transplantation (HCT). Therapeutic administration of corticosteroids is often required after HCT to treat acute and chronic graft versus host disease (GVHD). Although corticosteroids are known to induce T-cell apoptosis and suppress T-cell cytotoxic function, their effects on NK cells have not been well characterized. We investigated for differences between T cells and ex vivo expanded NK cells in their susceptibility to apoptosis and inhibition of proliferation following treatment with corticosteroids. T and NK cells were isolated by magnetic bead separation from peripheral blood mononuclear cells. NK cells were expanded in X-vivo 20 with 10% human AB serum, 500U/ml IL-2 and irradiated (100Gy) Epstein-Barr virus transformed B cells for 7 days. T cells were expanded for 5 days in RPMI 1640 supplemented with IL-2. Hydrocortisone was used in vitro at concentrations (0.5ug/mL to 20ug/mL) comparable to drug levels achieved in patients receiving 1mg/kg to 1 gram of methylprednisolone . Apoptosis, cellular proliferation and the cytolytic function of T and NK cells against K562 cells were evaluated by flow cytometry staining for annexin-V and 7-AAD, thymidine incorporation and ^{51}Cr-release assays respectively. Compared to T-cells, NK cells were resistant to the apoptotic and anti-proliferative effects of hydrocortisone; hydrocortisone concentrations of 0.5ug/ml and 20ug/mL inhibited T-cell proliferation by 60% ($p = 0.005$) and 80% ($p = 0.001$) respectively, whereas steroids at these same doses did not inhibit NK cell proliferation (table). In contrast to T-cells, NK cells were resistant to steroid-induced apoptosis; at steroid concentration of 20ug/mL 81% of T cells (grown in serum free media) stained positive for annexin-V versus 42% of NK cells (table). NK cell cytolytic function against K562 targets was not affected by steroid exposure; at an NK cell: K562 ratio of 1:1, NK cells treated with 0, 0.5ug/ml, and 20ug/ml of hydrocortisone lysed 35%, 33%, and 27% of K562 targets respectively (p = n.s.). These findings show that NK cells are resistant to the immunosuppressive effects of corticosteroids compared to T-cells and further suggest that corticosteroid administration following allogeneic HCT may not preclude a beneficial anti-tumor effect of adoptively infused donor NK cells.

Steroid Effect on Apoptosis and Proliferation of T and NK cells

Steroid Concentration	%T cell Apoptosis	% NK cell Apoptosis	P	T cell SI*	NK cell SI*	P
0 ug/mL	73%	45%		1	1	
0.5 ug/mL	77%	40%	0.009	0.4	1.07	0.004
20 ug/mL	81%	42%	0.02	0.16	0.98	0.005
200 ug/mL	95%	38%	0.06	0.01	0.69	0.05

SI*. Stimulation Index.

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IN SITU ACTIVATION OF HOST CD4$^+$ CD25$^+$ FOXP3$^+$ T CELLS: A NEW STRATEGY FOR CIRCUMVENTING RESISTANCE AND ESTABLISHMENT OF HEMATOPOIETIC ENGRAFTMENT AFTER MHC-MATCHED ALLOGENEIC HCT

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Treg infusions have been shown to suppress HVG and GVH in experimental allogeneic HCT. These findings have engendered enthusiasm for the administration of Tregs in the clinical setting. We hypothesized that activation/expansion of Tregs *in situ* may represent an approach that obviates the requirement to isolate and enrich Treg populations for use in such transplants. To address this question, we administered a complex of IL-2 and anti-IL2 mAb (IAC) to recipients following reduced intensity conditioning (RIC) and allogeneic MHC-matched HCT. A protocol was identified which significantly enhanced early donor chimerism, and importantly, facilitated long-term multi-lineage engraftment in these recipients. Administration of this complex relative to the time of HCT was crucial: post-HCT treatment with IAC more efficiently facilitated marrow engraftment compared to pre-HCT administration. Donor chimerism remained present >27 weeks post-HCT. One week post-HCT, the frequency of host Tregs as well as CD25 expression (MFI) were significantly elevated in IAC-treated vs. control recipients. To address a potential mechanism for these observations, we assessed host-anti-donor (tetramer$^+$) CD8 T cells that arise post-HCT and mediate resistance to engraftment. IAC but not control (PBS) treatment suppressed the emergence of these host tetramer$^+$ expressing CD8 T cells early post-HCT. Following *in vivo* administration of donor antigen into chimeric and non-chimeric recipients >6 months post-HCT, an increased frequency of tetramer$^+$ CD8 cells was observed only in non-chimeric marrow allograft recipients. Importantly, LPS and anti-CD3 responses by splenocytes from both groups of recipients were comparable. Hence, we conclude that the inhibition of host anti-donor reactive CD8 cells was not associated with global immune suppression in chimeric animals and may indicate tolerance in these recipients. In total, these findings are consistent with the notion that the transient activation/expansion of host Tregs *in situ* post-HCT can be explored as a new strategy to regulate host alloreactivity post-transplant. Notably, the *in situ* stimulation of recipient Tregs following RIC obviated the infusion of Tregs and may represent an alternative approach to, and/or complement – the adaptive transfer of Treg populations in clinical HCT.

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PRE-TRANSPLANT RISK ASSESSMENT IN ADULT ACUTE LYMPHOCYTIC LEUKEMIA

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Introduction: Hematopoietic cell transplantation (HCT) can be curative in hematological malignancies but the procedure is associated with significant risks. Several scoring systems have been developed to predict transplant-related mortality (TRM) and overall survival (OS), however none has become a standard in clinical decision making yet. In the present study we compared the prognostic value of the HCT Comorbidity Index (HCT-CI) (Sorror ML, Blood 2005), the Pretransplantation Assessment of Mortality score (PAM) (Parimon T, Ann Intern Med 2006), the EBMT risk score (ERS) (Gratwohl A, Lancet 1998) and the Karnofky-Index (KI) in 151 adult patients who received HCT for acute lymphocytic leukemia (ALL) from 1995 until 2007 at our center.

Methods: Risk scores were calculated as defined in the original publications based on data extracted from patient charts, discharge letters and computer databases. Median age was 31 years (16–67). Disease status was first complete remission (CR) (n = 72), higher CR (n = 31) and no CR (n = 48). Patients received high-dose (HD) (n = 138) or reduced intensity conditioning (RIC) (n = 13) and bone marrow (n = 41) or peripheral blood stem cells (n = 110) from related (n = 63) or unrelated (n = 88) donors. Graft-versus-host-disease (GVHD) prophylaxis was CSA/MTX for HD or CSA/MMF for RIC. OS was assessed by Kaplan-Meier curves and logrank statistics, cumulative incidence of relapse mortality and NRM was assessed by competitive risk analysis.

Results: Median follow-up was 48 months (5–144). OS at 1, 3 and 5 years was 62% (55–69), 45% (37–54) and 40% (31–49). Deaths were due to relapse (n = 47), GVHD (n = 15), infections (n = 17) or other causes (n = 8). According to HCT-CI criteria comorbid conditions were present in 98 patients with liver disease (n = 43), lung disease (n = 34) and infections (n = 34) being the most common. Median scores for HCT-CI, PAM, ERS and KI were 1 (0–10), 24 (15–36), 3 (0–6) and 90% (60–100), respectively. We found that the HCT-CI and PAM were not predictive for OS or NRM whereas the EBMT risk score and KI were able to predict outcome based on differences in relapse mortality (Table 1).

Conclusions: 65% of ALL patients undergoing HCT at our center had significant pre-transplant comorbidities as assessed by HCT-CI criteria. ERS and KI had discriminative potential for OS and relapse incidence, however prediction of NRM was not possible with the examined indices. Further research is needed to optimize risk assessment in ALL patients.