HAPLOIDENTICAL, UNMANIPULATED, G-CSF PRIMED BONE MARROW TRANSPLANTATION FOR PATIENTS WITH HIGH-RISK HEMATOLOGICAL MALIGNANCIES

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ABSTRACT

Eighty patients with high risk hematological malignancies underwent unmanipulated, G-CSF primed BMT from an haploidentical family donor. Patients were transplanted in 1st or 2nd complete remission (CR, standard-risk: n=45) or in >2nd CR or active disease (high-risk: n=35). The same regimen for GVHD prophylaxis was used in all cases. The CI of neutrophil engraftment was 93±0.1%. The 100-day CI of II-IV and III-IV grade of acute GVHD was 24±0.2% and 5±0.6%, respectively. The 2-year CI of extensive chronic GVHD was 6±0.1%.The 1-year CI of TRM was 36±0.3%. After a median follow-up of 18 months, 36 of 80 (45%) patients are alive in CR. The 3-year probability of OS and DFS for standard-risk and high-risk patients was 54±8% and 33±9% and 44±8% and 30±9%, respectively. In multivariate analysis, DFS was significantly better for patients with standard-risk disease and transplanted after 2007. We conclude that unmanipulated, G-CSF primed bone marrow transplantation from haploidentical family donor provides very encouraging results in terms of engraftment rate, incidence of GVHD and survival and represents a feasible, valid alternative for patients with high-risk malignant hematological diseases, lacking an HLA identical sibling and in need to be urgently transplanted.

INTRODUCTION

Although the best results with allogeneic hematopoietic bone marrow transplantation (BMT) are obtained in patients receiving the graft from a human leukocyte antigen (HLA) genotipically identical sibling, this type of donor is only available for less than 30% of patients. In the last 3 decades, for patients lacking an HLA-matched family donor the main alternative was an 8/8 HLA antigens matched unrelated donor (MUD) allocated mainly through the International Registries of volunteer donors[1,2]. However, despite the fact that, as of June 2012, the number of such volunteer donors exceeds 19 millions of individuals[3], almost two thirds of patients do not reach transplantation. The enormous variability of HLA polymorphisms and the time required for identifying a suitable donor are the 2 most important factors limiting the use of MUD transplant, especially for patients at high risk of disease progression and on urgency to be transplanted. For these patients, two potential alternative sources of graft remain available: umbilical cord blood

(UCB) and HLA haploidentical related donors [4,5]. UCB offers the advantages of ready availability and faster procurement of cryopreserved stem cells, no risk for the donors, low risk of transmissible infection, potentially reduced risk of graft versus host disease (GvHD) and less stringent criteria for donor-recipient HLA matching [6]. The results of UCB transplant in children and adults are well established [7-10] and comparable with those reported for MUD transplants [11, 12]. However, the low number of hematopoietic stem cells contained in a single cord blood unit limits its use for transplant and, despite several alternative strategies that are currently explored [13-16], it remains the main obstacle for successful UCB transplant, particularly in adults. Considering that virtually all patients have at least one HLA-haploidentical family member, when neither matched sibling donor nor MUD nor UCB are available transplant from haploidentical family donor represents a valid alternative for patients with high-risk hematological malignancies [5,17,]. Together with immediate donor availability, the more adjustable management of graft procurement, the cost savings for the donor search and, if indicated, the possibility of post-transplant cellular therapies represent other important advantages of haploidentical transplant. Historically, in haploidentical transplant the high risk of both graft failure and, on the other side, of acute GVHD has been overcome by infusing megadoses of ex-vivo T cell depleted CD34+ purified peripheral blood stem cells (PBSC) [18,19]. However, due to the almost complete T cell depletion, immune reconstitution is slow leading to high frequency of mainly viral and fungal infection complications as well high relapse rate. Recently, encouraging results have been reported with an alternative approach enabling to perform haploidentical transplants with an unmanipulated non T-cell depleted graft using a vigorous pre- and post-transplant pharmacologic GVHD prophylaxis [20-24]. Herein, we report the results of 80 patients with high-risk hematologic malignancies that underwent an unmanipulated BM graft from an haploidentical family donor primed with low dose granulocyte-colony stimulating factor (G-CSF) and that received an identical regimen for GVHD prophylaxis.

PATIENTS AND METHODS

Eligibility criteria

All patients affected by malignant hematological disease in active status or in complete remission (CR) but at high risk of progression were offered the option of haploidentical transplantation if they fulfilled the following criteria: 1) no available ≥8/10 HLA antigen matched unrelated donor in the preliminary search through the international volunteer donor registry; 2) no available cord blood unit suitable for transplant on the base of cellularity (nucleated cells $>3x10^7$/Kg recipient body weight) and HLA compatibility (at

least 4/6 Class 1 or 2 identical HLA antigens by molecular typing); 3) expected interval time to transplant of less than 3 months. This study includes data from 80 patients with hematological malignancies who received an allogeneic BMT from haploidentical related donor in 4 transplant Centers (Rome, Italy, n=38; Pescara, Italy, n=36; Ancona, Italy, n=4; Tel-Hashomer, Israel, n=2) between August 2005 and October 2010. The primary end points of the study were engraftment, acute GVHD, and 1-year TRM; the secondary end points were chronic GVHD, relapse, overall survival (OS) and disease free survival (DFS). The study was approved by the institutional review board (IRB) of each participating Institution. Informed consent for the treatment was obtained from all patients and donors or their legal guardians in accordance with the Declaration of Helsinki.

Patients

Patients (male, 65%) had a median age of 37 years (range, 5-71 years) with 29% of them aged more than 50 years (Table 1). They suffered from the following underlying diseases: acute myeloid leukemia (AML, n=45), acute lymphoblastic leukemia (ALL, n=15), chronic myeloid leukemia (CML, n=5), Hodgkin lymphoma (HL, n=5), myelodysplastic syndrome (MDS, n=3), plasma cell leukemia (PCL, n=3), myelofibrosis (MF, n=2) and Non-Hodgkin lymphoma (NHL, n= 2) . Patients transplanted in first (n=30) or second (n=15) CR were considered as standard-risk. The remaining 35 patients (44%) were considered as high-risk if they received transplant in ≥CR3 (n=3), in 2nd chronic phase of CML (n=4) or in refractory or recurrent active disease (AD, n=28). For the 21 patients with AML transplanted in CR1, the risk factors were:, refractoriness to first line chemotherapy (n=9), secondary leukemia (n=4), hyperleukocytosis with complex karyotype (n=4) , FLT-3/ITD positivity (n=3) and engraftment failure following autologous PBSC transplant (n=1). For the 8 patients with ALL transplanted in CR1, the risk factors were: Philadelphia chromosome positivity (n=4), refractoriness to first line chemotherapy (n=2), hyperleukocytosis with T- cell phenotype (n=2). Nineteen patients (23%) had been previously transplanted: 16 with autologous PBSC and 3 with allogeneic bone marrow (2 from HLA-identical sibling and 1 from MUD). The median interval from date of diagnosis to BMT for all patients was 11 months (range, 3 - 442 months).

Donors

Donors (male, 54%) had a median age of 39 years (range, 16-72 years) and were in order represented by siblings (47%), mothers (25%), sons (22%), fathers (5%) and cousin (1%). In the case of multiple available donors, the mother had priority followed by the youngest male adult donor within the family. Donor-recipient Cytomegalovirus (CMV) status and ABO matching were also considered for donor selection. The donor-recipient combinations were female to male in 30%, negative to CMV positive in 17% with a donor-recipient CMV negativity occurring in only 4% of cases and ABO minor and major incompatibility in 15% and 38%, respectively. Patients and their donors were typed for HLA-A,-B, -DR and C loci by at least intermediate-resolution DNA typing, and HLA-DRB1, DQB1 and DPB1 typing by high–resolution techniques . All donors were HLA identical for one haplotype and mismatched for 2 (n=28, 35%) or 3 (n=52, 65%) A, B, DR loci on the unshared haplotype (Table 1).

Conditioning regimen

A myeloablative conditioning (MAC) regimen was employed in 64 (80%) patients and a reduced intensity conditioning (RIC) in 16 (20%) patients. From April 2005 to April 2008, for the first 29 patients the MAC regimen included cytarabine 3 g/m^2/day i.v. in 2 divided doses for 3 days and cyclophosphamide 45 mg/kg/day for 2 days associated with 10 Gy total body irradiation in 4 fractions over 2 days (n=7) or treosulfan 14 g/m^2/day for 3 days (n=11) or oral busulfan 16 mg/kg in 4 days (n=11). In the same period of time, 3 patients received a RIC regimen including fludarabine alone at 160 mg/m^2 over 4 days because of persistent engraftment failure following autologous PBSC transplant (n=1) or thiotepa 5 mg/kg/day for one day followed by fludarabine 150 mg/m^2 over 3 days and melphalan 140 mg/m^2 for one day (n=2). Since May 2008, the 2 Centers of Rome and Pescara, contributing the highest number of patients in this study, changed the general strategy of allogeneic transplant by adopting an identical conditioning regimen not including TBI for the different sources of hematopoietic stem cells: HLA identical sibling, matched unrelated donor, umbilical cord blood or haploidentical donor. According to this new transplant policy, a uniform chemotherapy based conditioning regimen was employed in the myeloablative and reduced intensity version consisting, respectively, of Thiotepa 5 mg/kg/day at days -7 and -6, Busulfan 3.2 mg/kg/day in a single i.v. infusion over 3 hours combined with Fludarabine 50 mg/m^2/day i.v. in 1 hour at days -5, -4 and -3 (TBF-MAC) as recently reported by the Spanish group for cord blood transplant [25] or Thiotepa 5 mg/kg on day -6, Busulfan 3.2 mg/kg/day in a single i.v. infusion over 3 hours at days -5 and -4 and

Fludarabine 50 mg/m^2/day i.v. in 1 hour at days -5, -4 and -3 (TBF-RIC) . Thirty-five patients were conditioned with TBF-MAC and 13 received TBF-RIC .

GVHD prophylaxis

All patients received the same GVHD prophylaxis as reported by Ji et al [20] and consisting of a combination of 5 drugs with different points of attack: 1) antithymocyte globulin (ATG, Fresenius AG, Oberurse, Germany) infused i.v. at 5 mg/kg from day -4 to -1; 2) cyclosporine (CsA) given as continuous i.v. 12 hours infusion at 1,5 mg/kg/day from day -7 to -2 and at 3 mg/kg/day from day -1 until patients were able to tolerate oral medication. Then, CsA was given orally at 5-6 mg/kg/day in 2 divided doses. The CsA doses were adjusted on the basis of plasma levels (150 to 350 ng/ml) and hepatic and renal toxicity. Starting from day +180 and in absence of chronic GVHD, the CsA dose was progressively tapered by 5% every week and stopped by day +365; 3) methotrexate (MTX) was given i.v. at 15 mg/m^2 on day 1 and at 10 mg/m^2 at day 3, 6 and 11; 4) mycophenolate mofetil administered orally at 15 mg/kg/day in 2 divided doses from day 7 to day 100. The MMF doses were adjusted on the basis of marrow toxicity; 5) basiliximab (Simulect, Novartis Pharma AG, Basle, Switzerland), an anti-CD25 monoclonal antibody, given at fixed dose of 20 mg or 10 mg to patients with a body weight, respectively exceeding or inferior to 35 kg, as 30 min. i.v. infusion on day 0 (2 hours before graft infusion) and on day 4. Diagnosis and clinical grading of acute and chronic GVHD were established according to the standard criteria [26-28] .

Collection of hematopoietic cells

The day of transplant was designated as day 0. All donors were primed with 4 µg/kg/day granulocyte-colony stimulating factor (G-CSF, Filgrastim, Granulokine) in single daily subcutaneous injection for 7 consecutive days from -7 through -1. Bone marrow cells were harvested from the posterior iliac crests on day 0 with a target volume collection of 15 ml/kg recipient body weight. Fresh and unmanipulated bone marrow cells were infused into the recipient on the same day.

Evaluation of engraftment and donor chimerism

Neutrophil and platelet engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) of ≥0.5X10^9/L and an absolute platelet count of ≥25x10^9/L, respectively. Chimerism was evaluated either on bone marrow cells or peripheral blood cells on days 30, 90, 180 and 365. Sex-mismatched donor-recipient

chimerism was evaluated by cytogenetic G-banding or fluorescent in situ hybridization. Sex-matched donor-recipient chimerism was assessed by PCR-based analyses of polymorphic microsatellite regions by short number tandem repeats (STR). HLA typing was performed after transplantation as confirmation of engraftment.

Supportive therapy and Infection prophylaxis

All patients were hospitalized in rooms with high-efficiency particle-arresting (HEPA) air filtered rooms and received antinfectious prophylaxis with oral trimethoprim-sulfamethoxazole (day-10 to -2), fluconazole (from day -10), acyclovir (from day -1) and ciprofloxacin (from day -1). All blood products were irradiated with 2500 cGy. CMV and Epstein Barr virus were regularly monitored in the blood by PCR assays.

Definitions

Primary failure of engraftment was defined as no evidence of myeloid donor cells in recipient bone marrow at day 28 after transplantation. The incidence of acute GvHD was evaluated in all patients with evidence of engraftment. The incidence of chronic GvHD was evaluated in patients surviving more than 100 days after transplantation with allogeneic engraftment. Transplant-related mortality (TRM) was defined as death from any cause except relapse. Relapse was defined by molecular, cytogenetic or morphological evidence of the original hematological disease in peripheral blood, bone marrow or any extramedullary site. Overall survival was defined as time to death from all causes. Disease-free survival was defined as time to relapse or death in remission.

Statistical analysis

A descriptive analysis of all variables was performed including mean, median, standard deviation, range, minimum and maximum value for continuous variables, absolute and relative frequencies for categorical variables. Using parametric and non parametric statistical procedures (Chi-square test, Fisher exact test and rank correlation coefficient of Spearman), the possible interdependence between two or more variables was evaluated and a p-value of <0.05 was considered significant. OS and DFS were estimated by the product-limit method of Kaplan-Meier[29] and the curves of various subgroups were compared using the log-rank test [30].

Taking into consideration the corresponding competing risks, the probabilities of neutrophil and platelet engraftment, acute and chronic GvHD, TRM and disease relapse were estimated with the of cumulative incidence(CI) method[31] and the curves of various

subgroups were compared using the Gray's test[32]. Using the stepwise selection procedure for all the variables of interest (diagnosis, age, disease stage at transplant, donor type, conditioning, year of transplant), the Cox proportional hazard model[33] was used for multivariate analyses of OS and DFS . The joint effect of variables on TRM and relapse was evaluated using the multivariate model of Fine and Gray[34]. The analyzed data are referred to patients transplanted from August 2005 to October 2010.The analysis was conducted using two statistical software packages: SAS version 9.1.3 and R version 2.11.0

RESULTS

Marrow cell composition

A median of 7.4 (range, 1-29) x 10^8/kg total nucleated cells, 0.6 (range, 0.3-5) x 10^8/kg mononucleated cells, 2 (range, 0.7-11) x 10^6/kg $CD34^+$ cells, and 2.9 (range, 1-9.8) x 10^7/kg $CD3^+$ cells were infused into the recipients. No untoward side effect related to G-CSF administration or bone marrow harvest was reported by donors.

Engraftment

Six patients died of transplant-related complications before day 21 after transplantation without myeloid recovery. One patient showed primary engraftment failure and died of infection on day 29. Seventy-three (91%) patients achieved a trilineage engraftment with a median time of 21 days (range, 12-38 days) for ANC and 28 days (range, 14-185 days) for platelets. The 100-day CI for neutrophil and platelet engraftment was 93±0.1% and 75±0.2%, respectively (Figure 1). For all engrafted patients, the chimerism at 60 days was of full donor origin. The median number of red blood cell and platelet units from single donor transfused over the first 100 days post-transplant was 9 (range, 1-52) and 14 (range, 2-70), respectively.

Acute and chronic GVHD incidence and severity

Among 73 evaluable cases, no signs of acute GVHD were observed in 38 patients (52%). Acute GVHD occurred at a median time of 26 days (range, 12-130 days) after transplantation and was grade I in 14 patients (19%), grade II in 16 (22%), grade III in 2 (3%) and grade IV in 3 (4%). The CI of grade II-IV and grade III-IV acute GVHD at 100 days was 24±0.2% and 5±0.6%, respectively (Figure 2A).

No chronic GVHD was observed in 49 out of 59 evaluable patients (83%), 7 patients (12%) experienced a limited form and 3 (5%) an extensive form of chronic GVHD at a median time of 145 days (range, 100 to 445 days) after transplant. The CI of overall (limited + extensive) and only extensive chronic GVHD at 2 years was 17±0.3% and 6±0.1%, respectively (Figure 2B). Complete resolution of the clinical manifestations of chronic GVHD with discontinuation of any immunosuppressive treatment was observed in all 7 patients with the limited form and in 2 out of 3 patients with the extensive form, respectively. Only 1 patient continues to receive immunosuppressive therapy at time of the analysis.

Transplant-related mortality and complications

Twenty-seven patients (34%), 13 in the standard -risk group and 14 in the high-risk group, respectively, died from transplant-related complications at a median time of 76 days (range, 6-369 days). Causes of death included infections in 11 patients (14%), pneumonia in 5 (6%), multiorgan failure in 5 (6%), acute GVHD in 3 (4%), liver failure in 1 (1%), veno-occlusive disease (VOD) in 1 (1%), and central nervous system (CNS) disease complications in 1 (1%). The CI of TRM was 32±0.3% at 6 months and 36±0.3% at 1 and 3 years. Although the TRM was higher in the high-risk group as compared to the standard-risk group (45±0.1% vs 30±0.5% at 3 years), the difference was not statistically different . Furthermore, no difference was found for the 3-year CI of TRM between patients (n=35) prepared with TBF-MAC and patients (n=31) conditioned with other MAC (39±01% vs 32±0.7%; p=0.58). In multivariate analysis, no significant factor was found to affect TRM. In the first 6 months post-transplant, 56 patients developed CMV reactivation (CI: 70±0.27%), 38 bacterial septicemia (CI: 47±0.32%), 25 hemorrhagic cystitis (CI: 31±0.27%), 13 CNS complications (CI: 16±0.17%), 10 fungal infections (CI: 14±0.17%) and 5 VOD (CI: 6±0.07%) (Figure 3).

Relapse

Eighteen patients (10 in the standard-risk group and 8 in the high-risk group) relapsed after a median time of 180 days (range, 56 - 467 days) after transplantation. Ten patients were affected by AML, 7 by ALL, and 1 by PCL. The overall CI of relapse was 21±0.2% at 1 year and 28±0.3% at 5 years, respectively. The CI of relapse was not significantly different between patients in the standard -risk group and those in the high-risk group at 1 year (17±0.2% vs 28±0.7%; p=0.19) and 3 years (26±0.5% vs 28±0.7%; p=0.38), respectively. Although the difference was not statistically significant, the 3-year CI

of relapse for the 35 patients conditioned with TBF-MAC regimen was remarkably lower than for the 31 patients receiving other MAC (24±07% vs 39±0.8%; p=0.19). All leukemia relapses occurred in the marrow with no extramedullary relapses. Among the 18 relapsed patients, 14 died of leukemia progression or chemotherapy-related complications and 4 patients are currently surviving in CR: 2 following chemotherapy reinduction and 2 after a second haploidentical BMT from a different donor. In multivariate analysis, the TBF-MAC regimen was the only significant factor for lower relapse rate (Table 2).

Overall Survival (OS) and Disease Free Survival (DFS)

The 3-year probability of OS for all patients was 45±6%: 54±8% for patients in the standard -risk group and 33±9% for patients in the high-risk group (p=0.06) (Figure 4A). Although patient and donor characteristics were not statistically different before and later than 2007 (data not shown), the 3-year probability of OS was significantly better for the 54 patients transplanted after 2007 (58±7% vs 27±9%; p=0.04) (Figure 4B). Of these patients, 29 were at standard-risk and 25 at high-risk at time of transplant and the 3-year OS was 69±9% and 42±13%, respectively (p=NS) (Figure 4C). Most of these patients were conditioned with TBF regimen, MAC (n=35) or RIC (n=13) and 6 patients received other MAC (n=4) or RIC (n=2) regimen, 4 of these last patients are alive in continuous CR. The 1-year probability of OS was higher for 13 patients conditioned with TBF-RIC as compared to 35 patients conditioned with TBF-MAC (77±12% vs 51±9%; p=NS). At a median follow-up of 18 months (range, 6 to 74 months), 36 patients (45%) (20 AML, 5 ALL, 3 CML, 3 HD, 2 MDS , 2 MF, 1 NHL) are surviving in CR. Of these patients, 25 had standard-risk and 11 high-risk of disease at time of transplant. The 3-year probability of DFS was 38±6% for all patients: 44±8% for the standard-risk patients and 30±10% for the high-risk patients (p=NS) (Figure 4D). Among 28 patients transplanted with active disease, 12 (43%) died of transplant-related causes and 5 (18%) of relapse; 11 patients (39%) are alive and disease-free with a median follow-up of 14 months (range, 6 - 40 months). For the 54 patients transplanted later than 2007, the DFS was better than that of the 26 patients transplanted prior to 2007 (47±8% vs 23±8%, p=0.059) (Figure 4E). The DFS of the 29 standard-risk patients grafted after 2007 was higher, although not significantly different, than that of the 25 high-risk patients transplanted in the same period (54±10% vs 37±13%) (Figure 4F). In the Cox model, the risk category (standard-risk vs high-risk) and the year of transplant (2005-07 vs 2008-10) were the only two variables significantly affecting both OS ($p<0.03$ and $p<0.02$, respectively) and DFS ($p<0.05$ and $p<0.03$, respectively) (Table 2). Recipients of BM from haploidentical mothers, showed an advantage in terms of both

OS and DFS compared to patients transplanted from other related haploidentical donors, but this difference was not statistically significant with an HR of 1.2 (95% CI 0.59 – 2.49) and 1.32 (95% CI 0.66 – 2.49), respectively.

DISCUSSION

Following myeloablative conditioning and standard (MTX-CSA) GVHD prophylaxis, the outcome of patients undergoing allogeneic transplant from 2 or 3 HLA antigens mismatched family donor is particularly dismal due to the highest risk of graft rejection, acute GVHD and, consequently, TRM[35,36]. The incidence of primary graft failure significantly correlates with the degree of HLA incompatibility in the host-versus-graft direction being 12.3% among bone marrow transplants from HLA partially matched donor compared with 2.0% among recipients from an HLA-identical sibling[37]. In order to favor engraftment and prevent GVHD a number of different procedures have been adopted for patients undergoing an haploidentical transplant following either a myeloablative or a reduced intensity conditioning and using either manipulated (T-cell depleted) or unmanipulated (non T-cell depleted) graft with more intensive in vivo GVHD prophylaxis[19-24, 38-42]. However, in patients receiving a T cell depleted graft the risk of graft failure remains in the range of 9% to 15%, and the incidence of ≥II grade acute and chronic GVHD ranges from 8% to 59% and 7% to 14%, respectively. On the other hand, among recipients of unmanipulated graft the risk of graft failure is 0.4% to 13%, while the incidence of ≥II grade acute and chronic GVHD is 16% to 55% and 14% to 74%, respectively. Herein, we analyzed the clinical outcome of 80 patients with high-risk hematological malignancies who underwent allogeneic BMT from haploidentical donor. Our transplant strategy was based on the use of unmanipulated BM cells harvested from donors primed with low-dose G-CSF and on the administration of an intensive GVHD prophylaxis. Primary end-points of our study were engraftment, acute-GVHD and 1-year TRM. Several studies both in vitro as well as in experimental models and most importantly in the clinical setting have demonstrated that relevant quantitative and qualitative modifications in the marrow cell composition and function are induced by G-CSF priming. After G-CSF stimulation, the number of marrow CD34+ cells increases 1.4 to 1.7 fold, the number of colony-forming cells (CFCs) 3 fold and the number of long-term culture-initiating cells (LTC-IC) 50 to 90 fold [43]. Furthermore, G-CSF exerts an intense immunoregulatory effect on marrow T-cells by downregulating expression of adhesion and CD28/B7 molecules and by increasing the absolute number of DC2 antigen presenting cells favoring T-cell shift from Th1 to Th2-type cells and inducing an higher production of IL-4

and IL-10 anti-inflammatory cytokines [44-47]. In transplants from HLA identical sibling, engraftment of G-CSF primed BM cells is faster with an incidence of ≥II grade acute GvHD of only 6.3%[48]. In our study, the cumulative incidence of myeloid engraftment has been very high (93±0.1%) with a median time to engraftment in the range of that usually observed in patients transplanted from an HLA-identical sibling. Notably, only one evaluable patient did not achieve engraftment and died in aplasia at day 29. After an unmanipulated BMT from partially matched or haploidentical relatives, it is mandatory to use a highly effective regimen of GVHD prophylaxis in order to reduce incidence and severity of acute GVHD. For all our patients an identical regimen of GVHD prophylaxis was adopted according to the protocol reported by Ji et al[20] and based on the classical CSA and MTX combination with the addition of sequential immunosuppressive drugs given before (ATG Fresenius), at time (anti-CD25 monoclonal antibody basiliximab) and after (MMF) the G-CSF primed BM graft infusion. However, with respect to the experience of the Chinese group, the higher number of patients, the multicentric nature of our study and the use of the TBF conditioning regimen not including TBI provide relevant novelty to the present report. Indeed, the cumulative incidence of advanced grades acute and chronic GVHD was only 5% and 6%, respectively. Acute GVHD was the main cause of death in only 3 patients and all but one evaluable patients had complete resolution of the clinical manifestations of chronic GvHD and are free of immunosuppressive therapy. Most patients surviving 1 year after BMT returned to their full social and work activity. All the patients in our series had underlying diseases with very poor prognosis and were at high risk of developing clinical complications: of them 35% were transplanted in active disease phase, most were heavily pretreated with 23% having received a previous transplant and 29% aged more than 50 years including 7 patients older than 60 years. In this high-risk group of patients, a TRM occurring in one third of patients with most events observed within the first 6 months post-transplant is not surprising. No fatal infection occurred after the first year from transplant and no patient developed a post transplant lymphoproliferative disease. In multivariate analysis, no factor was found to be significantly associated with TRM. Using the combination of G-CSF primed unmanipulated bone marrow and peripheral blood as source of hematopoietic stem cells for 250 patients transplanted from mismatched/haploidentical donors, Huang et al.[23] have reported an excellent rate of engraftment of 99,6%, a CI of II-IV and III-IV grade acute GVHD of 45.8% and 13.4%, respectively, and a CI of overall and extensive chronic GVHD of 53.9% and 22.6%, respectively. In the same study, TRM at 3-years ranged from 19.4% to 59.8% depending on the diagnosis and the disease risk. Notably, the Chinese study included only acute

leukemia patients, 16% of whom with only 1 HLA antigen disparity, with a median age of 25 years,(range, 2 - 56) and higher proportion of patients receiving maternal grafts. In spite of these differences in patient risk between the 2 cohorts, the primary end points achieved in our study (engraftment, incidence of GVHD and TRM) are well comparable with the Chinese results. In particular, a clear reduction of the acute and chronic GVHD incidence can be observed in our patients with respect to that reported by the Chinese group (II-IV and III-IV grade acute GVHD: CI 24% vs 45.8% and 5% vs 13.4%, respectively; overall and extensive chronic GVHD: CI 17% vs 53.9% and 6% vs 22.6%, respectively). In our study, the graft composition consisting of only G-CSF primed bone marrow by excluding peripheral blood and the use of the anti CD25 monoclonal antibody might have contributed to a better prevention of the GVHD. The number of patients and the heterogeneity of diagnosis do not allow to evaluate the impact of our transplant procedure in preventing relapse for each single disease category, which was 21±0.2% at 1 year and 28±0.3% at 5 years for the whole group without significant difference between standard and high risk patients. Interestingly, 11 out of 28 patients transplanted with active disease are disease-free at 2 years after transplantation. The use of TBF-MAC was associated with a lower, although not statistically significant, risk of relapse and in multivariate analysis it was the only factor found to be significant in preventing relapse. This regimen initially proposed by Sanz et al.[25] in the setting of umbilical cord blood transplantation seems particularly effective also for recipients of an haploidentical unmanipulated BM transplant. Indeed, in multivariate analysis patients transplanted later than 2007, who were mostly conditioned with TBF regimen and who had characteristics not substantially different from those of patients transplanted before 2007 (data not shown) had a significantly better outcome. Moreover, despite the relatively low number of patients and the rather short follow-up, the observed 77±12% OS at 1-year for patients conditioned with the RIC version of TBF regimen is particularly encouraging. Overall, the results of this study are comparable with those reported for similar series of high risk patients lacking an HLA identical sibling and undergoing an allogeneic transplant from umbilical cord blood or matched unrelated donor [7-12]. A number of considerations can be drawn from this experience. Firstly, the transplant procedure consisting of a chemotherapy based regimen not including total body irradiation combined with an intensive in vivo GVHD prophylaxis requiring neither expensive laboratory facilities nor personnel with high expertise in cell manipulation allows to extend the practice of haploidentical transplant to all centers involved in an allogeneic transplant program. Furthermore, in comparison with UCB or MUD, haploidentical transplant provides an easier management of the transplant work-up and enables to save the relevant costs

related to the search for the graft acquisition. Finally, because of the prompt donor availability, the potential use of DLI after transplant for prevention or treatment of relapse is better guaranteed to the patients. To date, as no substantial differences in terms of patient outcomes are emerging from the retrospective studies of UCB, MUD and haploidentical transplants and in absence of prospective randomized trials, all three options should be considered in the algorithm of search for an alternative donor. In this context, the G-CSF primed, unmanipulated bone marrow transplantation from an haploidentical family donor represents a valid alternative for patients with high-risk malignant hematological diseases, lacking an HLA identical sibling donor and urgently requiring to be transplanted.

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Authorship:

Contribution: W.A. and P.D.B. contributed patients, designed the study, analyzed the data and wrote the manuscript; S.S., G.D.A., A.P., L.C., R.C., P.B., G.B., M.M., A.N. contributed patients and critically reviewed the manuscript; G.A. provided transfusional and supportive care; L.D.F. and M.C.R. provided cell culture and molecular data; L.S. provided laboratory data and guidance in infectious complications; S.A. and M.D.N. performed the statistical analysis; M.A. and F.P. provided the HLA typing; all authors have read and agreed to the final version of the manuscript.

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Table 1. Patient and Donor/Recipient characteristics

Patient age, years median (range)	**37** (5-71)
Age groups, n (%)	
<20 years	13 (16)
20 -50 years	44 (55)
>50 years	23 (29)
Patient gender, n (%)	
Male	52 (65)
Female	28 (35)
Malignant diseases:	
Standard Risk, n° (disease status)	**45**
Acute Myeloid Leukemia	34 (CR1=21; CR2=13)
Acute Lymphoblastic Leukemia	10 (CR1=8; CR2=2)
Plasma Cell Leukemia	1 (CR1=1)
High Risk, n° (disease status)	**35**
Acute Myeloid Leukemia	11 (CR3=2; AD=9)
Acute Lymphoblastic Leukemia	5 (CR3=1; AD=4)
Hodgkin Lymphoma	5 (AD=5)
Chronic Myeloid Leukemia	5 (CP2=4; AD=1)
Myelodysplastic Syndrome	3 (AD=3)
Non Hodgkin Lymphoma	2 (AD=2)
Myelofibrosis	2 (AD=2)
Plasma Cell Leukemia	2 (AD=2)
Donor age, years median (range)	**39** (16-72)
Donor gender, n (%)	
Male	43 (54)
Female	37 (46)
Donor/Recipient relationship, n (%)	
Sibling	38 (47)
Mother	20 (25)
Son	17 (22)
Father	4 (5)
Cousin	1 (1)
Donor/Recipient gender, n (%)	
male/male	28 (35)
female/male	24 (30)
male/female	15 (19)
female/female	13 (16)
Donor/Recipient CMV serostatus, n (%)	
positive/positive	55 (69)
negative/ positive	14 (17)
positive/negative	8 (10)
negative/negative	3 (4)
AB0 match, n (%)	
minor mismatched	12 (15)
major mismatched	30 (38)
matched	38 (47)
N° of HLA-A, -B, -DRB1 antigens mismatched	
2 Antigens	28 (35)
3 Antigens	52 (65)

CR, complete remission; **AD**, active disease; **CP**, chronic phase.

Table 2. Multivariate analysis of Relapse, Overall Survival and Disease Free Survival

	HR	95% CI	p-value
Relapse			
Conditioning *(TBF-MAC vs Other MAC)*	*1.837*	*1.014 - 3.330*	*0.0450*
Overall Survival			
Disease risk *(Standard-risk vs High-risk)*	*2.113*	*1.105 - 4.038*	*0.0236*
Years of transplant *(2005-07 vs 2008-10)*	*0.459*	*0.244 - 0.865*	*0.0161*
Disease Free Survival			
Disease risk *(Standard-risk vs High-risk)*	*1.878*	*1.020 - 3.456*	*0.0430*
Years of transplant *(2005-07 vs 2008-10)*	*0.510*	*0.279 - 0.929*	*0.0279*

FIGURE LEGEND

Figure 1. Engraftment. Cumulative incidence: Neutrophils (continuous line), platelets (dotted line) .

Figure 2. A: Acute graft-versus-host disease. Cumulative incidence: grade II-IV (continuous line), grade III-IV (dotted line). **B**: Chronic graft-versus host disease. Cumulative incidence: limited plus extensive (continuous line), extensive alone (dotted line).

Figure 3. Transplant related complications. Cumulative incidence: cytomegalovirus (**5 A**), bacterial infections (**5 B**), hemorrhagic cystitis (**5 C**), central nervous system (**5 D**), fungal infections (**5 E**), veno-occlusive disease (**5 F**).

Figure 4. Probability of Overall Survival according to: (**4 A**) disease risk: standard-risk patients (n=45, continuous line), high-risk patients (n=35, dotted line); (**4 B**) year of transplant: years 2005-2007 (n=26, continuous line), years 2008-2010 (n=54, dotted line); (**4 C**) 54 patients transplanted over the years 2008-2010: standard-risk patients (n=29, continuous line), high-risk patients (n=25, dotted line);

Probability of Disease Free Survival according to: (**4 D**) disease risk: standard-risk patients (n=45, continuous line), high-risk patients (n=35, dotted line); (**4 E**) year of transplant: years 2005-2007 (n=26, continuous line), years 2008-2010 (n=54, dotted line); (**4 F**) 54 patients transplanted over the years 2008-2010: standard-risk patients (n=29, continuous line), high-risk patients (n=25, dotted line).

Figure 1

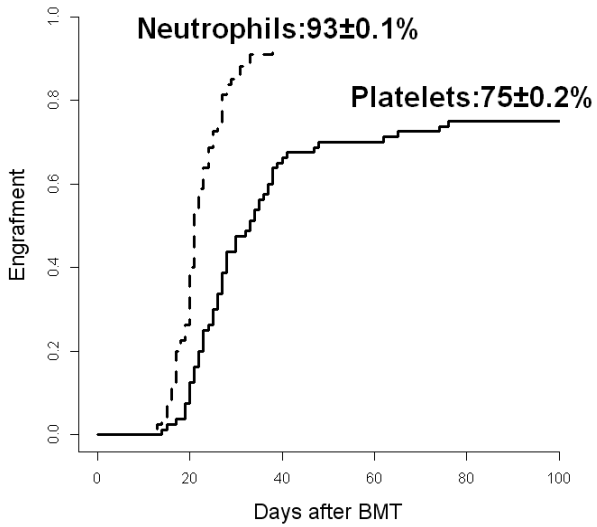

Neutrophils:93±0.1%
Platelets:75±0.2%
Engrafment
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Days after BMT

Figure 2

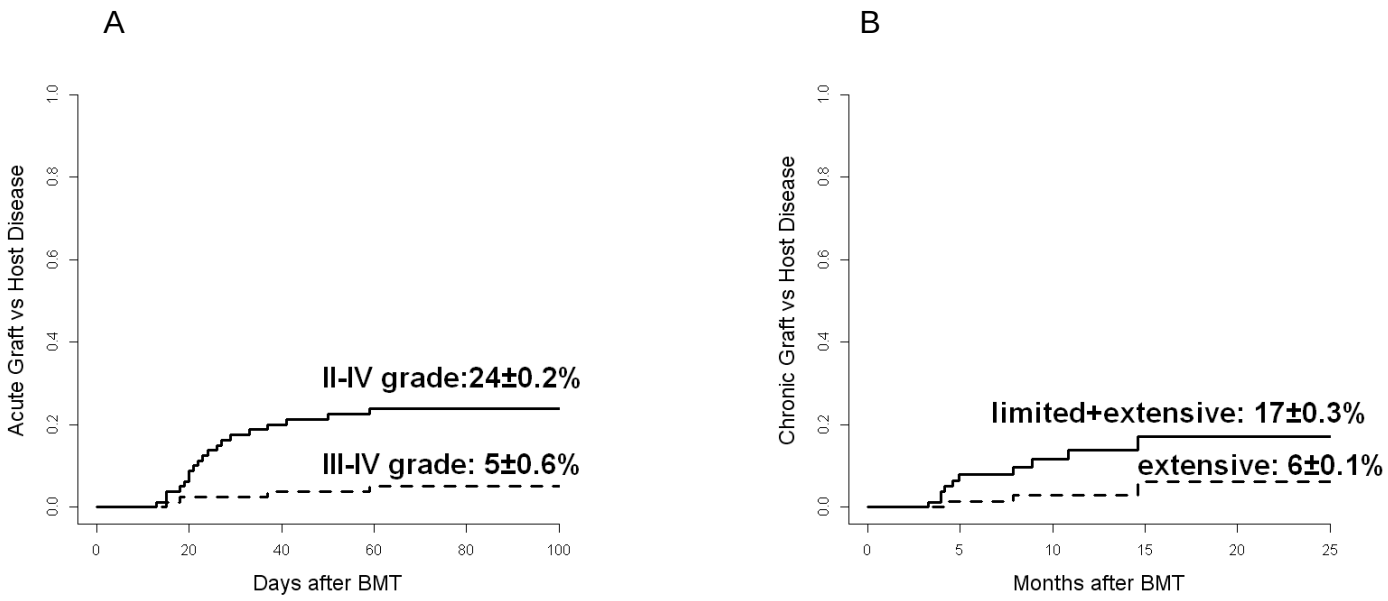

A
Acute Graft vs Host Disease
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Days after BMT
II-IV grade:24±0.2%
III-IV grade: 5±0.6%
B
Chronic Graft vs Host Disease
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
20
25
Months after BMT
limited+extensive: 17±0.3%
extensive: 6±0.1%

Figure 3

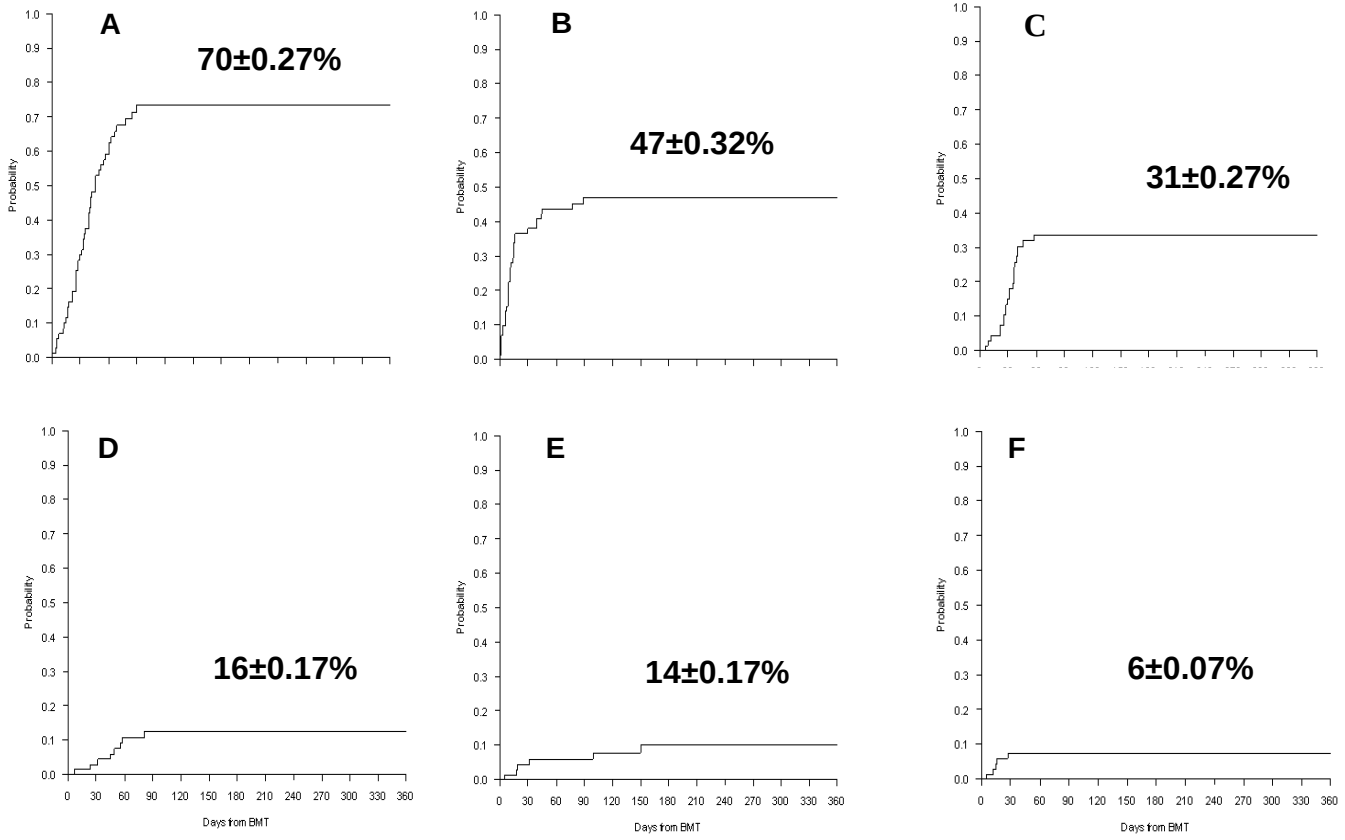
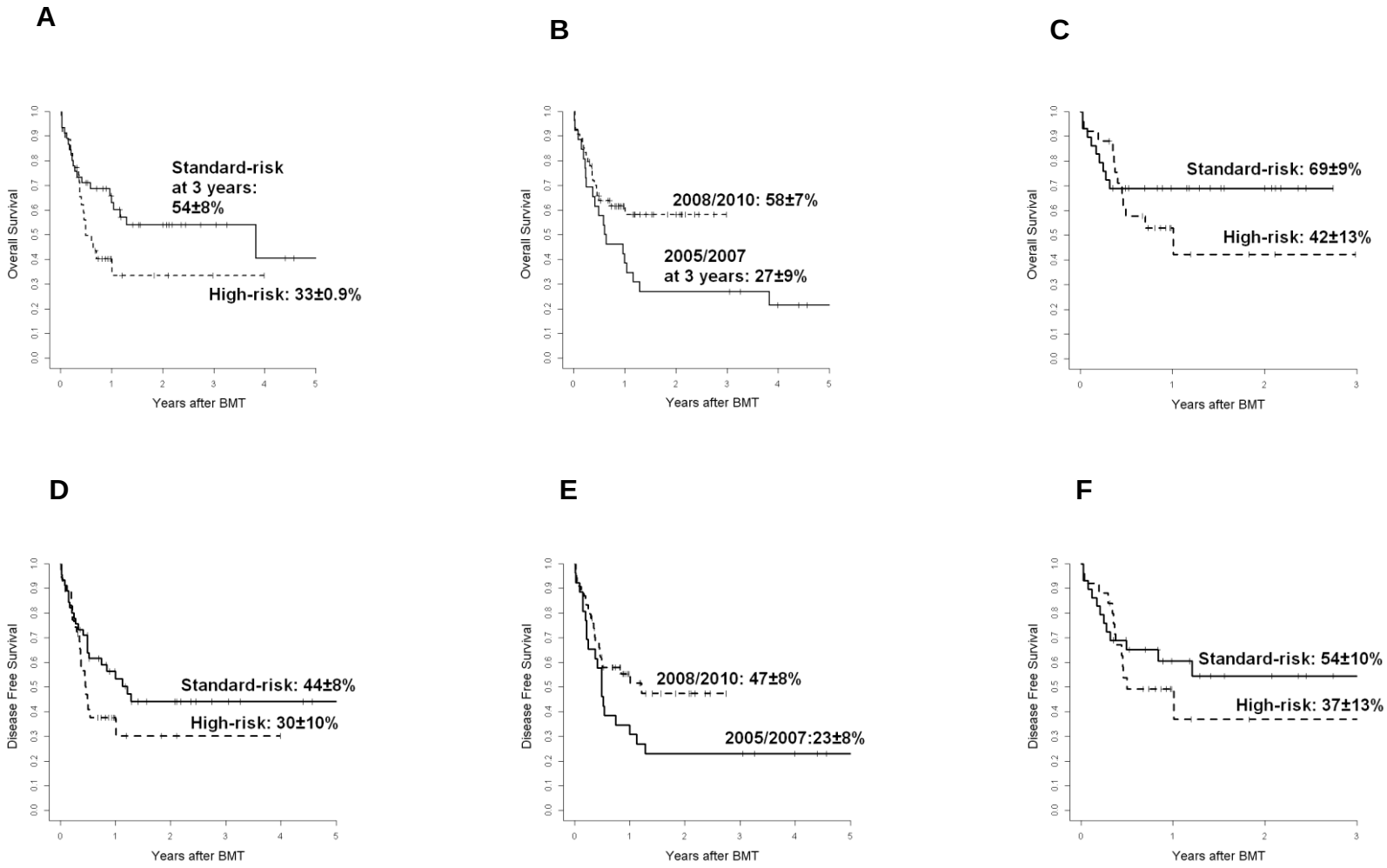

A
70±0.27%
B
47±0.32%
C
31±0.27%
D
16±0.17%
E
14±0.17%
F
6±0.07%
Probability
Days from BMT
0 30 60 90 120 150 180 210 240 270 300 330 360

Figure 4


A
Overall Survival
Standard-risk
at 3 years:
54±8%
High-risk: 33±0.9%
Years after BMT
B
Overall Survival
2008/2010: 58±7%
2005/2007
at 3 years: 27±9%
Years after BMT
C
Overall Survival
Standard-risk: 69±9%
High-risk: 42±13%
Years after BMT
D
Disease Free Survival
Standard-risk: 44±8%
High-risk: 30±10%
Years after BMT
E
Disease Free Survival
2008/2010: 47±8%
2005/2007:23±8%
Years after BMT
F
Disease Free Survival
Standard-risk: 54±10%
High-risk: 37±13%
Years after BMT