Feasibility and Outcome of Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant High-Dose Cyclophosphamide for Children and Adolescents with Hematologic Malignancies: An AIEOP-GITMO Retrospective Multicenter Study

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Abstract

Post-transplant high-dose cyclophospamide (PTCy) is a novel approach to prevent graft-versus-host disease (GvHD) and rejection in patients given haploidentical HSCT.

Thirty-three patients with high risk hematological malignances and lacking a match-related or unrelated donor were treated with PTCy haploidentical HSCT in five Italian AIEOP centers. A total of 19 patients had a non-myeloablative preparative regimen (NMA, 57%), 14 patients received a full myeloablative conditioning (MAC, 43%). No patients received serotherapy; GvHD prophylaxis was based on PTCy (50 mg/kg on day +3 and +4) combined with mycophenolate plus tacrolimus or cyclosporin-A.

Neutrophil (ANC) and platelets (PLT) engraftment was achieved on days +17 (14-37) and +27 (16-71). One patient had autologous reconstitution for anti-HLA antibodies. Acute GvHD II-IV, III-IV and chronic GvHD developed in 22% (95% CI, 11-42), 3% (95% CI, 0-21) and 4% (95% CI, 0-27). The 1-year overall survival (OS) rate was 72% (95% CI, 56-88), relapse incidence (RI) was 24% (95% CI, 13-44) and transplant related mortality (TRM) was 9% (95% CI, 3-26). The univariate analysis for RI risk showed how three significant variables, the mother as donor (P=0.02), the female donor gender (P=0.04) and the female patient gender (P=0.02), were significantly associated with a lower risk of relapse. The multivariate analysis for RI showed the mother as the only independent variable. Disease progression was the main cause of death.

The PTCy is a safe procedure also for children and adolescents who have already received several lines of chemotherapy. Among the different diseases a trend for better 1-year OS was obtained for non-acute leukemia patients. In particular, lymphoma patients had excellent 1-year OS (80%) even when transplanted in an advanced disease stage.

Introduction

Allogeneic stem cell transplantation (HSCT) is a key option for a variety of pediatric malignant and non-malignant diseases. When no related or unrelated compatible donor is found, haploidentical hematopoietic stem cell transplantation (haplo-HSCT) represents a valid alternative (1). Haplo-HSCTs were initially performed with conventional post-transplant immunosuppression which often caused a strong bidirectional alloreactivity which in turn caused high primary graft failure, hyperacute graft-versus-host disease (GvHD) and very poor outcomes (2,3). In the 1980s and, then, mostly, in the 1990s, the use of *ex vivo* T-cell depleted grafts were followed by high engraftment probability, very low GvHD occurrence together with high transplant related mortality mainly due to a impaired immune reconstitution (4-7).

Recently, starting from basic research two different approaches have gained scientific relevance: manipulated *ex-vivo* T-cell depleted haploidentical HSCT (8-12) and *in vivo* T-cell replete haploidentical HSCT followed by post-transplant high-dose cyclophosphamide (PTCy) (13-15). Initially, post-transplantation Cy was found to reduce the incidence and severity of GVHD following HSCT in rodent models. When this observation was tested in humans, a randomized clinical trial demonstrated that a lower dose of Cy (7.5 mg/ kg i.v. on days 1, 3, 5, 7, and 9 and then weekly) was inferior to cyclosporine-A in preventing acute GvHD after HLA-matched sibling HSCT (16). Subsequent studies in mouse models demonstrated that tolerance to minor histocompatibility antigens could be induced only when a single dose of ≥150 mg/kg Cy was given between 48 and 72 hours after alloantigen exposure. Moreover, tolerance was not induced if the same dose of Cy was given 24 or 96 hours after transplantation (17-19). From a biological point of view, hematopoietic stem cells (HSC) are relatively quiescent and express high levels of aldehyde dehydrogenase, which likely confer cellular resistance to cyclophosphamide. These findings may explain why high dose Cy is not marrow ablative and provided a rationale for its use. The Seattle and the John Hopkins groups were the first to use the PTCy protocol early after stem cell infusion to control GvHD by eliminating rapidly dividing donor derived T cells generated by the major HLA mismatch graft. This model has been shown to minimize GvHD without interfering with the stem cell pool or the non-dividing, resting T lymphocytes (in part due to a high level of aldeyde dehydrogenase [13, 14]), and more importantly it was tested in a large multicenter trial showing an acceptable incidence of acute and chronic GvHD, very low TRM together with a 1-year high relapse incidence (19). However, most of the current literature on haplo HSCT PTCy studies come from adult patients and there is a very limited experience in the pediatric setting.

The aim of this study was to test PTCy after haploidentical stem cell transplantation in a multicenter pediatric population, which lacks HLA-compatible related or unrelated donors. These pediatric patients are mostly referred to Italian Associazione Italiana di Ematologia e Oncologia pediatrica (AIEOP, the Italian Association of Paediatric Haemato-Oncology) centers by low income countries.

Patient and Methods

From January 2012 to February 2015, a total of thirty-three patients underwent a first haploidentical HSCT in 5 the AIEOP centers (Turin, Genoa, Verona, Pavia and Bologna). The patients' median age was 12 years (1-21), twenty were males (61%) and 13 females. The details of patient-, transplant- and donor characteristics are outlined in Table 1. A total of 5 patients had also experienced a failure of previous allogeneic HSCT (15%). The decision to proceed with haploidentical HSCT was based on the absence of compatible related or unrelated donor and/or urgency of the procedure. A total of 23 patients had haplo-HSCT for acute leukemia (69%) and only 8 had HSCT in first remission acute leukemia (CR1, 24%). A total of 5 patients had haplo-HSCT for lymphoma, none of them had HSCT in complete remission.

The mother was the preferred donor for 18 patients (54%). The bone marrow stem cell source was chosen for 30 patients (91%). The median number of nucleated cells, CD34+ and CD3+ were 4.9×10^8, 5.5×10^6 and 0.7×10^8, respectively. All transplants were performed in air-filtered rooms. Antimicrobial prophylaxis was started during conditioning and consisted of acyclovir 500 mg/m^2 tid from day -5, cotrimoxazole 5 mg/kg over two consecutive days per week until day -2 and after engraftment, antifungal prophylaxis with fluconazole unless contraindicated, otherwise echinocandine was given. Acyclovir was maintained for three months after calcineurin inhibitors stop, while antifungal and cotrimoxazole were stopped when CD4+ lymphocyte counts were above 200/µL. Twice weekly CMV PCR monitoring was started from day +15 until day +100 and weekly until day +180 or when clinically indicated. Weekly EBV and Adenovirus PCR monitoring was started from day +15 to day +100 or when clinically indicated. The aspergillus galactomannan antigen test was performed twice a week. Bacteria and fungal cultures were performed weekly or when clinically indicated. Piperacillin-tazobactam alone or in combination with an aminoglycoside was given as empirical therapy for febrile neutropenia, unless previous colonization for resistant bacteria was documented, appropriate antimicrobial therapy was started. First line pre-emptive CMV reactivation was based on iv gancyclovir, while foscarnet was preferred for low WBC patient counts. EBV reactivation was treated with Rituximab at 375 mg/m^2 for 4 doses at weekly intervals, while polyoma virus or adenovirus-related hemorrhagic cystitis were initially treated by supportive care only. The study was approved by the local Institutional Review Board and all parent or legal guardians signed the appropriate consent form.

Conditioning regimen and GvHD prophylaxis

Nineteen patients received a nonmyeloablative HSCT (57%, NMA) according to Luznik et al. (15), while the remaining patients received a myeloablative HSCT (43%, MAC) based on Busulfan or total body irradiation (TBI). The NMA protocol consisted of Fludarabine 150 mg/m^2 over 5 days together with Cyclophosphamide at 29 mg/kg over 2 days and a single TBI dose at 200 cGy on day -1. For 10 patients the MAC regimen was Busulfan-based with Thiotepa and Fludarabine, two patients received TBI (1200 cGy) together with Fludarabine, one patient had Treosulfan combined with Thiotepa and Fludarabine and finally one patient received Thiotepa together with Fludarabine and Etoposide.

For all patients, the GvHD prophylaxis was 50 mg/kg cyclophospahamide on days +3 and +4 and Mofetil Mycofenolate 15 mg/Kg tid from day +5 to day 30. A total of 20 patients also had Tacrolimus 0.01-0.03 mg/kg from day +5 to day +180, while the others received Cyclosporin-A (1-3 mg/kg) from day +5 to +180. One patient had no calcineurin inhibitors but steroids until day +30. G-CSF was started from day +5 until neutrophil counts were above $0.5 \times 10^3/\mu L$. Tacrolimus or Cyclosporin-A blood level concentration were monitored two-three times per week.

Human leucocyte antigen typing and compatibility

All HLA typings were performed in EFI accredited laboratories. All patients and donors were typed by high resolution molecular standard technique at HLA-A, HLA-B, HLA-C and HLA-DRB1 loci. A total of 25 patients were fully haploidentical with their donors, 8 patients had HSCTs from a non-full haploidentical donor. HLA phenotyping was performed by the same methods and similar protocols. Briefly, typing prior to PTCy haploidentical HSCT was performed using 2 different molecular test methods. Samples were PCR amplified with HLA locus-specific primers. Amplified samples were hybridized to panels of sequence specific oligonucleotide probes (SSOP) bound to plastic microspheres with a single tube hybridization for each HLA locus for each sample. The positive and negative probe reactions were captured by a Luminex Flow Analyzer and submitted to an HLA analysis program (One Lambda LabType) loaded with a library of the expected hybridization patterns of the known HLA alleles. High-resolution HLA typing to confirm allele level matching was performed by direct DNA sequencing of HLA locus-specific PCR amplified DNA, using dye terminator chemistry, with analysis on an ABI 3130xl Genetic Analyzer and Assign (Conexio Genomics, Australia) HLA sequencing analysis software.

Donors were initially chosen according to their medical history, NK alloreactivity (20) and the absence of antibodies directed against donor HLA.

Immunophenotypic analysis after engraftment

Analyzes of circulating lymphocytes were performed at regular intervals whenever possible. The time-points were day +60, day+120, day +180 and day +365. The following surface markers were analyzed: CD3+, CD3+CD4+, CD3+CD8+, CD3-CD56+, CD19+ in order to quantify T, NK and B cell reconstitution.

Definition and statistical analysis

Data were analyzed as May 31st 2015. Neutrophil engraftment was defined as the first of three consecutive days with ANC $>0.5 \times 10^3/\mu L$. PLT engraftment was defined as the first of three consecutive days of unsupported platelet counts over $50 \times 10^3/\mu L$. Acute GvHD was defined according to Seattle criteria (21), while for chronic GvHD the NIH criteria were preferred (22). OS was defined as the probability of survival irrespective of disease state, TRM was defined as the probability of dying with no previous occurrence of a relapse, which was its competing event. Relapse was defined as the probability of having had a relapse

before the last follow-up or death, whichever occurred first. Death without experiencing a relapse was its competing event.

The primary endpoint of the study was OS. The secondary endpoints were ANC and PLT engraftment, acute and chronic GvHD incidence, relapse incidence, transplant related mortality incidence and viral reactivation. The following variables were considered: diagnosis (acute leukemia vs. other diagnoses); disease status at HSCT (CR1-CR2 vs. others); patient gender (female vs. male); donor (mother vs. others); donor gender (female vs. male); stem cell source (BM vs. PBSC); conditioning regimen (NMA vs. MAC), total nucleated cells (≥median vs. <median); CD34+ cells (≥median vs. <median); CD3+ cells (≥median vs. <median); HLA (full haplo vs other); and finally according to NK alloreactivity evaluated according to allele discriminants and not on genotypic functional analysis.

Patient-, disease- and transplantation related variables are expressed as medians and ranges, or as percentages, as appropriate. The Kaplan Meier statistics were used to calculate OS (23), while differences among the curves were calculated by the log-rank test, the cumulative incidences of RI, TRM and GvHD were calculated by the competing risk analysis, the P values were calculated by the Gray test (24, 25). The Cox regression model was used for calculating multivariate analysis of OS curves (26). The multivariate analysis for competing risk was carried out with the application of the proportional hazard model for the sub-distribution of competing risks, we used the "R" software. Differences were considered statistically significant if P<0.05.

Results

The median follow-up was 383 days (61-1203). The median follow up for alive patients was 540 days (56-1212) and 100 days for deceased patients (49-395). For the entire patient population the 1-year OS was 72% (95% CI 56-88), the cumulative incidence of TRM was 9% (95% CI 3-26) and the RI was 24% (95% CI 13-44).

Engraftment

All patients except one, who had an autologous recovery, engrafted. The ANC and PLT engraftments were achieved on days +17 (14-37) and +27 (16-71). ANC engraftment was achieved on days +18 (14-37) and +17 (14-23 , P=NS) for NMA and MAC patients, while PLT engraftment was achieved on days +23 (116-69) and +35 (21-71, P=0.02) for NMA and MAC patients.

GVHD

The cumulative incidence of acute GvHD II-IV, acute GvHD III-IV and chronic GvHD were 22% (95% CI 11-42), 3% (0-21) and 4% (95% CI 0-27), respectively. One patient had an autologous reconstitution given by anti-donor HLA antibodies. He was treated with steroids, three Rituximab doses and two plasma-

exchange procedures and then successfully re-transplanted with his mother's marrow stem cells. Before the second haplo-HSCT the screening for anti-donor HLA antibodies was negative. The acute GvHD II-IV cumulative incidence was 17% (95% CI, 6-47) and 27% (95% CI, 11-62, P=NS) for NMA and MAC, acute GvHD III-IV CI was 0% and 7% (95% CI, 1-44, P=NS) for NMA and MAC, and chronic GvHD CI was 0% and 7% (95% CI, 1-47, P=NS) for NMA and MAC, respectively.

Immune reconstitution and chimerism

On day +60 the median numbers of CD4+/µL, CD8+/ µL, CD19+/ µL and CD3-CD56+/ µL were 132 (32-1159), 491 (16-4909), 46 (2-90) and 154 (12-704). Details of lymphocyte counts following NMA and MAC are outlined in Table 2. In brief, we had an higher statistically significant CD19+ and NK counts on day +60: 174/uL (10-717) vs. 17 (0-322, P=0.03) for NMA and MAC recipients and on day +120 NK cells 171/uL (42-315) vs. 70/uL (11-104, P=0.04) for NMA and MAC, respectively.

Full donor chimerism was observed on day +60 for all patients except for one who had AML relapse and for another patient having anti-HLA-antibody mediated autologous reconstitution. He was then successfully re-transplanted with his mother's marrow cells.

Viral reactivation

None of the patients developed CMV-, EBV- or Adenovirus-related diseases. The cumulative incidences of CMV, EBV and Adenovirus reactivation were 36% (95% CI, 22-49), 3% (95% CI 0-22) and 3% (95% CI, 0-22). When we analyzed the NMA and MAC recipients the cumulative incidences of CMV, EBV and Adenovirus reactivation were: 28% (95% CI, 13-58) vs. 8% (95% CI, 1-54, P=NS), 0% vs. 8% (95% CI, 1-54, P=NS) and 5% (95% CI, 1-37) vs. 8% (95% CI, 1-54, P=NS).

Overall survival

The 1-year OS was 72% (95% CI 56-88). As shown in Table 3, in this cohort, no variable reached statistical significance. A trend in favour of better OS was observed in patients who received the graft from their mothers, compared to other relatives, 82% (95% CI, 63-100) vs. 49% (95% CI, 19-79, P=0.09). Among the various diseases, lymphoma patients experienced an excellent 1-year OS (80%), also considering that none of them were in CR at HSCT.

Relapse Incidence

The 1-year RI was 24% (95% CI, 13-44). The mother-donor, the female-donor gender and the female patient gender were statistically associated with a reduced risk of relapse: 0% vs. 53% (95% CI, 33-86, P=0.02), 10% (95% CI, 3-37) vs. 46% (95% CI, 25-83, P=0.04) and 7% (95% CI, 1-50) vs. 35% (95% CI, 19-63, P=0.02). When we carried out the multivariate analysis the only independent variable was the mother-donor (RR 26.1 [95% CI, 6.5-104.9]). To exclude a possible unbalanced distribution of high-risk disease or status at transplantation we first analyzed the mother donor-group with the non mother-group for acute leukemia

patients only. Although a higher number of acute leukemia patients were distributed in the non-mother group, no statistical difference emerged. We then analyzed the acute leukemia status at HSCT considering early (CR1-CR2) vs. advanced disease (>CR3). Once again, despite a higher number of acute leukemia patients in the non mother-HSCT group, no statistical difference was observed. When we focused on RI only in the mother-group (18 patients), we found no differences in the 1-year RI in either the female or the male gender recipients (P=0.18).

Transplant related mortality

The 1-year TRM was 9% (95% CI 3-26). In Table 3 we report that no factor was statistically associated with lower TRM.

Cause of death

At the last follow-up, nine patients were dead: 5 patients died for disease progression (55%), one patient for idiopathic interstitial pneumonia (11%), one patient for septic shock (11%), one patient for cerebral hemorrhagic stroke (11%), one patient for infection complications following a second umbilical cord blood HSCT for leukemia relapse (11%). None of the patients died of acute or chronic GvHD.

Discussion

This paper reports our experience in the use of high-dose, post-transplantation cyclophosphamide for the prevention of graft rejection and GVHD after HLA-haploidentical bone marrow or peripheral blood stem cell transplantation.

Post-transplantation immunosuppression with high-dose Cy, tacrolimus/cyclosporin-A, and thrice daily MMF was associated with an acceptably low incidence of graft rejection, severe acute GVHD, and chronic GVHD, while allowing prompt engraftment. This is an important point also considering the different intensities of conditioning our patients received (MA and NMA). If the MA conditioning is considered the standard of care for the treatment of relapsed leukemia, then this study shows how a considerable number of patients can be cured with a NMA regimen even when an intensive conditioning regimen can not be offered.

With the exception of the single case of immunological rejection related to the presence of anti-HLA antibodies, all patients engrafted even after receiving standard doses of nucleated/CD34+ cells. This appears to be a clear advantage over the CD34 megadose required for the engraftment over manipulated CD34+ haplo-HSCT. If the engraftment and GvHD do not pose problems, even in the NMA settings, the role of transplanted and cyclophospamide-spared lymphocytes cannot be excluded unlike CD34+ selected HSCT.

In addition to the control of HLA-haploidentical alloreactivity, there was a suggestion of effective clinical immune reconstitution as demonstrated by the low incidence of viral reactivation and more importantly the

absence of CMV-, EBV- or Adenovirus-related diseases, irrespective of the conditioning regimen intensity. Immune reconstitution was relatively fast in our series: the median day CD4+ count on days +60 and +120 were 132/µL and 206/µL from haplo-HSCTs, respectively. Comparable values were found in recipients from sibling-HSCTs (27). As a direct consequence of this immune reconstitution, our patients did not experience CMV disease or fungal infections. There was no EBV-related post-transplant lymphoproliferative disorders and only one patient required Rituximab therapy for EBV increased viremia. Furthermore, hemorrhagic cystitis was only found in a minority of patients (17%) even considering GvHD prophylaxis with high-dose cyclophosphamide and the preparative regimen consisting of the administration of cyclophosphamide or MAC regimens. Notably the hemorrhagic cystitis developed irrespective of MAC or NMA administrated.

In our population of patients with high risk hematological malignancies relapse was the major cause of treatment failure (24%). While acute leukemia patients experienced a higher incidence of relapse, it has to be highlighted that 5 out of 22 (23%) patients had a previous allogeneic HSCT failure. Of these, two patients relapsed after PTCy haplo-HSCT, one died of pneumonia, while for two patients continue to be alive and leukemia-free at 24 and 7 months year after haploidentical-HSCT.

Patients transplanted from their mother's had a reduced risk of relapse together with a trend of better 1-year OS. Although, these patient-groups did not have a favorable risk profile regarding both the disease type and their status at HSCT, the multivariate analysis showed the mother as the only independent prognostic factor. HSCT from the mother provided better tumor control, suggesting that maternal grafts exerted a potent alloreactive effect that was preferentially active against the tumor-target, as it was not associated with a higher risk of GvHD. Because the tumor-control was independent from patient gender (as we observed when we only analyzed the mother donor-group), we can then argue that alloreactivity was not directed toward H-Y-mH antigens, but directed against the tumor target(s) (28). In brief, the mother's immune system, sensitized during the pregnancy by the father's haplotype, is able to build an anti-tumor reaction. Spared T lymphocytes is probably the crucial factor able to enhance this graft versus leukemia (GvL) effect after maternal haplo-HSCT. The beneficial effect seems to be related with the pregnancy, as no similar effect emerged in non-mother female donor to any recipients. Our data do not agree with Bashey and Solomon's suggestion that donor selection rule out the mother for male patients (29).

Particular benefit has been shown for patients with advanced lymphoma transplanted with active disease. In our case series, we had 5 lymphoma cases, none of which were in CR. Their 1-y OS was 80% and the only failure was for a relapse 717 days after PTCy, confirming previous data of the Baltimore and Genoa groups (19,30, 31).

While some studies have demonstrated a beneficial effect of NK cell alloreactivity on HSCT outcomes (32, 33), others have shown inferior rates of relapse and GvHD (34), and still others have reported that NK cell alloreactivity has no effect on HSCT outcomes (35). Our data report no significant advantage of KIR

alloreactivity on OS, however it has to be stated that our cohort consists of mixed hematological diseases. Reasons for these conflicting results include the heterogeneity in HSCT protocols employed, namely differences in inclusion criteria, the HSCT preparative regimen and graft content, HLA-incompatibility, and also the immunosuppressant given.

In conclusion, we observed that PTCy is a safe GvHD-prophylaxis which does not lose the GvL effect. In contrast to previous data (29), the mother-donor should be considered as the first option since the probability of relapse is dramatically lower compared to other relatives. Therefore, as our study clearly shows, engraftment issues and the achievement of full-donor chimerism are not real problems, even when an NMA regimen is chosen.

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Patients		N=33 (100%)
Gender	Male	20 (61%)
	Female	13 (39%)
Disease	ALL	15 (45%)
	AML	7 (21%)
	Dendritic Cell Leukemia	1 (3%)
	MDS	4 (12%)
	CML	1 (3%)
	Lymphoma	5 (15%)
Disease status	CR1	8 (24%)
	CR2	10 (30%)
	CR3	5 (15%)
	Other	10 (30%)
Donor relation	Mother	18 (54%)
	Father	10 (30%)
	Brother	3 (9%)
	Sister	2 (6%)
Sex mismatch	Female donor/Male recipient	13 (39%)
	Male donor/Female recipient	6 (18%)
	Male donor/Male recipient	7 (21%)
	Female donor/Female recipient	7 (21%)
Full haploidentical donor	Yes	25 (75%)
	No	8 (24%)
NK alloreactivity	Yes	17 (51%)
	No	16 (49%)
Conditioning regimen	RIC	19 (57%)
	MAC	14 (42%)
GVHD prophylaxis	Luznik-like	20 (61%)
	Others	13 (39%)
BM Stem Cell Source n=30	TNC	4,6 (1,9-11,1)
	CD34+	5,3 (1-12,9)
	CD3+	0,7 (0,4-1,2)
PBSC Stem Cell Source n=3	TNC	8,4 (8,3-11,1)
	CD34+	13,2 (6,8-18,7)
	CD3+	2,3 (1,5-2,8)

Table 1. Clinical data of patients receiving PTCy post-haploidentical HSCT. ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, CML: chronic myelogenous

leukemia, CR: complete remission, NK: natural killer cells, BM: bone marrow, PBSC: peripheral blood stem cells.

		NMA conditioning	MAC conditioning	P
CD4+/µL	Day +60	114 (62-212)	162 (95-792)	NS
	Day +120	213 (96-303)	168 (69-234)	NS
	Day +180	284 (128-458)	542 (531-553)	NS
	Day +365	628 (425-1062)	na	na
CD8+/ µL	Day +60	446 (116-767)	1425 (16-2392)	NS
	Day +120	593 (241-694)	208 (30-1596)	NS
	Day +180	694 (210-862)	1093 (882-1304)	NS
	Day +365	896 (613-1049)	na	na
CD19+/ µL	Day +60	174 (10-717)	17 (0-322)	0.03
	Day +120	274 (108-614)	123 (0-391)	NS
	Day +180	352 (153-494)	178 (130-244)	na
	Day +365	517 (355-1268)	na	na
CD3-56+/ µL	Day +60	173 (88-415)	150 (19-506)	NS
	Day +120	171 (42-315)	70 (11-104)	0.04
	Day +180	186 (116-263)	na	na
	Day +365	177 (57-496)	na	na

Table 2. Lymphocyte count following PTCy haplo-HSCT

		OS (95% CI)	P	RI (95% CI)	P	TRM (95% CI)	P
Disease	Acute leukemia	58% (35-81)	NS	26% (13-41)	NS	13% (4-37)	NS
	Other	90% (71-100)		10% (1-64)		0%	
Patient gender	Female	82% (59-100)	NS	7% (1-50)	0.02	8% (1-50)	NS
	Male	66% (44-88)		35% (19-63)		10% (2-37)	
	Other	90% (71-100)		10% (1-64)		0%	
Disease status	CR1/CR2	65% (39-90)	NS	22% (9-52)	NS	11% (3-41)	NS
	Other	71% (47-91)		26% (11-61)		7% (1-44)	
Donor	Mother	82% (63-100)	NS	0%	0.02	17% (6-46)	NS
	Other	49% (19-79)		53% (33-86)		0%	
Donor gender	Female	78% (59-97)	NS	10% (3-37)	0.04	15% (5-42)	NS
	Male	51% (19-82)		46% (25-83)		0%	
HLA	Full haplo	62% (39-85)	NS	23% (10-49)	NS	14% (5-39)	NS
	Other	71% (38-100)		37% (15-92)		0%	
KIR alloreactivity	Yes	78% (56-100)	NS	14% (4-51)	NS	7% (1-47)	NS
	No	54% (25-82)		37% (20-70)		12% (3-46)	
Conditioning	NMA	81% (61-100)	NS	22% (9-53)	NS	5% (8-37)	NS
	MAC	61% (34-88)		27% (11-61)		13% (4-48)	
Stem Cell Source	BM	74% (57-91)	NS	23% (12-47)	NS	10% (3-29)	NS
	PBSC	50% (0-100)		33% (7-100)		0%	
TNC	>median	59% (30-88)	NS	37% (20-70)	NS	6% (1-43)	NS
	<median	74% (56-93)		12% (3-43)		12% (3-43)	
CD34+	>median	72% (45-100)	NS	40% (21-74)	NS	0%	NS
	<median	66% (42-91)		12% (3-43)		17% (6-49)	
CD3+	>median	66% (39-93)	NS	28% (12-65)	NS	7% (1-47)	NS
	<median	69% (44-94)		40% (14-100)		0%	

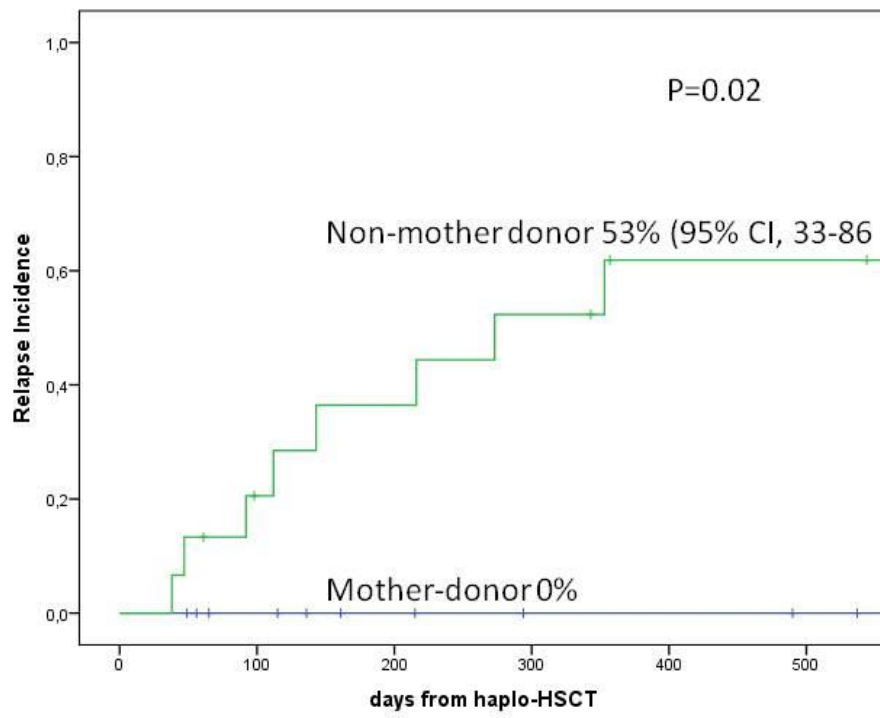Table 3. Univariate analysis of OS, RI and TRM following T cell replete HSCT.

Fig. 1. 1-year relapse incidence for patients given PTCy haplo-HSCT