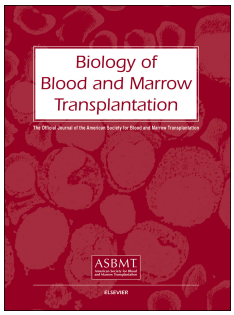
Accepted Manuscript



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

Massimo Berger, MD PhD, Edoardo Lanino, MD, Simone Cesaro, MD, Marco Zecca, MD, MD, Elena Vassallo, MD, Maura Faraci, MD, Massimiliano De Bortoli, MD, Veronica Barat, MD, Arcangelo Prete, MD, Franca Fagioli, MD

PII: S1083-8791(16)00096-3

DOI: 10.1016/j.bbmt.2016.02.002

Reference: YBBMT 54194

To appear in: *Biology of Blood and Marrow Transplantation*

Received Date: 8 October 2015

Accepted Date: 1 February 2016

Please cite this article as: Berger M, Lanino E, Cesaro S, Zecca M, Vassallo E, Faraci M, De Bortoli M, Barat V, Prete A, Fagioli F, Feasibility and Outcome of Haploidentical Hematopoietic Stem Cell Transplantation with Post-Transplant High-Dose Cyclophosphamide for Children and Adolescents with Hematological Malignancies: an AIEOP-GITMO Retrospective Multicenter Study, *Biology of Blood and Marrow Transplantation* (2016), doi: 10.1016/j.bbmt.2016.02.002.

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

Massimo Berger MD PhD [1], Edoardo Lanino MD [2], Simone Cesaro MD [3], Marco Zecca MD [4] MD, Elena Vassallo MD [1], Maura Faraci MD [2], Massimiliano De Bortoli MD [3], Veronica Barat MD [1], Arcangelo Prete MD [5] and Franca Fagioli MD [1]

[1] Pediatric Onco-Hematology and Stem Cell Transplant Division, City of Health and Science, Regina Margherita Children's Hospital, Turin, Italy

[2] Department of Pediatric Hematology-Oncology, IRCCS G. Gaslini, Genoa, Italy

[3] Pediatric Hematology-Oncology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy

[4] Pediatric Hematology-Oncology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy

[5] Pediatric Oncology and Hematology Unit "Lalla Seràgnoli", Department of Pediatrics, University of Bologna Sant'Orsola-Malpighi Hospital, 40138 Bologna, Italy

Correspondence to Massimo Berger, MD PhD

Pediatric Onco-Hematology and Stem Cell Transplant Division, City of Health and Science, Regina Margherita Children's Hospital, Turin, Italy

Tel +390113135449, Fax +300113135487, Email; massimo.berger@unito.it

Abstract word count: 251

Text word count: 3576

Tables: 3, Figures: 3

Running title: Post-transplant cyclophosphamide for Haploidentical Hematopoietic Stem Cell Transplantation

Key words: PTCy-haplo HSCT, Pediatric, Hematological Malignancies

Abstract

Post-transplant high-dose cyclophosphamide (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 malignancies 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 (MA, 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), progression free survival (PFS) rate was 61% (95% CI, 43-80), CI of relapse 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. Disease progression was the main cause of death.

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.

Introduction

Haploidentical Hematopoietic Stem Cell Transplantation (Haplo-HSCT) was 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 (1-3). 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 showed 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. 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 (13, 14, 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 were mostly referred to the Italian Associazione Italiana di Ematologia e Oncologia pediatrica (AIEOP, the Italian Association of Pediatric Haemato-Oncology) centers by low-income countries.

Patient and Methods

From January 2012 to February 2015, a total of thirty-three patients underwent a haploidentical transplant in five 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 a compatible related

or unrelated donor and/or the 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.

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 then monitored 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. Bacterial 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.

The non-myeloablative (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 total body irradiation dose at 200 cGy on day -1. For 10 patients the MA 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. The choice of a myeloablative or a NMA conditioning was based on the patient performance status together with the center's policy.

For all patients, the GvHD prophylaxis was 50 mg/kg cyclophosphamide on days +3 and +4 and Mofetil Mycofenolate 15 mg/Kg tid from day +5 to day +35. A total of 20 patients received 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 0.5x10^3/µL for three consecutive days. The Tacrolimus or Cyclosporin-A blood level concentrations 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 and NK alloreactivity (20), thereafter according to 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 of May 31st 2015. Neutrophil engraftment was defined as the first of three consecutive days with ANC >0.5x10^3/µL. PLT engraftment was defined as the first of three consecutive days of unsupported platelet counts over 50x10^3/µL. 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, Progression Free Survival (PFS) was defined as the probability of survival without disease recurrence, transplant related mortality (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, PFS, cumulative incidence of relapse (RI), TRM 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. MA), 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. Kaplan Meier statistics were used to calculate OS (23), while differences among the curves were calculated by the log-rank test, the cumulative incidences (CI) of RI, TRM and GvHD were calculated by the competing risk analysis, the P values were calculated by the Gray test (24, 25). Differences were considered statistically significant if $P<0.05$.

Results

The median follow-up was 383 days (61-1203). The median follow up of alive patients was 540 days (56-1212) and 100 days of deceased patients (49-395). The median number of TNC, CD34+ and CD3+ transplanted cells in the NMA and MA were: 7 $x10^6$ (2.3-11.1) vs. 3.7$x10^6$ (1.9-6.8, P<0.00), 9.3$x10^6$ (1.5-18.7) vs. 3.9 $x10^6$ (1-14.8, P=0.004), 0.8$x10^8$ (0.4-2.3) vs. 0.5 $x10^8$ (0.1-0.9, P=0.02). No significant differences in terms of acute leukemia patients or disease status at HSCT were found among patients who underwent NMA or MA regimens.

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 MA patients, while PLT engraftment was achieved on days +23 (116-69) and +35 (21-71, P=0.02) for NMA and MA patients.

GVHD

The CI 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 following graft rejection mediated by anti-father HLA antibodies. This patient 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. Acute GvHD II-IV CI was 17% (95% CI, 6-47) and 27% (95% CI, 11-62, P=NS) for NMA and MA, acute GvHD III-IV CI was 0% and 7% (95% CI, 1-44, P=NS) for NMA and MA, and chronic GvHD CI was 0% and 7% (95% CI, 1-47, P=NS) for NMA and MA, 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 MA

are outlined in Figure 1 (day +365 values were omitted due to the low number of data at this time-point). In brief, we had higher statistically significant CD19+ and NK counts on day +60: 174/µL (10-717) vs. 17/µL (0-322, P=0.03) for NMA and MA recipients and on day +120 NK cells 171/µL (42-315) vs. 70/µL (11-104, P=0.04) for NMA and MA, respectively.

Full donor chimerism was observed on day +60 for all patients except for one who experienced early AML relapse and for another patient who had anti-HLA-antibody mediated graft rejection.

Viral reactivation

None of the patients developed CMV-, EBV- or adenovirus-related diseases. The CI of CMV, EBV and adenovirus reactivation were 36% (95% CI, 22-49), 3% (95% CI 0-22) and 3% (95% CI, 0-22), without any significant differences between NMA and MA recipients.

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 MA regimens. Notably, hemorrhagic cystitis developed irrespective of MA or NMA administered.

Overall survival

The 1-year OS was 72% (95% CI 56-88) (Figure 2). As shown in Table 2, in this cohort, no variable reached statistical significance. A trend in favor 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 different diagnosis, lymphoma patients (n=5) had excellent survival 80% (95% CI, 60-99).

Progression Free survival

The 1-year PFS was 61% (95% CI, 43-80) (Figure 2). A trend in favor of better PFS was observed in female patients, 82% (95% CI, 59-100) vs. 53% (95% CI, 24-72, P=0.03). A trend for a better PFS was observed in the mother donor group compared to the non-mother donor group (82% [95% CI 63-100] vs. 45% [21-65], P=NS).

Relapse Incidence

The 1-year CI of relapse 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 [Figure 3]), 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). To exclude a possible unbalanced distribution of high-risk disease or status at transplantation we first compared 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 differences emerged. We then analyzed the acute leukemia status at HSCT considering early

(CR1) vs. advanced disease. Despite a higher number of acute leukemia patients in the non mother-HSCT group, no statistical difference was observed (Table 3). When we focused on RI only in the mother-group (18 patients), we found no differences in the 1-year RI risk 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 2 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 infections complicating a second umbilical cord blood HSCT performed after leukemia relapse (11%). None of the patients died of acute or chronic GvHD.

Discussion

Haploidentical stem cell transplantation has gained great interest over the last two decades and in 2015 despite numerous publications, only few included a pediatric patient population (26-29) with a different platform of haplo-HSCT. However, these studies have proved that this type of HSCT can be offered to a large proportion of pediatric patients irrespective of the underlying disease.

This paper reports our experience with the use of high-dose PTCy for the prevention of graft rejection and GVHD after haploidentical bone marrow or peripheral blood stem cell transplantation in childhood hematologic malignancies.

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 MA conditioning is considered the standard of care for the treatment of relapsed leukemia, then this study shows how some patients can be successfully treated with an NMA regimen even when an intensive conditioning regimen cannot 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 pose no problems, even in NMA settings, then 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+ counts on days +60 and +120 were 132/µL and 206/µL from haplo-HSCTs, respectively. Comparable values were found in recipients from sibling-HSCTs (30). As a direct consequence of this immune reconstitution, our patients did not experience CMV disease or fungal infections. There were no EBV-related post-transplant lymphoproliferative disorders and only one patient required Rituximab therapy for EBV increased viremia.

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 two patients continue to be alive and leukemia-free at 24 and 7 months after haploidentical-HSCT.

Patients transplanted from their mothers had a reduced risk of relapse together with a trend of better 1-year OS. These patient groups did not have a favorable risk profile regarding both the disease type and phase at HSCT. 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 observed when analyzing the mother donor-group separately), we can argue that alloreactivity was not directed toward H-Y-mH antigens, but directed against the tumor target(s) (31). 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 are probably crucial 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 the non-mother female donor to any recipients. Our data do not agree with Bashey and Solomon's suggestion that donor selection rules out the mother for male patients (32,33). However these data have to be taken very cautiously because they were only from 18 transplants, and they have to be confirmed in a well-designed study with a higher sample size.

Particular benefit has been shown for patients with advanced lymphoma transplanted with active disease. In our small case series, five patients suffered from lymphoma (three Hodgkin and two non-Hodgkin lymphoma), none of which in CR as demonstrated by positive PET scans at the time of transplant. Their 1-y OS was 80%, and the only failure was for a relapse 717 days after PTCy, confirming the Baltimore and Genoa groups data (19,34).

While some studies have demonstrated a beneficial effect of NK cell alloreactivity on HSCT outcomes (35,36), some have shown inferior rates of relapse and GvHD (37), and others have reported that NK cell alloreactivity has no effect on HSCT outcomes (38). While our data report no significant advantage of KIR alloreactivity on OS, it has to be stated that our cohort is limited and 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 (32,33), our data suggest that the mother 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.

The study is limited by the relatively small number of children and the short follow-up, and needs to confirmed in a large cohort of patients.

References

1) Thomas' Hematopoietic Cell Transplantation: Stem Cell Transplantation, Fourth Edition. Editor(s): Frederick R. Appelbaum, Stephen J. Forman, Robert S. Negrin, Karl G. Blume. Published Online: 5 MAR 2009. Print ISBN: 9781405153485 Online ISBN: 9781444303537 DOI: 10.1002/9781444303537

2) Kekre N, Antin JH. Hematopoietic stem cell transplantation donor sources in the 21st century: choosing the ideal donor when a perfect match does not exist. Blood. 2014 Jul 17;124(3):334-43. doi: 10.1182/blood-2014-02-514760. Epub 2014 Jun 9.Review. Erratum in: Blood. 2015 Feb 5;125(6):1048

3) Falk PM, Herzog P, Lubens R et al. Bone marrow transplantation between a histocompatible parent and child for acute leukemia. Transplantation 1978; 25:88-90

4) Dupont B, O'Reilly RJ, Pollack MS et al. Use of HLA genotypically different donors in bone marrow transplantation. Transplant Proc 1979; 11:219-24

5) O'Reilly RJ, Kernan NA, Cunningham I et al. Allogeneic transplants depleted of T cells by soybean lectin applutination and E rosette depletion. Bone Marrow Transplant 1988; 3:3-6

6) Aversa F, Tabilio A, Terenzi A et al. Successful engraftment of T-cell-depleted haploidentical “three loci” incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 1994; 84: 3948: 55

7) Aversa F, Terenzi A, Carotti A, Felicini R, et al. Improved outcome with T-cell depleted bone marrow transplantation for acute leukemia. J Clin Oncol 1999; 17:1545-50

8) Aversa F, Tabilio A, Velardi A, Cunningham I, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. NEJM 1998; 339: 1186-1193

9) Locatelli F, Bauquet A, Palumbo G, Moretta F, Bertaina A. Negative depletion of α/β T cells and CD19+ B lymphocytes: a novel frontier to optimize the effect of innate immunity in HLA-mismatched hematopoietic stem cell transplantation. Immunol Lett 2013; 155: 21-3

10) Bertaina A, Merli P, Rutella S et al. HLA-haploidentical stem cell transplantation after removal of α/β+ T and B cells in children with nonmalignant disorders. Blood 2014; 124:822-6

11) Reisner Y, Hagin D, Martelli MF. Haploidentical hematopoietic transplantation: current status and future perspectives. Blood 2011; 118: 6006-6017

12) Bader P, Soeresen J, Jarisch A and al. Rapid immune recovery and low TRM in haploidentical stem cell transplantation in children and adolescents using CD3/CD19-depleted stem cells. Best Pract Res clin Haematol 2011; 24: 331-7

13) Owens AH Jr, Santos GW. The effect of cytotoxic drugs on graft-versus-host disease in mice. Transplantation 1971; 11: 378-382

14) Jones RJ, Barber JP, Vala MS et al. Assessment of aldhehyde dehydrogenase in viable cells. Blood 1995; 85. 2742-2746

15) Luznik L, O'Donnell P, Symons H, et al. HLA-haploidentical bone marrow transplantation for hematological malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant 2008: 14: 641-650

16) Santos GW, Tutschka PJ, Brookmeyer R, et al. Cyclosporine plus methylprednisolone versus cyclophosphamide plus methylprednisolone as prophylaxis for graft-versus-host disease: a randomized, double-blind study in patients undergoing allogeneic marrow transplantation. Clin Transplant. 1987;1:21–28

17) Mayumi H, Umesue M, Nomoto K. Cyclophosphamide-induced immunological tolerance: an overview. Immunobiology. 1996;195:129–39

18) Nomoto K, Eto M, Yanaga K, Nishimura Y, Maeda T. Interference with cyclophosphamide-induced skin allograft tolerance by cyclosporin A. J Immunol. 1992;149:2668–74

19) Luznik L, O'Donnell PV, Ephraim JF. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical BMT. Semin Oncol 2012; 39: 6-19

20) Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002; 295: 2097-2100

21) Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-vs-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation 1974; 18: 295-304

22) Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11:945-56

23) Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. J Am Stat Assoc 1958; 53: 457-481

24) Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risk: new representation of old estimators. Stat Med 1999; 18:695-706

25) Gray R. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Statist 1988; 16:1141-1154

26) Im HJ, Koh KN, Seo JJ. Haploidentical hematopoietic stem cell transplantation in children and adolescents with acquired severe aplastic anemia. Korean J Pediatr. 2015 Jun;58(6):199-205. doi: 10.3345/kjp.2015.58.6.199. Epub 2015 Jun 22. Review. PubMed PMID: 26213547; PubMed Central PMCID: PMC4510352

27) Balashov D, Shcherbina A, Maschan M, et al. Single-Center Experience of Unrelated and Haploidentical Stem Cell Transplantation with TCRαβ and CD19 Depletion in Children with Primary Immunodeficiency Syndromes. Biol Blood Marrow Transplant. 2015 Nov;21(11):1955-62. doi: 10.1016/j.bbmt.2015.07.008. Epub 2015 Jul 15. PubMed PMID: 26187864.

28) Kruchen A, Stahl T, Gieseke F, et al. Donor choice in haploidentical stem cell transplantation: fetal microchimerism is associated with better outcome in pediatric leukemia patients. Bone Marrow Transplantation 50, 1367-1370 doi:10.1038/bmt.2015.136

29) Arcese W, Picardi A, Santarone S, et al. Haploidentical, G-CSF-primed, unmanipulated bone marrow transplantation for patients with high-risk hematological malignancies: an update. Bone Marrow Transplant. 2015 Jun;50 Suppl 2:S24-30. doi:10.1038/bmt.2015.91. PubMed PMID: 26039203.

30) Berger M, Figari O, Bruno B, et al. Lymphocyte subsets recovery following allogeneic bone marrow transplantation (BMT): CD4+cell count and transplant-related mortality. Bone Marrow transplant 2008; 41: 55-62

31) Montagna D, Daudt L, Locatelli F, et al. Single cell cloning of human, donor-derived antileukemia, T-cell lines for in vitro separation of graft-vs-leukemia effect from graft-vs-host reaction. Cancer Res 2006; 66:7310-7316

32) Bashey A, Solomon SR. T-cell replete haploidentical donor transplantation using post-transplant CY: an emerging standard-of-care option for patients who lack an HLA-identical sibling donor. Bone Marrow Transplantation 2014; 49: 999-1008

33) Huang XJ. Donor Selection For Haploidentical Hematopoietic Stem Cell Transplantation: Who Is The Better – Donor-Recipient Risk Factor Analysis. Blood 2013; 122: 705.

34) Bacigalupo A, Dominietto A, Ghiso A et al. Unmanipulated haploidentical bone marrow transplantation and post-transplant cyclophosphamide for hematologic malignanices following a myeloablative conditioning: an update. Bone Marrow Transplant 2015; 50: Suppl 2 S37-9

35) Hsu KC, Keever-Taylor CA, Wilton A, et al. Improved outcome in HLA-identical sibling hematopoietic stem cell transplantation for acute myelogenous leukemia (AML) predicted by KIR and HLA genotypes. Blood. 2005:2004–2012

36) Zhao Xy, Huang Xj, Liu Ky, Xu Lp, Liu Dh. Prognosis after unmanipulated HLA-haploidentical blood and marrow transplantation is correlated to the numbers of KIR ligands in recipients. European Journal of Haematology. 2007;78:338–346

37) Ruggeri L, Mancusi A, Capanni M, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. 2007;110:433–440

38) Gagne K, Brizard G, Gueglio B, et al. Relevance of KIR gene polymorphisms in bone marrow transplantation outcome. Human Immunology. 2002;63:271–280

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 (HL and NHL)	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	NMA	19 (57%)
	MA	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 HSCT. ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, CML: chronic myelogenous leukemia, HL: Hodgkin lymphoma, NHL: non-Hodgkin lymphoma, CR: complete remission, NK: natural

killer cells, BM: bone marrow, PBSC: peripheral blood stem cells. NMA: nonmyeloablative condition, MA: myeloablative conditioning

		OS (95% CI)	P	PFS (95% CI)	P	RI (95% CI)	P	TRM (95% CI)	P
Disease	Acute leukemia	58% (35-81)	NS	58% (28-73)	NS	26% (13-41)	NS	13% (4-37)	NS
	Other	90% (71-100)		90% (71-100)		10% (1-64)		0%	
Patient gender	Female	82% (59-100)	NS	82% (59-100)	0.03	7% (1-50)	0.02	8% (1-50)	NS
	Male	66% (44-88)		53% (24-72)		35% (19-63)		10% (2-37)	
Disease status	CR1/CR2	65% (39-90)	NS	63% (41-85)	NS	22% (9-52)	NS	11% (3-41)	NS
	Other	71% (47-91)		63% (38-89)		26% (11-61)		7% (1-44)	
Donor	Mother	82% (63-100)	NS	82% (63-100)	NS	0%	0.02	19% (0-38)	NS
	Other	49% (20-79)		45% (21-65)		53% (33-86)		0%	
Donor gender	Female	78% (59-97)	NS	73% (52-94)	NS	10% (3-37)	0.04	15% (5-42)	NS
	Male	51% (19-82)		51% (19-82)		46% (25-83)		0%	
HLA	Full haplo	62% (39-85)	NS	57% (21-93)	NS	23% (10-49)	NS	14% (5-39)	NS
	Other	71% (38-100)		58% (35-81)		37% (15-92)		0%	
KIR alloreactivity	Yes	78% (56-100)	NS	78% (56-100)	NS	14% (4-51)	NS	7% (1-47)	NS
	No	54% (25-82)		48% (22-68)		37% (20-70)		12% (3-46)	
Conditioning	NMA	81% (61-100)	NS	71% (49-91)	NS	22% (9-53)	NS	5% (8-37)	NS
	MA	61% (34-88)		59% (25-82)		27% (11-61)		13% (4-48)	
Stem Cell Source	BM	74% (57-91)	NS	63% (45-81)	NS	23% (12-47)	NS	10% (3-29)	NS
	PBSC	66% (33-100)		66% (33-100)		33% (7-100)		0%	
TNC	>median	59% (30-88)	NS	55% (20-75)	NS	37% (20-70)	NS	6% (1-43)	NS
	<median	74% (56-93)		74% (56-93)		12% (3-43)		12% (3-43)	
CD34+	>median	72% (45-100)	NS	60% (36-91)	NS	40% (21-74)	NS	0%	NS
	<median	66% (42-91)		66% (42-91)		12% (3-43)		17% (6-49)	
CD3+	>median	66% (39-93)	NS	66% (39-93)	NS	28% (12-65)	NS	7% (1-47)	NS
	<median	69% (44-94)		57% (25-79)		40% (14-100)		0%	

Table 2. Univariate analysis of OS, PFS, RI and TRM following T cell replete HSCT. CR1: first complete remission, CR2: second complete remission, KIR: killer immunoglobulin receptors,

NMA: nonmyeloablative condition, MA: myeloablative conditioning, BM: bone marrow, PBSC: peripheral blood stem cells, TNC: total nucleated cells, NS: not significant

	Mother donor N=18	**Non-mother donor N=15**	**P**
Patient age	12 (1-19)	9 (1-21)	NS
Acute leukemia	10/18	13/15	NS
CR1	3/10	6/13	NS
Bone marrow stem cells	17/18	13/15	NS
NMA/MA	8/10	10/5	NS
TNC x 10^8/Kg	4.2 (1.9-9.5)	7 (3-11)	0.003
CD34+ x 10^6/Kg	4.6 (1-14.8)	10 (1-18)	0.03
CD3+ x 10^8/Kg	0.5 (0.2-0.9)	0.9 (0.1-2.3)	NS
Full haplo Yes/No	14/18	11/15	NS
NK alloreactivity Yes/No	10/8	7/8	NS

Table 3. Distribution of patients' characteristics at haplo-HSCT. CR1: first complete remission, NMA: nonmyeloablative conditioning, MA: myeloablative conditioning, TNC: total nucleated cells.

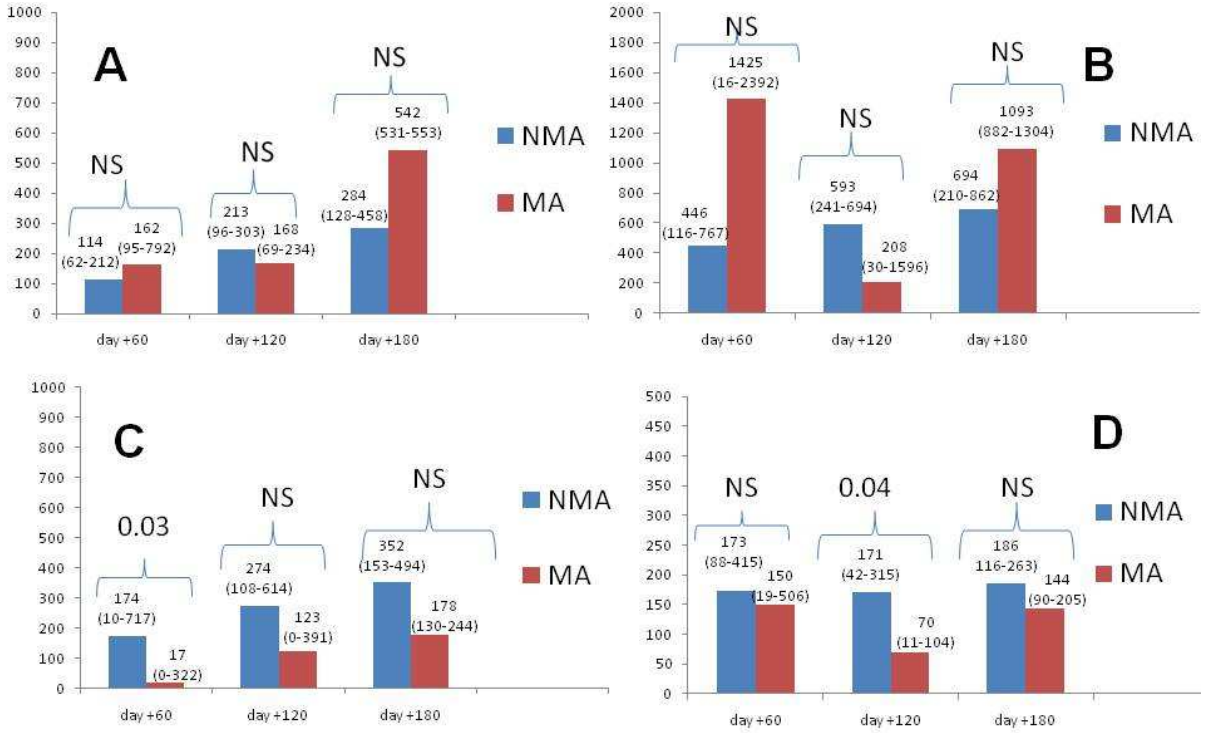


Fig. 1. Immunological reconstitution following PTCy Haplo-HSCT. A: CD3+CD4+/μL, B: CD3+CD8+/μL, C: CD19+/μL, D: CD3-CD56+/μL. NMA: nonmyeloablative conditioning, MA: myeloablative conditioning.

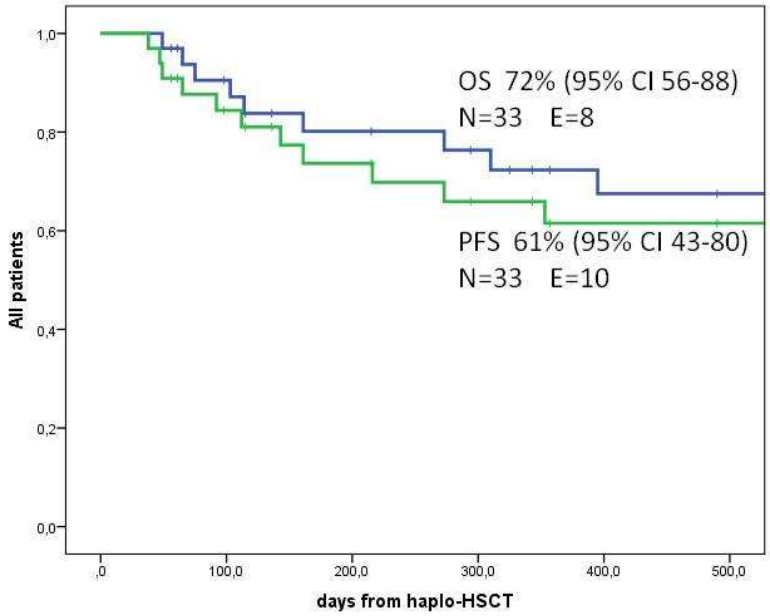


Figure 2. 1-year Overall survival (OS) and Progression free survival (PFS) for all patients given PTCy

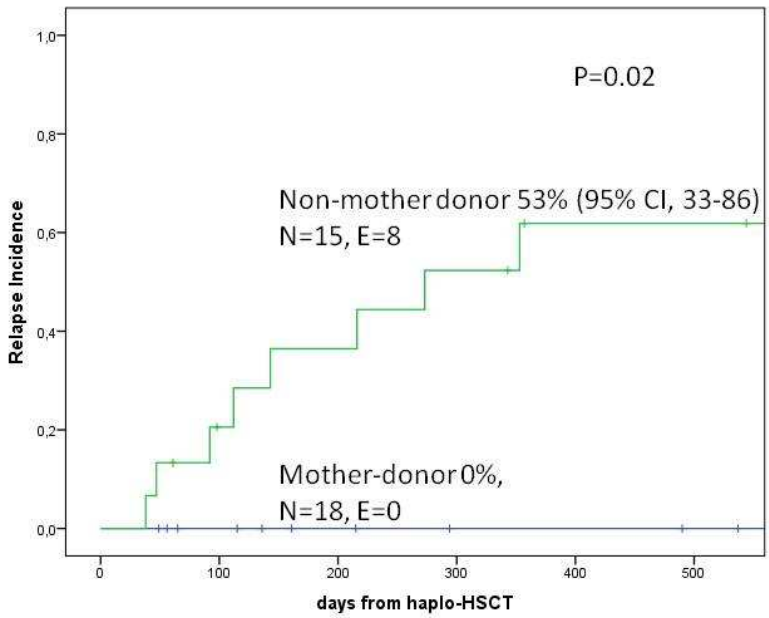


Fig. 3. 1-year Relapse Incidence for patients given PTCy haplo-HSCT.
N= number of patients, E=events

Highlights for review

- Thirty-three children with high risk hematological malignancies and lacking a match-related or unrelated donor were treated with PTCy haploidentical HSCT in five Italian AIEOP centers
- 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) of children. The 1-year overall survival (OS) rate was 72% (95% CI, 56-88), progression free survival was 61% (95% CI, 43-80), 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 gender (P=0.02), were significantly associated with a lower risk of relapse. When we then considered the relapse incidence in the mother-donor group only, no differences emerged between the recipient gender, showing a possible role of parental haplotype as a target of immunological reaction against malignancies.