cells during collection
- Damage to the cells during storage
- Damage to cells during manipulation
Suggested reading:


