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Characterization of haploidentical stem cell grafts after negative depletion of B-cells and α/β+ T cells

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) from a HLA-haploidentical relative is a suitable option for patients lacking a compatible donor. We have developed a novel method of ex vivo T/B-cell depletion based on the selective elimination of α/β+ T cells through labeling with a biotinylated anti-TCR-α/β Ab, followed by incubation with anti-biotin and anti-CD19 Ab conjugated to paramagnetic beads (Miltenyi Biotec, Germany). Here, we report the results of graft manipulation using this methodology.
Methods: Twenty-two children entered the study, 16 with hematological malignancies and 6 with non-malignant disorders. Cell therapy products contained up to $60x10^9$ white blood cells (WBC). Graft aliquots were used to enumerate residual α/β+ T cells and B cells, as well as other immune effector cells (type 1 and 2 DC precursors, NK cells, invariant NKT cells, classical CD14+CD16- and non-classical CD14+CD16+ monocytes).
Results: Median recovery of CD34+ HSC and median number of infused CD34+ HSC were 99.3% (range 55.4-100) and $14.7x10^6$/kg (range 7.9-37), respectively. The graft contained a median of $9.9x10^6$ CD3+ T cells/kg (range 3.9-16) and $0.08x10^6$ B cells/kg (range 0.002-0.32). The log-depletion of α/β+ T cells was 4.1 (range 3.33-4.96); the median number of transplanted α/β+ T cells was $42x10^3$/kg (range 3-100.9). Patients received $28.5x10^6$ CD56+ NK cells/kg (range 13.6-192.0) and $9.1x10^6$ γ/δ+ T cells/kg (range 3.7-106.0). The grafts were also enriched in DC1 and DC2 (0.11% of BDCA-1+ cells, range 0.02-0.43; 0.61% of BDCA-3+ cells, range 0.02-1.17); 0.66% of BDCA-2+ cells, range 0.25-1.28, and 0.28% of BDCA-4+ cells, range 0.02-1.16), as well as in non-classical CD14+CD16+ monocytes (10.0%, range 4.7-17.5), which may predict a reduced incidence of GVHD after allogeneic HSCT. All pts rapidly engrafted, with the median time to reach 500 neutrophils and 50,000 platelets per μl of blood being 12 days (range 10-16) and 13 days (range 12-18), respectively. Only 2 pts developed skin grade I/II acute GVHD, while no pt had visceral acute GVHD. With a median follow-up of 6 months (range 2-8), no pt died of transplant-related complications.
Conclusions: The results of immunomagnetic removal of α/β+ T cells and B cells were robust and reproducible. The grafts were enriched in CD34+ HSC, as well as in immune effector cells implicated leukemia and GVHD control, such as γ/δ+ T cells, NK cells, DC1, DC2 and non-classical monocytes.

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Effect of volume reduction of cord blood units before storage on transplantation outcomes: a retrospective analysis of Eurocord-EBMT and Netcord

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Background: Cord blood banking process is subjected to a wide range of procedures variability, despite international standards aimed at standardizing the process. Only single bank analyses of laboratory variables have been reported so far. Eurocord and Netcord have launched a retrospective analysis aimed at assessing whether the major variables associated with the banking process had an impact on transplantation outcome.
Methods: With the aim of analyzing the banking procedures, we selected an homogenous population of patients who 1: received a single CBT with a TNC > 3.0x10e7/kg at freezing, 2: had Acute Leukemia in remission, 3: were transplanted in EBMT Centers after 1997, 4: had availability of clinical follow-up, and 5: bank could be identified. A specific questionnaire was circulated to the banks, aimed to assess the variables associated to banking of the selected cord blood units (CBU), from processing to shipment. In particular, CBU volume reduction (VR) technologies (analyzed as below or above 30mL after processing) and cells viability assessment in thawed sample were investigated. Questionnaires were answered by 38/48 Banks worldwide and 677 patients met the eligibility criteria.
Results: Table 1 reports patient, disease and transplant characteristics. VR was performed in 399 out of 677 patients and its extension (≤/>30 mL) did not influence any clinical endpoints. Therefore, all multivariate analyses were performed including VR (yes/no). Technologies for viability assessment on a thawed sample were different across the banks (32% Trypan-Blue, 33% Acridine Orange, 34% 7-ADD) and were not analyzed for outcomes. Neutrophil recovery was 87% for patients given either a VR-CBU or an unmanipulated CB. NRM at 100 days and at two years were 14% and 29% for patients given a VR CBU, and 19% and 31% for those without VR, respectively (p=ns). VR was not associated with any outcome differences in a multivariate analysis which included CB age, year of CB collection, year of Tx, patient age, diagnosis, status at Tx, Nr of mismatches, cells dose, CMV status.
Conclusions: In this Registry study, manipulation of the CBUs aimed at volume reduction was not shown to influence the clinical outcome, indicating a satisfactory validation of the associated technologies across the banks. Cells viability assessment methodology varied among banks. Further efforts to standardize the quality controls before CBU release are needed.

Table 1. Patients, CBU and transplant characteristics according to volume reduction

		Volume reduction		Total (n)	p
		no	yes		
		median (range)			
Year of transplant		2004 (1997-2010)	2006 (1997-2010)		**<0,0001**
Year of collection		1998 (1993-2007)	2001 (1993-2010)		**<0,0001**
Recipient's age in years		9,4 (0,09-68,2)	6,8 (0,3-64)		**0,004**
TNC x10^7/Kg		5,53 (3,03-26,09)	6,03 (3,02-29,3)		**0,04**
		n(%)			
Age category	children	198 (71%)	300 (75%)	498 (74%)	0,23
	adult	80 (29%)	98 (25%)	178 (26%)	
Diagnosis	ALL	171 (62%)	234 (59%)	405 (60%)	0,46
	AML	107 (38%)	165 (41%)	272 (40%)	
CR at UCBT	no	168 (60%)	205 (51%)	373 (55%)	**0,02**
	yes	110 (40%)	194 (49%)	304 (45%)	
Disease status at Transplant	CR1	110 (40%)	194 (49%)	304 (45%)	
	CR2	137 (49%)	175 (44%)	312 (46%)	
	>=3rd CR	31 (11%)	30 (7%)	61 (9%)	
Conditioning	MAC	215 (80%)	335 (86%)	550 (84%)	**0,03**
	RIC	54 (20%)	53 (14%)	107 (16%)	
HLA matching	6/6 or 5/6	138 (51%)	235 (62%)	373 (57%)	
	4/6 or 3/6	132 (49%)	145 (38%)	277 (43%)	**0,006**
Patient CMV status	pos	136 (50%)	210 (58%)	346 (55%)	
	neg	136 (50%)	153 (42%)	289 (45%)	**0,05**

Abbreviations: CBU means cord blood unit; TNC, total nucleated cell count after processing; ALL, acute lymphoid leukemia; AML, acute myeloid leukemia.