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Long-Term Thymic Function and Reconstitution of the T Cell Compartment after T Cell-Replete Haplo-Identical Allografting

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INTRODUCTION: Post-transplant cyclophosphamide (PTCY) has expanded the application of haploidentical stem cell transplantation (haplo-HSCT). Thymic function may play a pivotal role in long-term clinical outcomes.

OBJECTIVES: To evaluate the kinetics of long-term immune thymus-dependent reconstitution after PTCY haplo-HSCT.

METHODS: Twenty-nine patients (median age 53) underwent T-cell replete haplo-HSCT with PTCY. Blood samples were collected before conditioning and at 1, 3, 6, 12, 18, 24 months after transplantation. Analyses of CD4 and CD8 T-cell subsets by flow-cytometry were correlated by generalized linear models with Real-Time PCR quantification of signal joint T-cell receptor excision DNA circles (sjTRECs), specific marker of naive T-cells thymopoiesis. A) Naive; b) central; c) memory; and d) revertant CD4 and CD8 T-cells were defined as follows: a) CD45RA+CD62L+; b) CD45RO+CD62L+; c) CD45RO+CD27-; and d) CD45RA+/45RO+, respectively. SjTRECs real-time PCR was performed on genomic DNA (100 ng) extracted from sorted CD4 and CD8 T-cells.

RESULTS: Following PTCY induced T-cell depletion, a constant gradual increase in absolute numbers of all CD4 and CD8 T cell subsets and of sjTRECs copies from the first month up to two years post-transplant was observed (**Figure 1**). Overall, at two years, CD4 and CD8 T-cell levels and sjTRECs levels were however lower than those observed in healthy donors. sjTRECs kinetics was associated with the increase in naive T-cells (overall, $p < 0.008$, and p 0.048 in CD4 and CD8 T cells respectively). This correlation clearly suggests that most naive T-cells derive from thymic re-education of donor precursor stem cells. Furthermore, an increase in CD4 revertant memory T-cells was also significantly correlated with sjTRECs kinetic (p 0,041). Central and effector memory T-cells showed a faster thymic-independent expansion. Interestingly, sjTRECs levels and thymic dependent immune-reconstitution were higher in a cohort of 63 adult patients undergoing HSCT from HLA identical donors (manuscript in preparation). Clinical parameters and thymic function were correlated starting at 6 months after HSCT. By multivariate analysis, low pre-transplant TRECs values, moderate-severe chronic GVHD, age older than 50 years were significantly associated with lower thymic output after haplo-HSCT.

CONCLUSIONS: Active thymic function despite age-dependent involution, substantially contributes to T-cell reconstitution after haplo-HSCT. Chronic GVHD and older age are however significantly correlated with lower thymic activity. Overall, lower production of sjTRECs after haplo-HSCT as compared after HLA identical sibling HSCT may partly be due to a higher degree of "mismatching" of MHC molecules during thymic re-education.

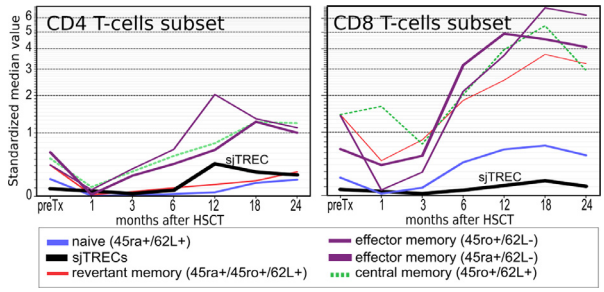

Figure 1. Standardized value of CD4 and CD8 T-cell subsets and sjTRECs reconstitution at different time - points after HSCT. (1 = donor median value).

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Mechanisms of CD8$^+$ T-Cell Resistance to Post-Transplantation Cyclophosphamide (PTCy)

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Despite the lymphocyte reducing effects of PTCy after hematopoietic cell transplantation (HCT), CD8$^+$ T-cell numerical reconstitution is rapid and diverges from CD4$^+$ T-cell reconstitution. To understand this rapid reconstitution and explore possible mechanisms of CD8$^+$ T-cell resistance to PTCy, we used mixed lymphocyte culture (MLC) of healthy donor CD3$^+$ T cells with irradiated HLA-mismatched CD3$^-$ peripheral blood mononuclear cells as previously published (Kanakry CG et al., *STM*, 2013). Accordingly, cells in MLCs were treated on day 3 with the active Cy analog mafosfamide (Maf) 7.5 ug/ml or from days 0-7 with cyclosporine (CsA) 600 ng/ml or rapamycin (Rap) 15 ng/ml. We found that all drugs reduced CD8$^+$ T-cell numbers at day 7. However, the relative impact on CD8$^+$ subsets differed by treatment. Maf preserved a similar distribution of memory and naïve subsets compared with untreated MLCs, unlike CsA and Rap which preferentially spared naïves. Percentages of mucosal associated invariant T (MAIT) and phenotypically stem cell memory CD8$^+$ T cells were increased after Maf. Proliferation was reduced after Maf but persisted at lower levels, distinct from CsA or Rap, which abolished proliferation.

Mechanisms of resistance to Cy include expression of aldehyde dehydrogenase (ALDH), the major *in vivo* detoxifying enzyme for Cy. Multidrug transporter (MDR) effluxing also may play a role. At day 3 of MLC, all assessed CD8$^+$ T-cell subsets increased expression of ALDH1A1 from undetectable expression in donor T cells. In a Rhodamine-123 efflux assay, all CD8$^+$ T-cell subsets also increased MDR activity at day 3 compared with donor cells. Both ALDH1A1 expression and Rhodamine-123 effluxing declined at day 7. Increased effluxing by CD8$^+$ T cells also was seen in clinical HCT from donor and recipient day 0 to recipient day +3 blood samples. In MLC, pharmacologic inhibition of ALDH with diethylaminobenzaldehyde or MDR with PK11195 sensitized CD8$^+$ T cells to Maf, with combined inhibition leading to death of nearly all cells, suggesting both mechanisms may contribute to CD8$^+$ T-cell resistance.

To dissect the underlying molecular mechanisms, we explored the relative impact of proliferative state and cytokines on MDR activity. Effluxing was largely restricted to those cells that either had not proliferated or had proliferated in MLC only a