Topics to be presented today:

*Introduce and explain indications for:*

- Bone Marrow Processing
- CD34 flow testing and reporting
- CD34 positive selection of stem cell products for the purpose of T cell depletion
- Cord Blood thaw and wash prior to infusion
Types of HPC Products used for BMT

- **Apheresis**: 200 ml
- **Bone Marrow**: 1 L
- **Cord Blood**: 25 ml
Steps in BMT Cell Processing:

1) Collection  2) Processing for Freezing & Storage  3) QC testing  4) Thaw & Infusion
Bone Marrow Harvesting

- Performed in theatre under general anaesthesia, collected by trained doctors
- HPC(M) is obtained by multiple aspirations from the iliac crest or sternum
- Maximum marrow can be removed 20ml/Kg body weight
- This process takes approx. 2 hours
- Target TNC dose >2 x10^8/Kg
- Common anti-coagulant is ACD-A
- Ratio 1:5, example
  - 100mls ACDA + 500mls BM, total 600 mls in 1 bag
- BM is filtered in BMT Lab to remove fat, clots and bone spicules, in a closed bag system
Bone Marrow Processing in Lab

- Common machine used for BM processing is the Cobe 2991 machine
- Operates on centrifugal principals
  - Spin BM for 10mins at 3000rpms
  - Multiple spins for 1-2 L BM
  - 2-3 hours
- Centrifugation into 3 layers; RBC/Buffy coat cells/plasma
Processing BM on the COBE 2991

- COBE centrifuge bowl
- Buffy coat
- Plasma
- Red cells
Why Bone Marrow Process?

- Debulk BM in preparation for cryopreservation, want to freeze down BM buffy coat cells

- Plasma Depletion (or volume reduction)
  - Adult donation for a pediatric patient, large volume of collected cells, remove some of the plasma to obtain a safe infusion volume of <20 ml/Kg.

- RBC Depletion
  - Major ABO incompatibility of donor graft to patient
  - Starting BM volume for adults is 1-2 L
  - Typically have a starting RBC volume of 300 to 500 mls
  - After RBC depletion on Cobe 2991, have approx 90% removal of RBC, >75% TNC recovery and >80% CD34 recovery
  - Target is to infuse a safe incompatible RBC volume of < 0.5 ml/Kg in graft
Challenges with the Cobe 2991:

• Machine is reaching its retirement (>25 years old)
• Manual, hands-on
• No walk-away
• Typical 1.5 L marrow will require >3 hrs of processing on Cobe 2991
• Intimidating for new lab staff in training
New Marrow Processing:

- Spectra Optia: Commonly used for Apheresis collections
Processing time on the Optia:

• Optia = 40-75 mins
• Cobe 2991 = > 3 hrs for 1.5L marrow
• Bone Marrow MNC and not BM buffy
• Operator adjustments - Optia 1/10 compared to Cobe 2991 10/10
## Results:

<table>
<thead>
<tr>
<th>Median Cell Parameters</th>
<th>Published Results N=30 (28/20 Allog donors)</th>
<th>Westmead’s Results N=8 2015-current</th>
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<tbody>
<tr>
<td>Volume reduction</td>
<td>93%</td>
<td>91%</td>
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<tr>
<td>MNC recovery</td>
<td>79%</td>
<td>75%</td>
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<tr>
<td>RBC reduction</td>
<td>98.8%</td>
<td>98%</td>
</tr>
<tr>
<td>CD34 recovery</td>
<td>88%</td>
<td>89%</td>
</tr>
<tr>
<td>CD3 recovery</td>
<td>79%</td>
<td>78%</td>
</tr>
<tr>
<td>Neut &gt; 0.5 (days)</td>
<td>20</td>
<td>19.5</td>
</tr>
<tr>
<td>PLT &gt; 20 (days)</td>
<td>19.6</td>
<td>25</td>
</tr>
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</table>
Summary on BM Processing:

• The Spectra Optia can effectively volume reduce and RBC deplete marrow before transplantation
• Fast cell processing
• Small volume for infusion (<100 ml), great volume reduction > 90% reduction
• Produces consistent results with CD34 recovery
• Extreme RBC reduction >96%, great for major ABO incompatible donor marrow grafts
• Safe for adults and pediatrics for BMT
Apheresis Collection

- MNC collected by apheresis machines
- Time approx 4 hours
- 10-12 L blood volume processed for an adult
- Average volume of PBSC collected
  - 200 to 250 ml (adult)
  - 40-100 ml (paediatrics)
- Target: To collect >2x10^6 CD34/Kg
- Request for 100 mls plasma
- ACD-A added as anti-coagulant (1:12)
- Requires good venous access
- Can collect on multiple days to achieve CD34 target if donor is fit
Optimum Transport of Fresh HPC Products is 2 - 8 °C, not at RM temp.
How we perform volume reduction for next cell processing steps:
Testing of stem cell products for CD34
Why a CD34+ Assay?

- CD34+ cells: typically represent 1% of TNC in HPC (range 0.2 – 5%), thus need an accurate and reproducible assay.
- The number of CD34+ cells infused for transplantation is a clinically important variable for engraftment.
- Collection Target for infusion:
  - Min >2 million CD34/patient weight
  - Aim >5 million CD34/patient weight
- Good internal QC for the monitoring of the laboratory practices for collecting and processing of HPC products.
Add 50µl buffer to tube

Add 10 µl HPC sample (1-3x10^6 cells) by reverse pipetting

Vortex to disperse bead

Lyophilized Bead pellet with known # of beads

10 mins incubation RT

Viable CD34 Assay
Add 450µl buffer or warm lysing solution (NH₄Cl)

Flow Cytometer

CD34 count-30 mins
International ISHAGE Gating Strategy for CD34 counts:

**Enumerating Viable CD34+ cells**

\[
\text{#CD34+ events in R6} \times \text{beads per tube}^* \times \frac{\text{#beads in R7}}{\text{HPC vol } \muL}
\]

\[
\frac{3101 \times 49,944}{8535} = 369 \text{ CD34+ cells } / \muL \text{ or } 369 \text{ CD34+ cells/Kg}
\]

* Number given by manufacturer
Accurate Assay to Enumerate viable CD34+ cells in HPC Products

**HARVESTED Apheresis**

- No Dead cells

**THAWED Apheresis**

- No Dead cells

- Dead cells

- Dead CD34+ cells
CD34 Positive Cell Selection:

**CD34+ Cell Selection:**
- The purification and isolation of CD34+ human stem cells from Apheresis products

**Clinical Applications:**
- TCD of allogeneic grafts, to reduce GVHD
  -Predominantly used in the pediatric MUD setting
Why CD34 Positive Cell Selection?

- Allogeneic transplantation
- Removal of donor T cells to prevent GvHD
- Goal: Prevention or reduction of GVHD
- Laboratory positive selection of CD34+ selection cells
How does the CD34 Selection device work?

Based on immunomagnetic bead technology

**CliniMACS**

- **CD34+**
- Anti-CD34 Ab + bead
- Magnet

(50nm bead)
# CD34 Flow Cytometry Results

## Pre-Selection

- **CD34**: $2.8 \times 10^6$/kg
- **CD3**: $6300 \times 10^5$/kg

## Post-Selection

- **CD34**: $2.1 \times 10^6$/kg
- **CD3**: $0.2 \times 10^5$/kg

Mean CD34 Recovery > 75%  Mean Purity > 90%

**HPC, Apheresis**

![Flow Cytometry Plots](chart.png)
CD34 + Selection Process: Costs

- $8.5K to $15K
- Requires special lab staff training
- Not all BMT labs perform this procedure
The CliniMACS Plus system has been used in our laboratory for over 15 years to remove T-cells from stem cell grafts of the unrelated donors for paediatric CHW patients undergoing BMT.

Multiple equipment - 2 F/T staff and approx. 5hrs
New Technology: The CliniMACS Prodigy

CliniMACS® plus

CliniMACS® Prodigy
Fully automated closed system cell processing:
Results:

CD34 Recovery %

<table>
<thead>
<tr>
<th></th>
<th>CliniMACS</th>
<th>Prodigy</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>66</td>
<td>9</td>
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<tr>
<td>CD34 Recovery %</td>
<td>69</td>
<td>67</td>
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</table>

P value: 0.4953

CD34 Purity %

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</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>66</td>
<td>9</td>
</tr>
<tr>
<td>CD34 Purity %</td>
<td>82</td>
<td>76</td>
</tr>
</tbody>
</table>

P value: 0.1152
Results:

CD3 or T Cell Log Depletion

- ClinMACS
- Prodigy
- n = 63
- n = 9
- P value 0.0117

CD19 or B Cell Log Depletion

- ClinMACS
- Prodigy
- n = 57
- n = 9
- P value 0.0099
Why we like the Prodigy:

- The recovery and purity of CD34+ cells was comparable to the CliniMACS Plus
- The Prodigy offers a single device for CD34 cell selection, unlike the CliniMACS Plus which requires other equipment
- The Prodigy process is fully automated and this translates into a reduction in the time required of experienced laboratory staff
- Our results suggest that the Prodigy can be used for the routine clinical application of CD34 selection to HSCT products
The process of receiving, testing and preparing a CB unit for transplantation:

1) Cord Blood Banks

2) Transport

3) Transplant Center Laboratory

4) CB Infusion

Communication:
• CBB and TC Registry
• Clinical BMT Team
• Transplant Center Lab Staff
• Courier company
Cryopreserved CB units come in different bags and vary in the type of processing performed prior to freezing.

1 bag

Old bag
Not RBC depleted before freezing

New bag
Plasma and RBC depleted before freezing

3 bags
History of our Lab Practice on CB units prior to Infusion:
As of 2013, n=112 CB units for 90 patients

- **2000-** washed all UCB units
  - Antonenas V et al, BMT 2004 - mean TNC cell loss of 27%, (range 11-41% n=21)

- **2005-** CB units were routinely thawed with **no washing and infused immediately**
  - No lab processing, infuse at bedside - as per marrow and apheresis infusions
  - CB infusions for Adults (single and double)
  - But noticed serious adverse reactions for some paediatric patients and noticed 'thickness' of thawed CB for some infusions

- **2010- present-** CB units are routinely **thawed and diluted prior to infusion**
  - As described by Barker et al, BBMT 2009;15(12):1596-602 and
  - As described by Regan, D et al, BBMT 2006

- **Washing** was ONLY performed for:
  - CB units that have **NOT** been volume / red blood cell reduced prior to freezing or
  - For paediatric patient weights <20 Kg
The Thaw and Wash Method:

- Traditional wash method - developed by Rubenstein 1995
  - CB products were not red cell or plasma reduced, cryopreserved in large volumes, thus greater amounts of DMSO for paediatric infusions
- Same solution mixture as used for the dilution method
  Dextran-40 and Albumex-4 solution (1:1) volumes
- Centrifugation is required
- Reduces DMSO toxicity and the free haemoglobin
- Restores osmolarity & extends cell viability
- Decreases CB infusion volume
- Infuse within 2 hrs of thawing
Why wash all the non RBC-depleted units?

NMDP and WMDA NOTICE:  
Adult CB infusion resulting in Death (March 2013)

- Patient death following a double CB infusion  
  M/39, CML transplant date March 2013  
  History of cardiac risk factors

- CB unit #1- **red cell replete** B+ and patient O+ in 105 mls in 2 bags- infused directly

- CB unit #2- **red cell replete** O+ 75 ml in 1 bag-thawed and infused
Thaw and Wash Method:

After Centrifugation of a diluted RBC-depleted CB unit

After Centrifugation of a diluted Non RBC-depleted CB unit
The Challenge of Washing CB units:

- Most labs are unfamiliar with the wash method
- Difficult to see the separated layers after centrifugation
- Some cell loss is associated
- Intimidating wash-tubing sets sometimes provided by the CBB’s
- Unfamiliar with the SOP provided by CBB’s as to how to wash unit
How should transplant centres treat CB units for infusion?

- Best and easy method is to perform the thaw and wash method on all CB units
- We have seen a decrease in reported adverse reactions
- **MUST** Wash all non RBC-depleted CB units
Acknowledgements

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