TCD-BM + Tregs (4.5×10^5) compared to recipients of allogeneic HCT only ($p < 0.05$). We next examined the effects of pre-HCT infusion of Tregs: Tregs (4 or 1.5×10^5) were transplanted 24 hr. post-conditioning either 3 days pre-HCT or co-transplanted with (day 0) donor TCD-BM. Although no difference was observed using higher Treg doses, pre-infusion - but not co-transplant of low Treg doses was able to induce chimerism in these recipients. One month post-HCT of TCD-BM alone, the frequency of circulating host anti-donor tetramer$^+$ (the immunodominant epitope H60) CD8$^+$ cells in these recipients rejecting the marrow graft was clearly greater compared to recipients of TCD-BM + Tregs both of which engrafted ($p < 0.0008$). However, the host anti-donor CD8 levels were equivalent in recipients of TCD-BM alone and TCD-BM + Day 0 low doseTreg administration which failed to engraft. Notably, transplants using unmanipulated *host* Tregs failed to support engraftment. These findings indicate that donor Tregs support chimerism via suppression of host anti-donor antigen-specific T cells. Finally, the persistence of donor cells in circulation as well as in lymphoid tissues 3 months post-HCT indicated that long-term, stable chimerism was established by this pre-HCT Treg regimen. Together, our results demonstrate that donor Tregs promote MHC-matched allogeneic marrow engraftment by suppressing host anti-donor responses. Thus, the strategy of pre-HCT Treg infusion is consistent with the notion that antigen-driven expansion of donor anti-host reactive Treg cells can enhance hematopoietic engraftment. We are currently investigating host Treg expansion in MHC-matched HCT recipients.

AUTOLOGOUS TRANSPLANTS

18

UTILITY OF CO-MORBIDITY ASSESSMENT IN PREDICTING TRANSPLANT-RELATED TOXICITY FOLLOWING AUTOLOGOUS HEMATOPOIETIC TRANSPLANTATION FOR MULTIPLE MYELOMA

Labonte, L.[1], Iqbal, T.[2], Zaidi, M.A.[1], McDiarmid, S.A.[2], Huebsch, L.B.[2], Tay, J.[2], Allan, D.S.[1,2]. [1] Ottawa Health Research Program, Ottawa, ON, Canada; [2] University of Ottawa/The Ottawa Hospital, Ottawa, ON, Canada.

Background: Several randomized studies have demonstrated the benefits and favorable toxicity following autologous hematopoietic stem cell transplantation (AHSCT) for patients with multiple myeloma (MM) who are less than 55 yrs. Treatment related toxicity may increase for older patients with co-morbidities and the benefits of AHSCT become debatable. Decision tools for identifying myeloma patients most suitable for AHSCT are needed to avoid the risk of excess toxicity. **Methods:** Data regarding co-morbidities and toxicity following transplant were extracted from the hospital records and BMT database from 126 patients with MM undergoing AHSCT between 1994 and 2006. All patients consented to the use of medical information for research purposes. Co-morbidity was assessed using the Charlson Co-morbidity Index (CCI), the Haematopoietic Cell Transplantation Co-morbidity Index (HCT-CI) and a modified Pretransplantation Assessment of Mortality (mPAM). Transplant-related toxicity was assessed using length of hospital stay (LOS) following the transplant, the total number of organs having any toxicity according to the Seattle criteria, cumulative toxicity using the scores in 8 organ systems, and non-relapse mortality at 100 days. **Results:** Any co-morbidity using the CCI (score > 0) was associated with prolonged LOS (OR 4.05, 1.76 – 9.29 for LOS greater than 18 days), more organ systems with any toxicity (OR 2.27, 0.99 – 5.22 for at least one organ), and greater cumulative organ toxicity (OR 2.2, 1.06 – 4.49 for total score ≥2). Likewise, any co-morbidity using the HCT-CI (score > 0) was also associated with longer LOS (OR 5.34, 2.24 – 12.75), increased organ systems with any toxicity (OR 2.5, 1.08 – 5.80) and cumulative overall toxicity (OR 2.22, 1.08 – 4.55). A score of ≥24 using the mPAM was associated with increased LOS (OR 3.03, 1.35 – 6.77) but not with an increased number of organs with any toxicity or with cumulative toxicity. None of the co-morbidity assessment scales was associated with non-relapse mortality due to low incidence of transplant-related death in this group (2 deaths or 1.6%). **Conclusion:** Any co-morbidity identified using the CCI and HCT-CI was associated with greater toxicity following autologous transplant for multiple myeloma. None of the scales was associated with increased mortality in this group. In discussing AHSCT for patients with myeloma, assessment of co-morbidities using the CCI or HCT-CI may assist in determining the risk of toxicity.

GRAFT PROCESSING

19

PHARMACOLOGIC INHIBITION OF ALDEHYDE DEHYDROGENASE (ALDH) CAUSES THE EXPANSION OF HEMATOPOIETIC STEM CELLS (HSCs) WITH ENHANCED ENGRAFTMENT CAPACITY

Muramoto, G.G., Himburg, H., Meadows, S., Salter, A., Chao, N.J., Chute, J.P. Duke University, Durham, NC.

Delayed engraftment and graft failure are persistent obstacles which limit the application of umbilical cord blood (CB) transplantation in adults. We recently demonstrated that inhibition of ALDH with diethylaminobenzaldehyde (DEAB) induced the expansion of human HSCs capable of repopulating NOD/SCID mice (PNAS 2006 103:11707). In this study, we utilized syngeneic CD45.1/CD45.2 mice to determine whether inhibition of ALDH could accelerate the engraftment of transplanted HSCs in vivo. Culture of murine c-kit+sca-1+lin-CD34- (34-KSL) cells with 100 μM DEAB coupled with thrombopoietin, SCF and Flt-3 ligand (TSF) for 7 days resulted in a 22.6-fold expansion of total cells, compared with 360.0-fold expansion with TSF alone ($p = 0.001$). However, DEAB-culture maintained a significantly higher percentage of lin- (mean 97.1%) and KSL cells (mean 14.1%) at day 7 as compared to TSF alone (42.6% and 4.7%, respectively; $p < 0.001$ and $p = 0.03$), indicating that inhibition of ALDH preferentially maintained more primitive progenitors in culture than TSF alone.

We then transplanted FACS-sorted donor CD45.1+ BM 34$^-$KSL cells or their progeny following culture with TSF alone or TSF + DEAB at limiting doses into lethally irradiated CD45.2$^+$ congenic recipients. At 4 weeks post-transplantat, DEAB-cultured progeny showed significantly accelerated engraftment in recipient mice, as compared to day 0 34$^-$KSL cells or their progeny following culture with TSF alone. Using Poisson statistical analysis, the repopulating cell frequency was found to be 1 in 48.4 for the DEAB-cultured progeny (95% confidence interval: 27.3 – 86.0) as compared to 1 in 99.6; CI: 50.4 – 197.0) within the TSF-cultured progeny. Unmanipulated BM 34$^-$KSL cells had not yet shown significant levels of hematopoietic engraftment at week 4 (1 in 1319.4; CI 187.2 – 9298.8). DEAB-cultured progeny also demonstrated significantly higher B220$^+$ and Thy 1.2$^+$ lymphoid contribution, as compared to day 0 BM 34$^-$KSL cells ($p < 0.001$ and $p < 0.001$), and significantly higher level of Mac-1$^+$/Gr-1$^+$ myeloid reconstitution ($p < 0.001$), compared with the progeny of TSF alone. These data demonstrate that pharmacologic inhibition of ALDH facilitates the differential expansion of hematopoietic progenitors with enhanced early engraftment capacity. This strategy can be particularly useful to expand human CB grafts to overcome the delays in engraftment which currently limit this otherwise attractive source of HSCs for adult transplantation.

20

HIGHER CD34+ CELL DOSES ARE ASSOCIATED WITH DECREASED RELAPSE RATES FOLLOWING UNRELATED DONOR ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH TRENDS TO IMPROVED DISEASE-FREE SURVIVAL

Nakamura, R.[1], Auayporn, N.[1], Smith, D.D.[2], Palmer, J.[2], Sun, J.Y.[1], Schriber, J.[3], Pullarkat, V.[1], Parker, P.[1], Rodriguez, R.[1], Stein, A.[1], Rosenthal, J.[1], Wang, S.[4], Karanas, C.[1], Senitzer, D.[1], Forman, S.J.[1]. [1] City of Hope National Medical Center, Duarte, CA; [2] City of Hope National Medical Center, Duarte, CA; [3] City of Hope/Samaritan Hematopoietic Cell Transplantation Program, Phoenix, AZ; [4] City of Hope National Medical Center, Duarte, CA.

Peripheral blood stem cells (PBSC) have been increasingly used in the matched unrelated donor (MUD) transplant setting, but the impact of CD34+ cell dose on outcomes in this setting have not been well characterized. We analyzed a consecutive case-series of 181 patients who underwent MUD-PBSC transplantation at City of Hope from August 2000 to December 2004. Eighty-three patients were conditioned with a full-intensity regimen (TBI-Cy: 46, TBI-VP16: 28, Bu-Cy: 9) and 98 received a reduced-intensity regimen (Flu-Mel: 97, Flu-Bu: 1). The two-year overall survival (OS), disease-free survival (DFS), relapse, and 100-day transplant-related mortality (TRM) probabilities were 52%±4%, 47%±4%, 29%±4%, and 15 ± 3% respectively. There was a highly significant correlation between CD34+ cell dose infused and absolute lymphocyte count on day 30 post-transplant ($r = 0.378$, $p < 0.0001$). By univariate analysis, a CD34+ cell dose $\geq 4.2 \times 10^6$/kg (above the lowest quartile: p25) was associated with significantly lower relapse risk (HR = 0.67; p = 0.013), with a trend toward improved disease-free survival (HR = 0.84; p = 0.12) but not overall survival (HR = 0.91; p = 0.46). There was no significant association between higher CD34+ cell dose and TRM or acute/chronic GVHD. By multivariate analysis, cellular composition of the PBSC grafts remained significant in influencing transplant outcomes (Table 1). Further subset analysis of low risk group (n = 35) versus high-risk disease (n = 146) showed that the CD34+ dose ≥ p25 was significantly associated with reduced relapse in high-risk disease (p = 0.015), whereas the p-value was not significant in low-risk disease (p = 0.5). Among 18 patients with an iKIRL mismatch in the GVH direction, the log-rank p-value for comparing < p25 of CD34+ cell dose versus those who received higher doses was $p < 0.0001$ with respect to time to relapse. This is in contrast with p = 0.041 among the others. We also performed a stratified analysis in patients lacking iKIRL (n = 56) versus not (n = 125). When stratified by CD34+ cell dose, time-to-relapse was not significant among the patients who did not lack iKIRL but was significant in patients lacking iKIRL (p = 0.0030). In summary, we demonstrate that the higher CD34+ cell dose reduces relapse in MUD-PBSCT, in particular, for high-risk diseases. The data also suggest that the protective effect of higher CD34+ cell dose may be linked to an NK cell-mediated GVL effect.

Table 1.

	HR	p-values
OS		
10/10 HLA match (baseline) vs. others	2.23 (95%CI: 1.46–3.43)	0.0002
Lymphocyte dose in the graft (continuous)	0.20 (95%CI: 0.05–0.64)	0.005
MNC dose in the graft: p25 (baseline) vs. others	1.34 (95%CI: 1.01–1.82)	0.04
DFS		
10/10 HLA match (baseline) vs. others	1.91 (95%CI: 1.28–2.87)	0.001
Lymphocyte dose in the graft (continuous)	0.35 (95%CI: 0.14–0.81)	0.01
Good risk disease (baseline) vs. others	1.73 (95%CI: 1.01–3.17)	0.04
Relapse		
CD34+ quartile: p25 (baseline) vs. others	0.64 (95%CI: 0.47–0.88)	0.01
Recipient age (continuous)	1.02 (95%CI: 1.00–1.05)	0.02
TRM		
10/10 HLA match (baseline) vs. others	2.46 (95%CI: 1.44–4.30)	0.001
Full-intensity (baseline) vs. reduced-intensity conditioning	1.76 (95%CI: 1.03–3.10)	0.04

MNC: mononuclear cell.

21

PREPARATION OF HIGHLY PURIFIED REGULATORY T CELLS FOR CLINICAL APPLICATIONS

Sheehan, K.[1], Armstrong, R.[1], Tudisco, C.[1], Hou, J.-Z.[2], Negrin, R.[1]. [1] Stanford University Medical Center, Stanford, CA; [2] University of Pittsburgh Medical Center, Pittsburgh, PA.

Naturally occurring regulatory T cells (Treg) may limit or prevent the immune mediated morbidity associated with allogeneic transplants. The administration of Treg along with specified doses of conventional T cells (Tcon) has been proposed as an alternative to current T cell reduction strategies. This approach may provide patients with immune protection and graft-versus-tumor activity during post-transplant immune reconstitution while minimizing the risk of severe graft-versus-host responses when the donor and recipient are mismatched.

In order to establish the optimal dose of Treg to be administered with Tcon, highly purified Treg are required. Isolation of Tregs for clinical application is complicated by the lack of an exclusive surface marker suitable for selection and the relatively rare representation (<1%) of the cells in peripheral blood. Treg are characterized by surface expression of CD4, CD25, CTLA-4, and GITR among other markers and by intracellular expression of the transcriptional factor FoxP3.

We conducted a feasibility study of a cell selection strategy that combines immunomagnetic enrichment of $CD25^+$ cells with high flow cytometric speed cell sorting of $CD4^+CD25^{bright}CD127^{dim}$ cells. System operations took into consideration selection efficiency, effect on cell viability and functionality as well as the risk of contamination both from external sources and product cross-contamination. Using this approach, we have obtained cells that are >95% $FoxP3^+$ by intracellular staining and showing titratable suppression in MLR assays. Recovery of the target cell population on the Influx cell sorter was >90% at 20,000 events per second and without detectable microbial contamination. In addition, more than 100×10^6 $FoxP3^+$ Tregs were routinely obtained from individual G-CSF mobilized donor apheresis collections.

GVH/GVL

22

DISCOVERY OF LEUKEMIA ASSOCIATED MINOR HISTOCOMPATIBILITY ANTIGENS USING $CD8^+$ T CELL CLONES ISOLATED BY PRIMARY IN VITRO STIMULATION OF NAÏVE T CELLS

Bleakley, M.[1], Nishida, T.[1], Otterud, B.[2], Riddell, S.[1]. [1] Fred Hutchinson Cancer Research Center, Seattle, WA; [2] University of Utah, Salt Lake City, UT.

Allogeneic hematopoietic stem cell transplantation (HCT) is curative for many patients with leukemia in part due to a graft versus leukemia (GVL) effect mediated by donor T cells specific for recipient minor histocompatibility antigens (miH) on leukemic cells. Unfortunately, the GVL effect fails to completely eradicate leukemia in some patients, and additional strategies for preventing and treating post HCT relapse are required. Immunotherapy targeting miH selectively expressed on hematopoietic cells, including leukemia, could be used to augment the GVL effect without inducing GVHD but too few human miH have been molecularly characterized for this approach to be broadly applied. The identification of additional leukemia associated miH is essential. We developed a new strategy that employs primary in vitro stimulation of donor naïve $CD8^+$ T cells (T_N) with recipient APC in microcultures, allowing generation of a broad repertoire of miH specific CTL for gene discovery. Using this approach, >100 miH specific $CD8^+$ CTL lines were derived from 19 HLA identical sibling recipient-donor pairs and further characterized using a panel of target cells from unrelated individuals to determine the HLA restricting allele and population frequency of the miH. CTL directed against multiple distinct miH were