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Clofarabine and Treosulfan as Conditioning for Matched Related and Unrelated Hematopoietic Stem Cell Transplantation: Results from the Clo3o Phase II Trial

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can be curative for patients with hematologic malignancies. The ideal conditioning regimen before allo-HSCT has not been established. We conducted a Phase II study to evaluate the tolerability and efficacy of clofarabine and treosulfan as conditioning regimen before allo-HSCT. The primary objective was to evaluate the cumulative incidence of nonrelapse mortality (NRM) on day +100. Forty-four patients (36 with acute myelogenous leukemia, 5 with acute lymphoblastic leukemia, 3 with myelodysplastic syndromes) were enrolled. The median patient age was 47 years, and the median duration of follow-up was 27 months. The conditioning regimen was based on clofarabine 40 mg/m^2 (days -6 to -2) and treosulfan 14 g/m^2 (days -6 to -4). Allogeneic hematopoietic stem cells were derived from a sibling (n = 22) or a well-matched unrelated donor (n = 22). Graft-versus-host disease (GVHD) prophylaxis consisted of antithymocyte globulin, rituximab, cyclosporine, and a short-course of methotrexate. The regimen allowed for rapid engraftment and a 100-day NRM of 18%, due mainly to bacterial infections. The incidences of grade II-IV acute GVHD and chronic GVHD were 16% and 19%, respectively. The rates of overall survival (OS), progression-free survival, and relapse at 2 years were 51%, 31%, and 50%, respectively. Significantly different outcomes were observed between patients with low-intermediate and patients with high-very high Disease Risk Index (DRI) scores (1-year OS, 78% and 24%, respectively). Our findings show that the use of treosulfan and clofarabine as a conditioning regimen for allo-HSCT is feasible, with a 78% 1-year OS in patients with a low-intermediate DRI score. However, 1-year NRM was 18%, and despite the intensified conditioning regimen, relapse incidence remains a major issue in patients with poor prognostic risk factors.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative option for patients with hematologic malignancies [1,2]. The ideal conditioning regimen before allogeneic HSCT has not yet been established. Reduced-intensity conditioning (RIC) regimens emerged more than 15 years ago with the aim of decreasing the toxicity and morbidity related to HSCT [3,4]. Unfortunately, the trade-off for RIC has been an increase in disease recurrence and a high incidence of chronic graft-versus-host disease (cGVHD), with its considerable impact on late nonrelapse mortality (NRM) and quality of life [5,6]. Progressively, the concept of RIC switched to the concept of reduced-toxicity conditioning, based on the combination of fludarabine and an alkylating agent, which currently represent the backbone of conditioning regimens for HSCT performed worldwide.

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Treosulfan is a water-soluble bifunctional alkylating cytotoxic agent often considered as an alternative agent to busulfan in conditioning regimens and characterized by a low nonhematologic toxicity profile, broad stem cell toxicity, and immunosuppressive as well as antileukemic activity. In the last decade, the combination of treosulfan and fludarabine proved to be feasible and efficient in several types of malignancies, including acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) [7–10].

Clofarabine is a second-generation purine nucleoside analog that requires intracellular phosphorylation for activation and is resistant to deamination. Along with inhibiting DNA polymerase, clofarabine also acts as an inhibitor of cellular ribonucleotide reductase. Clofarabine has significant documented antileukemic activity, particularly in patients with relapsed acute lymphoblastic leukemia (ALL) [11], and it is approved for treating pediatric ALL patients after at least 2 previous regimens. Clofarabine also has been studied in patients with relapsed AML [12]. Direct induction of apoptosis by activation of caspase 9 and direct interaction with the mitochondrial membrane may also play a role in this superior antileukemic effect. Hand-foot syndrome and reversible liver function abnormalities are the 2 main adverse events of the drug described in the literature [13].

With the aim of identifying a better reduced-toxicity conditioning regimen, in terms of both efficacy and tolerability, we investigated a novel combination of drugs. Here we present the results of a prospective, multicenter Phase II trial (Clotreo; EudraCT 2008-006972-31; ethics approval, November 27, 2008; first enrollment November 23, 2009) that evaluated the use of a conditioning regimen with clofarabine replacing fludarabine, in combination with treosulfan and antithymocyte globulin, in 44 patients with acute leukemia or high-risk MDS.

METHODS

Patients and Donors

This prospective Phase II study was conducted in 4 Italian bone marrow transplantation centers: Milan, Udine, Torino, and Bolzano. The study was approved by the Institutional Review Board of each participating center. Both pediatric and adult patients were included (age range, 1-70 years), each with an available HLA-matched related or unrelated donor. HLA compatibility among donor-recipient pairs was assessed by 10-loci molecular typing (HLA-A, -B, -C, -DRB1, -DQB1), with no more than a 2-allele disparity allowed.

Additional eligibility criteria included creatinine clearance >50 mL/minute, alanine aminotransferase ≤2.5 times the upper limit of normal, Karnofsky Performance Status ≥80%, and Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) score <4 according to Sorror et al [14]. Patients who had undergone previous HSCT were excluded. Between November 2009 and November 2013, we enrolled 44 patients (median age, 47 years), including 36 with AML, 5 with ALL, and 3 with MDS. Comorbidities at time of transplantation were evaluated according to the HCT-CI. Patients were stratified by disease type and status at the time of transplantation, according to the Disease Risk Index (DRI), as validated by Armand et al [15]; 27 patients were in the low-intermediate risk group, and the other 17 were in the high-very high risk group.

Conditioning Regimen and GVHD Prophylaxis

For conditioning, all patients received treosulfan 14 g/m^2 for 3 days (days -6 to -4) and clofarabine 40 mg/m^2 for 5 days (days -6 to -2).

GVHD prophylaxis was investigational and consisted of in vivo T cell depletion using Thymoglobulin (Sanofi Genzyme, Cambridge, MA) for 3 days (days -4 to -2) at 2 different dosages according to HLA matching: 1.5 mg/kg/day for patients with a 10/10-matched donor and 2.5 mg/kg/day for patient-donor pairs with any mismatch. All patients received a single dose of rituximab at 200 mg/m^2 on day -1 for in vivo B cell depletion, as prophylaxis against GVHD and post-transplantation lymphoproliferative disorder; cyclosporine from day -1 (to a target plasma level of 150-250 ng/mL) and short-course methotrexate (15 mg/m^2 on day +1, 10 mg/m^2 on days +3 and +6) with folinic acid rescue were also used for GVHD prophylaxis.

In the absence of GVHD or disease relapse, cyclosporine was tapered to discontinuation, starting at month +3 after HSCT in patients with a high-very high DRI and at month +6 in patients with low-intermediate DRI, with the aim of maximally exploiting the graft-versus-leukemia effect exerted by the donor's immune system, selectively in those patients with the greatest probability of disease recurrence.

Donor Graft

Peripheral blood stem cells were obtained from donors using standard mobilization protocols and apheresis techniques. A median of 6.0×10^6 CD34$^+$ cells/kg (range, 1.3-14.4 $\times$ 10^6 CD34$^+$ cells/kg) were infused. If peripheral blood mobilization was not possible and in all pediatric patients, bone marrow (BM) was the stem cell source.

Supportive Care

Microbial, fungal and viral prevention, together with the treatment of infectious complications were performed according to institutional transplant guidelines, following international recommendations [16–18]. Allogeneic recipients have been screened for the presence of cytomegalovirus (CMV) in peripheral blood samples 1 time/week from HSCT to at least 100 days after HSCT. Diagnostic tests to determine the need for preemptive treatment included the detection of CMV DNA by quantitative polymerase chain reaction (PCR). Ganciclovir was used first-line for preemptive treatment of CMV. For prevention of Epstein-Barr virus (EBV)-related post-transplantation lymphoproliferative disease, patients were monitored every 2 weeks for EBV DNA load using a blood EBV PCR assay. Testing for galactomannan in serum was performed weekly using PCR-based diagnostics.

Acute GVHD (aGVHD) was graded according to consensus criteria [19], and cGVHD was classified according to National Institutes of Health criteria [20]. GVHD was treated according to institutional protocols, with consideration of the European Society for Blood and Marrow Transplantation-European LeukemiaNet recommendations [21].

Evaluation of Response

Neutrophil engraftment was defined as a neutrophil count ≥.5 $\times$ 10^9/L for more than 3 consecutive days; platelet engraftment was defined as a platelet count ≥20 $\times$ 10^9/L for more than 3 consecutive days in the absence of transfusions. Toxicity after allo-HSCT was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3. Post-transplantation disease follow-up comprised monthly marrow evaluations for the first 3 months after HSCT, and every 3 months thereafter for the first year. Response and relapse were determined by standard hematologic criteria. Hematopoietic chimerism was assessed on BM aspirate samples by in-parallel short-tandem repeats.

Statistical Analysis

Primary objective was evaluation of the cumulative incidence of NRM on day +100 to assess the feasibility of this regimen. According to Simon's 2-stage design method, the total number of patients needed to assess an expected NRM of 20% was 45. Categorical variables are expressed as proportions, and continuous variables are expressed as median and range. Comparisons between groups were performed using the chi-square test for categorical variables and the Mann-Whitney *U*test for continuous variables. Outcomes were calculated from the date of transplantation. Progression-free survival (PFS) was defined as the probability of being alive and progression-free at any time; NRM was defined as death without evidence of disease progression or relapse. Disease progression or relapse was treated as a competing event in the NRM analyses. The incidence rates of aGVHD and cGVHD were estimated considering disease progression or relapse as a competing event. Only patients alive at day +100 after transplantation were evaluated for cGVHD. The cumulative incidence of engraftment was calculated using death before engraftment as a competing event. The Kaplan-Meier method was used for survival analyses, hazard ratios were estimated with their respective 95% confidence intervals (CIs). The cumulative incidence method with competing risks was used for the NRM, relapse/progression, GVHD and engraftment analyses. *P* values ≤ .05 were considered significant. All the outcomes were calculated on an intention-to-treat basis. The statistical software packages SPSS version 16.0 (SPSS, Chicago, IL) and R version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria) were used.

RESULTS

Between November 2009 and November 2013, we enrolled 44 patients (median age, 47 years), including 36 with AML, 5 with acute lymphoblastic leukemia, and 3 with MDS. Observation ended in March 2015; 4 patients were lost to follow-up. Patients were enrolled in 4 Italian bone marrow transplantation centers: Milan (n = 28), Udine (n = 12), Torino (n = 3), and Bolzano (n = 1).

Patient, disease and transplantation characteristics are summarized in Table 1.

Engraftment and chimerism

Overall, 93% of the patients achieved primary engraftment, with a median time of 14 days (range, 10-27) for neutrophil recovery, and 12 days (range, 7-117) for platelet recovery. The median time for the platelet count to reach 50×10^9/L was 16 days (range, 11-156). A total of 3 patients died before neutrophil recovery. One patient experienced secondary graft failure 2 months after allo-HSCT, concomitant with a severe invasive fungal infection; he was rescued by a BM-derived stem cells boost from the original donor. At the time of this report, the patient was alive, in good clinical condition, in complete remission (CR), and with normal peripheral blood counts.

Table 1
Patient, Disease, and Transplantation Characteristics

Characteristic	All	MRD HSCT	MUD HSCT
Patient demographics			
Total number	44	22	22
Age at HSCT, yr, median (range)	47 (13-69)	43 (13-61)	50 (16-69)
Male sex, n (%)	22 (50)	9 (41)	13 (59)
Disease diagnosis, n (%)			
AML	36 (82)	17 (77)	19 (86)
MDS	3 (7)	3 (14)	0
ALL	5 (11)	2 (9)	3 (14)
Status at transplantation, n (%)			
First CR	16 (36)	8 (36)	8 (36)
Other CR	9 (20)	4 (18)	5 (23)
Active disease	16 (36)	7 (32)	9 (41)
Upfront	3 (8)	3 (14)	0
HCT-CI, n (%)*			
0	15 (34)	10 (45)	5 (23)
1-2	12 (27)	5 (23)	7 (32)
3-4	17 (39)	7 (32)	10 (45)
DRI, n (%)†			
Low	1 (2)	1 (4)	0
Intermediate	26 (59)	14 (64)	12 (55)
High	13 (30)	5 (23)	8 (36)
Very high	4 (9)	2 (9)	2 (9)
CMV serostatus (host/donor), n			
Negative/negative	2	0	2
Negative/positive	2	1	1
Positive/negative	11	3	8
Positive/positive	29	18	11
Donor-recipient HLA matching, n (%)‡			
MRD			
10/10	–	22 (100)	–
MUD			
8/10	–	–	1 (4)
9/10	–	–	7 (32)
10/10	–	–	14 (64)

MRD indicates matched related donor; MUD, matched unrelated donor.
* Comorbidities at time of transplantation were evaluated according to the HCT-CI by Sorror et al [14].
† DRI according to Armand et al [15].
‡ Donor-recipient HLA matching at 4-digits for 5 HLA loci (HLA-A, -B, -C, -DRB1, -DQB1).

Full donor chimerism was documented in 100% of assessed patients at day +30 and confirmed at the subsequent evaluations in patients who maintained complete disease remission.

Toxicity

Table 2 summarizes all adverse events graded >2 according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 observed during conditioning and after HSCT. Overall, reversible hepatic damage and body weight gain were the most frequent side effects, the latter occurring in 14 of 44 subjects (32%), due mainly to liquid retention and thus usually managed with maximal diuretic stimulation. Skin rash after clofarabine administration was frequently observed but was reversible and of low severity in the vast majority of cases; only 3 patients presented with severe cutaneous lesions. Five patients experienced an increase in creatinine level, with a maximal severity grade of 2.

In terms of infectious events, febrile neutropenia occurred in >70% of patients, and 8 patients sustained septic shock, which was ultimately fatal in 7 patients. Pneumonitis was reported in 5 patients and was the cause of death in 1 case. One patient was diagnosed with proven fungal pneumonia, which was successfully treated with a surgical lobectomy. Seven patients experienced EBV reactivation, but only 2 of them required treatment; no EBV-related lymphoma occurred. Reactivation of CMV occurred in 23 patients (52%), with onset at a median of 28 days (range, 6-52 days) from transplantation.

Finally, 1 patient died during the conditioning regimen from a massive cerebral hemorrhage.

Other adverse events were infrequent and of lower severity (Table 2).

Overall, the NRM at 100 days (study primary endpoint) was 18% (95% CI, 7%-30%). Interestingly, no later events attributable to NRM were reported, translating to the same 18% NRM at 1 year (Figure 1).

aGVHD and cGVHD

Grade II, III, and IV aGVHD occurred in 1 patient, 4 patients, and 1 patient, respectively. The cumulative incidence of grade II-IV aGVHD was 16% (95% CI, 4%-28%), and the cumulative

Table 2
Toxicities

Adverse Event	n (%)	Maximum CTCAE Grade
Febrile neutropenia	32 (73)	3
Liver enzymes	12 (27)	4
Septic shock	8 (18)	5
Mucositis	6 (14)	4
Pneumonia	5 (11)	5
Skin lesions*	3 (7)	4
CNS infection	3 (7)	4
Hematuria/cystitis	2 (5)	3
Nausea	1 (2)	3
Pleural effusion	1 (2)	3
VOD	1 (2)	3
DVT	1 (2)	3
Arrhythmia	1 (2)	3
CNS bleeding	1 (2)	5
Microangiopathy	1 (2)	3
Hypocalcemia	1 (2)	3

CTCAE indicates common terminology criteria for adverse events; CNS, central nervous system; DVT, deep vein thrombosis.
* Rash, erytrodermia, ulcerations.

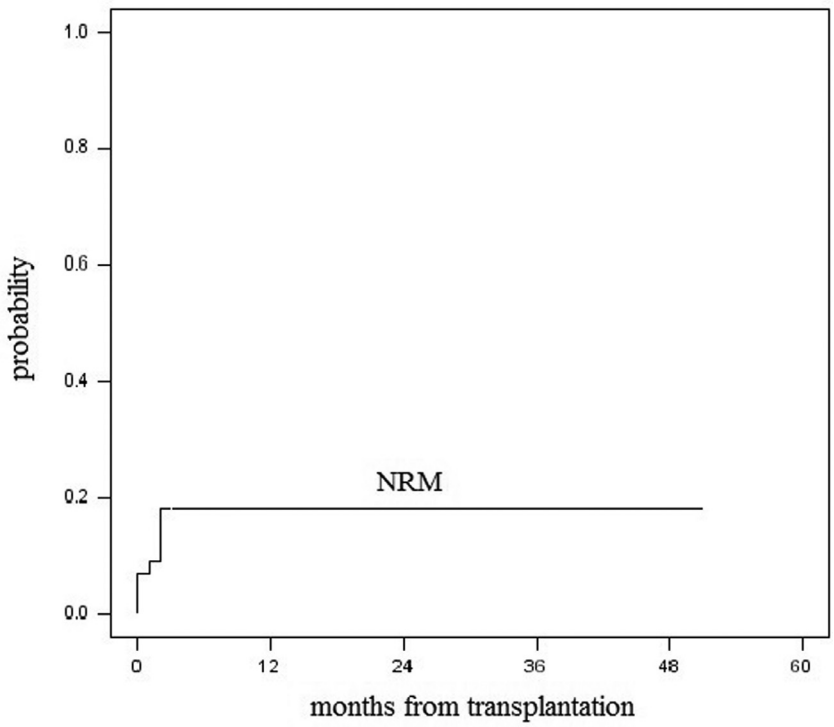

Figure 1. NRM of all 44 patients undergoing allogeneic hematopoietic stem cell transplantation conditioned with treosulfan and clofarabine.

incidence of grade II-IV aGVHD was 13% (95% CI, 2%-24%). The median time of diagnosis was at 54 days post-transplantation (range, 11-111 days). The cumulative incidence of cGVHD (all severity) was 19% (95% CI, 5%-33%). Overall, 7 patients experienced cGVHD, including 5 after allo-HSCT from an HLA-matched related donor and 2 after an unrelated donor allo-HSCT.

Outcomes

The median duration of follow-up after allo-HSCT was 27 months (range, 0-61 months).

The 1-year overall survival (OS), PFS, and relapse incidence were 60% (95% CI: 52-68), 41% (95% CI: 34-48) and 41% (95% CI: 26-56), respectively. The corresponding figures at 2 years were 51% (95% CI: 43-59), 31% (95% CI: 24-39) and 50% (95% CI: 34-67) respectively (Figure 2A). At 1 year, disease relapse was observed in 18 patients (41%) within a median time of 3.4 months (range, 1-9.5 months) after transplantation. Three additional relapses occurred at 12.5, 20.6 and 20.9 months.

As expected, patients with a high-very high DRI displayed significantly lower OS and PFS rates at 1 year compared with those with a low-intermediate DRI: 24% (95% CI, 11%-37%) versus 78% (95% CI, 70%-86%) and 9% (95% CI, 1%-17%) versus 59% (95% CI, 50%-69%), respectively ($P < .0001$) (Figure 2B), and this was consistently related to a higher relapse rate in patients affected by advanced disease: 68% (95% CI, 41%-95%) versus 26% (95% CI, 9%-43%) ($P = .002$) (Figure 2C).

Five patients with overt disease relapse received either 1 (n = 2) or 2 (n = 3) donor lymphocyte infusions, with a documented response in only 1 of them. Five additional patients underwent a second allo-HSCT from a different donor, typically a haploidentical related donor, after disease relapse.

DISCUSSION

Currently, allogeneic HSCT is the potentially curative treatment for the majority of hematologic malignancies. However, major advances in the field of HSCT during the last decade [22] have expanded the applications of transplantation to nonmalignant diseases, such as genetic and autoimmune disorders [2,23].

Novel conditioning regimens are warranted, aimed at reducing HSCT-related toxicity while retaining maximal antimalignancy effect. Although advances in transplantation approaches over the past few decades have led to markedly improved outcomes after allo-HSCT, mortality due to disease recurrence has remained largely unchanged [22]. However, approaches for reducing relapse, such as more intensive conditioning regimens, could also increase toxicities without improving overall outcomes [24].

The combination of fludarabine with an alkylating agent has become increasingly popular over the last decade. Available safety and efficacy data favor such a combination over double-alkylating agent regimens such as busulfan and cyclophosphamide [25].

Treosulfan is a new-generation alkylating agent with a myeloablative effect on committed and non-committed stem cells, as extensively investigated in preclinical studies. Moreover, it has potent immunosuppressive activity, which makes it an alternative option in conditioning regimens before allo-HSCT. Of note, treosulfan's toxicity profile is favorable, due to its limited extramedullary toxicity.

The combination of fludarabine and treosulfan has been explored in patients ineligible for standard myeloablative conditioning, and data are rapidly emerging. This regimen is associated with consistent engraftment and favorable survival in the range of 40%-80%. Promising results have been seen in patients with MDS and leukemia in remission.

Whereas fludarabine acts primarily as an immunosuppressant, the more recently synthetized purine nucleoside analog clofarabine has demonstrated greater antileukemic activity. Clinical trials using clofarabine within the conditioning

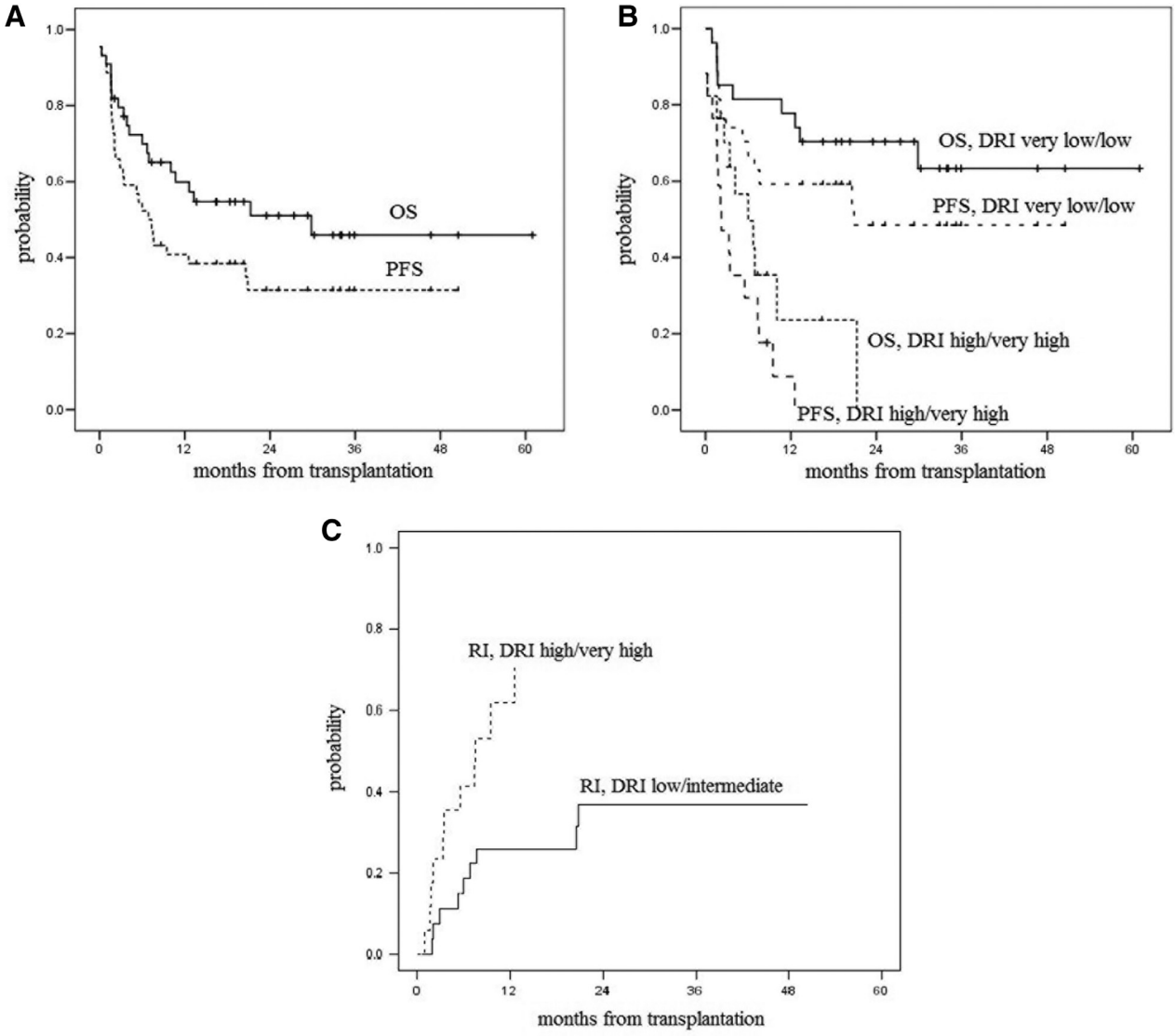

Figure 2. OS, PFS, and relapse incidence. (A) OS and PFS of all 44 patients undergoing allo-HSCT conditioned with treosulfan and clofarabine. (B) OS and PFS curves, stratified by DRI [15]: high-very high versus low-intermediate. (C) Relapse incidence (RI) stratified by DRI.

regimen before allo-HSCT allowed elucidation of its role in terms of antileukemic potential and immunosuppressive and engrafting-promoting activity. The first published trial using clofarabine (in association with cytarabine) in transplantation conditioning reported an unacceptably high rate of poor donor chimerism [26]. Recently, Kebriaei et al [27] reported encouraging data on the combination of clofarabine and busulfan as a conditioning regimen for HLA-matched allo-HSCT in patients with ALL. They registered a trend toward higher NRM in patients older than 40 years. Of note, 6 out of 107 patients developed veno-occlusive disease (VOD), and reversible elevation of liver enzymes was observed in 85% of patients. These transplantation-related complications prompted us to explore the combination of clofarabine and a reduced-toxicity alkylating agent, such as treosulfan.

Here we report results of a multicenter trial (Clo3o; EudraCT 2008-006972-31) investigating a new conditioning regimen based on clofarabine substituted for fludarabine. In this study, clofarabine was combined with treosulfan, and both engraftment and chimerism data were acceptable. Other authors have reported good engraftment even with clofarabine and busulfan-based conditioning, inferring that the immunosuppressant potential of clofarabine is sufficient to guarantee engraftment and full donor chimerism [28–31]. In the literature, clofarabine-based conditioning regimens display a NRM at 1 year ranging from 26% to 32%. Of note, all studies included either busulfan or melphalan in the conditioning schedule [32–34]. In our study, clofarabine and treosulfan were associated with a low incidence of severe hepatic toxicities, such as VOD. The NRM was 18% at 1 year, comparable to that reported for other myeloablative conditioning regimens in high-risk populations [35]. Although in line with our primary endpoint, recently a lower incidence of NRM was reported for patients in CR using a combination of busulfan and fludarabine [25].

By incorporating clofarabine in the conditioning regimen, our main aim was to improve disease control and reduce relapse incidence, but we observed the opposite results. While patients with a low-intermediate DRI group had an encouraging 59% PFS at 1 year, and a 79% OS, those with a high-very high DRI had a PFS of only 9% and OS of 24% at 1 year. All patients treated in our trial, regardless of HLA matching,

underwent in vivo T cell and B cell depletion, with Thymoglobulin and rituximab, respectively, along with cyclosporine and short-course methotrexate. The Thymoglobulin dose was adjusted according to HLA disparity. This investigational immunosuppressive regimen resulted in a lower than expected aGVHD and cGVHD incidence. Indeed, in vivo T cell depletion has been recently shown to lower the incidence of cGVHD in patients in CR from acute leukemia who received peripheral blood stem cells from an HLA-identical sibling [36]. However, the trade-off of this highly immunosuppressive GVHD prophylaxis package was a disappointingly high relapse rate, an appreciable rate of infection (including CMV reactivation), and 3 deaths before engraftment. Accordingly, to reduce the relapse incidence, high-risk patients may benefit from a less aggressive GVHD prophylaxis, namely a modulation of in vivo T cell depletion, especially when receiving a graft from a fully HLA-matched sibling donor. Moreover, in the last decade, post-transplantation cyclophosphamide (PT-Cy) in association with other immunosuppressive agents (sirolimus, cyclosporine, or tacrolimus) or alone has emerged as a promising pharmacologic strategy in the setting of allo-HSCT [37–40]. Recently, PT-Cy was also tested in the HLA-matched donor setting, providing low mortality and acceptable severe GVHD [40–43]. This strategy was able to provide long-term survival in patients with high-risk diseases as well, paving the way for further investigations in large prospective trials.

To our knowledge, this is the first study combining clofarabine and treosulfan as conditioning before allo-HSCT, demonstrating a low incidence of severe hepatic toxicities, even if the low numbers and heterogeneity of our population preclude us from drawing final conclusions. Nevertheless, we failed to demonstrate the superiority of clofarabine-treosulfan over the fludarabine-treosulfan combination, mainly in terms of disease control. The considerable relapse incidence in patients with poor prognostic risk factors remains a major issue. Options for decreasing the risk of disease relapse are currently limited, with little chance of further implementing the efficacy and safety profiles of current conditioning regimens. Given these limitations, prophylactic or preemptive post-HSCT strategies targeting minimal residual disease will play a dominant role in future clinical trials.

The dataset supporting the conclusions of this article is available from the San Raffaele Research Institute (Trial Office, Stem Cell Program), Via Olgettina 60, Milan, Italy.

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