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Background: Original Performance Qualification on Sorvall ST40R Centrifuge for plasma depletion on Hematopoietic progenitor cell (HPC) product was set at 300g Revolution Centrifugal Force (RCF) 10 minutes at 20°C to achieve ≥ 80% Total Nucleated Cell (TNC) recovery. Cellular Therapy Lab (CTL) noticed the spun down product has unclear supernatant plasma, loosely packed cellular sedimentation with difficulty for separation. CTL has decided to re-validate the process to find an optimal RCF for a clear & defined separation between plasma and Buffy coat, maintaining ≥ 80% TNC recovery.

Study Design and Methods: Research performed on practice recommendations and reference materials lead to select three variables of RCF: 700g, 1000g, and 1300g for this study. Ten samples were collected from different sources: single donor whole blood; pooled whole blood; pre HPC,Apheresis peripheral blood; HPC product and Donor Lymphocyte product. Sample size varied from 21ml — 120ml. Each sample was divided into three equal portions centrifuged for 10 minutes at 20°C at the selected RCF. White blood cell (WBC) count was tested by the Sysmex cell counter. Viability was tested with Trypan blue method. The following data and test results were recorded for each portion pre and post centrifugation: sample volume, WBC, packed cell volume, plasma volume. Post plasma WBC, calculated TNC, Viability%, TNC % recovery, Clarity of spun sample, and Appearance of Buffy-coat. One way analysis of Variance was run to assess the P value for statistical significant difference. Optimal RCF was selected after this study. Validation of the selected RCF was performed on 5 HPC products from prior transplant recipients.

Result: The study found no statistically significant difference among the 3 RCF variables with P=0.67. The difference between Pre & Post Viability was less than 3% which indicates minimal cell damage during centrifugation. Observation found hazy plasma and loose Buffy Coat with 700g, clear and defined with 1000g and 1300g. CTL selected RCF 1300g for plasma depletion. Validation study on 7 processes found post TNC plasma range of 0.01-0.10% of pre TNC, difference in Pre & Post Viability is 0-1%, five patients with successful absolute NC and platelet engraftment. Results of retro study compared to 7 random processes using prior RCF 300g found P= 0.352.

<table>
<thead>
<tr>
<th>RCF selection Result: TNC % Recovery</th>
<th>RCF 700g</th>
<th>RCF 100g</th>
<th>RCF 1300g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>91-106</td>
<td>91-100</td>
<td>93-100</td>
</tr>
<tr>
<td>Median</td>
<td>95.5</td>
<td>98.5</td>
<td>97.0</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>4.22</td>
<td>2.62</td>
<td>2.41</td>
</tr>
</tbody>
</table>

Comparison Result: Prior RCF 300g Implemented RCF 1300g

<table>
<thead>
<tr>
<th>TNC % Recovery</th>
<th>Prior RCF 300g</th>
<th>Implemented RCF 1300g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>85-93</td>
<td>85-98</td>
</tr>
<tr>
<td>Median</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>3.13</td>
<td>3.99</td>
</tr>
</tbody>
</table>

Conclusion: The study found no significant difference in TNC % recovery using 700g, 1000g and 1300g. There is defined cell
pellet and clear plasma with higher RCF for ease of separation which help to harvest the final concentrated cellular product for cryo-preservation; especially using small volume bag.

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Optimal Cryopreservation Conditions to Preserve Viability, Proliferation, and Lytic Function of NK Cells: Protective Factors of Serum in Cryopreservation

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Introduction: Natural killer (NK) cells are a promising tool for adoptive immunotherapy in many disease settings. The availability of large quantities of functionally effective NK cells represents one of the major challenges for immunotherapeutic trials, and a major objective of our laboratory is to enhance NK cell number and function ex vivo for subsequent adoptive immunotherapy. We previously published a robust approach for ex vivo propagation of NK cells that has been validated for healthy donor (Denman, 2012) and patient peripheral blood (Liu, 2013), cord blood (ASH 2012), and embryonic/pleuripotent stem cell sources (Knorr 2013) and has the potential to generate far more NK cells than can be infused at one time. Cryopreservation of NK cells provides significant advantages over infusion of fresh products by 1) reducing timing constraints in matching GMP manufacturing to patient need, 2) enabling multiple infusions from single large-scale expansions, and 3) ensuring uniform characteristics for aliquots, all of which are essential for generating off-the-shelf products. However, NK cells are known to have poor survival and function after cryopreservation, and this was recently confirmed in clinical trials using standard clinical GMP cryopreservation media containing Plasmalyte, dimethyl sulfoxide (DMSO), and human serum albumin (HSA). In contrast, our preclinical data with expanded NK cells cryopreserved in media, fetal bovine serum (FBS) and DMSO showed excellent function and no significant difference between fresh and frozen cells (Liu, 2013). Therefore, we hypothesized that serum may provide protective factors for NK cells during cryopreservation.

Methods: NK cells from four healthy donors were expanded with weekly addition of irradiated K562 Clone9.mbl21 feeder cells as previously described. After three weeks, cells were cryopreserved with freezing media of 40% Plasmalyte and 10% DMSO, with varying ratios of HSA and human AB serum (hAB) comprising the remainder. Cryopreserved NK cells were thawed and assessed for cell recovery, viability, proliferation, and function at 3, 24, 48, 72,120, 168 hours after thawing. Cytotoxicity was determined against 721.221 target cells.

Results: A linear relationship was observed between hAB content and recovery, viability, and cytotoxicity, plateauing at 40% hAB. Unstimulated proliferation after thawing was associated with higher hAB content, but re-stimulation with Clone9.mbl21 was similar in all conditions.

Conclusion: In the present study, we aimed to establish the benefit of including serum in cryopreservation of expanded NK cell. We confirmed a significant effect in preserving viability, cytotoxicity, and proliferation, justifying its use in clinical trials.

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Background: Allogeneic transplantation is associated with both GvL and GvHD. Elimination of donor T cells results in less GvHD, but also less GvL and engraftment. To eradicate infused T cells if GvHD develops, our suicide gene based therapy uses a CD34-Herpes Simplex Virus-I-thymidine kinase (CD34-TK75) chimera (Rettig et al, Mol Ther 2003 and J Immunol 2006). We enrich and purify retrovirally transduced cells via cell surface expression of the extracellular domain of CD34. Modified TK75 both mediates cell suicide upon treatment with ganciclovir and enables tracking of modified cells that entrap the substrate. [18F]CT/CT.

Methods: Following production and release testing of CD34-TK75 transduced and affinity-purified T cells (88-98.8% CD34+), we began a phase I pilot and feasibility study. Cells were infused as a DLI (0.1–1.3 x 10^6 CD34-TK75+ T cells/kg) into 8 patients (relapsed after allo-HSCT). Since no toxicities were observed during treatment of our first 2 patients, we initiated trafficking studies using [18F]FHBG-PET/CT imaging at baseline, d15, and d30 after infusion of CD34TK75+ T cells in patients 3–8.

Results: No acute toxicities were associated with administering either genetically modified T cells or [18F]FHBG. Using real-time quantitative PCR, we detected the CD34-TK75 transgene in the circulating CD34-TK75+ T cells of all but one patient. GvHD developed in only one patient (grade III GI) who did not respond to a 10 day treatment with I.V. ganciclovir and enables tracking of modified cells that entrap the substrate. [18F]CT/CT.

SHAPE V MERGEFORMAT

Baseline scan
14-Day scan
30-Day scan
TK04 (no GvHD) ANT ANT POST POST POST
18CT/CT.

[18F]-Fhbng-PET/CT Imaging of CD34-TK75+ T Cells in Allogeneic HSCT Recipients after Donor Lymphocyte Infusion (IND#11917; Clinicaltrials.Gov Identifier: NCT00871702)
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