Umbilical cord blood transplantation from unrelated donors in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia

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ONLINE SUPPLEMENTARY METHODS

Patients

From June 1999 to December 2011, 45 consecutive Ph+ ALL patients undergoing myeloablative single-unit UCBT from an unrelated donor at 10 different institutions within the Grupo Español de Trasplante Hematopoyético (GETH) and the Rome Transplant Network belonging to the Gruppo Italiano Trapianto Midollo Osseo (GITMO) were included in the study. From 2006, patients were included in 2 subsequent prospective trials: TSCU-GETH2005 (8 patients) and TSCU-GETH/GITMO2008 (24 patients). All data records were obtained from the GETH/GITMO database, which were completed by each center.

Eligibility criteria

All consecutive patients with Ph+ ALL were considered to require an urgent hematopoietic stem cell transplantation after achieving a CR, and if the probability of having a suitable unrelated donor (HLA identical in HLA-A, -B, -C and DRB1 or one-locus mismatched in some centers) available within 3 months was low, patients were considered candidates for an UCBT. Patients were required to have an ECOG performance status score of 2 or lower, serum bilirubin lower than 2 mg/dL, serum creatinine lower than 1.6 mg/dL and no symptomatic cardiac or pulmonary disease. The institutional review board approved the protocol and written informed consent was obtained from all patients according to the Declaration of Helsinki. TSCU-GETH/GITMO2008 clinical trial was registered on the EudraCT with code 2008-000927-24.

Cord blood unit selection

Graft selection algorithm was reported elsewhere in detail. (11) Briefly, UCB units were required to be ≥ 4/6 HLA matched with the recipient (HLA class I antigens [A and B] considering the antigen level, refer to as low-resolution typing and class II antigen [DRB1] considering allele level resolution DNA typing, refer to as high-resolution typing). Once HLA-matching criteria was met, TNC and CD34+ cell counts from the information provided by the different UCB banks were considered. A total nucleated cell dose (TNC) ≥ 1.5 x 10^7/kg recipient's body weight was required until 2005, while TNC ≥ 2 x 10^7/kg and CD34+ cell dose ≥ 1 x 10^5/kg recipient's body weight were required in 2006 and 2007. For patients included in the GETH/GITMO-2008 protocol, the total cryopreserved cell doses were the primary variables used for selecting the most appropriate UCB unit(s), irrespective of the recipient's body weight; thus, the minimum required total cell counts were TNC > 150 x 10^7 and CD34+ cells > 70 x 10^5.

Conditioning regimen and graft-versus-host-disease prophylaxis

All patients received thiotepa, busulfan, cyclophospamide or fludarabine, and ATG as reported elsewhere. (11) Until March 2005, 13 patients received thiotepa (10 mg/kg), busulfan (12 mg/kg PO or 9.6 mg/kg IV), cyclophosphamide (120 mg/kg) and ATG (Lymphoglobuline®, Merieux, Lyon, France; 60 mg/kg in the first 4 patients or Thymoglobulin®, Genzyme Transplant, Cambridge, MA; 6-8 mg/kg in the remaining patients). From March 2005, the remaining 32 patients received the same preparative regimen but substituting cyclophosphamide by fludarabine (150 mg/m2).

For graft-versus-host disease (GvHD) prophylaxis, all patients received cyclosporine combined with either long course prednisone in the first 25 patients (0.5 mg/kg/d on days +7 to +14, 1 mg/kg/d on days +14 to +28, with slow tapering until discontinuation on day +180) or micofenolate-mofetil (MMF) (15 mg/kg/12h until day +28) in the following 20 patients.

Supportive care and GvHD treatment

Patients were nursed in HEPA-filtered rooms. Prophylaxis of busulfan-induced neurologic toxicity with diphenylhydantoin, clonazepam, or lorazepam was administered according to the current protocols at each site. G-CSF was administered i.v. or subcutaneously at 5 µg/kg/day from day +7 until neutrophil engraftment and stopped when neutrophil count was >1.0 x 10^9/L for 3 consecutive days. Antibacterial and antifungal prophylaxis was given following institutional policies. Recommendations were made to dispense oral ciprofloxacin during neutropenia or until the start of broad-spectrum antibiotics and a mold-active azole (itraconazole or voriconazole) until day +100 or whenever the patient was given steroid for the treatment of GvHD. Prophylaxis against *Pneumocystis jiroveci* comprised cotrimoxazole from day -10 to day -2, which was then restarted after engraftment on 2 days a week and maintained for a minimum of 1 year or until immunosuppressive therapy was stopped. Inhaled pentamidine was used if cotrimoxazole was contraindicated. All blood products were irradiated and leukocyte depleted. CMV-seropositive patients received high-dose acyclovir 500 mg/m^2/8 h i.v. until neutrophil recovery. Thereafter, some institutions followed a preemptive approach with PCR monitoring, whereas others administered valganciclovir prophylactically. Non-specific immunoglobulin was administered i.v. at a dose of 500 mg/kg weekly until day +100 and then monthly during the first year after transplantation.

Patients who developed acute GvHD received first-line therapy with methylprednisolone at doses ranging from 2 to 20 mg/kg/day followed by gradual tapering, according to institutional policy. Chronic GvHD was treated with prednisone 1 mg/kg/d.

Disease evaluation

Pre-transplant disease status was assessed during the 30 days prior to UCBT and included clinical history and physical examination, complete blood count with differential cells count, bone marrow aspiration for morphology, immunophenotyping studies by flow cytometry, conventional cytogenetic and molecular analysis.

For patients in CR, minimal residual disease (MRD) was assessed by qualitative or quantitative polymerase chain reaction (PCR) of p190 BCR/ABL mRNA. In addition, according to each center's standard practice, flow cytometry studies, conventional cytogenetics and/or fluorescence in situ hybridization (FISH) were also used for MRD assessment before UCBT and implemented in the post-transplant disease evaluation. PCR was performed on each participating center using TaqMan technology in accordance with the guidelines approved in the Europe Against Cancer Program. (12) Patients under continuous tyrosine kinase inhibitor therapy stopped at least 7 days before starting the conditioning regimen. No patients received TIT prophylaxis after UCB transplant.

After transplant, disease status was assessed by bone marrow aspiration morphology on day +28, when clinically indicated or when granulocyte recovery occurs. Thereafter, bone marrow samples for qualitative or quantitative PCR were obtained at 3, 6 and 12 months after transplant and then each 3-6 months or when clinically indicated. A tyrosine kinase inhibitor (TKI) was given after engraftment at the physicians´ discretion. However, the generally accepted practice before starting TKI maintenance included the patient's hematopoietic engraftment, their oral tolerability and the potential risks of drug interactions.

Definitions

Complete remission was defined according to standard morphological criteria as outlined by the International Working Group. (13) CR was considered to have been achieved when all the following criteria were fulfilled: less than 5% blasts in bone marrow, no leukemic blasts in the peripheral blood, recovery of peripheral blood neutrophil counts to at least 1.5×109/L and platelet counts to at least 100×10^9/L. A negative molecular status was defined as the absence or less than $1x\ 10^{-4}$ of *BCR-ABL* transcripts copies assessed by qualitative or quantitative PCR, respectively. Myeloid engraftment was defined as an absolute neutrophil count of $0.5\ x\ 10^9$/L for at least 3 consecutive days. Platelet engraftment was defined as a platelet count of $20\ x\ 10^9$/L or higher, without transfusion support, for 7 consecutive days. Patients who survived more than 28 days after transplantation and who failed to achieve myeloid engraftment were considered as graft failures. The time to myeloid or platelet engraftment was defined as the time required to reach the first day of engraftment. Secondary graft failure was defined as the loss of the engraftment. Diagnosis of acute GvHD (aGvHD) was based on the classical clinical presentation with

confirmatory pathological findings in all patients. Acute and chronic GvHD were assessed and graded according to published criteria. (14, 15) Acute renal failure was defined as a decrease of at least 50% of base-line GFR. Hematological relapse was defined based on marrow morphology and/or supported by flow cytometry and cytogenetic studies. Non-relapse mortality (NRM) was defined as death from any cause without evidence of relapse. For event-free survival (EFS), hematological relapse, death and graft failure were considered as treatment failure.

Statistical analysis

The primary endpoint of this study was long-term EFS after UCBT. Secondary endpoints were engraftment, regimen-related toxicity, overall survival (OS), relapse rate, and NRM. The probabilities of engraftment, NRM, GVHD, and relapse were estimated by the cumulative incidence method (marginal probability). (16) For cumulative incidence (CI) analyses of engraftment and relapse, death in CR was considered as a competing cause of failure. Relapse and death were considered competing events for GVHD, whereas relapse was the competing event for NRM. Unadjusted time-to-event analyses were performed using the Kaplan–Meier estimate, (17) and for comparisons, the log-rank tests. (18) All tests of significance were two-sided, with a significance level of $P < 0.05$. The follow-up of the patients was updated on January 15, 2013. Multivariate analyses were not done due to the small sample size. The variables considered for EFS, NRM, OS, GvHD, engraftment and relapse univariate analysis were: age, UCBT protocols, GvHD prophylaxis, conditioning regimens, sex, recipient body weight, recipient CMV serology, disease status at transplantation, MRD at transplantation, prior TKI before transplantation, HLA compatibility considering low-resolution typing for class I and high-resolution typing for class II antigens, ABO blood group mismatch, donor–recipient sex match, and the numbers of TNCs and CD34+ cells/kg at time of freezing and infusion. To analyze the impact of TKI maintenance after transplant, we included into the analysis the patients who were alive and disease-free 6 months after UCBT. Statistical analysis were conducted using R version 2.12.2 (The CRAN project) with packages, survival v2.36-10, Design 2.3-0, prodlim v1.2.1 and cmprsk v2.2-2.(19)

ONLINE SUPPLEMENTARY Table 1.

Table 1. Univariate analysis of patients' and transplant's characteristics for NRM, EFS and OS at median follow-up of 5 years.

Variables	NRM		EFS		OS	
	Cum.Inc. % (95%C.I.)	P value	K-M % (95%C.I.)	P value	K-M. % (95%C.I.)	P value
Recipient age, in years		0.6		1		0.65
• <20 (n= 11)	36 (8-65)		34 (14-80)		44 (22-87)	
• ≥20 (n= 34)	29 (8-45)		36 (22-60)		45 (29-68)	
Conditioning regimen		0.02		0.03		0.02
• Fludarabine-based (n=32)	22 (8-36)		46 (30-70)		54 (37-78)	
• Cyclophosphamide-based (n=13)	54 (27-81)		15 (4-55)		23 (9-62)	
Acute GVHD prophylaxis		0.14		0.79		0.3
• PDN (n= 25)	40 (21-59)		35 (20-60)		39 (24-65)	
• MMF (n= 20)	20 (2-38)		37 (17-77)		53 (32-87)	
Protocols		0.08		0.09		0.07
• 1999-2004 (n=13)	54 (27-81)		15 (4-55)		23 (9-62)	
• TSCU-GETH2005 (n=8)	25 (0-55)		50 (25-100)		50 (25-100)	
• GETH/GITMO2008 (n=24)	21 (5-37)		45 (26-76)		57 (38-85)	
Status Disease		0.18		0.43		0.23
• CR1 (n= 35)	26 (11-40)		37 (22-61)		48 (32-71)	
• Others (n= 10)	50 (19-81)		30 (12-77)		30 (12-77)	
MRD status by PCR techniques		0.15		0.07		0.07
• Positive (n= 22)	43 (22-64)		24 (11-51)		30 (15-58)	
• Negative (n= 23)	22 (5-39)		46 (27-80)		60 (40-88)	
MRD status in patients in CR1		0.49		0.19		0.24
• Positive (n=12)	33 (7-60)		25 (9-67)		34 (15-78)	
• Negative (n= 22)	23 (5-40)		45 (26-78)		59 (39-87)	
TNC infused per recipient body weight		0.71		0.2		0.47
• *<2.5x10^7/ Kg* (n=20)	30 (10-50)		23 (9-58)		40 (22-73)	
• *≥2.5 x10^7/ kg* (n=20)	35 (14-56)		48 (30-77)		51 (32-82)	
CD34+ infused per recipient body weight		0.71		0.58		0.44
• *<1.4x10^5/ Kg*	33 (13-53)		38 (21-68)		50 (32-79)	
• *≥1.4x10^5/ Kg*	37 (15-59)		31 (15-64)		38 (21-71)	
HLA compatibility		0.54		0.65		0.62
• 4/6 (n= 28)	29 (12-45)		42 (26-67)		50 (34-75)	
• *5/6* (n= 15)	33 (9-57)		26 (10-66)		33 (15-78)	
• *6/6* (n= 2)	50 (0-100)		50 (13-100)		50 (13-100)	

Abbreviations: **NRM**, non-relapse mortality: **EFS**, event free survival: **OS**, overall survival: **Cum.Inc**., cumulative incidence: **K-M**, Kaplan-meier test: **GvHD**, graft versus host disease: **TNC**, total nucleated cells: **MRD**, minimal residual disease: : **CR1**, first complete remission.