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Graft-Specific HLA-Antibodies Do Not Influence Unit Dominance and Do Not Prevent High Rates of Sustained Donor Engraftment in Recipients of Double-Unit Cord Blood (CB) Transplantation

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Background: While HLA-antibodies (Ab) have an established role in failed engraftment after single-unit CB transplantation (CBT), their role in double-unit CBT (DCBT) engraftment is more controversial.

Methods: We evaluated the influence of pre-transplant HLA-Ab on sustained donor neutrophil engraftment & unit dominance in 82 recipients (median age 48, range 2-69) of 4-6/6 HLA-A,B antigen, DRB1 allele matched double-unit CB grafts, & transplanted for hematologic malignancies from 7/2008-7/2012. HLA-Ab were measured by the American Red Cross Blood Service using Single Antigen Luminex beads & analyzed by HLA Fusion software. The cut-off for positive results was a normalized mean fluorescence intensity value > 1000.

Results: 28/82 (34%) patients were positive for HLA-Ab [16 (19.5%) had Ab without graft specificity, & 12 (14.5%) had Ab with graft specificity]. These patients were more likely to be female with acute leukemia & CMV seropositive. All patients with graft-specific Ab received myeloablative conditioning; 5 had Ab against class I HLA, 6 against class II, & one patient had Ab to both Class I/II. Moreover, of these 12 patients, 6 had Ab against one unit & 6 had Ab against both units. Neutrophil engraftment according to Ab is summarized in the Table.

Table
Sustained Donor Neutrophil Engraftment According to HLA-Ab

1A) Myeloablative Conditioning (n = 67)		
	Median (Range) Time to Neutrophil Recovery (≥0.5)	N (%) Sustained Engraftment
No Ab (n = 41)	23 days (range 12-38)	39/40 (1 non-evaluable)
Ab: not graft-specific (n = 14)	23 days (range 12-37)	14/14
Ab: graft-specific (n = 12)	31 days (range 13-40)	11/12
Against engrafting unit (n = 3)	28 days (range 24-31)	3/3
Against non-engrafting unit (n = 3)	31 days (range 25-32)	3/3
Against both units (n = 6)	33 days (range 13-40)	5/6
1B) Non-Myeloablative Conditioning (n = 15)		
	Median (Range) Time to Neutrophil Recovery (≥ 0.5)	N (%) Sustained Engraftment
No Ab (n = 13)	10 days (range 8-20)	13/13
Ab: not graft specific (n = 2)	19 days in 1 patient (other had autologous recovery)	1/2
Ab: graft specific (n = 0)	-	-

64/66 (97%) evaluable myeloablative DCBT recipients (Table 1A) engrafted at a median of 24 days (range 12-40); one patient without Ab & one with Ab against both units had primary graft failure, both in the setting of early onset multi-organ failure. Both patients were 100% donor in the marrow with one unit but did not recover counts. Of the 6 patients with Ab to one unit, 3 engrafted with that unit & 3 with the opposite unit. Of the 6 patients with Ab against both units, one had clinical graft failure as described above, & the 5 others had sustained donor engraftment (4 with one unit & one with both). In engrafting myeloablative recipients, the median time to neutrophil recovery was 8 days slower in patients with graft-specific Ab, but the engrafting unit in these patients also had the lowest infused dose: median CD34+ cell dose/kg if no Ab 0.83 x 10^5/kg, non-specific Ab 1.09 x 10^5/kg, & Ab specific to graft 0.65 x 10^5/kg. In non-myeloablative recipients, 15/16 (94%) engrafted (Table 1B). The single patient with rejection/ autologous recovery had Ab that were not graft specific.

Conclusions: 11/12 double-unit CBT recipients with graft-specific Ab engrafted successfully. While myeloablative recipients with graft-specific Ab engrafted more slowly, this may be explained by their lower infused CD34+ cell dose. While multivariate analysis of larger series will be required to further evaluate the effect of graft-specific Ab on engraftment speed, currently the presence of graft-specific Ab should not preclude DCBT, & whether their presence should influence graft selection is unclear. Moreover, there is no suggestion that HLA-Ab influence unit dominance after DCBT.

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The Effect of Donor Characteristics On Graft Vs. Host Disease (GVHD) and Survival After Unrelated Donor Transplantation for Hematologic Malignancy

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The Effect of Donor Characteristics on Graft vs. Host Disease (GVHD) and Survival after Unrelated Donor Transplantation for Hematologic Malignancy.

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We analyzed 6,349 adult unrelated donor transplantations performed in 1988–2006 to reexamine the effect of donor age and other donor characteristics including donor-recipient HLA matching on transplant outcomes.

Patients had a hematologic malignancy (ALL, AML, CML, MDS) and approximately 40% were in first complete remission or first chronic phase. 93% of transplantations used donors younger than 50 (median 35.8, range 18-61). The majority of patients received myeloablative preparative regimens (88%) and bone marrow grafts (62%). All patients received calcineurin inhibitor containing GVHD prophylaxis and 20% received *in vivo* T cell depletion. We identified three donor characteristics associated with overall survival; donor age, high resolution donor-recipient HLA-match and ABO blood group match. Risk adjusted 5-year survival rates were 37%, 33% and 29% with donors aged 18-32, 33-50, and >50 years, respectively ($p<0.0001$). Corresponding hazard ratios were 1.13 ($p=0.0004$) for donors aged 33−50 years and 1.29 ($p<0.001$) for donors >50 years compared with donors aged 18 − 32 years. Mortality risks were higher with one (HR 1.24, $P < .0001$) and two (HR 1.62, $P < .0001$) HLA-mismatches compared with HLA-matched transplants and minor (HR 1.10, $P = .002$) or major (HR 1.13, $P = .001$) ABO blood group mismatch compared with ABO-matched transplants. A subanalysis investigated the possibility that the association with donor age may be due to underlying genetic disparity between donor and recipient. That is, recipients with rare HLA genotypes tend to have fewer matched donors to choose from and thus more likely to receive a graft from an older donor. Recipient genotypes were assigned to quartiles using frequency data from 4 million NMDP donors as a reference. The lowest frequency quartile was associated with donors older than 50 ($p<0.0001$) and higher rates of HLA mismatching ($p<0.001$) but no association was found between genotype frequency and overall mortality. Acute GVHD risks were associated with donor age and HLA match and, donor parity, the only donor characteristic associated with chronic GVHD (table). In summary, the data recommend the consideration of donor age and ABO blood group match to maximize survival when selecting among comparably HLA-matched adult unrelated stem cell donors for treatment of a hematologic malignancy.

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Higher Infused CD34+ Dose Positively Influence Platelet Recovery After Cord Blood Transplantation

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Background: Umbilical cord blood transplantation (UCBT) is associated with delay in platelet recovery. However, the underlying factors influencing this delayed recovery remain poorly understood. With the aim of identifying factors that influence platelet engraftment, we retrospectively analyzed data from 68 consecutive myeloablative UCBT recipients transplanted between April 2006 and March 2012.
Methods: Forty-two (62%) patients received high-dose TBI (1320 cGy), cyclophosphamide and fludarabine (FLU); while 26 (38%) received Treosulfan, FLU, and a single fraction of 200cGy TBI. Graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine and mycophenolate mofetil. Platelet recovery was defined as the first day of a platelet count $\geq$20 and $\geq$50 x 10^9/L without transfusion for 7 days. Cumulative incidence (CI) curves were used to estimate the probabilities of platelet engraftment in the first 100 days post- transplant. A proportional hazards regression model was used to evaluate the association between $CD34^+$ cell dose and platelet engraftment. Factors considered as potential predictors or confounders included age at transplant, sex, race, body mass index (BMI), disease risk, CMV seropositivity, presence of minimal residual disease at transplant (MRD), number of UCB units infused, acute GVHD, total nucleated cells (TNC) and $CD34^+$ cells.
Results: Median age and BMI were 36 years (range, 1-63) and 26 kg/m^2 (range, 16-41), respectively. The majority of patients (88%) received 2 cord blood units (n=60). Twenty-seven (40%) had MRD and 46 (68%) were CMV seropositive at time of transplant. Median total infused cell doses were: 3.9 x 10^7 TNC/kg (range: 2.2-11.2) and 0.25 x 10^6 $CD34^+$ cells/kg (range: 0.09 − 1.46). The 100-day CI of platelet engraftment was 65% (95% CI: 53-76%) for platelets $\geq$20 10^9/L and 59% (95% CI: 47-71%) for platelets $\geq$50 10^9/L. In univariate analysis higher $CD34^+$ infused dose was significantly associated with platelet engraftment [HR=1.4 (95% CI: 1.0-1.9, $P = .03$)]. Furthermore, platelet engraftment was suggestively slower among patients with higher BMI [HR=0.9 (95% CI: 0.9-1.0, $P = .06$)] while presence of aGVHD grade III-IV was associated with higher rate of engraftment [HR=2.1 (95% CI: 0.8-5.9, $P = .07$)]. No significant associations were found between all the others factors analyzed and platelet recovery.In multivariable analysis the association between larger $CD34^+$ dose and higher rate of engraftment remained statistically significant [HR=1.9 (95% CI: 1.3-2.8, $P < .001$]. In addition, CMV seropositivity [HR=0.4 (95% CI: .0.2-0.9, $P = .02$)] became significantly associated with a slower rate of engraftment.
Conclusions: These results indicate that the infused $CD34^+$ dose is a strong independent predictor of platelet engraftment. Furthermore we should expect an earlier sustained platelet recovery among CMV seronegative patients.

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Ultra-Low Dose IL-2 Expands Natural Regulatory T Cells and CD56bright NK Cells in Patients and Healthy Donors and Is Associated with Clinical Improvement in Chronic Graft Versus Host Disease

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Low dose interleukin-2 (IL-2) can increase regulatory T cells (T_{reg}) and has therapeutic benefit in steroid refractory cGVHD. To further define immunological changes underlying the response to IL-2 and to determine tolerability, we performed phenotypic characterization and functional analysis of T_{reg} and natural killer (NK) cells in individuals given IL-2. Six healthy volunteers received subcutaneous ultra low dose IL-2 (0.2MIU/m^2/day) for 5 days without significant side effects. Two patients with steroid and calcineurin-refractory cGVHD received the same dose of IL-2 daily for 4 or 8 weeks, after HLA identical myeloablative stem cell transplant (SCT) for AML. Patient 1 (50 year old male, 3 yr post SCT) had skin and fascial GVHD affecting the lower limbs. Patient 2 (44 year old female, 2 yr post SCT) had upper gastrointestinal cGVHD. Both patients had a prompt and durable partial response by