units homozygous for the *CCR5*-delta32 allele will provide an improved probability of finding an appropriately HLA-matched donor for a patient with HIV infection in need of a HCT.
Methods: *CCR5* genotype analysis is performed on DNA extracted from cord blood using a PCR based assay. Biomathematicians at the NMDP developed estimates regarding the probability of providing an adequately HLA matched cord blood unit.
Results: We have tested about 10,000 cryopreserved cord blood units and have identified 81 homozygous *CCR5*-delta32 units. Testing an additional 25,000 cord blood units from Caucasians is expected to increase the special inventory to about 300 units which is projected to provide for Caucasians an adequately HLA matched cord blood unit with an adequate cell dose for 73.61% of pediatric patients and 27.92% of adults. The initial population for transplantation is patients in need of a HCT for a hematologic malignancy or other indication, and who are also infected with HIV, although some selected patients with AIDS who have no other illness may also ultimately be considered for transplantation.
Conclusions: Cord blood transplantation has a unique role in providing for long term control or possible cure of HIV infection.
Disclosure: No relevant conflicts of interest to declare.

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LONG TERM OUTCOMES FOR ALEMTUZUMAB BASED REDUCED INTENSITY CONDITIONING TRANSPLANT FOR MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKAEMIA

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Haematopoietic Stem Cell Transplantation (HSCT) remains the only curative therapy for patients with myelodysplastic syndromes (MDS) and acute myeloid leukaemia with tri-lineage dysplasia (TLD-AML). Reduced intensity conditioning (RIC) has expanded this approach to older patients and to those with comorbidities. Previously published data from our institution has shown excellent overall survival and low rates of GVHD at one year with alemtuzumab-based RIC-HSCT. Herein we report the long-term follow-up data of 237 patients with high risk MDS and TLD-AML treated at our institution from 1999 to June 2010.

All patients received a conditioning protocol consisting of fludarabine (150mg/m^2), busulphan and alemtuzumab (100mg) from sibling (n = 57) or matched unrelated donors (MUD) (n = 180). 30 patients received 4 days of busulphan (total dose 12.8mg/kg iv or 16mg po) while the remainder received 2 days (total dose 6.4mg/kg iv 8mg po). The majority of patients receiving an unrelated transplant had a fully matched donor (n = 128), with 45 having a 1-antigen mm donor and 7 having a 2-antigen mm donor. The median age of the cohort was 56 years (range:19-72) and median follow-up for survivors was 5.2 years (range:0.12-11.4). OS and DFS for the entire cohort was 44% and 34% respectively at five years with no significant difference between sibling and MUD transplants. NRM was 23% at one year and 31% at five years. Relapse at five years was 51%. Acute GVHD occurred in 35% and chronic GVHD in 27%. The rate of extensive de-novo chronic GVHD was very low at 10%. Outcomes were similar for those receiving four doses of busulphan despite a higher proportion of patients not in CR at the time of HSCT in this group.

On multivariate analysis, age remained the only significant factor with regard to OS, DFS and NRM. For patients aged less than 60 vs greater than 60 years at five years OS was 55 vs 23%, DFS was 46 vs 15% and NRM 27 vs 50%. To our knowledge this analysis represents the largest series of patients receiving alemtuzumab based RIC for MDS and TLD-AML. Long-term outcomes remain excellent for younger patients. Novel strategies are required for the prevention of relapse and to improve outcomes in older patients.

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RESULTS OF PHASE II CLINICAL TRIAL MPD-RC 101: ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CONDITIONED WITH FLUDARABINE/MELPHALAN IN PATIENTS WITH MYELOFIBROSIS

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The Myeloproliferative Disorder-Research Consortium (MPD-RC) designed the first US prospective phase II study of reduced intensity allogeneic hematopoietic stem cell transplantation (HSCT) in patients with primary myelofibrosis (PMF) or MF secondary to essential thrombocythemia (ET-MF) or polycythemia vera (PV-MF). Between May 2007 and March 2011, 66 patients were enrolled into MPD-RC 101 study and transplanted from related (n = 32) or unrelated (n = 34) donors using a fludarabine/melphalan ± ATG regimen. Of 66 patients, 63 were at intermediate/high risk according to Lille score system and 3 low risk patients had thrombocytopenia. Recipients of related and unrelated HSCT were comparable with respect to age (median: 54 vs 55 years), gender, Lille score, time from diagnosis to transplant, presence of Jak-2 V617F mutation, splenomegaly and splenectomy. Donors were HLA matched in 94% of related and 74% of unrelated transplants. Engraftment of neutrophils and platelets occurred in 31/32 related and 26/34 unrelated transplants. Five secondary graft failures occurred (4 among unrelated recipients). Median time to ANC $>$ 0.5 x 10^9/L and platelets $>$20 x 10^9/L engraftment was: day 22 and 28 in the related, and day 18 and 28 in the unrelated cohort, respectively. Acute GVHD grade II-IV occurred in 37% related (grade III-IV: 12%) and 42% unrelated transplants (grade III-IV: 21%). In patients with ≥ 6 months follow-up, there were 7 CR, 8 PR, and 11 CI according to the IWG criteria among the 28 patients in the related group, and 5 CR, 1 PR, and 5 CI among the 16 patients in the unrelated group. After a median follow-up of 24 months for survivors in the related group, 78% of the patients are alive, TRM was 18% and relapse-related mortality was 3%. In the unrelated group, 44% of the patients are alive after 12 months follow-up, TRM was 53% and 3% died due to relapse. Median survival time has not been reached in the related group and is 7 months in the unrelated group (hazard ratio 4.2, 95% CI: 1.7-10.1, p$<$0.001). Survival in unrelated transplants was not associated with PBSC or BM HSCT, HLA matching, diagnosis, Jak-2 mutation. In this prospective study a reduced intensity allogeneic HSCT with Flu/Mel regimen was very effective in myelofibrosis patients transplanted from related donors. In unrelated transplants, a high rate of primary or secondary graft failure led to a high rate of TRM. For these patients a different conditioning regimen may be required.

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IDENTIFICATION AND CHARACTERIZATION OF H-Y SPECIFIC ALLOGENEIC B CELLS FOLLOWING SEX-MISMATCHED TRANSPLANTATION

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H-Y allo-antibody develop in association with chronic graft-versus-host disease (cGVHD) following sex-mismatched transplant. We hypothesize that H-Y specific B cells contribute to cGVHD pathogenesis and have developed H-Y specific FACS stain for their isolation and characterization.

In order to identify the immune dominant H-Y epitope(s), we developed a high-throughput H-Y microarray. Five H-Y proteins and their 95% identical X-chromosome homologs (H-X) were printed on epoxy glass slides along with fifty 15-19 amino acid overlapping disparate H-Y peptides. Normal male donors have negligible H-Y antibodies. H-Y seropositive thresholds were mean result of 31 healthy males + 3 SD. The H-X homologue is scored using its corresponding H-Y antigen threshold.

Plasma samples from 32 male patients with female donors (F→M) who developed cGVHD, had sera tested for H-Y specific IgG and IgM, 6, 12,18 months after HCT. Twenty-two of 32 (69%) had anti-DBY (aka DDX3Y) antibodies by microarray and ELISA (R2 = 0.85). DBY-2 was recognized by 17/32 (36%) overall and accounted for 77% of DBY responders.

We used this immune dominant peptide to FACS stain DBY-2 specific B cells. DBY-2 biotinylated peptide was used to capture the antigen specific B cells and was later detected by streptavidine-Qdot along with markers of B cell development. Ten healthy males had no detectable DBY-2 staining. Peripheral blood mononuclear cells (PBMCs) collected days 90, 180, 270, and 365 post HCT from 25 F→M patients and stained for DBY-2 in relation to DBY IgG and IgM in plasma (Table). Seven of 11 (64%) had DBY-2 specific B cells detected as early as 90-180 days. Further, 3 of 7 (43%) of DBY and DBY-2 IgG seronegative patients tested persistently DBY-2 B cell positive. Day 90 and 180 detected DBY-2 B cells showed naïve phenotype (IgD+IgM+CD38+CD27-). In contrast, >30% DBY-2 B cells at 1-year were activated CD27+. The range of DBY-2 B cells at 1y was 0.3-0.8 % of CD19+ cells.

This is the first identification of human allogeneic B cells and surprisingly they develop months before subsequent allo-IgG and are detected in 40-50% of seronegative F→M. Immune dominant DBY-2 B cell isolation now enables allogeneic B cell functional studies.

Table. H-Y antibodies and B cells detected in 32 male patients with female donors

F→M HCT n = 32	Protein ELISA	Peptide ELISA		DBY-2 B cells	
	DBY IgG	DBX-2 IgG	DBY-2 IgM	d180	d365
DBY IgG pos n = 22	22/22 (100%)	0/22	6//22 (27%)	7/11 (64%)	7/11 (64%)
DBY-2 IgG pos n = 17	12/17 (71%)	0/17	5/17 (29%)	4/11 (36%)	5/13 (39%)
DBY-2 IgG neg n = 15	10/15 (67%)	1/15 (7%)	1/17 (7%)	3/7 (43%)	4/7 (56%)
Healthy Male	0/31	0/31	0/31	0/10	0/10

ELISA measurements were performed 1 y after transplant.

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CYTOREDUCTION WITH ALEMTUZUMAB IN PATIENTS WITH ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA PRIOR TO ALLOGENEIC STEM CELL TRANSPLANTATION – RESULTS OF A PROSPECTIVE CLINICAL TRIAL (NCT 00337519)

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The majority of patients (pts) with chronic lymphocytic leukemia (CLL) with an indication for allogeneic hematopoietic cell transplantation (HCT) suffer from refractory disease. Lower tumor load prior to HCT may be associated with better outcome. Thus, cytoreductive pre-treatment needs to be considered. Alemtuzumab has proven activity in refractory disease. On the other hand, pre-treatment with alemtuzumab has been associated with inferior outcome after HCT in a retrospective analysis. We present results from a phase II trial where we systematically investigated treatment with alemtuzumab followed by a washout period and subsequent reduced intensity conditioning (RIC) prior to allogeneic HCT.

Methods: All pts were scheduled for one month of treatment with 30 mg alemtuzumab 3 times weekly. The washout period between the last dose of alemtuzumab and the day of HCT was increased from two weeks to one month during the study. Antibody levels were measured on the day of HCT and T-cell engraftment was monitored. RIC consisted of fludarabine (150 mg/m^2) and busulfan (8 mg/kg). Cyclosporine (CSA) and methotrexate (MTX) were administered as GVHD-prophylaxis.

Results: 62 pts with relapsed or refractory CLL and a median age of 56 years were included. Two pts failed to reach HCT due to progressive disease under therapy. Donors were HLA-identical siblings (N = 26) or HLA-compatible unrelated donors accepting one mismatch (N = 34). No primary or secondary graft failure occurred. Interference of persisting antibody levels with T-cell engraftment could be demonstrated even after a washout period of one month in 4 out of 30 pts (ASH 2009, 114:3351). The cumulative incidence of acute GVHD grades II to IV at day +100 was 42%, and that of extensive chronic GVHD at one year was 56%. With a median follow-up of 35 months (16 to 61 months) 3-year overall survival and progression-free survival were 62% (95% CI, 48% to 76%) and 52% (95% CI, 38% to 66%). Day +100 non-relapse mortality was 2%. At 3 years non-relapse mortality and relapse incidence were 24% (95% CI, 12% to 36%) and 24% (95% CI, 13% to 35%), respectively.

Conclusions: The favorable long-term results of this study argue in favor of that strategy for patients with refractory disease. However, unless in vivo T-cell depletion is the goal, a washout period of a minimum of one month after the last dose of alemtuzumab should be kept and T-cell engraftment needs to be monitored.

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SAFETY AND EFFECTIVENESS OF INTRAVENOUS PALIVIZUMAB FOR PREVENTION OF RESPIRATORY SYNCYTIAL VIRUS INFECTION FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Respiratory syncytial virus (RSV), a single-stranded RNA virus of the Paramyxoviridae family, is one of the most common causes of respiratory infections during childhood. Although most children are infected with this virus by 2 years of age resulting in the production of neutralizing antibodies, life-long immunity is not induced. Nosocomial spread is common, even among immunocompetent individuals. Following allogeneic HSCT, progression from upper respiratory tract to lower tract disease occurs in 40-60% of cases and is generally fatal if left untreated. In a prior study at our institution, 16 of 21 patients infected during the first post HCT month developed pneumonia. In an effort to prevent RSV infection in unrelated and HLA-mismatched transplant recipients, intravenous palivizumab (15 mg/kg infused at 20-40 mg/minute) was administered to 60 of 142 patients <21 years of age transplanted between 2005 through 2009. Patients who received prophylactic palivizumab were younger, more likely to have been transplanted after 2007, to be the recipient of an HLA-mismatched donor transplant, and/or to have GVHD. Patients received a median of 3 doses of palivizumab at 4-6 week intervals during the RSV season. All doses were well tolerated and resulted in an RSV neutralizing antibody titer of 1:640->1:1280. An RSV infection developed in 6 of 60 patients who received prophylaxis and 17 of 82 who did not, (p = 0.04). Of the 17 patients who developed RSV in the absence of prophylaxis, 9 were treated with palivizumab, and none developed pneumonia. This retrospective study demonstrates that IV palivizumab is safe and suggests it can prevent infection. Future studies evaluating the number of doses needed to prevent disease, the optimum population to target, and the cost effectiveness of this strategy are warranted.