**IMMUNE RECONSTITUTION**

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**TIME SENSITIVE PARAMETERS OF IMMUNE RECONSTITUTION MEASURED BETWEEN DAY 100 AND 1 YEAR PREDICT SURVIVAL AFTER UNRELATED CORD BLOOD TRANSPLANT (UCBT): THE DYNAMIC IMPACT OF DENDRITIC CELLS, TREGS, AND THYMICYCLOVERECOVERY**

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**Objective:** Reconstitution of dendritic cells (DC) and lymphocytes is critical for protective immunity, however, the tempo of their recovery is variable and their impact at specific time points on UCBT outcome is not defined. This study aims to analyze the impact of cellular immune reconstitution within the first year.

**Methods:** Between 07/05 and 12/07, 93 children with full donor chimerism following myeloablative conditioning were longitudinally assessed at Day (D) 100, 180, 270 and 365 for reconstitution of DC and T cell subsets, B and NK cells by 4 color FACS to determine thresholds associated with overall survival (OS). Parameters were dichotomized at the median (M) of each time point and hazard ratios estimated by Cox proportional hazards analysis.

**Results:** Patients were transplanted for non-malignant (N = 63) and malignant diseases (N = 30). M age was 2.1 years, 58% male, 36% were 4/6, 42% were 5/6 and 23% were 6/6 HLA matched. The M infused TNC and CD34+ cell dose was 7.3x10^7/kg and 1.9x10^5/kg, respectively. 22 patients died within 2 years: OS at 2-years ~ 76%. At D100, lower percentage and absolute numbers of regulatory T cells (CD4+/CD62L+/CD3+/CD4+/Tregs) was associated with death (p = 0.04 and p = 0.02). A larger proportion of activated” HLA-DR expressing CD8+ T cells predicted death at D100 with marginal significance at 180 and 270 while absolute numbers significant at D270. At D180, a period when thymic function may resume, a rise in ‘recent thymic emigrants’ was associated with survival (RTF, CD45RA+/CD62L-+T cells), p = 0.01 and p = 0.04. Higher levels of ‘plasmacytoid’ CD123+ DC were consistently associated with better OS after D100 (p < 0.05). Major lymphocyte subsets, B, NK, CD3+, CD4+ T cells were NOT associated with superior OS except at D180 with all lymphocytes combined, exceeding an ALC of ~ 1800/ul (p = 0.04). Table 1 depicts the Hazard Ratios for the above immune parameters with cut-offs at the median values.

**Conclusions:** Our data demonstrate that different immune cell subsets will have unique kinetics and dynamics of recovery that confer shifting power in predicting outcome at different time points within the first year. Nevertheless, by the second half of the first year, superior pDC and RTE recovery along with a reduced activation state (%HLA-DR) are independently associated with better OS. These results should facilitate the development of risk models for the early identification of those at highest risk of death and who may benefit from novel immunotherapies.

**Table 1. Cellular Factors of Immune Reconstitution Associated with Death After UCBT**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time Point (Day)</th>
<th>Cut-Off Level</th>
<th>Hazard Ratio</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Lymphocyte Count/ul</td>
<td>180 ≤ 1180</td>
<td>1.0000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45RA+ / CD62L+ (RTF) (CD4+)</td>
<td>180 ≤ 0.175 (0.1052-0.958)</td>
<td>0.0417</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45RA+ / CD62L+ (RTF) (CD4+)</td>
<td>180 ≤ 0.240 (0.079-0.7308)</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hazard Ratio’s and (p-values) at each time point

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**207 RAPID CHIMERISM – SWITCH OF LYMPHOCYTES AND PHENOTYPIC CONVERSION OF NAIVE T CELLS EARLY AFTER CORD BLOOD TRANSPLANTATION**

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**Background:** To understand the pathogenesis of allogeneic immune reaction early after cord blood transplantation (CBT), we analyzed the kinetics of donor-chimerism and T-cell phenotype during early period after CBT.

**Methods:** Analysis of chimerism was performed on CD3, CD4, CD8, and CD56 fractions of peripheral blood by HLA-Flow method on 1, 2, 3, 4, and 8 weeks after CBT. T-cell phenotype was analyzed by flow-cytometry on 2, 4, and 8 weeks after CBT in engrafted patients.

**Results:** Forty-eight patients were enrolled in this study. The patients had a median age of 57.5 years (range, 19-71 years). All except for two patients received fludarabine-based conditioning. GVHD prophylaxis was mainly composed of tacrolimus and mycophenolate mofetil (n = 35). Forty-one patients achieved neutrophil engraftment at a median of 20 days (range, 13-44 days). Using HLA-Flow method for the chimerism analysis (n = 32), the engrafted patients (n = 28) showed rapid switch to donor-type dominant chimerism; the median percentages of donor-derived CD4+ T-cells, CD8+ T-cells and NK cells at one week were 89.8% (range, 19.1-100%), 91.7% (range, 9.3-100%) and 95.3% (range, 23.5-100%), respectively, and all the patients reached complete donor chimerism by 3 weeks. In phenotypical analysis (n = 42), the proportions of naive phenotype (CD45RA+ CCR7+) in CD4+ and CD8+ T-cells at 2 weeks decreased rapidly to a median of 55% (range, 10-90%) and 20% (range, 1-88%), respectively.

**Conclusions:** We observed rapid chimerism-switch of lymphocytes along with early T-cell differentiation from naive to memory phenotype after CBT. These findings may explain the mechanisms behind severe immune-mediated complications following CBT.