Phase II prospective study of treosulfan-based reduced-intensity conditioning in allogeneic HSCT for hematological malignancies from 10/10 HLA-identical unrelated donor

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Abstract Different RIC regimens were evaluated prior to allo-HSCT in different hematological malignancies. We conducted this prospective study in adult patients with various hematological malignancies in order to evaluate the toxicity and efficacy of treosulfan-based conditioning, followed by allo-HSCT from 10/10 HLA-identical unrelated donors. Conditioning included treosulfan 12 g/m^2/day i.v. (day −6 to day −4), fludarabine 30 mg/m^2/day i.v. (day −6 to day −2), and ATG 2.5 mg/kg/day (day −2 to day −1). PBSC were used as HSC source. We included 56 patients (29 AML, 9 MM, 8 MDS, 6 CLL, 3 ALL, and 1 CML) with a median age of 57 years (18–65.5). Fifty-four (96%) patients engrafted; the cumulative incidence of aGVHD grade ≥II at 3 months reached 31%. The cumulative incidence of cGVHD at 18 months was 34% limited and 8% extensive. The median overall survival (OS) was not reached with a 3-year probability of 52%. The cumulative incidence of relapse at 3 years was 25%, and the cumulative incidence of transplant-related mortality (TRM) at 12 and 24 months was 20% and 23%, respectively. Treosulfan appears to be a good alternative for conditioning of MUD transplant patients with promising results in terms of OS, relapse, and TRM.

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) nowadays represents a curative approach for a number of hematological malignancies. The growing number of international volunteer donor registries increased the availability and use of hematopoietic stem cell (HSC) from unrelated donors and allowed to obtain similar results to those after allo-HSCT from related donors [1, 2]. This was especially due to the important development of human leukocyte antigen (HLA) knowledge and typing techniques leading to the improvement in donor selection, and was also due to the better management of transplantation complications [3–5]. The major focus in the allo-HSCT settings was to find the optimal balance between the graft-versus-host disease (GVHD) and the graft-versus-leukemia (GVL) effect, one of the main factors was by adjusting the conditioning intensity. Dose-intensive standard myeloablative conditioning (MAC) regimens followed by allo-HSCT can lead to a lower relapse incidence and prolonged remissions [6–8]. However, these results are associated with an increased risk of transplant-related mortality (TRM) [9, 10] even among young patients and those who have less comorbidities. On this account, different reduced-intensity conditioning (RIC) regimens were developed to overcome high rates of TRM and confer better transplantation outcomes [11, 12], but the main issue was the higher relapse rate after RIC compared to MAC. Treosulfan is a prodrug of a bifunctional alkylating agent possessing both myelotoxic and immunosuppressive properties; it has previously been evaluated in the treatment of solid tumors including ovarian cancer [13]. Treosulfan associated to fludarabine was recently used as conditioning regimen in many clinical studies prior to allo-HSCT for the treatment of different hematological malignancies leading to promising results either in terms of toxicity or efficacy [14–22]. Treosulfan-based conditioning can probably offer a less toxic regimen than MAC with a full intensity and efficacy. Dose range finding and phase II clinical trials revealed treosulfan doses of 3×12 to 3×14 g/m^2 as feasible and effective when combined with fludarabine for additional pre-transplant immunosuppression [14, 23]. This multicenter prospective phase II trial was designed to evaluate the efficacy and toxicity of treosulfan, fludarabine, and anti-thymocyte globulin (ATG) association as conditioning regimen followed by matched unrelated donor (MUD) allo-HSCT in patients with various hematological malignancies in stable disease or in remission, but considered ineligible for MAC. Our main objective was to study the overall survival (OS) at 1 year. Secondary objectives were: engraftment, GVHD incidence, chimerism analysis, event-free survival (EFS), and toxicity.

Design and methods

Criteria for eligibility

This phase II prospective study included adult patients aged 18 years or older presenting hematological malignancies that would gain benefit from allo-HSCT. Accepted diagnoses were: chronic myeloid leukemia (CML) in first chronic phase (CP) resistant to imatinib, in second CP, or in complete remission (CR) after blast crisis; multiple myeloma (MM) or chronic lymphocytic leukemia (CLL) in stage B or C, in partial remission after relapse post-autologous HSCT; acute lymphocytic or myeloid leukemia (ALL, AML) patients in CR1 at high risk or >CR1, and myelodysplastic syndromes (MDS) with poor prognostic factors. Patients with poor performance status (Karnofsky score <70%) or having one of the following criteria: HIV seropositivity, inadequate renal or liver function, severe heart failure, severe concomitant neurological or psychological disease, pregnancy or lactation were not considered as eligible. The trial was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the local ethics committees at the participating institutions. Written informed consent was obtained for all participants.

Donor selection

All unrelated donors had to be 10/10 HLA identical on HLA-A, -B, -C, -DRB1, and -DQB1 on allelic level. Donors were mobilized for peripheral blood stem cells (PBSC) collection using G-CSF 10 μg/kg/day for five consecutive days.

Conditioning and GVHD prophylaxis

Based on previously conducted dose range finding and phase II clinical trials and considering the eligible patient population of this clinical protocol (aged patients with intensive pretreatment and comorbidities), a dose of 3×12 g/m^2 treosulfan was recommended. In detail, the conditioning included: treosulfan (provided by medac GmbH, Hamburg, Germany) 12 g/m^2/day i.v. (day −6 to day −4), fludarabine (Schering GmbH Berlin, Germany) 30 mg/m^2/day i.v. (day −6 to day −2), and anti-thymocyte globulin (ATG; Genzyme Inc.) 2.5 mg/kg/day (day −2 to day −1). Anticonvulsive prophylaxis was not administered. GVHD prophylaxis used ciclosporine-A oral (5 mg/kg/day) or i.v. (3 mg/kg/day) from day −1; beyond day 21, doses were adjusted according to disease response and chimerism results. In case of minor ABO incompatibility, methotrexate 15 mg/m^2 was administered i.v. at day 1, then 10 mg/m^2 at days 3, 6, and 11 [24].

Chimerism analysis

Chimerism analysis was performed on marrow and/or blood samples (total white blood cells and/or CD3+ cells) using polymerase chain reaction based on informative polymorphic short tandem repeat with an accuracy of ±5%, at 1, 2, 3, 4, 6, 9, and 12 months after transplantation.

Adverse event reporting

Adverse events were recorded from the start of the conditioning regimen up to 12 months after transplantation according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE version 3.0).

Endpoint description

Neutrophil recovery was defined by the first day of an absolute neutrophil count ≥500 cells/mm^3 for three consecutive days; platelet recovery was defined by the first day of a count ≥50,000 cells/mm^3 for seven consecutive days without any transfusion support. GVHD was reported and graded according to published criteria [25]. Chronic GVHD was diagnosed according to standard criteria in patients who survived at least 90 days after transplantation [26]. TRM was defined as death from any cause other than relapse occurring after transplantation. Relapse was defined on the basis of morphologic evidence of hematopoietic disease in bone marrow or other sites. OS was defined as the time from transplantation to any cause of death, and EFS was defined as survival from transplantation to disease progression or death.

Statistical analysis

Cumulative incidence curves were used to estimate incidence over time for neutrophil and platelet recovery, acute and chronic GVHD, TRM, and relapse. OS and EFS were estimated by the Kaplan–Meier method with log-rank test for univariate analysis [27]. Cox proportional hazards regression models were used to assess the influence of disease- and transplant-related variables on OS and EFS [28]. This multivariate analysis studied the following variables: age, cytogenetics, type of disease, number of previous treatments, sex matching, ABO compatibility, cytomegalovirus (CMV) matching, disease status at transplantation, interval diagnosis–allo-HSCT, and CD34+ infused cell number. Statistical analysis was performed with R statistical software (version 2.9.2).

Results

Patients

Between February 2005 and June 2009, 56 patients from five French centers were enrolled, 30 (54%) males and 26 (46%) females with a median age of 57 years (range, 18–65). Patients were chosen by doctors to be treated in this study because they were either heavily pretreated or had comorbidities disabling them to receive full-intensity conditioning regimen. There were 38 (68%) myeloid malignancies [29 AML (13 in CR1 and 16 CR2), 8 MDS (1 CR1, 2 PR1, 3 stable disease, and 2 in relapse), and 1 CML in CR after blast crisis] and 18 (32%) lymphoid malignancies [9 MM in partial remission (PR), 6 CLL (2 CR1 and 4 PR), and 3 ALL (1 CR1 and 2 CR2)]. Among 45 explored patients for cytogenetics, 24 (53%) showed normal karyotype and 21 (47%) were categorized with "poor prognosis" (11 AML, 4 MM, 3 ALL, 2 MDS, and 1 CML) according to commonly known unfavorable cytogenetics in each underlying disease. Before transplantation, 23 (41%) patients received one treatment line, 22 (39%) two lines, and 11 (20%) more than two lines. For sex matching, 48% of patients were sex-mismatched. For CMV serological status (donor/recipient), 41% were −/−, 25% +/+, 4% +/−, and 30% −/+. For ABO matching, 46% were compatible 29% had major incompatibility and 25% minor incompatibility. The median time between diagnosis and allo-HSCT was 15 months (4–168). All patients received PBSC with a median number of infused CD34+ cells of 6.5×10^6/kg (range, 1.4–17.2). Patient characteristics are shown in Table 1.

Hematopoietic recovery, chimerism results, and response rate

After transplantation, 54 (96%) patients engrafted with a median time to neutrophil recovery and platelet recovery of 16 days (range, 4–86) and 11 days (range, 4–82), respectively. The cumulative incidence of neutrophil recovery at day 60 and 90 after transplantation was 93% [95% confidence interval (CI), 80–97] and 95% (95% CI, 91–98), respectively. The cumulative incidence of platelet recovery at day 60 and 90 after transplantation was 85% (95% CI, 80–90) and 87% (95% CI, 83–92), respectively. Complete donor chimerism, defined as having at least 95% of donor cells, was observed in 90% of evaluated patients at 1 month; it increased to 95% at 4 months and finally reached 100% at 6 months. At day 90 post-allo-HSCT, among 46 (82%) evaluable patients, 31 (67%) were in CR, 7 (15%) were in PR, and 8 (17%) were in less than PR.

Toxicity

The whole procedure was well tolerated by the majority of patients; the most frequent adverse event was infection with

Table 1 Patient characteristics

Gender: male/female	30 (54%)/26 (46%)
Patient median age (years)	57 (18–65)
Donor median age (years)	36 (20–54)
Median follow-up (months)	13 (1–57)
Median interval diagnosis–allo-HSCT (months)	15 (4–168)
Disease status according to disease type	
Acute myeloid leukemia, n=29 (52%)	13 CR1, 16 CR2
Multiple myeloma, n=9 (16%)	9 PR
Myelodysplastic syndromes, n=8 (14%)	1 CR1, 2 PR2, 3 SD, 2 relapse
Chronic lymphocytic leukemia, n=6 (11%)	2 CR, 4 PR
Acute lymphoblastic leukemia, n=3 (5%)	1 CR1, 2 CR2
Chronic myeloid leukemia, n=1 (2%)	CR after blast crisis
Previous treatment lines	
1 line	23 (41%)
2 lines	22 (39%)
>2 lines	11 (20%)
Prognostic factors according to cytogenetics (45 explored)	
Normal	24 (53%)
Poor (unfavorable): AML, MM, ALL, MDS, and CML	21 (47%) (11, 4, 3, 2, 1)
Median number of infused CD34+ cells (×10^6/kg)	6.5 (1.4–17.2)
CMV matching (donor–recipient))	
Negative–negative	23 (41%)
Negative–positive	17 (30%)
Positive–negative	2 (4%)
Positive–positive	14 (25%)
Sex matching (donor–recipient)	
Male/male	19 (34%)
Male/female	16 (28%)
Female/female	10 (18%)
Female/male	11 (20%)
ABO compatibility	
Compatible	26 (46%)
Minor incompatibility	14 (25%)
Major incompatibility	16 (29%)

62% of grade ≥II, septicemia of grade ≥II was observed in 27% of patients, and in all other grades, III–IV, adverse events did not reach the 10% level. There were three (5%) Epstein–Barr virus (EBV)-induced lymphoma, one patient died from lymphoma, and two have been treated with rituximab and are in CR at the last follow-up. Details of the different adverse events are shown in Table 2.

Acute and chronic GVHD

Seventeen patients developed acute GVHD ≥grade II (eight grade II, two grade III, and seven grade IV) with a cumulative incidence at 3 months of 31% (95% CI, 25–38). The cumulative incidence of chronic GVHD at 12 months was: 32% (95% CI, 25–39) limited and 6% (95% CI, 2–10)

extensive and at 18 months was 34% (95% CI, 27–47) limited and 8% (95% CI, 5–12) extensive.
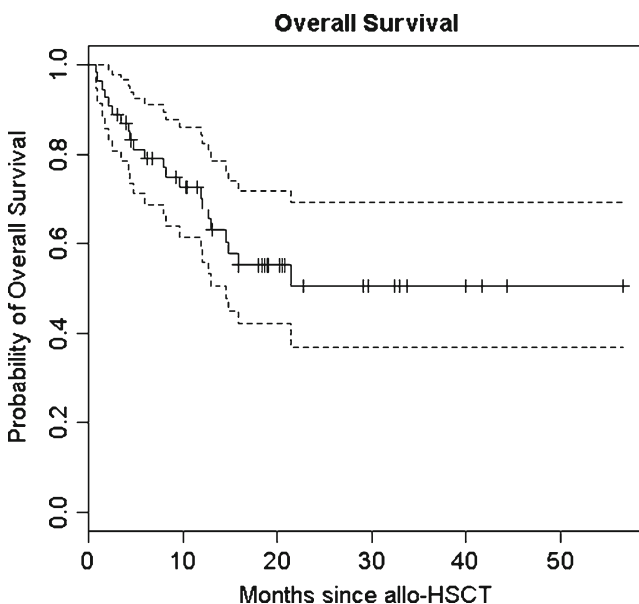
Overall survival, event-free survival, and relapse incidence

After a median follow-up of 13 months (range, 1–57), the median OS was not reached with a 3-year probability of 52% (95% CI, 38–71), Fig. 1. When we studied the impact of disease status at transplantation on OS, we found a better, but not significant OS rate at 3 years for patients in CR1 (65%) versus those in CR2 (44%; p=0.25). Patients with active chronic GVHD appeared to benefit from the GVL effect and thus had a better OS rate when studied in univariate analysis [hazard ratio (HR)=0.2; 95% CI, 0.1–0.6; p=0.002] with a lower relapse incidence (relapse incidence at 1 year,

Table 2 Different adverse events according to the NCI-CTCAE version 3.0

	Grade 1	Grade 2	Grade 3	Grade 4
Allergy/immunology	–	1 (2%)	–	–
Bone pain	–	3 (5%)	–	–
Cardiovascular (arrhythmia)	–	4 (7%)	2 (4%)	–
Cardiovascular (general)	–	2 (4%)	–	2 (4%)
Constitutional symptoms	9 (16%)	6 (11%)	3 (5%)	–
Dermatology/skin	11 (20%)	2 (4%)	–	–
Diarrhea	7 (12%)	3 (5%)	2 (4%)	–
Febrile neutropenia	5 (9%)	6 (11%)	2 (4%)	–
Gastrointestinal	6 (11%)	3 (5%)	2 (4%)	–
Hemorrhage	–	–	–	2 (4%)
Hepatic	1 (2%)	8 (14%)	3 (5%)	2 (4%)
Infection	5 (9%)	25 (45%)	7 (12%)	5 (9%)
EBV-induced lymphoma	–	–	3 (5%)	–
Metabolic/laboratory	3 (5%)	6 (11%)	4 (7%)	–
Multiorgan failure	–	–	–	1 (2%)
Nausea and vomiting	4 (7%)	7 (12%)	2 (4%)	–
Neurology	5 (9%)	3 (5%)	2 (4%)	1 (2%)
Pain	8 (14%)	8 (14%)	1 (2%)	–
Pulmonary	3 (5%)	–	1 (2%)	1 (2%)
Renal/genitourinary	6 (11%)	10 (18%)	3 (5%)	–

15% (95% CI, 14.6–15.4) in patients with active chronic GVHD versus 40.7% (95% CI, 40–41) in patients with no active GVHD, $p=0.06$. The median time of EFS was 15 months (range, 8–57) with a 3-year probability of 47% (95% CI, 35–64), Fig. 2. The cumulative incidence of relapse at 3 years after transplantation was 25% (95% CI, 19–31), Fig. 3.

Transplant-related mortality and causes of death

The cumulative incidence of TRM at 12, 24, and 36 months was 20% (95% CI, 16–27) and 23% (95% CI, 16–29) (same at 24 and 36 months), respectively, Fig. 4. At the last follow-up, 22 patients had died, 7 due to relapse and 15 due to TRM (5 pneumonia, 4 GVHD, 1 EBV-induced lymphoma, and 6 other toxicities).

Multivariate analysis

The multivariate analysis studying age, cytogenetics, type of disease, number of previous treatments, sex matching, ABO compatibility, CMV matching, disease status at transplantation, interval diagnosis/allo-HSCT, and CD34+ infused cell number showed only a negative significant impact of minor ABO incompatibility on OS HR=5.05 (95% CI, 1.8–14); $p<0.001$. There was no significant impact of studied factors on both relapse incidence and TRM.

Discussion and conclusion

Our study showed that the combination of treosulfan, fludarabine, and ATG represents a suitable option as a new conditioning regimen leading to promising results in terms of safety and efficacy. Engraftment was achieved in 96% of patients; in terms of toxicity, there was no unexpected adverse

Fig. 1 Probability of overall survival. *Dashed lines are 95% CI*
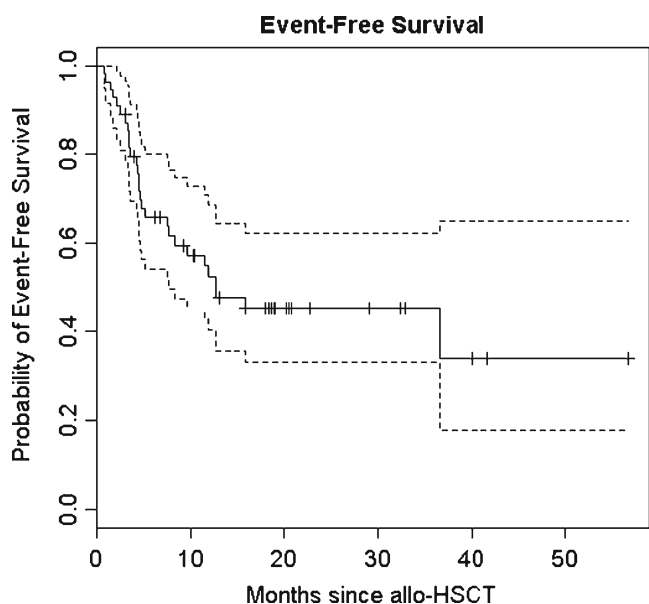
Fig. 2 Probability of event-free survival. *Dashed lines are 95% CI*

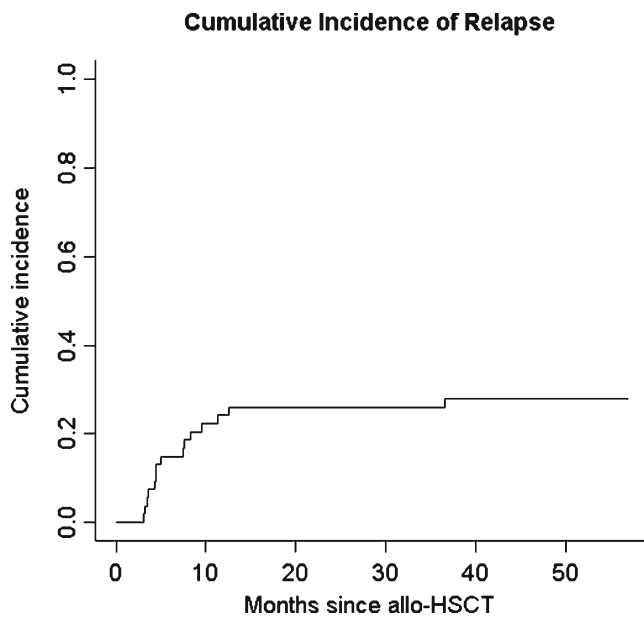## Cumulative Incidence of Relapse

Fig. 3 Cumulative incidence of relapse

event observed within the entire population; infection and septicemia were the most frequent events, and all other adverse events were comparable to those observed in other treosulfan-based allo-HSCT trials [14–22]. We did not observe any increase in hepatic, cardiac, CNS, or pulmonary toxicities as reported in studies that used either MAC or RIC based on busulfan, melphalan, or total body irradiation [11, 29–32]. Nevertheless, we observed three cases of EBV infections that lead to later lymphoma development. The first patient was heavily pretreated for an ALL resistant to different types of chemotherapies, and then received a first allo-HSCT, relapsed 3 months later, and was then included in our study
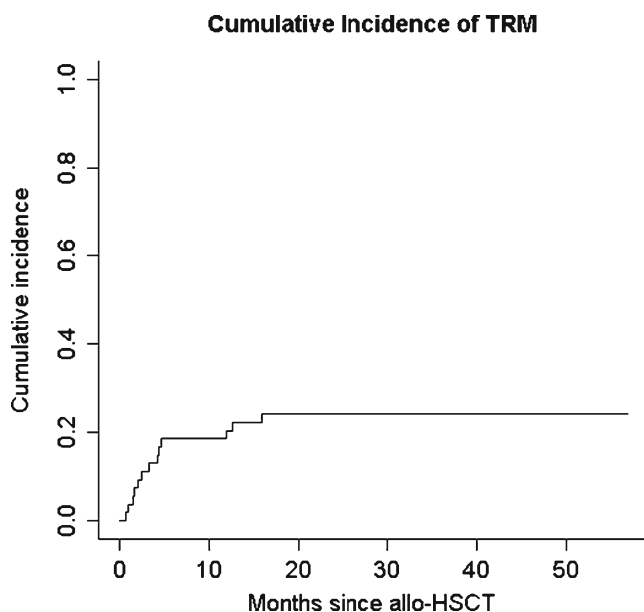
Cumulative Incidence of TRM

Fig. 4 Cumulative incidence of TRM

after being put in CR. One month after second allo-HSCT, the patient was EBV+, and the EBV-induced lymphoma diagnosis was confirmed; a few days later, treatment with rituximab was initiated, and then CHOP was added for resistance. The patient died 40 days later from its lymphoma. The second patient was also heavily pretreated for an AML, received induction, then two courses of consolidation chemotherapy, autografted and relapsed 3 years later, put in CR again, was then included in this study. EBV-induced lymphoma appeared 2 months later; a treatment with rituximab has been initiated, and the patient was in CR for his lymphoma at the last follow-up. The last patient was an AML diagnosis who received induction and consolidation chemotherapies, with maintenance of very bad hematopoietic, development of a MDS 2 years later, treated in this study, and developed an EBV-induced lymphoma 3 months later; treatment with rituximab leads to a CR at the last follow-up. This was also observed with a same incidence rate in the prospective study by Casper et al., where two additional fatal cases were observed in the MUD patient's arm [14]. Similarly, EBV-induced lymphoproliferative disorders have been described after allo-HSCT and were observed after T cell depletion [26, 33]; in our case, in vivo ATG was used, and lymphoma development is more attributable to patient history than to our study treatment procedures. Interestingly, despite PBSC as HSC source and MUD, GVHD incidence was acceptable either for acute or for chronic forms, and reached 31% at 3 months and 32% at 1 year, respectively. The use of ATG has probably contributed to a better immunological modulation and leads to a lower GVHD incidence. Mohty et al. assessed the role of ATG in patients receiving allo-HSCT from MUD after myeloablative conditioning for hematological malignancies and found a beneficial effect of its use especially in lowering the GVHD incidence [34]. In survival analyses, we showed a probability of 52% at 3 years; this was not influenced by disease type or its related factors, which suggests that this conditioning regimen can be effective in different types of hematological malignancies with a remarkable GVL effect with the plateau phase beyond 2 years; this was comparable to non-myeloablative conditioning results already described [35]. At 3 years, the relapse incidence reached 25%, and TRM rate was acceptable taking into account the unrelated settings as well as the selected patient population. It was 20% at 1 year and 23% at 3 years; no direct treosulfan-related mortality was observed. Regarding the negative impact of ABO mismatching on OS in our study, we can say that its role in allo-HSCT is still controversial. Klumpp et al. [36] found a lack of effect of ABO mismatching on transplant outcomes while Kanda et al. [37] found in a large meta-analysis a marginally lower OS in recipients of minor ABO mismatched grafts from unrelated donors. In our study, and in view of the small sample size, no significant conclusion about this can be drawn, and large prospective studies are needed to assess this impact. Nemecek

et al. [38] evaluated in a prospective study the efficacy and safety of treosulfan 14 g/m^2/day i.v. (day −6 to day −4) plus fludarabine 30 mg/m^2/day i.v. (day −6 to day −2) in AML, ALL, and MDS patients receiving allo-HSCT from related and unrelated donors. In this study, 60 patients were included, and no difference in outcome was observed between transplantations from MUD or siblings in multivariate analysis. Casper et al. [14] reported results in 55 patients with various hematological malignancies, who were conditioned using treosulfan 10 (n=20), 12 (n=18), or 14 (n=17) g/m^2/day i.v. (day −6 to day −4) plus fludarabine 30 mg/m^2/day i.v. (day −6 to day −2) and transplanted from either HLA identical or one mismatched sibling donor, or from HLA-identical unrelated donor. They reported higher incidence of grade IV NCI CTC adverse events compared to our results, with 25%, 22%, and 29% for the three studied treosulfan doses, respectively. Grade II–IV aGVHD at day 100 was also higher than what we have observed with 42% including 5% of grade III–IV. At 2 years, the OS probability and relapse incidence for the whole population were 64% and 31%, respectively. Treosulfan, fludarabine, and ATG conditioning combination have been evaluated by Kröger et al. [16] in secondary AML and MDS patients receiving allo-HSCT from related or unrelated donors. Acute grade II–IV GVHD was seen in 23% of patients, and chronic GVHD reached 36%. The 2-year OS and relapse incidence were 36% and 21%, respectively, and the TRM rate was 28% at 100 days. No statistically significant difference was observed between transplants from related and unrelated donors due to the small patient number in the studied arms (n=6 versus n=20, respectively). Recently, Blaise et al. evaluated in a retrospective study the outcomes of fludarabine, oral busulfan, and ATG in conditioning allo-HSCT for hematological malignancies from matched HLA siblings [39]. The 5-year OS, PFS, and TRM were 60%, 54%, and 25%, respectively, but a high GVHD incidence was observed with 43% and 81%, respectively, for acute and chronic forms. The authors associated this outcome with an insufficient immunomodulation with the low ATG doses administered. Other busulfan/fludarabine-based conditioning studies in MDS or secondary AML were performed. The German Cooperative Study Group reported 37 patients with MDS and secondary AML transplanted from related or unrelated donors after using fludarabine-, busulfan-, and ATG-based conditioning [40]. The TRM rate reached 45% in the MUD arm and 12% in the sibling arm.

We showed in this prospective phase II study that treosulfan-based conditioning can offer the desired low toxicity of a RIC with a full-intensity antileukemic activity not only to patients ineligible for a standard conditioning regimen either due to increased age or because of other comorbidities but can also be considered a promising alternative treatment for other populations with different hematological malignancies.

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