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.

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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.

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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.

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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