and decrease human error. The AutoXpress Platform are transitioning to automated systems to standardize methods.

TNC and MNC were measured both pre- and post-process-since collection, and a clotting score of 0 to 2 

ing AXP had a volume of 40–130 mL, an age of less than 48 hours 

on the volume, age, and the degree of clotting. Units processed us-

laboratory were allocated to either ficoll or AXP processing based 

umbilical cord and transported to CBR’s processing facility in Tuc-

at enrollment, and after delivery, cord blood was collected from the 

CBU at Cord Blood Registry (CBR). Collection kits were provided 

from 1414 consenting mothers who elected to preserve and bank 

Methods:

covery rates regardless of variability in collection volume.

the use of the AXP system in a directed donation family cord blood 

bag at a uniform volume of 21 mL.

separated into composite cell populations and the TNC fraction is 

maintained MNC recovery of greater than 98%.

decreases the labor and time required for CBU processing while 

system yields the highest published cell recovery rate to date and 

evaluating the differences between processing centers. The AXP 

lection volume, percent recovery becomes particularly important in 

for stem cell dose if the sample is used in transplant. Because limited 

tumour burden in some patients, including those with “negative” 

use of autologous PBPCs “negative” for contaminating tumour cells 

non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM).

Background: Relapsed disease remains a major obstacle follow-

ing autologous hematopoietic stem cell transplantation (HSCT) for 

non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM).

Studies regarding the role of residual tumour cells collected in au-

tografts in earlier relapse and reduced survival have been inconclu-

sive. The impact of residual disease detected by sensitive molecular 

methods in autologous PBPCs remains uncertain and is addressed 

in this study. Methods: Patients undergoing autologous HSCT 

for NHL and MM at our institution between June 2001 and January 

2006 were enrolled (n = 158). Aliquots of freshly collected PBPC 

collections were assessed for the presence of clonal IgH gene rear-

rangements using qualitative semi-nested PCR. Patients with detec-

table clonal IgH gene rearrangements were designated “positive” and compared with “negative” patients without detect-

able IgH gene rearrangements, and time to next treatment were determined for all patients. All out-

comes were compared using the method of Kaplan and Meier.

Results: In comparison to patients with “positive” PBPC grafts, 

patients “negative” for detectable disease had no improvement in 

overall survival for MM (p = 0.91) and for NHL (p = 0.82). Further 

analysis based on tissue histology in patients with NHL revealed no 

significant difference in overall survival between patients with “posi-

tive” grafts compared with “negative” PBPC collections (aggressive 

histology NHL, p = 0.74; indolent NHL, p = 0.29). There was also 

no significant improvement in progression-free survival among pa-

tients with NHL (p = 0.85) or MM (p = 0.91). Conclusion: The 

use of autologous PBPCs “negative” for contaminating tumour cells 

does not improve overall survival in MM or NHL. Further-

more, the absence of detectable clonal IgH rearrangements using 

sensitive PCR did not correlate with a reduction in progression-

free survival. Our results suggest that disease relapse cannot be ad-

equately explained by the reinfusion of PBPCs containing residual 

tumour cells. It is possible that high dose chemotherapy regimens 

used in autologous HSCT are not sufficiently eliminating residual 

tumour burden in some patients, including those with “negative” 

PBPC collections. Taken together, our results suggest that strate-

gies aimed at removing tumour cells from autologous PBPC grafts 

in patients with MM and NHL may have marginal benefit.

<table>
<thead>
<tr>
<th>Table 1. Factors Predictive of Neutrophil and Platelet Engraftment in Multivariate Analysis of Graft/Recipient Characteristics</th>
<th>AXP Stem Cell Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multivariate Model</strong></td>
<td><strong>Mean Post-</strong></td>
</tr>
<tr>
<td><strong>Neutrophil</strong></td>
<td><strong>Processing</strong></td>
</tr>
<tr>
<td><strong>Engraftment</strong></td>
<td><strong>(mL)</strong></td>
</tr>
<tr>
<td>Pre-cryopreservation</td>
<td>CD34+ (0.0046)</td>
</tr>
</tbody>
</table>

IgH GENE REARRANGEMENTS IN PBPC MONONUCLEAR CELLS DOES NOT INFLUENCE SURVIVAL OR RELAPSE FOLLOWING AUTOLOGOUS TRANSPLANTATION FOR B-CELL LYMPHOPROLIFERATIVE DISEASES

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Foster, J1, Yang, L2, Benibahemi, B3, Martin, L2, Hatberrri, M2, McDargt, S1, Huebch, L1, Sablof, M1, Atkins, H1, Benczuckier, L1, Guileci, A3, Allam, D1. 1 University of Ottawa, Ottawa, ON, Canada; 2 Canadian Blood Services, Ottawa, ON, Canada.

Background: Relapsed disease remains a major obstacle follow-

ing autologous hematopoietic stem cell transplantation (HSCT) for 

non-Hodgkin’s lymphoma (NHL) and multiple myeloma (MM).

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gies aimed at removing tumour cells from autologous PBPC grafts 

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expression and production of IL-15, IL-12 and IL-18 in activated CB (Qian/Cairo, Blood, 1997; Lee/Cairo, Blood 1996; Sawarri/Cairo, Br J Haem, 2005) may contribute to diminished CB cellular immunity and delayed immune reconstitution after UCBT. NK (CD3–/CD56+) effector cells recognize target cells and express activating and inhibitory receptors. NK cells may also play a role in allograft and tumor cytotoxicity. We reported the ability to EvE cryopreserved, thawed, recyopreserved, rethawed CB with increased NKbright/dim subsets expressing KIRs and NCR with enhanced in-vitro and in-vivo cytotoxicity (Ayello/Cairo, BBMT, 2006). In this study, we compare 2 vs 7 d expansion and activation of NK and NKT subsets expressing NKRPs and the production of IL-15, IL-18 and IFN-γ. Rethawed CB cells were cultured with anti-CD3, IL-2, IL-7 and IL-12; 2–7 d. Days 3, CD3, CD16, CD56, CD94, NKG2A, NKG2D, Nkp46, KIR2DS4, KIR3DL1, LAMP-1 and granzyme-B expression were determined by flow cytometry; IL-15, IL-18 and IFN-γ protein by ELISA. Non-adherent total cells were significantly increased (6.2 × 214 × 10^6 vs 5.8 ± 57 × 10^6, p < 0.001) with no change in NKdim subset; but significant increased NKT subset (71.8 ± 6.0 vs 2.97 ± 0.3%, p < 0.001). NKNKhigh subsets were decreased (3.3 ± 1.1 vs 13.4 ± 1.4%, p < 0.01); whereas, NKNKbright subset was significantly increased (10.6 ± 1.4 vs 0.7 ± 1.1%, p < 0.05). NK KIR3DL1 NKdim subset was not significantly increased, but NK KIR3DL1 NKbright subset was increased (17.1 ± 12.1 vs 1.1 ± 0.5%, p < 0.05). CD94/NKG2A expression was decreased (7.8 ± 1.3 vs 22.7 ± 1.0%, p < 0.001.) while CD94/NKG2D subsets were increased (24.8 ± 0.1 vs 3.1 ± 0.4%, p < 0.001; 19.0 ± 0.6 vs 1.0 ± 0.1%, p < 0.001, respectively), as well as CD26 (65.3 ± 2.2 vs 12.95 ± 1.5%, p < 0.001), granzyme-B (33.6 ± 0.61 vs 25.8 ± 1.79%, p < 0.001), and IL-15, IL-18 and IFN-γ protein production (7.6 vs 47 ± 183 ± 8.8 pg/ml, p < 0.001; 37.3 ± 7.6 vs 21 ± 1.4 pg/ml, p < 0.05). In summary, BM MNC may be thawed at time of UCBT, recyopreserved, rethawed, and activated for up to 7 d to yield increased NK and NKT NKdim NKKIR2DS4 NKKIR3DL1 NKNKdim NKKIR2DS4 NKKIR3DL1 expression but did not enhance NK cell cytotoxicity of tumor targets. The decline in NKG2D expression in lenalidomide treated cells may have offset any potential increase in NK cell cytotoxicity expected to occur as a consequence of upregulating granzyme B and TRAIL. Prior studies showing in vivo enhancement in NK cell numbers and cytotoxicity after treatment with lenalidomide may be related to the effects of this drug on other cellular populations which indirectly enhance NK cell function in vivo.

**Processing cryopreserved UCB cord blood for adult transplantation with minimal manipulation and cell loss: a single institution experience**

Parthasarathy, M., Rodriguez, T., Smith, S., Lu, S., Stiff, P. Loyola University Medical Center, Maywood, IL.

Hematopoietic Stem Cells from Umbilical Cord Blood (UCB) are increasingly being used as an alternative to marrow or peripheral blood stem cells in adults. Most Cord Blood Centers have recommend a post thaw washing step to remove the cryoprotectant DMSO (dimethylsulfoxide). As the total nucleated cell (TNC) dose is considered one of the most important factors for successful engraftment, a re-exploration of the need to wash thawed UCB cells has been underway in many centers. To control for all UCB unit variables (cryo bags, volumes etc.) and standardize thawing process we established a methodology and made it our standard operating procedure (SOP) after proper validation. Rather than washing, our protocol uses a 4:1 dilution method that minimizes cell loss and decreases DMSO concentrations to <2%. The diluent medium consists of 8% human albumin in10% dextrose. Units are thawed in a 37°C water bath and then diluted over 12 minutes with three rates set to deliver 10%, 30% and 60% of the volume at 5%, 8% and 10% per minute respectively. After completion of dilution a sample is removed from the bag to perform viability, sterility testing and TNC count and the cells are infused into the patients over 15 minutes. Data was collected over 5 years (2002 to 2007) for 45 UCB transplants. All samples were negative for Sterility testing and there were no adverse reaction during infusion. The recovery and engraftment rates are as follows: Conclusion: The thaw dilution process without wash yielded a superior %TNC recovery and %viability. All but one patient achieved ANC engraftment. There were 7 (15%) early deaths due to infection. The ANC engraftment and 100 day survival was not significantly different between the 2 cell dose groups, but the platelet engraftment was delayed in the group who received a TNC dose <1.5 × 10^7/kg. We now target units with a cell dose of ≥1.5 × 10^7 TNC with a minimum match of 4/6, but given the prompt ANC engraftment seen, we continue to accept units of <1 × 10^7/kg processed in this manner.

**Results N = 46 Values reported as Median (Range)**

<table>
<thead>
<tr>
<th>TNC dose</th>
<th>% Post-thaw TNC</th>
<th>% Post-thaw</th>
<th>Days to Recovery</th>
<th>Days to ANC &gt; 500</th>
<th>Engraftment Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>×10^7/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7 (0.9-4.4)</td>
<td>46 (82-100)</td>
<td>99 (96-99)</td>
<td>18 (11-38)</td>
<td>49 (18-87)</td>
<td>65%</td>
</tr>
<tr>
<td>≥1.5</td>
<td>19</td>
<td>21 (13-38)</td>
<td>63 (38-87)</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>&gt;1.5</td>
<td>27</td>
<td>16 (11-24)</td>
<td>37 (18-59)</td>
<td>57%</td>
<td></td>
</tr>
</tbody>
</table>