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**ORIGINAL ARTICLE**

# Haploidentical, G-CSF-primed, unmanipulated bone marrow transplantation for patients with high-risk hematological malignancies: an update

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Ninety-seven patients affected by high-risk hematological malignancies underwent G-CSF primed, unmanipulated bone marrow (BM) transplantation from a related, haploidentical donor. All patients were prepared with an identical conditioning regimen including Thiotepa, Busilvex, Fludarabine (TBF) and antithymocyte globulin given at myeloablative (MAC = 68) or reduced (reduced intensity conditioning (RIC) = 29) dose intensity and received the same GvHD prophylaxis consisting of the combination of methotrexate, cyclosporine, mycofenolate-mofetil and basiliximab. Patients were transplanted in 1st or 2nd CR (early phase: $n = 60$) or in >2nd CR or active disease (advanced phase: $n = 37$). With a median time of 21 days (range 12–38 days), the cumulative incidence (CI) of neutrophil engraftment was 94 ± 3%. The 100-day CI of III–IV grade acute GvHD and the 2-year CI of extensive chronic GvHD were 9 ± 3% and 12 ± 4%, respectively. Overall, at a median follow-up of 2.2 years (range 0.3–5.6), 44 out of 97 (45%) patients are alive in CR. The 5-year probability of overall survival (OS) and disease-free survival (DFS) for patients in early and advanced phase was 53 ± 7 vs 24 ± 8% ($P = 0.006$) and 48 ± 7 vs 22 ± 8% ($P = 0.01$), respectively. By comparing MAC with RIC patient groups, the transplant-related mortality was equivalent (36 ± 6 vs 28 ± 9%) while the relapse risk was lower for the MAC patients (22 ± 6 vs 45 ± 11%), who showed higher OS (48 ± 7 vs 29 ± 10%) and DFS (43 ± 7 vs 26 ± 10%). However, all these differences did not reach a statistical significance. In multivariate analysis, diagnosis and recipient age were significant factors for OS and DFS. In conclusion, this analysis confirms, on a longer follow-up and higher number of patients, our previous encouraging results obtained by using MAC and RIC TBF regimen as conditioning for G-CSF primed, unmanipulated BM transplantation from related, haploidentical donor in patients with high-risk hematological malignancies, lacking an HLA-identical sibling or unrelated donor and in need to be urgently transplanted.

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## INTRODUCTION

To date, following the encouraging results obtained during the last years in particular by the use of unmanipulated graft,[1–7] haploidentical hematopoietic stem cell transplant (haplo-HSCT) represents a valid alternative for patients affected by high-risk hematological malignancies who lack an HLA-identical sibling. Several retrospective studies comparing transplants from volunteer-unrelated donor, cord blood (CB) and haploidentical related donor have not shown any substantial difference in terms of outcome between the three HSC sources.[5,8–14]

Haplo-HSCT includes procedures based either on the use of T-cell depleted (TCD)[15,16] PBSC or T-cell repleted unmanipulated bone marrow (BM) combined or not with PBSC.[1,6,17–19] The main advantage of TCD is represented by the low incidence of GvHD in absence of long-lasting immunosuppressive therapy administered after transplant. However, the traditional TCD procedure using CD34 selected cells is associated with a delayed immunological recovery leading an increased risk of transplant-related mortality (TRM).[20] More recently, the introduction of partial TCD methods including the combined use of T regulatory and T conventional cells[21] or α/β T-cell depletion by maintaining the γ/δ T-cell fraction in the graft has considerably improved the post transplant immune reconstitution by preserving the anti-infective and anti-leukemia activity.[22–26] However, these last experiences are referred to a limited number of patients, mostly children,[27] and need a longer follow-up so that definitive conclusions can be drawn. Finally, TCD procedures, requiring either expensive laboratory facilities or personnel with high expertise in cell manipulation, do not allow to easily extend this practice to other transplant Centers.

On the other hand, the feasibility of unmanipulated haplo-HSCT using G-CSF primed BM alone[1,6] or in combination with PBSC[18] represents the main reason of its increasing spread to transplant Centers worldwide.

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In T-cell repleted haplo-HSCT, the GvHD prophylaxis consists of a necessarily intensive immunosuppressive therapy.[6,28] Combined with other drugs, the use of high dose post transplant cyclophosphamide (CTX) over 2 days[19,29] or the administration of basiliximab, a monoclonal anti-CD25 Ab, at days 0 and +4 after transplant[28] are both associated with favorable outcomes in the context of both myeloablative (MAC) and reduced intensity conditioning (RIC) regimen.

Rome Transplant Network (RTN), a Joint Accreditation Committee-ISCT & EBMT (JACIE) accredited metropolitan transplant program, and the transplant program of Pescara Hematologic Center promoted a transplantation protocol with G-CSF primed, unmanipulated BM for patients with high-risk hematological malignancies lacking an HLA-identical sibling, for whom neither an unrelated donor from the International Registry nor a CB unit were available in adequate time. All patients received the same GvHD prophylaxis, but at the beginning of this experience the conditioning regimens were slightly different. Over the years, we changed our general transplant policy and since the end of 2007 a unique conditioning regimen was established for any type of HSC source: HLA-identical sibling, volunteer-unrelated donor, CB and Haplo transplant. The first analysis of our pilot clinical trial was conducted on the first 80 haploidentical transplant patients receiving the same GvHD prophylaxis but different conditioning regimens.[6] The median follow-up was 1.5 years (range 0.5–6.2 years). In multivariate analysis, the use of Thiotepa, Busilvex, Fludarabine (TBF)-MAC regimen and the year of haplo-HSCT after 2007 were identified as significant favorable factors in preventing relapse and for both overall survival (OS) and disease-free survival (DFS), respectively. To prospectively confirm the encouraging results obtained in the pilot trial and to verify the indications arising from multivariate analysis, a unique conditioning regimen, TBF-MAC or RIC according to the age and/or the Sorror comorbidity index, was adopted. Overall, 134 patients including the first 80 patients previously analyzed have been transplanted from an haploidentical donor. Herein, we report the results of 97 patients with a median follow-up of 2.2 years (range 0.3–5.6 years), receiving a unique conditioning regimen (TBF-MAC = 68; TBF-RIC = 29) and an identical GvHD prophylaxis, who were transplanted with an unmanipulated BM from a haploidentical, G-CSF primed family donor.

## PATIENTS AND METHODS

From January 2008 to June 2013, 97 consecutive patients, affected by hematological malignanices, underwent G-CSF primed BM transplantation from haploidentical related donors at 2 italian transplant Centers.

Patients were selected according to the following criteria: (1) diagnosis of malignant hematological disease in active status or in CR but at high risk of progression; (2) unavailability of $\geqslant$8/10 HLA Ag-matched unrelated donor through the international registry; (3) unavailability of single unrelated CB unit matched at low (class I) and high resolution (class II) typing for 5/6 HLA Ags and containing $>3x10^7$/kg total nucleated cells (TNC) and $\geqslant 1\times10^5$/kg CD34+ cells by recipient body weight or matched for 4/6 HLA Ags and containing $>3.5\times10^7$/kg TNC and $\geqslant 2\times10^5$/kg CD34 + cells; (4) an expected interval time to transplant from an unrelated donor of $>$3 months.

Patients with $>$2 performance status according to ECOG criteria, of age $>$70 years or affected by uncontrolled infections and/or severe heart, liver, renal or psychiatric disease were considered not eligible.

The primary end points of the study were engraftment, chimerism, acute GvHD and 1-year TRM; the secondary end points were maintenance of long-term engraftment, chronic GvHD, relapse, OS and DFS. The study was approved by the institutional review board (IRB) of both Institutions. Informed consent for the treatment was obtained from all patients and donors or their legal guardians in accordance with the Declaration of Helsinki.

**Table 1.** Patient characteristics

| *Patient characteristics* (n = *97*) | |
|---|---|
| Median age, year (range) | 44 (5–67) |
| Pediatric patients $<$ 16 year, *n* (%) | 4 (4%) |
| Patients $>$55 year, *n* (%) | 20 (21%) |
| *Patient sex,* n *(%)* | |
| Male | 52 (54%) |
| Female | 45 (46%) |
| *Disease* | |
| *Early phase,* n *(status)* | 60 |
| AML | 46 (CR1 = 32; CR2 = 14) |
| ALL | 10 (CR1 = 7; CR2 = 3) |
| CML | 1 (CP1 = 1) |
| MM | 1 (CR1 = 1) |
| HL | 1 (CR2 = 1) |
| MDS | 1 (CR1 = 1) |
| *Advanced phase,* n *(status)* | 37 |
| AML | 11 (CR $>$ 2 = 2; AD = 9) |
| ALL | 4 (CR $>$ 2 = 1; AD = 3) |
| CML | 3 (CP2 = 3) |
| HL | 7 (AD = 7) |
| MFI | 2 (AD = 2) |
| MDS | 3 (CR $>$ 2 = 1; AD = 2) |
| NHL | 6 (AD = 6) |
| Plasm. Leuk. | 1 (AD = 1) |
| *Malignancies,* n *(%)* | |
| Myeloid | 67 (69%) |
| Lymphoid | 30 (31%) |
| *Previous transplant,* n *(%)* | |
| Autologous | 22 (23%) |
| Allogeneic | 2 (2%) |

Abbreviations: AD = active disease; CP = chronic phase; HL = Hodgkin Lymphoma; MDS = myelodisplastic syndrome; MFI = myelofibrosis; MM = multiple myeloma; NHL = Non-Hodgkin Lymphoma; Plasm. Leuk. = plasma cell leukemia.

### Patients

Patients had a median age of 44 years (range 5–67 years) and 20 of them were $>$55 years. Patients in first or second CR were considered in early phase at time of transplant, while patients in $>$2 CR, PR or with active/resistant disease were considered in advanced phase. The majority of patients ($n$ = 71) were affected by acute leukemia, most of them by AML ($n$ = 57). For AML patients transplanted in CR1, high-risk factors were: refractoriness to first line chemotherapy, secondary leukemia, complex karyotype, FLT-3/ITD positivity and persistence of minimal residual disease after consolidation, while high-risk factors for ALL transplanted in CR1 were: refractoriness to first line chemotherapy, Ph positivity and hyperleukocytosis. Overall, 60 (62%) and 37 (38%) patients underwent haplo-HSCT in early or advanced phase, respectively. Twenty-four (25%) of the cases had received a previous transplant as autologous ($n$ = 22) or allogeneic ($n$ = 2). Further details concerning underlying disease and patient characteristics are described in Table 1.

The median time between diagnosis and haplo-HSCT was 11 months (range 4–119), while the median time between the start-up of allogeneic donor search and haplo-transplant was 5.3 months (range 1.9–46.7).

### Donors

Donors eligibility was independently evaluated by transplant and blood bank physicians, according to the JACIE criteria. In case of multiple available donors, the mother aged $\leqslant$70 years without comorbidity contraindicating BM collection and/or G-CSF administration had priority. The youngest male adult donor within the family represent the second choice. Donor/recipient CMV status and ABO matching were also considered for donor selection. Before the BM harvesting, two autologous blood transfusions of the donor were collected and stored.

**Table 2.** Donor/recipient characteristics

| *Donor/recipient characteristics* | |
|---|---|
| Median donor age, year (range) | 41 (18–70) |
| *Patient sex,* n *(%)* | |
| Male | 56 (58%) |
| Female | 41 (42%) |
| *Donor/recipient kinship,* n *(%)* | |
| Sibling | 38 (39%) |
| Offspring | 32 (33%) |
| Mother | 22 (23%) |
| Father | 5 (5%) |
| *Donor/recipient sex,* n *(%)* | |
| Male–male | 30 (31%) |
| Male–female | 26 (27%) |
| Female–male | 22 (23%) |
| Female–female | 19 (19%) |
| *Donor/recipient CMV serostatus,* n *(%)* | |
| Negative–negative | 5 (5%) |
| Negative–positive | 14( 14%) |
| Positive–negative | 8 (8%) |
| Positive–positive | 70 (72%) |
| *Donor/recipient AB0 match,* n *(%)* | |
| Minor mismatched | 20 (21%) |
| Major mismatched | 25 (26%) |
| Matched | 52 (53%) |
| *HLA-A, B, DRB1 mismatched Ags,* n *(%)* | |
| 2 | 37 (38%) |
| 3 | 60 (62%) |

Donors (male, 58%) had a median age of 41 years (range18–70 years) and in order were represented by sibling (39%), offspring (33%), mother (23%) and father (5%). The donor/recipient combinations were female to male in 23%, negative to CMV positive in 14% with a pair's CMV negativity occurring in only 5% of cases and AB0 minor and major incompatibility in 21% and 26%, respectively. The HLA-A, B, DRB1, DQB1, DPB1 and C loci were determined by at least intermediate-resolution DNA typing, in all cases. All donors were HLA-identical for one haplotype and mismatched for 2 ($n=37$, 38%) or 3 ($n=60$, 62%) A, B, DR loci on the unshared haplotype (Table 2).

## Conditioning regimen

An identical chemotherapy-based conditioning regimen consisting of a combination of TBF was adopted.[30] It was administered according to a MAC schedule (TBF-MAC: thiotepa 5 mg/kg per day at days −7and −6, busulfan 3.2 mg/kg per day in a single IV infusion over 3 h and fludarabine 50 mg/m$^2$ per day IV in 1 h at days −5, −4 and −3) or at reduced intensity (TBF-RIC) by deleting one dose of thiotepa and busulfan, respectively. The conditioning regimen included the antithymocyte globulin (ATG-Fresenius, Neovii Biotech GmbH, Grafelfing, Germany) given at dose of 5 mg/kg per day on days −4 through −1. Overall, 68 patients (70%) were conditioned with TBF-MAC and 29 (30%) with TBF-RIC.

## GvHD prophylaxis

Regardless of the conditioning regimen (RIC or MAC), the GvHD prophylaxis was identical for all the patients and consisted of five drugs combination: (1) pre-transplant antithymocyte globulin; (2) cyclosporine given by continuous IV infusion at 1.5 mg/kg per day from day −7 to −2 and increased to 3 mg/kg per day from day −1 until oral intake at 5–6 mg/kg per day in two daily doses. The cyclosporine dose was adjusted on the basis of plasma levels (150–350 ng/ml), hepatic and renal toxicity. From day +180, cyclosporine was weekly tapered by 5% of the dose until discontinuation; (3) IV methotrexate was administered at 15 mg/m$^2$ on day +1 and at 10 mg/m$^2$ on day 3, 6 and 11; (4) basiliximab (Simulect, Novartis Pharma AG, Basle, Switzerland), an anti-CD25 monoclonal Ab, given as 30 min IV infusion on day 0 (2 h before graft infusion) and on day +4 at a fixed dose of 20 or 10 mg according to the patient body wt, respectively, exceeding or less than 35 kg; (5) mycophenolate-mofetil, administered orally at 15 mg/kg per day in 2 daily doses from day +7 to day +100.

## BM harvest

All donors were primed with 4 μg/kg per day granulocyte-CSF given as single SC injection for 7 consecutive days, from −7 to −1. On day 0, BM was harvested from the posterior iliac crests for a target vol of 15–20 ml/kg donor body wt. Fresh and unmanipulated BM cells were infused into the recipient on the same day.

## Evaluation of engraftment and donor chimerism

Myeloid engraftment was defined as the first of 3 consecutive days with an ANC $\geqslant 0.5\times10^9$/L, whereas platelet engraftment was defined as the day with a platelet $\geqslant 20\times10^9$/L in absence of transfusion support for a week. Hematopoietic chimerism was evaluated by cytogenetic G-banding or FISH for sex mismatched patient–donor pairs or by PCR-based analyses of polymorphic microsatellite regions by STR for sex matched pairs, using peripheral blood samples from the donors and recipients. After haplo-HSCT, recipient BM samples were drawn monthly for the first 3 months and every 3–6 months for the additional 1–2 years.

Primary graft failure was defined as the absence of hematological recovery in patients surviving >21 days with no evidence of myeloid donor cells in recipient's BM at day 28 after transplantation.

## Supportive therapy and Infection prophylaxis

All patients were hospitalized in rooms with HEPA air filter and received antinfectious prophylaxis with oral trimethoprim–sulfamethoxazole pre-haplo-HSCT from day −10 to −2 and from the hematopoietic recovery until the achievement of CD4+ T-cell counts $>200$–$400\times10^6$/L; (2) fluconazole from day −10 to day +100; (3) acyclovir from day −1 to immunological recovery; (4) ciprofloxacin from day −1. All blood products were irradiated with 2500 cGy. Granulocyte-colony stimulating factor (filgastrim or lenograstim) was administered from day +1 until the achievement of a sustained and durable PMN engraftment. CMV and Epstein Barr virus were regularly monitored in the blood by PCR assays.

## Definitions

The incidence of acute and chronic GvHD were evaluated in all patients with evidence of engraftment or surviving >100 days and they were classified by Glucksberg criteria[31,32] or Seattle-National Institutes of Health (NIH) criteria,[33] respectively. TRM was defined as death from any cause except relapse. Relapse was assessed by molecular, cytogenetic or morphological evidence of the original hematological disease in peripheral blood, BM or any extramedullary site. OS and DFS were defined as time to death from all causes and time to relapse or death in remission, respectively.

## Statistical analysis

The data from clinical assessments were summarized using descriptive techniques, including mean, median, SD, range, minimum and maximum value for continuous variables, absolute and relative frequencies for categorical variables.

Using parametric and non-parametric statistical procedures ($X^2$-test, Fisher exact test and rank correlation coefficient of Spearman), the possible interdependence between two or more variables was evaluated. For all statistics a $P$-value of $<0.05$ was considered as statistically significant.

The cumulative incidence (CI) of neutrophil and platelet engraftment, acute and chronic GvHD, TRM and disease relapse were estimated with competing risk analysis,[34] considering relapse or TRM as competing events for engraftment and acute or chronic GvHD. Relapse and TRM were considered as reciprocal competing risks. The curves of various subgroups were compared using the Gray test.[35] DFS and OS curves were estimated and plotted by the Kaplan–Meier product-limit method[36] and significant differences were tested using the log-rank test.[37]

The Cox proportional hazard model[38] was applied to investigate the multivariate effect on OS, DFS, TRM and relapse of all the variables object of the study. All the analysis were conducted using software SAS 9.3.1 (SAS

Institute Inc., Cary, NC, USA) and R version 2.15.0 (Free Software Foundation's GNU General Public License).

## RESULTS

### Graft composition

The median dose of TNC, CD34+ and CD3+ cells infused were: 7.4 x$10^8$/kg (range 2–29), $2\times10^6$/kg (range 0.6-11) and $3\times10^7$/kg (range 0.9–17), respectively. No side effect related to donor rh-G-CSF priming and/or BM harvesting was observed.

### Engraftment

The 100-day CI of neutrophil and platelet engraftment was 94 ± 3% and 84 ± 4%, respectively (Figure 1), with a median time of 20 days (range 12–38) for absolute neutrophils count and 27 days (range 14–180) for platelets. At day 60, a full donor chimerism was detected in all evaluable patients. No significant difference was observed between patients receiving MAC or RIC conditioning regimen.

### Acute and chronic GvHD

Overall, aGvHD was absent in 41 (48%) out of 86 evaluable patients, while it was of grade I in 12 (14%), grade II in 22 (26%), grade III in 4 (5%) and grade IV in 7 (8%) patients. The median time to aGvHD was 26 days (range, 8–170) with a 100-day CI of grade II–IV and III–IV of 31 ± 5% and 9 ± 3%, respectively (Figure 2a).

No signs of c-GvHD were observed in 63 (81%) out of 78 evaluable patients, of whom 9 (11%) and 6 (8%) developed a limited and extensive form of c-GvHD, respectively, at a median time of 258 days (range 60–660) after transplant. The CI of overall and only extensive c-GvHD at 2 years was 25 ± 6% and 12 ± 4%, respectively (Figure 2b).

### TRM and complications

Overall, 31 patients (32%), 14 (23%) of 60 in early and 17 (46%) of 37 in advanced disease status, died of transplant-related complications at a median of 76 days (range 9–527). The infections were the main cause of TRM accounting for 48% of all deaths. Most of the events occurred within 6 months after transplant with a CI of TRM for all patients of 20 ± 4% at 100 days, 30 ± 5% at 6 months, 31 ± 5% at 1 year and 34 ± 5% at 5 years. TRM was significantly lower for patients who received haplo-HSCT in early phase of disease with respect to patients transplanted in advanced phase: 13 ± 4 vs 29 ± 8% at 100 days ($P = 0.048$), 22 ± 5 vs 41 ± 8% at 6 months ($P = 0.046$) and 25 ± 6 vs 49 ± 8% at 5 years ($P = 0.02$) (Figure 3). No statistical difference was found in terms of 5-year CI of TRM between patients conditioned with TBF-RIC and those prepared with TBF-MAC regimen: 28 ± 9 vs 36 ± 6% ($P$ = NS). The multivariate analysis did not show any factor significantly affecting TRM.

### Relapse

Overall, the CI of relapse was 14 ± 4% at 6 months, 19 ± 5% at 1 year and 30 ± 5% at 5 years from transplantation. The relapse occurred after a median time of 180 days (range 27–1217) in 23 patients, 12 of whom transplanted in early and 11 in advanced disease status. No significant difference of relapse rate was observed among patients conditioned with TBF-MAC or RIC. However, although the difference was not statistically significant, the 5-year CI of relapse was remarkably lower for patients

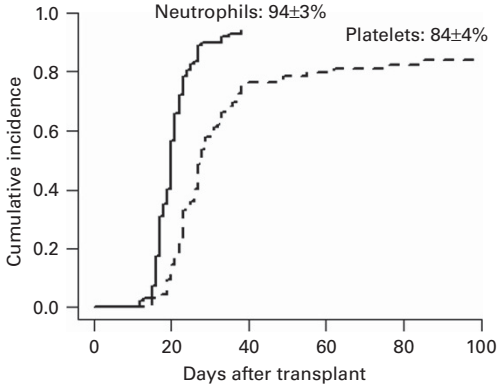


**Figure 1.** Engraftment. CI for neutrophils (continuous line) and platelets (dotted line).

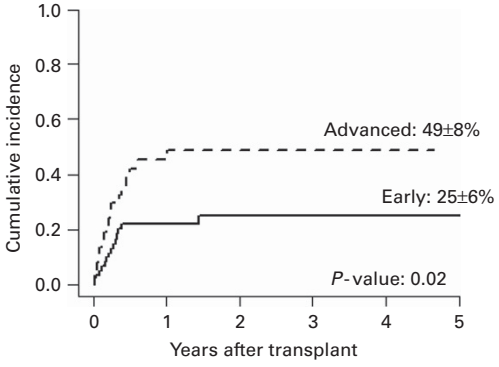


**Figure 3.** Transplant-related mortality. CI of TRM according to the disease status at transplant: patients in early phase ($n = 60$, continuous line) and patients in advanced phase ($n = 37$, dotted line).

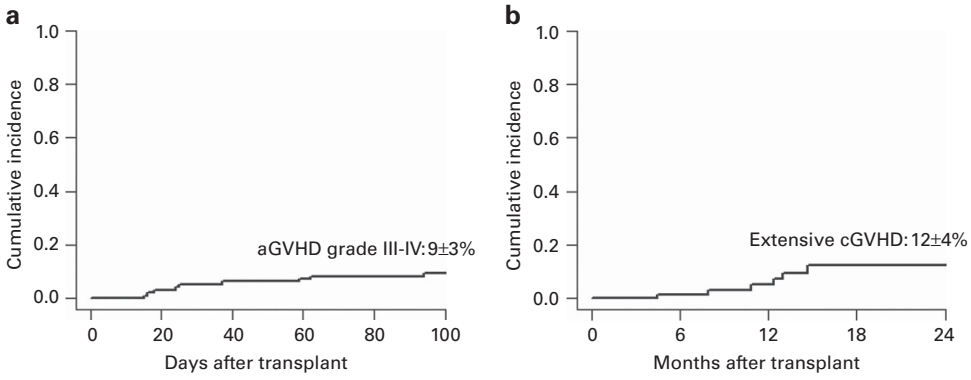


**Figure 2.** Acute and chronic GvHD. (**a**) CI of grade III–IV acute GvHD. (**b**) CI of extensive chronic GvHD.

receiving MAC than for those receiving RIC (22 ± 6 vs 45 ± 10%, $P$ = NS). The 5-year CI of relapse was not significantly different between patients who received haplo-HSCT in early or advanced phase of disease (26 ± 7 vs 36 ± 9%, $P$ = NS) (Figure 4). These data were confirmed on the cohort of 68 patients conditioned with only TBF-MAC regimen (18 ± 7 vs 27 ± 10%, $P$ = NS).

Sixteen (70%) out of 23 relapses occurred in the BM, while 5 (22%) were extramedullary and 2 (8%) occurred in both BM and extramedullary site. Among the 23 relapsed patients, 15 died of disease progression, 3 of treatment-related complications, 1 patient died in CR of unknown cause and 4 patients are currently alive. Among the four surviving patients, three are in CR (one after donor lymphocyte infusion and two after a 2nd haplo-HSCT from different donor) at 24, 34 and 44 months from relapse, respectively, and one patient has stable disease at 5 months after relapse. As for the TRM, multivariate analysis for relapse, has not identified any statistically significant factor.

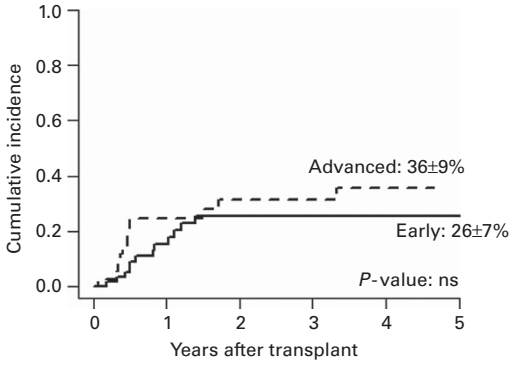


**Figure 4.** Relapse. CI of Relapse according to the disease status at transplant: patients in early phase ($n$ = 60, continuous line) and patients in advanced phase ($n$ = 37, dotted line).

## OS and DFS

The 5-year probability of OS was 42 ± 6% significantly higher for patients transplanted in early vs those transplanted in advanced phase of disease (53 ± 7 vs 24 ± 8%, $P$ = 0.006) (Figure 5a). When the analysis was restricted to patients conditioned with TBF-MAC, the superiority of OS remained significant for patients transplanted in early phase, (64 ± 8 vs 25 ± 10%, $P$ = 0.0008) (Figure 5b). Although not statistically different, the 5-year probability of OS was better for patients conditioned with TBF-MAC rather than for those who received TBF-RIC (48 ± 7 vs 29 ± 10%, $P$ = NS).

The 5-year probability of DFS was 37 ± 6% for all patients: 48 ± 7% for the early and 22 ± 8% for advanced disease status ($P$ = 0.01) (Figure 6a). DFS was higher for the 43 patients transplanted in early phase following TBF-MAC regimen than for the 25 patients in advanced phase at transplant (55 ± 9 vs 25 ± 10%, $P$ = 0.02) (Figure 6b). Moreover, the DFS was better, although not statistically significant, for patients treated with TBF-

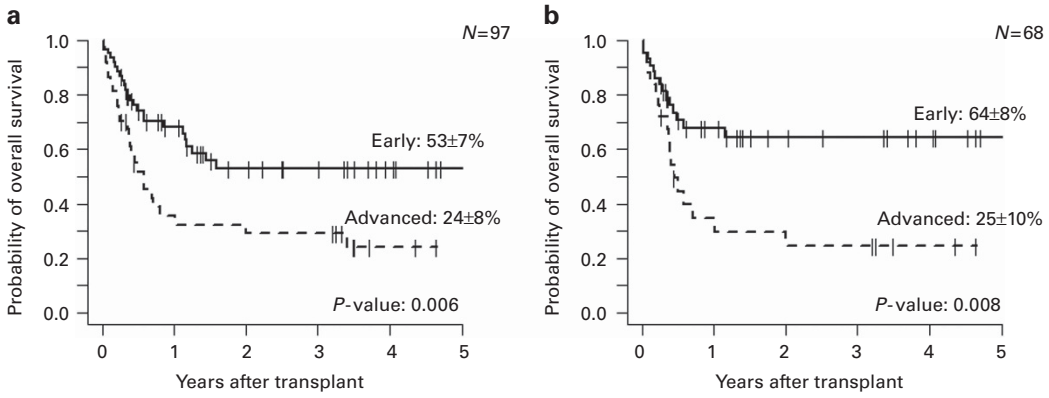


**Figure 5.** Overall survival. (**a**) Probability of OS according to the disease status at transplant: patients in early phase ($n$ = 60, continuous line) and patients in advanced phase ($n$ = 37, dotted line). (**b**) Probability of OS in TBF-MAC group according to the disease status at transplant: patients in early phase ($n$ = 43, continuous line) and patients in advanced phase ($n$ = 25, dotted line).

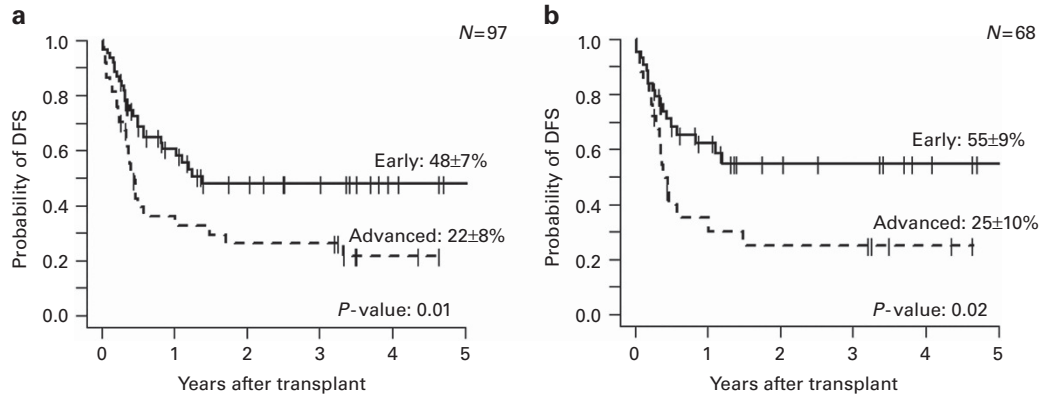


**Figure 6.** Disease free survival. (**a**) Probability of DFS according to the disease status at transplant: patients in early phase ($n$ = 60, continuous line) and patients in advanced phase ($n$ = 37, dotted line). (**b**) Probability of DFS in TBF-MAC group according to the disease status at transplant: patients in early phase ($n$ = 43, continuous line) and patients in advanced phase ($n$ = 25, dotted line).

**Table 3.** Multivariate analysis of OS and DFS

| *Parameter* | P-*value* | *Hazard ratio* | *95% HR CI* | |
|---|---|---|---|---|
| *Analysis of maximum likelihood estimates for OS* | | | | |
| Recipient age (continuous) | 0.0212 | 1.025 | 1.004 | 1.046 |
| Diagnosis, myeloid vs lymphoid | 0.0025 | 2.711 | 1.420 | 5.175 |
| Disease status, early vs advanced | 0.0672 | 1.724 | 0.962 | 3.088 |
| *Analysis of maximum likelihood estimates for DFS* | | | | |
| Recipient age (continuous) | 0.0175 | 1.025 | 1.004 | 1.046 |
| Diagnosis, myeloid vs lymphoid | 0.0036 | 2.629 | 1.371 | 5.043 |
| Disease status, early vs advanced | 0.1030 | 1.599 | 0.910 | 2.810 |

Abbreviations: CI = confidence interval; DFS = disease-free survival; HR = hazard ratio; OS = overall survival.

MAC than for patients prepared with TBF-RIC (43 ± 7 vs 26 ± 10%, $P$ = NS).

OS and DFS were remarkably, but not significantly, higher for patients aged ⩽44 years (OS: 52 ± 7 vs 25 ± 10%, $P = 0.10$; DFS: for OS 48 ± 7 vs 19 ± 9%, $P = 0.068$).

In multivariate analysis (Table 3), the variables significantly affecting both OS and DFS were: recipient age, as continuous variable ($P = 0.0212$ for OS and $P = 0.0175$ for DFS), and myeloid vs lymphoid disorder ($P = 0.0025$ for OS and $P = 0.0036$ for DFS). The disease status at transplant (early vs advanced phase) was not statistically significant ($P = 0.0672$ for OS and $P$-value = 0.1030 for DFS). However, patients in advanced stage of disease had mortality risk 1.724 higher in respect to patients in early phase (95% confidence interval (95% CI) = 0.962–3.088) and a risk 1.599 higher in terms of DFS (95%CI = 0.910–2.810).

## DISCUSSION

Transplantation of hematopoietic stem cells from partially matched family donors is a promising therapy for patients with high-risk hematological malignancy. Following the indication provided by the previous analysis of results on the first 80 patients,[6] our experience with haploidentical, unmanipulated BM transplantation was carried on with the enrollment of a total of 134 patients. Of these 134 patients, 97 received a G-CSF primed, haploidentical, unmanipulated BM transplant following a uniform, MAC or RIC regimen and an identical GvHD prophylaxis. The present report focused the transplant results on these last patients.

Despite the use of TBF-RIC in presence of two to three HLA-mismatched Ags with the donor, no primary graft failure occurred and a stable engraftment with full donor chimerism similar to those obtained using TBF-MAC was achieved within 30 days in all evaluable patients. This result is particularly encouraging if we consider the 13% graft failure reported by the Baltimore group in haploidentical, unmanipulated BM recipients prepared with a RIC regimen consisting of CTX, fludarabine and 2 Gy TBI association and aGvHD prophylaxis including high dose CTX given after graft.[19] We confirm the low incidence of acute and, on a considerably longer follow-up, of chronic GvHD with most patients surviving 1 year after bone marrow transplantation coming back to their full social and work activity.

Most patients in advanced phase at time of transplant had an active disease and had been heavily pretreated, so the high rate of TRM observed in this population is not surprising, but it leads to recommend a more careful selection of patients at highest risk of transplant mortality. Among the patients transplanted in early disease phase, the TRM mainly due to infection complications was significantly lower and occurred in most of cases within 6 months after transplant. As suggested by a matched pair analysis recently produced,[39] where we compared HLA-identical sibling with haploidentical transplants (data not shown), a more aggressive and stringent antinfectious policy directed in particular against CMV reactivation is required during the early period after haploidentical transplant.

Although the CI of relapse was not statistically different between patients transplanted in early and advanced disease phase or between recipients TBF-MAC or TBF-RIC and no significant factor was found in multivariate analysis, the risk of relapse was remarkably lower for patients transplanted in early phase or conditioned with the TBF-MAC regimen.

Taking into account the longer follow-up of our patients, the relapse rate is well comparable with that reported by Raiola *et al*.[7] for patients receiving an identical TBF-MAC regimen and aGvHD prophylaxis including high dose post-transplant CTX. In light of the recently reported observation on the concomitant loss of the unshared haplotype in a substantial proportion of relapsing patients,[40,41] the immuno-biological mechanism of relapse occurring after unmanipulated, haploidentical transplant needs to be better understood. However, Zeidan *et al*.[42] have recently reported a 30% successful rate of durable responses achieved in 40 relapsing patients by using escalating dose of donor lymphocyte infusions. Such results are particularly encouraging and lead to plan a careful monitoring of minimal residual disease after transplant with a view of an early pre-emptive donor lymphocyte infusion therapy, which is better guaranteed for the prompt donor availability otherwise than in volunteer-unrelated donor or CB transplant setting.

In multivariate analysis, the younger recipient age and the myeloid nature of the hematological disorders resulted in significant favorable factors related to either OS or DFS. Although in the Cox model the early disease phase did not rich the level of statistically significance and TBF-MAC did not enter into the model, either factors favorably affected both OS and DFS.

In conclusion, from our updated analysis on patients receiving a uniform conditioning regimen and an identical GvHD prophylaxis including the monoclonal anti-CD25 monoclonal Ab Basiliximab, we can confirm on a long follow-up that the G-CSF primed, unmanipulated BM 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. This transplant procedure enables to save the relevant costs related to the search for the graft acquisition from other sources and, avoiding expensive laboratory facilities and personnel with high expertise in cell manipulation, can be worldwide extended to transplant centers. To date, it is mandatory to include haploidentical transplant in the algorithm of search for an alternative donor.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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