decreases overall survival. Several risk factors for graft failure have been reported, such as reduced intensity conditioning, HLA-mismatch, T-cell depleted grafts etc. However, in the early post-transplant period following myeloablative conditioning there is a lack of tools for early detection of patients at risk for subsequent graft failure. For this purpose, we retrospectively evaluated all patients who after myeloablative conditioning received peripheral blood cells (PBSC) at our centre during 1995 to 2007 (n = 219). The indications for transplantation were: AML (43%), ALL (23%), CML (17%), myelodysplastic syndrome (6%), lymphoma (5%), metabolic disorders (4%), and multiple myeloma (2%). Graft failure was defined as absolute neutrophil count (ANC) $<0.5 \times 10^9$/L or less than 5% donor cell chimerism. Moreover, primary graft failure was set to day 28 post-transplant, and patients who died prior to that day were excluded from further analyses. Three patients experienced graft failure within 100 days post-transplant (2 primary and 1 secondary graft failure), which means that the incidence of graft failure was 1.4%. In univariate analysis, there was a tendency that the total nucleated cell dose was associated with primary graft failure ($P = 0.06$). In contrast, when analyzing risk factors for graft failure within 100 days post-transplant the total nucleated cell dose seemed to be unimportant ($P = 0.17$). Interestingly, in subanalyses the risk of graft failure within 100 days post-transplant was markedly increased in those patients who still had an ANC less than 0.2×10^9/L at day 16 post-transplant (OR = 30, $P < 0.01$). In conclusion, we suggest that in patients with less than 0.2×10^9/L in ANC 16 days post-transplant one must consider interventions to avoid subsequent graft failure. For this purpose, strategies such as administration of hematopoietic growth factors (e.g. G-CSF) or donor lymphocyte infusions need further evaluation.

348

IN-VIVO T-CELL DEPLETION USING THYMOGLOBULIN (THYMO) ALLOWS SUCCESSFUL ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) FROM MISMATCHED, UNRELATED DONORS (MM-URD): POTENTIAL INFLUENCE OF GRAFT SOURCE ON OUTCOME

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Historically, allo-SCT using MM-URD has been associated with poor outcomes, due primarily to high rates of GVHD. Decreasing the rate of GVHD, without increasing relapse, should lead to better outcomes and wider use of this life-saving procedure. Between 6/1/05 and 9/30/09, 88 patients (pts) received a first allo-SCT from an unrelated adult volunteer donor at Mayo Clinic Arizona/Phoenix Children's Hospital. This report focuses on the subset of 69 pts transplanted for malignancy who received at least one dose of Thymo (dose range 2.5-10 mg/kg, dependent on degree of HLA mismatch) as part of their GVHD prophylactic regimen (19 excluded, 9 with non-malignant disease, 10 who did not receive Thymo due to MD preference). Forty-two pts were HLA-identical (10/10) with their unrelated donor by high resolution typing, while 27 were mismatched at one or more loci as follows: 1 allele mismatch (7); 1 antigen (Ag) mismatch (11); 2 allele mismatch (2); 1 Ag and 1 allele mismatch (5); and 2 Ag mismatch (2). The median age was 37 (0.5-75). Conditioning was ablative in 48, reduced intensity in 21; graft source was PBSC in 47, marrow in 22. In addition to Thymo, all pts received a calcineurin inhibitor (or sirolimus) plus methotrexate or MMF for GVHD prophylaxis. The outcomes are shown in the Table. The estimated 2-yr overall survival (OS) did not differ between the matched (61.1%) and mismatched pts (60.5%). The rates of aGVHD grades II-IV (48.6% matched vs. 39.9% mismatched) and relapse (28.6% matched vs. 17.5% mismatched) also were not significantly different. Pts who received mismatched *marrow* grafts had relatively poor outcomes (2 yr OS 21.9%) due to increased relapse and higher than expected NRM, which did not appear to be explained by higher disease risk. By design, these pts received relatively high doses of Thymo (7.5-10 mg/kg), and such high doses may have led to excessive T-cell depletion of marrow grafts, abrogating the graft vs. malignancy effect and leading to increased infectious mortality. In contrast, pts receiving mismatched *PBSC* grafts (who received similar doses of Thymo), had good outcomes (2 yr OS 91.5%) and low relapse rates (2 yr rel 0%). We conclude that 1) using PBSC as graft source and in vivo T-cell depletion, mismatched unrelated SCT can be safely performed with excellent outcomes in adult and pediatric pts with hematologic malignancy; and 2) modification of the Thymo dose will be necessary to achieve similar success using marrow grafts.

Outcomes by Match Grade and Graft Type

Match Grade	Graft	N	2 Yr OS	2 Yr Rel	aGVHD II-IV	aGVHD III-IV
Matched	Either	42	61.1%	28.6%	48.6%	8.3%
Matched	PBSC	30	57.3%	30.4%	46.2%	8.1%
Matched	Marrow	12	68.2%	25.4%	53.9%	8.8%
Mismatched	Either	27	60.5%	17.5%	39.9%	14.6%
Mismatched	PBSC	17	91.5%	0.0%	41.8%	16.3%
Mismatched	Marrow	10	21.9%	50.5%	37.1%	12.2%

349

PERIPHERAL BLOOD CHIMERISM CAN REPLACE MARROW CHIMERISM ANALYSES FOLLOWING ADULT ALLOGENEIC STEM CELL TRANSPLANT

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Chimerism defines the amount of donor versus recipient hematopoiesis following allogeneic stem cell transplant (SCT). PCR-based analyses of short tandem repeats (STRs) are commonly used and are accurate and applicable to allogeneic transplant recipients. These analyses are performed on peripheral blood and marrow aspirates, but it is not known if it is necessary to analyze both. We performed a retrospective analysis of 42 consecutive adult allogeneic SCT recipients at our institution with available chimerism studies. PCR and capillary electropheresis of microsatellite loci were performed at 30, 60, and 90 days after SCT on both unfractionated blood and unfractionated marrow aspirate. Full donor chimerism (FDC) was defined as 95% or greater donor chimerism. PCR analyses of STRs for chimerism performed on unfractionated blood did not differ from results obtained on unfractionated marrow aspirate at 30, 60, or 90 days post transplant ($P < 0.0001$). Peripheral blood PCR-based chimerism analyses provide similar information as marrow aspirate analyses. Using peripheral blood alone saves the expense of an additional analysis on marrow aspirate and prevents an uncomfortable procedure. These findings provide unique results suggesting larger studies in the adult population are needed to further delineate the role of chimerism analyses following allogeneic SCT.

350

T-CELL DEPLETED ALLOGRAFTS FROM UNRELATED DONORS CONFER A LOW RISK OF RELAPSE ON PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment option for an expanding spectrum of patients (pts) with a greater variety of diseases and graft sources, cytoreductive regimens, cellular therapies, and supportive care.

T cell depletion (TCD) has successfully reduced the risk of graft versus host disease (GVHD) but concerns about relapse and infections have limited its application.

Methods: Between 7/2001 and 12/2005, 35 pts underwent TCD HSCTs as treatment for hematologic malignancies using HLA-matched (HLA-M)(20 pairs) or mismatched (-MM)(15 pairs) unrelated donors. The conditioning regimen, hyperfractionated total body irradiation (1375 cGy), fludarabine (25 mg/m^2) x 5d, and thiotepa (5 mg/kg) and antithymocyte globulin × 2d, was designed to reduce toxicity, while preserving immunosuppression for engraftment. Donors were ≥8 of 10 HLA matched by DNA SSOP analyses. Pts received TCD-peripheral blood stem cells (PBSC) (n = 29) or TCD-bone marrow (BM) (n = 6). PBSCs were CD34+ selected (Isolex 300i columns) and BMs agglutinated by soybean lectin, after which both underwent sheep erythrocyte-rosette sedimentation. The median age was 40.5yrs (18-63). Diseases included AML (standard or high risk) and ALL (high risk) CR1, AML CR2, ALL ≥CR2, acute biphenotypic leukemia, CML-CP, MDS, T-PLL. Median followup is 52 mos (37-83).

Results: All evaluable pts engrafted neutrophils, and 31/34 evaluable pts engrafted platelets. The 100d non-relapse mortality was 20% with infection causing >50% of deaths. 9% developed only acute GVHD grade II-III and 29% chronic GVHD, with no significant difference between HLA-M and -MM subsets. Estimated 4 yr DFS and OS is 56% and 59%, with DFS of 75% for pts with standard risk and 41% for high risk disease. Two high risk disease pts relapsed at 31 and 38 mos. Infectious deaths were: 2 viral, 1 toxoplasma, 1 fungal, 2 bacterial. Median time to achieve normal CD3 + CD8+ and CD3 + CD4+ counts were approximately 4-6 and 6-9 mos posttransplantation, respectively. Only 4 pts, who had been treated with steroids, had CD3 + CD4+ counts <100 cells/ul by 1 yr. 50% of patients reached a normal PHA proliferative response in vitro within 1 yr.

Conclusions: Outcomes with this cytoreductive regimen followed by TCD PBSC or BM are similar to those reported for TCD matched related donor, and for unmodified transplants but with a much lower incidence of GVHD. Relapse rates, with followup of >3 yrs, remain remarkably low.

351

ALLOGENEIC HEMATOPOETIC CELL TRANSPLANTATION IN PATIENTS POSITIVE FOR HEPATITIS B SURFACE ANTIGEN

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The incidence of liver related morbidity and mortality after allogeneic hematopoietic cell transplantation (Allo-HCT) in patients positive for hepatitis B surface antigen (HBsAg +) may increase, but it has been not well analysed. The aim of this study is to determine the frequency of HBV reactivation and to evaluate the effect of HBV reactivation on early transplant-related complications in a retrospective single center cohort. We detected HbsAg+ 23 patients (3.8%) who underwent allo-HCT (n = 680) within 10 years. Median age was 33 years. Twenty-two were male and one female. Liver function tests were normal and HBV-DNA negative in 20 patients prior to the transplant. Myeloablative (n = 20) or fludarabine based-reduced intensity conditioning regimen (n = 3) were used. Lamivudin prophylaxis was initiated 100 mg p.o o.d. in 14 patients with conditioning regimen and was continued until 6-12 months after the cessation of immunosuppresion at post-transplant period. Six patients had HBsAg+ donor, 5 seropositive (antiHBs with antiHBc IgG, 3 antiHBs alone and 6 were negative HBV naive. We could not obtain enough data about HBV serology of 3 donors. HBV reactivation was observed in 7 patients after median 4.5 months (range, 0.87-23.5) after the Allo-HCT. All the patients with HBV reactivation had received myeloablative conditioning. There was an increase of serum ALT (2.5×UNL- 54 UNL) level with positivity for HBV-DNA during reactivation. The reactivation was observed only in one patient during lamivudine prophylaxis 7.6% vs 60%. p = 0.012) while one patient experienced a reactivation in 2 months after cessation of prophylaxis. Two patients with HBV reactivation had donors positive for HBsAg. Lamivudin treatment was given in 5 patients after reactivation. Lamivudin resistance was observed in two patients, who received second line antiviral treatment. One of those patients died of fulminant hepatic failure. The incidences of acute or chronic liver graft versus host disease were not affected from HBV reactivation. The reactivation HBV is one of the most undesired complications of chemo/immunosuppressive treatment in HBsAg positive Allo-HCT recipients. The prophylaxis with lamivudine could decrease the frequency of HBV reactivation. But the development of the lamivudine resistance is another problem in some patients requiring long-term treatment. In conclusion, our study has shown that HBsAg+ recipients did not have a strict limitation for allo-HCT.

352

QUANTITATIVE ANALYSIS OF LINEAGE-SPECIFIC CHIMERISM FOR MONITORING POST-HAEMATOPOIETIC STEM CELL TRANSPLANTATION ENGRAFTMENT

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Haematopoietic stem cell transplantation (HSCT) is an established treatment for various hematological disorders. The monitoring of post-transplant engraftment has become a routine diagnostic assay in the clinical laboratory. PCR-based methodologies utilizing STR analysis and commercial multiplex assays are frequently used. However, these commercial kits were originally formulated for forensic applications and do not completely fulfill the needs of diagnostic chimerism testing, especially for non-myeloablative bone marrow transplants, in which treatment depends on the status of lineage-specific chimerism. We utilize the Beckman-Coulter GenomeLab™ Human STR Primer Set (Fullerton, CA) in our clinical laboratory. This kit consists of twelve STR markers in a single multiplex PCR reaction and has proven highly informative, sensitive and rapid in routine clinical testing. For use with this kit, DNA is extracted from peripheral blood, bone marrow, and/or CD3/CD33/CD56-enriched fractions sorted on the BD Biosciences FACSAria™ Cell Sorting System (San Jose, CA) and extracted with the Qiagen Blood Kit or Qiagen Micro Kit (Valencia, CA). 60 blood samples from multiple College of American Pathologists (CAP) proficiency panels were evaluated. These panels are challenges proctored by CAP and participated in by up to 80 US labs 3 times per year. Correlation of results were excellent ($r^2 = 0.99$). This method has been in use by our laboratory since the summer of 2005 with approximately 4500 patient sample studies. In our experience no pre-transplant/donor combination has yielded a non-informative result, even between siblings. We have found the STR-based method to be a rapid, specific, sensitive, and cost-effective diagnostic assay for monitoring donor cell engraftment after HSCT transplantation, making it a responsive tool for rapid clinical decision making. The assay is streamlined to provide rapid turn-around-time and requires a minute amount of DNA making it an excellent tool for patients with low white blood cell counts. This methodology is also suitable for detection of maternal cell contamination and for verifying pathology specimen identification.

353

CONFIRMATION OF UNRELATED CORD BLOOD TRANSPLANT AS A TREATMENT STRATEGY FOR SEVERE APLASTIC ANEMIA

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The St. Louis Cord Blood Bank (SLCBB) serves to collect, process, cryopreserve, and distribute umbilical cord blood for human transplantation. To date, nearly 1,600 units have been distributed globally for the treatment of more than 70 diseases