a significant proportion of patients with high-risk hematological disease. This is the first report containing data on long-term toxicity and disease control after any strategy of donor graft manipulation to selectively reduce HLA-mismatched alloreactivity.

39

FEASIBILITY OF UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION IN CONGENITAL CHILDHOOD DISEASES

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An HLA-matched sibling donor has been the initial choice for children requiring allogeneic hematopoietic cell transplant (HCT). However, less than 30% HCT patients have a matched related donor (MRD). In the past decade, umbilical cord blood (UCB) transplantation has emerged as an attractive alternative for patients without a MRD. Recent studies have shown the advantages of using UCB over bone marrow as an alternative graft source for children with acute leukemias. However, there is less information available regarding the utilization of unrelated UCB transplantation for children with non-malignant diseases. We report the use of an unrelated UCB myeloablative transplantation in fifty-five consecutive children with a median age of 2.6 years (range, 0.2–40.6 years) with Wiskott-Aldrich syndrome, Chediak-Higashi syndrome, hemophagocytic lymphohistiocytosis, langerhans cell histiocytosis, osteopetrosis, Diamond-Blackfan anemia, Hurler syndrome, Maroteaux-Lamy, α-mannosidosis, cerebral X-linked adrenoleukodystrophy, metachromatic leukodystrophy and globoid-cell leukodystrophy transplanted over 11.5 year period (1994–2006). Patients received grafts matched at 6 (14%), at 5 (56%), or at 4 HLA alleles (30%). The median total nucleated cell dose and the median $CD34^+$ cell dose were 5.4×10^7/kg and 4.2×10^5/kg, respectively. The median time to neutrophil recovery was 20 days (range, 10–45 days) and the incidence of neutrophil recovery by day 42 was 86%. The incidence of platelet recovery by 6 months was 73%. In the group of immune or hematological disorders, 6 of 10 patients achieved complete donor chimerism by day 21. In the metabolic disorders group, 8 of 13 patients achieved a complete donor chimerism at a median of 21 days and 3 additional patients at a median day of 95 days. In the leukodystrophy group, 6 of 12 patients achieved completed donor chimerism by day 21 and 4 patients achieved complete donor chimerism at a median of 120.5 days. The incidences of grade II-IV and grade III-IV acute GvHD were 34% and 12%, respectively. Chronic GvHD was observed in only 5% of cases. The overall survival was 62% at 2-years. These results demonstrate the usefulness of unrelated UCB as an alternate stem cell source for patients lacking an HLA matched related or unrelated donor. The use of unrelated UCB transplantation creates new opportunities in the treatment of non-malignant diseases requiring expedient HCT in order to prevent irreversible disease progression.

40

OUTCOMES OF A PROSPECTIVE TRIAL OF NMDP-FACILITATED UNRELATED DONOR (UD) PBSC HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR LEUKEMIA AND MYELODYSPLASIA: COMPARABLE SURVIVAL REGARDLESS OF REGIMEN INTENSITY AND IMPROVED SURVIVAL WITH HIGHER CELL DOSES

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We report outcomes of 932 recipients (rcpts) of UD PBSC HCT facilitated by NMDP from 1999 through 2003 (median f/u 3.3 yrs). Indications were AML (419 rcpts), ALL (185 rcpts), CML (134 rcpts), and MDS (194 rcpts). Preparative regimens included myeloablative (MA, N = 611), reduced intensity (RI, N = 160), and non-myeloablative (NMA, N = 161). Distributions of HLA-match grade, CMV status, Karnofsky scores (KS), and donor characteristics were similar between the preparative regimens, however, fewer rcpts with advanced disease received NMA ($p = 0.035$), while more rcpts with coexisting diseases received RI and NMA regimens ($p < 0.001$). The age of rcpts receiving RI and NMA regimens was substantially higher than rcpts receiving MA regimens (median RI 56 yo, NMA 57 yo, MA 38 yo, $p < 0.001$). Optimal cell dose cutpoints for TNC, MNC and CD34+ were determined based on Martingale residuals from Cox regression analyses. For MA rcpts, CD34+ counts $>3.8 \times 10^6$/kg improved day +25 neutrophil and day +60 platelet engraftment; higher infused TNC doses ($>6.9 \times 10^8$/kg) predicted decreased grade III-IV aGVHD, while improved overall survival (OS) and reduced TRM (RR 0.55) were seen with MNC doses $>4.4 \times 10^8$/kg. For RI and NMA rcpts, OS was higher and TRM was decreased in those receiving $>3.8 \times 10^6$ CD34+cells/kg. Of note, cGVHD was not increased with higher cell doses in rcpts of any type of preparative regimen. Additional predictors of improved OS included early disease, and for MA rcpts only, HLA-matched donors, KS ≥ 90, and CsA-based GVHD prophylaxis. Three year OS and DFS of rcpts receiving MA, RI, and NMA approaches were similar (33, 35, and 32% OS; 33, 30, and 29% DFS: MA, RI, and NMA, respectively). Higher risk of relapse at 3 yrs in RI and NMA approaches (35, 37 vs. 24% RI, NMA, MA, respectively, $p < 0.001$) was offset by higher 3 yr TRM using MA regimens (43 vs. 34, 34% MA, RI, NMA, respectively, $p = 0.008$). Sub-analyses of 1) rcpts with AML-CR1, 2) rcpts with AML/MDS/CML (excluding ALL), or 3) rcpts between the ages of 40–60 with AML/MDS also showed similar survival with MA vs. RI vs. NMA approaches. In summary, rcpts of UD PBSC HCT receiving preparative regimens differing in intensity experienced similar survival. Higher cell doses resulted in more rapid engraftment, less severe aGVHD (MA rcpts), and better 3 year OS (37 vs. 18%, MA; 36 vs. 21% RI/NMA, $p < 0.001$), but did not increase the risk of cGVHD.

IMMUNE RECONSTITUTION

41

PRE-TRANSPLANT ADMINISTRATION OF KERATINOCYTE GROWTH FACTOR AFFECTS PERIPHERAL T-CELL HOMEOSTASIS THROUGH INCREASED RECENT THYMIC EMIGRANT EXPORT AND AFFECTS THE COURSE OF MURINE CHRONIC GRAFT-VS.-HOST DISEASE

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Accelerated recovery of thymic function following allogeneic hematopoietic stem cell transplantation (allo-HSCT) not only provides a sufficiently broad repertoire of T-cell responses to pathogens, but also is thought to play a role in affecting the outcome of graft-vs.-host disease (GVHD) through the restoration of central tolerance and/or the production of regulatory cell populations that may blunt the effect of donor-derived alloreactive T-cell populations. Keratinocyte growth factor (KGF) has been shown in murine models to accelerate thymic function and ameliorate acute GVHD, but it is unclear whether the latter involves a thymic-dependent mechanism of increased T-cell production and/or cytoprotection of epithelial cells in target organs of GVHD. We examined the effect of pre-transplant administration of KGF in the B10.D2 into BALB/c murine model of chronic GVHD (cGVHD). KGF treated mice had significantly increased thymic function as assessed by enumeration of thymocyte populations, analysis of thymic cytoarchitecture, and enumeration of peripheral T-cell subsets and recent thymic emigrants (RTE). Significantly, enhanced export of RTE by KGF decreased peripheral T-cell homeostatic expansion and downregulated expression of activation markers, suggesting that RTE effectively compete with post-thymic T-cells for limited cytokines and contact-dependent niches post-allo-HSCT. Parallel experiments in thymectomized recipients receiving KGF exhibited no changes in cell cycle profiles or activation profiles of peripheral T-cells. Pre-transplant KGF administration improved clinical cGVHD outcomes in both thymus-intact and thymectomized recipients. However, there were no observable differences in the course of cGVHD between KGF treated thymus intact and thymectomized mice, suggesting that enhanced thymic function by KGF did not provide for any additional benefit to the cytoprotective effects of KGF. One contributing factor for this observation was

the inability of KGF treatment to increase peripheral regulatory T-cell numbers. In summary, pre-transplant administration of KGF can accelerate thymic recovery post allo-HSCT and that the increased export of newly generated T-cells can blunt peripheral expansion of post-thymic T-cells. However, this thymus-dependent effect of KGF is insufficient to further ameliorate cGVHD. Nevertheless, the results suggest a potentially important role of KGF in immune reconstitution and modulation of cGVHD post-allo-HSCT.

42

IN VIVO EXPANSION OF CD4+FOXP3+ REGULATORY T CELLS MAY CONTRIBUTE TO CONTROL OF ACUTE GVHD AFTER HLA-MISMATCHED ALLOANERGIZED HSCT

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Many strategies have been explored to selectively remove alloreactive donor T cells to prevent Graft-versus-Host Disease (GvHD) without impairing immune reconstitution after hematopoietic stem cell transplantation (HSCT). An alternative approach is allostimulation of donor T cells with costimulatory blockade (CSB) rendering allospecific cells anergized (hyporesponsive to subsequent alloantigenic challenge). Murine and human data suggest that induction of alloanergy involves cell-mediated suppression, requiring the presence of CD4+CD25+ regulatory T cells (Tregs). We conducted a pilot study of haploidentical alloanergized HSCT after CSB, and measured reconstitution of Treg by intracellular flow cytometry. 5 patients (pts; 4 acute leukemia, one marrow failure) underwent cyclophosphamide/TBI-conditioned haploidentical HSCT with cyclosporine and methotrexate GvHD prophylaxis. Donor bone marrow was incubated with irradiated recipient peripheral blood mononuclear cells and anti-B7.1/2 antibodies for 48 hours to induce alloanergy, washed and infused. All pts engrafted with very rapid reconstitution of T cell subsets, NK cells and immunoglobulins. All evaluable patients had a marked relative increase in peripheral blood CD4+FOXP3+ cells at D + 20–60. CD4+FOXP3+ cells had a memory Treg phenotype (CD25+ CD45RO+CTLA4+ CD127Lo) and were predominantly HLA DR- differentiating them from activated T cells. Despite receiving high doses of donor T cells (median 1.8 (CD4) and 3.1 (CD8) $\times$ 10^7/kg) and achieving full donor chimerism, only 2 pts developed acute GvHD, both Grade II, resolving after short courses of corticosteroids. All evaluable patients also had an increase in CD4+ T effector (Teff) cells with an activated phenotype (CD25+HLADR+FOXP3-) at D + 30–50. Although the antigenic specificity of Teff was not determined, cytokine secretion may have led to reversal of anergy and expansion of alloreactive Teff cells. The marked in vivo expansion of Treg may represent one mechanism of suppressing alloreactive Teff and achieving immunological control of acute GvHD without impairing immune reconstitution in pts receiving HLA-mismatched alloanergized donor T cells. We are using a modification of this strategy in a clinical trial of delayed infusion of escalating doses of alloanergized donor T cells after CD34-selected haploidentical HSCT, to determine the optimal dose of alloanergized donor T cells that abrogates acute GvHD without impairment of immune reconstitution.

43

ADMINISTRATION OF rhIL-7 INCREASES TCR REPERTOIRE DIVERSITY THROUGH PREFERENTIAL EXPANSION OF NAIVE T CELLS

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Interleukin-7 (IL-7) is a multifunctional cytokine with critical and non-redundant roles in thymopoiesis and peripheral T-cell homeostasis. We previously reported preliminary results of the first Phase I study of recombinant human IL-7 (rhIL-7), demonstrating that two weeks of alternate day treatment with rhIL-7 produced a marked increase in the number of CD4+ and CD8+ T cells. This increase was maintained in follow up assays at 6 to 12 weeks post treatment. Furthermore, rhIL-7 therapy disproportionately increased CD27+CD45RA+ naive cells, which represent the most diverse elements of the mature T cell receptor (TCR) repertoire, at the expense of CD27-CD45RA+/- effector populations, which are often oligoclonal. In CD8 T cells, the proportion of naive cells increased by 8–39% of total cells. Because of the extent of this population shift, we hypothesized that rhIL-7 treatment would lead to an overall increase in TCR repertoire diversity in CD4+ and CD8+ T-cells. We assessed TCR diversity using spectratype analysis on sorted CD4 and CD8 populations before and one week after rhIL-7 therapy (day 21) in six subjects. For each patient, we determined the divergence of spectratypes in 22 BV families from Gaussian-like normal donor standards and then compared the global diversity of pre and post spectratypes by Wilcoxon paired non-parametric analysis. We determined that rhIL-7 therapy induced a statistically significant increase ($P < .05$) in repertoire diversity in either the CD4+, CD8+, or both T-cell populations in 4 of the 6 patients. This enhancement in diversity was particularly remarkable in that three of these donors were over 60 years of age, and a fourth patient had reduced lymphocyte populations due to recent chemotherapy. Given the short duration of therapy, the age of the patients and the very modest change in TREC we observed, we believe this enhancement in diversity was due primarily to differential population expansion, not IL-7 induced thymic output. Consistent with this interpretation, we observed that a higher percentage of naive T cells than effector T cells remained in cycle (Ki-67+) and maintained elevated levels of anti-apoptotic Bcl-2 during IL-7 therapy. We therefore propose that rhIL-7 has the potential to induce T-cell growth and enhance repertoire diversity, even in lympho-depleted patients with limited thymopoietic capacities, by expanding naive T cell populations.

44

THE $CD4^+CD25^+FOXP3^+$ COMPARTMENT FOLLOWING CONDITIONING AND TRANSPLANT: HOST TREG CELLS EXPAND AND COMPRISE THE PREDOMINANT COMPONENT FOR SEVERAL MONTHS DURING RECONSTITUTION POST-HCT

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The capacity of $CD4^+CD25^+Foxp3^+$ (Treg) cells to regulate adaptive and innate immune responses has led to studies investigating their use in novel strategies to regulate allogeneic T cell responses during hematopoietic stem cell transplants (HCT). A fundamental clinical concern post-HCT is the reconstitution of the lymphoid compartment, particularly T cells which can be exceptionally delayed. We have previously found that host Treg cells can regulate resistance to engraftment following HCT, demonstrating that such cells survive and function at least transiently in recipients. The present studies investigated the residual host Treg compartment following varying levels of conditioning (3.0 – 14Gy TBI), and transplant. We found that recipient $CD4^+CD25^+Foxp3^+$ cells: 1) can survive ablative as well as reduced intensity conditioning, 2) undergo expansion (BrdU uptake/cell numbers) and 3) contribute greatly to the Treg compartment for several months post-HCT during which time donor derived Treg cells gradually arise and cede this compartment. Within the first 3 weeks post-lethal conditioning and HCT, 95% of the splenic $CD4^+FoxP3^+$ cells are positive for BrdU, vs. ~40% in normal mice. Using Thy1.1 congenic mice, the vast majority of these cells were found to be resistant (host) Tregs. Two months post-HCT, almost 30% of the compartment was still of host origin. To assess the functional capacity of the residual Treg cell compartment, we examined development of autoimmune disease following transplant of IL-2R$\beta^{-/-}$ BM into syngeneic recipients. Autoimmune disease was prevented in B6-wt but not T cell deficient recipients. Interestingly, the failure to transfer autoimmune disease following IL-2R$\beta^{-/-}$ HCT into B6-$CD4^{-/-}$ recipients was associated with the presence of a peripheral $CD8^+FoxP3^+$ population not detected in B6-wt mice. This finding