POST THAW CD34 RECOVERY AND VIABILITY OF HEMATOPOIETIC PROGENITOR CELLS USING A SIMPLIFIED DILUTION METHOD AND A MODIFIED GATING AND ANALYSIS STRATEGY.

Nicole L. Prokopishyn PhD
Director
Cellular Therapy Laboratory
Nicole.prokopishyn@cls.ab.ca
How do we determine if a cryopreserved product will be an effective/successful graft when it is thawed on day of transplant?
Cryopreservation of HPC, Apheresis Products for AUTOLOGOUS Transplant

HPC(A) Product Collected

+ 20% DMSO solution

HPC(A)

80 mL

80 mL

80 mL

QC

QC

Cryopreserved via Controlled Rate Freezing
Stored in Vapour Phase Liquid Nitrogen
Retrospective Analysis of THAW HPC(A) Products for AUTOLOGOUS Transplant

Pre-Freeze CD34 Dose = 5 x 10^6/kg

Post-Thaw CD34 Dose = ???

CD34 cell enumeration

Retrospective Analysis
Cells have already been transplanted

Post-Thaw CD34 Dose = 4.8
96% recovery

Post-Thaw CD34 Dose = 0.5
10% recovery
Prospective Analysis of THAWED of HPC(A) Products for AUTOLOGOUS Transplant

Pre-Freeze CD34 Dose = $5 \times 10^6$/kg

THAW

CD34 cell enumeration

Calculated CD34 Dose
ReCon Solution

10% LMD40
5% Human Serum Albumin

> Dilute thawed cells 1:4 with ReCon immediately after thaw

- Reduces negative effects of DMSO on the cells upon thaw
- Maintains stability of cells
  - >4 hours without loss of CD34 cell number of CD34 Viability
Flow Cytometry Analysis

- CD45-FITC, CD34-PE antibodies and 7-Aminoactinomycin D staining.
- Stem cells were enumerated using a single platform analysis by adding Flow Count beads™ and analyzed on a Beckman Coulter FC 500 Flow cytometer.
- Modified protocol based on ISHAGE
  - Validated against standard ISHAGE protocol using fresh and frozen/thawed HPC(A) and HPC(M).
80 mL sample

CD34 cell enumeration

Calculated CD34 Dose

80 mL ReCon

THAW

QC

THAW

QC

CD34 cell enumeration

ReCon
Calculated QC Vial Results Representative of CD34 cells in Infusion Bags Post-Thaw

<table>
<thead>
<tr>
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<th>QC Vial (n = 48)</th>
<th>Infusion Bag (n = 48)</th>
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<tbody>
<tr>
<td>CD34 % Recovery</td>
<td>92.2 (+/- 15.2)</td>
<td>89.4 (+/- 11.5)</td>
</tr>
<tr>
<td>CD34 % Viability</td>
<td>92.1 (+/- 6.7)</td>
<td>96.4 (+/- 7.4)</td>
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</tbody>
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Prospective Analysis of Cryopreserved HPC(A) identifies products with inferior CD34 recovery & viability

Mean = 88.9%
SD = 19.9%
Majority of HPC(A) Products have excellent post-thaw CD34 Recovery & Viability

- 75 - 100%
- 50 - 74%
- 25 - 49%
- 0 - 24%

Patient Diagnosis:
- Waldenstrom Macroglobulinemia
- Cryoglobulinaemia
- Amyloidosis
- Multiply Myeloma

- 72

- 3
- 1

Patient Diagnosis:
- Multiply Myeloma
- Lymphoma
- Neuroblastoma
Summary

- QC Vials are a good representative of infusion bags
- Thaw & dilution in ReCon solution allows for CD34 enumeration allows for assessment of graft quality prior to transplant.
- HPC(A) products from specific patients may be prone to poor recovery post-thaw
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