

ORIGINAL ARTICLE

Reduced-toxicity conditioning with treosulfan, fludarabine and ATG as preparative regimen for allogeneic stem cell transplantation (alloSCT) in elderly patients with secondary acute myeloid leukemia (sAML) or myelodysplastic syndrome (MDS)

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We investigated a dose-reduced conditioning regimen consisting of treosulfan and fludarabine followed by allogeneic stem cell transplantation (SCT) in 26 patients with secondary AML or MDS. Twenty patients were transplanted from matched or mismatched unrelated donors and six from HLA-identical sibling donors. The median age of the patients was 60 years (range, 44–70). None of the patients was eligible for a standard myeloablative preparative regimen. No graft-failure was observed, and leukocyte and platelet engraftment were observed after a median of 16 and 17 days, respectively. Acute graft-versus-host disease (GvHD) grade II–IV was seen in 23% and severe grade III GvHD in 12% of the patients. No patients experienced grade IV acute GvHD. Chronic GvHD was noted in 36% of the patients, which was extensive disease in 18%. The 2-year cumulative incidence of relapse was 21%. The relapse rate was higher in patients beyond CR1 or with intermediate two or high risk MDS ($P = 0.02$). The treatment-related mortality at day 100 was 28%. The 2-year estimated overall and disease-free survival was 36–34%, respectively. No difference in survival was seen between unrelated and related SCT.

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Introduction

Allogeneic stem cell transplantation is the treatment of choice for the majority of young patients with MDS or

secondary acute myeloid leukemia who have a histocompatible sibling. Disease-free survival ranges from 29 to 40% with a corresponding nonrelapse mortality of 37–50% and a rate of relapse ranging from 23–48% if the donor is an HLA-identical sibling.^{1–3} If unrelated donors were used, after standard myeloablative conditioning the treatment-related mortality exceeded more than 50%.⁴ Despite an improvement in the results of allogeneic stem cell transplantation during the past decade mainly due to a lower treatment-related mortality, there is still a high morbidity, which makes allogeneic stem cell transplantation after standard conditioning only appropriate for younger patients with good performance status.² Recently, dose-reduced or nonmyeloablative conditioning regimens offer new possibilities for patients who were not eligible for standard conditioning because of age, performance status or prior transplantation.^{5–7} As the median age of onset of MDS and sAML is between 60 and 70 years of age, dose-reduced conditioning followed by allogeneic stem cell transplantation seems to be an attractive treatment approach for elder patients. Several small studies using mainly busulfan-based reduced conditioning have been reported for MDS or sAML with encouraging results.^{8–12} More recently, treosulfan as a prodrug of a bifunctional alkylating cytotoxic agent has been shown to be a safe drug within a toxicity-reduced conditioning regimen in combination with fludarabine¹³ or cyclophosphamide¹⁴ for allogeneic hematopoietic stem cell transplantation.¹³ In preclinical models, treosulfan has shown broad stem cell toxicity, and it could induce apoptosis in acute myeloid leukemia cells.^{15,16} Therefore, we conducted a prospective study investigating a treosulfan-based dose-reduced conditioning regimen for patients with sAML or MDS who were not eligible for a standard myeloablative conditioning regimen mainly due to advanced age.

Patients and methods

Patients' eligibility

Patients up to 70 years of age with cytologically proven primary or secondary myelodysplastic syndrome or secondary acute myeloid leukemia were included in the

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protocol. All patients had to be ineligible for standard conditioning regimen due to a high risk of regimen-related toxicity and mortality. These high-risk features were: age >55 years, severe liver or lung toxicities after prior chemotherapies or persistent severe organ dysfunctions, or relapse after prior autologous stem cell transplantation. Related and unrelated donors were allowed, and one antigen-mismatch was accepted. One patient with one antigen and one allele mismatch was treated according to the protocol and included in this analysis. HLA-A- and -B-antigens were typed by serological methods; HLA-DRB1- and -DQB1-alleles were typed with sequence-specific oligonucleotide probes. The treatment plan was approved by the local ethics committee, and all patients gave written informed consent.

Patient characteristics

Twenty-six patients were enrolled in the study, 18 male and eight female patients with a median age of 60 years (range, 44–70) (Table 1). The donors were 20 females and six males with a median age of 35 years (range, 21–56). Fifteen patients had secondary acute myeloid leukemia, seven of 15 were in CR1 and eight of 15 were in >CR1 and 11 patients had MDS, mostly in advanced disease. Two patients had relapsed to prior autologous stem cell transplantation for acute myeloid leukemia and one patient developed secondary AML after autologous stem cell transplantation for non-Hodgkin lymphoma. The median number of blasts in bone marrow aspirate at time of transplantation was 10% (range, 0–18). Eighteen patients received a fully HLA-matched stem cell graft, while eight patients received a mismatch stem cell graft. The stem cell source was mainly peripheral blood stem cells ($n=24$), only one patient received bone marrow, and another patient received bone marrow plus peripheral blood stem cells in combination as stem cell source (Table 1).

Cytogenetic analysis was available in 19 cases and showed normal karyotype ($n=9$), complex karyotype ($n=4$), -7 ($n=2$), $\text{del } 5$ ($n=1$), or other abnormalities ($n=3$).

Twenty patients received stem cells from a matched or mismatched unrelated donor, and six patients from HLA-identical sibling. Nineteen patients had a positive CMV status prior transplantation, and seven patients had a negative CMV serostatus at time of transplantation (Table 1).

Therapy plan

Conditioning consisted of treosulfan ($30\text{--}42\text{ g/m}^2$, given in daily single doses on day -6 to 4 in a dose of $10\text{--}14\text{ g/m}^2$: 30 g/m^2 : $n=20$, 36 g/m^2 : $n=3$ and 42 g/m^2 : $n=3$). Fludarabine (150 mg/m^2 intravenously (i.v.) given divided from day -7 to -3), antithymocyte globulin (ATG; rabbit, Fresenius, Bad Homburg, Germany, $n=20$), given at a median dose of 30 mg/kg (range, $30\text{--}90$) or thymoglobulin (Genzyme GmbH, Neu-Isenburg, Germany, $n=4$) given at a dose of 7.5 mg/kg , followed by allogeneic blood stem cell transplantation on day 0. Two patients did not receive ATG, while four patients received granulocyte-colony-stimulating factor (Lenograstim, Chugai, Germany) i.v. at

Table 1 Patient characteristics

Number of patients	$n=26$
Median patient age (years)	60 years (range, 44–70)
Median donor age (years)	35 years (range, 21–56)
<i>Recipient gender</i>	
Male	$n=18$
Female	$n=8$
<i>Donor gender</i>	
Female	$n=20$
Male	$n=6$
<i>Diagnosis at transplantation</i>	
MDS	$n=11$
RA	$n=4$
RAEB	$n=4$
RAEB-t	$n=3$
sAML	$n=15$
CR ₁	$n=7$
≥CR ₂	$n=6$
Untreated/refractory	$n=2$
<i>International prognostic score (IPSS) of MDS (n=11)</i>	
Intermediate 1	$n=6$
Intermediate 2	$n=4$
High	$n=1$
Median number of BM-blasts at stem cell transplantation	10% (range, 0–18)
Median age of donor	39 years (range, 20–62)
<i>Donor</i>	
Unrelated	$n=20$
Related	$n=6$
<i>HLA-status</i>	
Matched	$n=18$
Mismatched	$n=8$
<i>Stem cell source</i>	
PBSC (peripheral blood stem cells)	$n=24$
BM (bone marrow)	$n=1$
PBSC + BM	$n=1$
Median number of transplanted CD34 ⁺ cells per kg BW	5.99 (range, 1.17–19.4)
<i>CMV status of recipient</i>	
Positive	$n=19$
Negative	$n=7$

a dose of $5\text{ }\mu\text{g/kg}$ after allogeneic transplantation from day +5 and continued until sustained neutrophil engraftment. In case of incomplete chimerism after withdrawal of all immunosuppression, donor lymphocyte infusion was allowed and scheduled with an escalating regimen. However, none of the patients received donor lymphocyte infusion due to mixed chimerism.

Supportive care

The graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine A (3 mg/kg , given from day -1 to 100 after transplantation). The dose of cyclosporine A was adjusted to serum level ($200\text{--}300\text{ ng/ml}$), tapered from day

100, and discontinued between day 120 and 140, if no signs of GvHD were observed. In patients who received stem cells from unrelated donors, cyclosporine A was tapered between day 140 and 180. Methotrexate (10 mg/m²) was given i.v. on day +1, +3 and +6 after transplantation. One patient received mycophenolat mofetil instead of methotrexate due to pretransplant elevated liver enzymes. One patient with two HLA-mismatches received FK506, methotrexate and mycophenolat mofetil. All patients were nursed in single room equipped with air filtration with Hepa-filters. Antibiotic prophylaxis consisted of ciprofloxacin, antifungal prophylaxis of fluconazole or of itraconazole or voriconazole in case of prior mycotic infections. Aciclovir was given as herpes prophylaxis from day +1 until day 100. CMV-seropositive patients with unrelated donors received CMV-prophylaxis with gancyclovir after stable engraftment until day 100. *Pneumocystis carinii*-prophylaxis consisted of either trimethoprim or sulphamethoxazole on 3 days weekly or of monthly pentamidine-inhalation. All blood products were CMV-negative and irradiated before infusion. Weekly monitoring of blood and urine for CMV-antigen by polymerase-chain-reaction (PCR) and short-term cultures of CMV-lower matrix protein pp65-positive leukocytes were performed. In case of CMV-reactivation/-infection, gancyclovir treatment was initiated (5 mg/kg i.v. twice daily) and discontinued after negative test results were obtained. Regimen-related toxicity was graded according to the Bearman-Score.¹⁷ A maximum for each organ system was recorded. Toxicities were defined as regimen related toxicity (organ toxicity) versus GvHD related toxicity.

Study objective

The objective of the study was to evaluate engraftment, toxicity, overall and disease-free survival of toxicity-reduced conditioning consisting of treosulfan and fludarabine followed by allogeneic stem cell transplantation in patients with secondary AML or MDS who were not eligible for a standard conditioning. Engraftment was defined as a leukocyte count of more than $1 \times 10^9/l$ for 3 consecutive days and a nontransfused platelet count of more than $20 \times 10^9/l$. Chimerism analysis was performed by allele-specific multiplex-PCR-technique as recently reported.¹⁸ Mixed chimerism was defined as presence of at least 5% of recipient's DNA in the presence of donor DNA. For high-sensitive Y-chromosome-specific real-time-PCR, complete donor chimerism was defined as more than 99.8% donor cells. Standard criteria were used for grading of acute and chronic GvHD.¹⁹

Statistical methods

Survival curves for overall and disease-free survival were estimated by the Kaplan–Meier method. The log-rank test was performed for statistical analysis for time-dependent analysis of survival, and a *P*-value <0.05 was considered significant. Overall survival was calculated from transplantation until death from any cause, and progression- or event-free survival was calculated from transplantation until progression or event from any cause. Estimation of relapse incidence and treatment-related mortality incidence

was carried out using the proper estimation of cumulative incidence curve.

Results

Toxicity

Major toxicity was mucositis grade I (*n* = 10) and grade II (*n* = 7). Severe grade III toxicity was observed for cardiac (*n* = 2), pulmo (*n* = 1), liver (*n* = 1), and CNS (*n* = 2) (Table 2). In three cases, lethal grade IV-toxicity was seen: liver failure (*n* = 1), CNS bleeding (*n* = 1) and in one patient combined toxicity of pulmonary and liver toxicity resulting in gastrointestinal bleeding. Overall, 14 patients died during follow-up. Causes of death were relapse (*n* = 4), sepsis (*n* = 4), toxicity (*n* = 3), GvHD and infections (*n* = 2), and secondary carcinoma (CUP syndrome: carcinoma of unknown primary) (*n* = 1). Nine patients died of treatment-related causes. The treatment-related mortality at day 100 was 28% (95%CI: 10–46). Of the patients with lethal toxicity, one had liver failure due to fulminant hepatitis, one died of CNS bleeding, and one died of pulmonary and hepatic toxicity combined with gastrointestinal bleeding. Of the four patients with lethal sepsis, two died 4–6 days after allogeneic transplantation, while two patients died of *Aspergillus* or *Mucor* sepsis on day 21 and 142, respectively.

Engraftment

The median number of transplanted CD34⁺ cells/kg BW was 5.99×10^6 . (range, 1.17–19.4). All patients became neutropenic ($<0.5 \times 10^9/l$), and all became thrombocytopenic ($<20 \times 10^9/l$), requiring a median of 11 platelet transfusions (range, 2–24), a median of 12 erythrocyte transfusions (range, 3–39). The median time until leukocyte ($>1 \times 10^9/l$) and platelet ($>20 \times 10^9$) engraftment was 16 days (range, 7–39) and 17 days (range, 5–42), respectively. The median duration of neutropenia ($<0.5 \times 10^9/l$) and of thrombocytopenia ($<20 \times 10^9$) was 16 days (range, 8–27). Twenty-two patients were evaluable for engraftment. Four patients died prior engraftment. No primary graft failure was observed. One patient had secondary graft failure and was retransplanted after conditioning with fludarabine and campath, but died due to acute GvHD. Chimerism analysis was available in 16 patients. Thirteen of them reached a full donor chimerism at a median of 34 days (range, 19–209). Three patients had only mixed chimerism after transplantation, but died during follow-up due to GvHD or infections. Therefore, no DLI was performed for mixed chimerism.

Table 2 Toxicity according to Bearman scale

	Skin (n)	Cardiac (n)	Pulmo (n)	Liver (n)	CNS (n)	Stomatitis (n)
Grade 0	22	22	24	10	23	9
Grade I	4	2	0	13	0	10
Grade II	0	0	0	0	0	7
Grade III	0	2	1	1	2	0
Grade IV	0	0	1	2	1	0

Graft-versus-host disease

None of the grade IV-GvHD was observed. Fourteen patients did not show any signs of acute GvHD. Six patients had mild acute grade I-GvHD. Grade II-GvHD was seen in three patients, and grade III-GvHD was seen in three patients. The overall rate of GvHD grade II–IV was 23%. Chronic GvHD was evaluated in 11 patients, seven patients did not show any signs of chronic GvHD, while two patients experienced limited chronic GvHD (18%), and two patients experienced extensive chronic GvHD (18%), resulting in an overall incidence of chronic GvHD of 36%.

Overall and disease-free survival

After a median follow-up of 8 months (range, 2–22), the 2-years estimated event-free survival was 34% (95% CI: 12–56), and the 2-years overall survival was 36% (95% CI: 14–58) (Figures 1 and 2). Overall survival did not differ between related and unrelated donors (25% vs 39%; $P=NS$), but due to the limited number of patients no definitive conclusion can be drawn. The 2-years cumulative incidence of relapse was 21% (95% CI: 3–39) (Table 3). The incidence of relapse was higher in patients with $>CR_1$, intermediate-2- and high-risk MDS in comparison to patients with CR_1 and intermediate-1-MDS (43% vs 0%; $P=0.02$) (Figures 3 and 4).

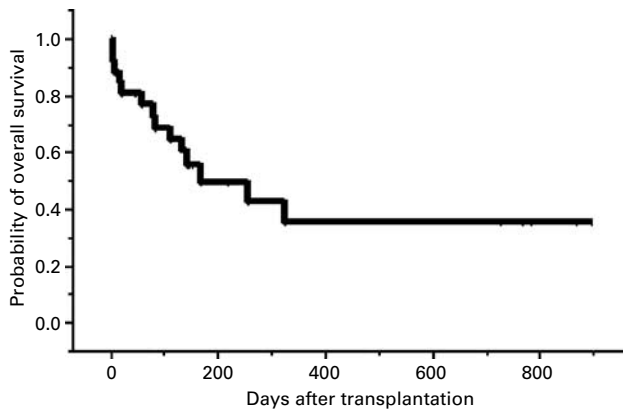


Figure 1 Overall survival.

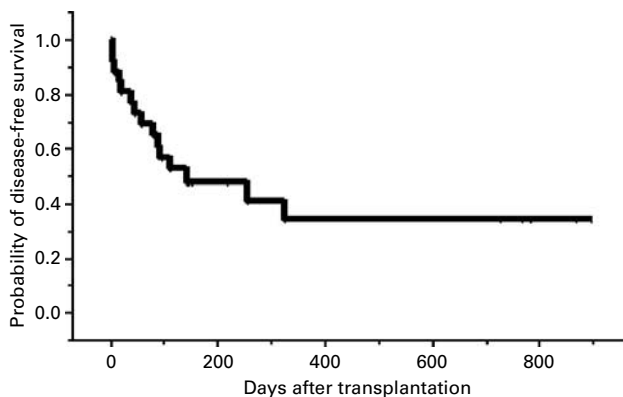


Figure 2 Disease-free survival.

Table 3 Results

Median days of leukocyte engraftment ($>1.0 \times 10^9/l$)	16 (range, 7–39)
Median days of platelet engraftment ($>20 \times 10^9/l$)	17 (range, 5–42)
Median number of transfused platelet units	11 (range, 2–24)
Median number of transfused erythrocyte units	12 (range, 3–39)
<i>Acute GvHD (n = 26)</i>	
Grade 0	n = 14 (54%)
Grade I	n = 6 (23%)
Grade II	n = 3 (23%)
Grade III	n = 3 (23%)
Grade IV	n = 0
<i>Chronic GvHD (n = 11)</i>	
None	n = 7 (64%)
Limited	n = 2 (18%)
Extensive	n = 2 (18%)
2-year overall survival	36% (95% CI: 14–58)
2-year event-free survival	34% (95% CI: 12–56)
2-year cumulative incidence of relapse	21% (95% CI: 3–39)
Day 100 treatment related mortality	28% (95% CI: 10–46)

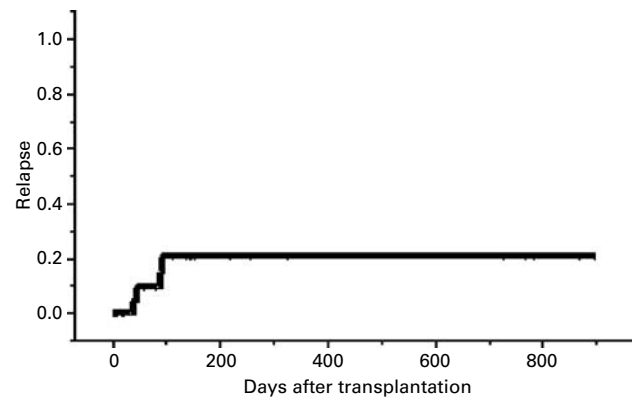


Figure 3 Cumulative incidence of relapse.

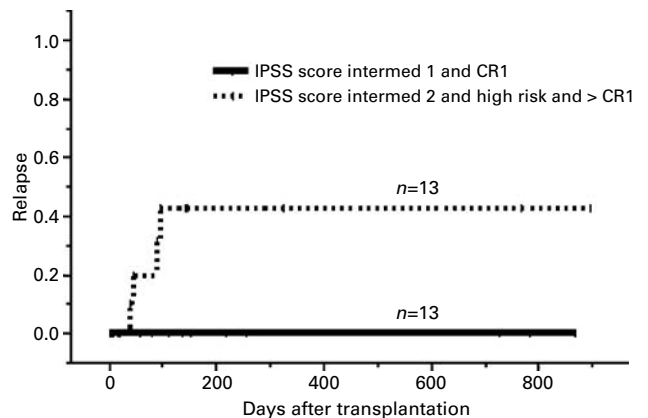


Figure 4 Cumulative incidence of relapse according to IPSS score and CR_1 .

Discussion

The current study shows that treosulfan in combination with fludarabine as dose-reduced conditioning for patients with secondary AML or MDS prior allogeneic stem cell transplantation is a feasible and effective regimen enabling engraftment in 100% of the evaluable patients. Treosulfan (L-threitol-1,4-bis-methanesulfonate; dihydroxybusulfan) is a prodrug of a bifunctional alkylating cytotoxic agent, which showed in clinical models a high stem cell toxicity.²⁰ The dose-limiting toxicity using i.v. formulation without stem cell support has been claimed to be 10 g/m².²¹ By using autologous hematopoietic stem cell support the maximum tolerated dose has been 47 g/m², and the dose-limiting factors were mucositis, diarrhea or skin toxicity.²² A phase II study using treosulfan, 30 g/m², in combination with fludarabine followed by allogeneic hematopoietic stem cell transplantation resulted in a generally low extramedullary toxicity. In this study, 30 patients with different hematological diseases were included, and the overall survival and disease-free survival were 73–49%, respectively. Beside fever and infections, the major toxicity was liver toxicity with low to moderate increase of liver enzymes. Therefore, the cumulative incidence of treatment-related mortality at 1 year was only 20%.¹³ More recently, a higher dose of treosulfan (36–42 g/m²) in combination with cyclophosphamide (120 mg/m²) was investigated in patients with hematological malignancies who were not eligible for a standard myeloablative conditioning regimen. In this study, which includes 18 patients, rapid engraftment with full donor chimerism was observed in all evaluable patients, and the nonrelapse mortality at 1 year was 22%. The overall survival and progression-free survival were estimated at 1 year of 67 and 56%, respectively.¹⁴ These encouraging results for those patients with increased risk for standard myeloablative preparative regimens and the recent report on *in vitro* activity of treosulfan against acute myeloid leukemia¹⁶ were the rationale for conducting a phase II study in patients with MDS or secondary acute myeloid leukemia. In contrast to the treosulfan-based studies mentioned above, in our study the organ toxicity regarding especially liver toxicity and stomatitis was somewhat higher, and two patients died due to organ toxicity. Other causes of treatment-related mortality were attributed to infectious complications, mainly sepsis ($n = 4$) or GvHD-associated infectious complications ($n = 2$) or bleeding ($n = 1$). Of those patients who died of sepsis, two of them died 4–6 days after transplantation without engraftment, which could be attributed to pre-existing infections. Two patients died of *Aspergillus* sepsis on day +21 and +142 after allogeneic stem cell transplantation. The relatively long median duration of neutropenia ($<0.5 \times 10^9/l$) of 16 days is most probably due to a strong stem cell toxicity of treosulfan on both primitive and committed stem cells¹⁵ and may explain the relatively high infectious complication. The observed higher toxicity and treatment-related mortality may also be explained by the fact that all patients have sAML/MDS mostly in advanced stage or refractory disease. Furthermore, in our trial the median age of the patients was 60 years, while in the other reported treosulfan-based studies the median age was

significantly younger with 44–49 years, respectively. Indeed, a higher treatment-related morbidity and mortality for 34 patients with advanced disease have been recently reported for a treosulfan-based preparative regimen.²³ In this trial, multiorgan failure reached 20%, veno-occlusive disease 6%, and infectious complications associated with GvHD 12%. In contrast to the organ-related toxicity, the incidence of grade II–IV acute GvHD and of chronic GvHD was relatively low with 23–36%, respectively. No grade IV acute GvHD was seen and the incidence of extensive chronic GvHD was only 18%. The incorporation of antithymocyte globulin with the preparative regimen may attribute to the relatively low incidence of GvHD. Similar to other published treosulfan-based allogeneic stem cell transplantation studies, we observed an ensured engraftment with no primary graft-failure. Other studies, mostly using dose-reduced conditioning regimens in MDS or secondary AML consisted of a busulfan/fludarabine-based regimen. The German Cooperative Study Group reported 37 patients with MDS and secondary AML who were transplanted from related and unrelated donors after using fludarabine/busulfan (8–10 mg/kg) and ATG. They observed a treatment-related mortality of 27%, which was higher in those patients transplanted from an HLA-matched unrelated donor (45 vs 12%). This higher treatment-related mortality in matched unrelated donors using fludarabine/busulfan-based regimens was confirmed by the study of Ho *et al.* investigating the busulfan/fludarabine/alemtuzumab regimen in 62 patients with MDS. They observed a 1-year treatment-related mortality of only 15%, which, however, was lower in siblings in comparison to unrelated donors (5 vs 21%). A similar low treatment-related mortality of only 5% was reported for busulfan/fludarabine-based regimens by the Spanish group, but this study group used only matched sibling-donors.^{8,10,11} In our study, the treatment-related mortality did not differ between unrelated ($n = 20$) and related ($n = 6$) donors. The 2-year overall and event-free survival of 36–34%, respectively, after treosulfan/fludarabine-regimen is comparable to those reported for the busulfan/fludarabine regimens which was reported at 3 years to be 39–38%, respectively.¹⁰ These data support the hypothesis that allogeneic stem cell transplantation using reduced conditioning is now a feasible treatment approach for those patients with sAML or MDS who are not eligible for standard conditioning mainly due to advanced age. Whether dose-reduced conditioning has similar results in comparison to the standard conditioning in patients who were eligible for standard transplantation is currently unknown, and for this reason the EBMT has recently launched a prospective randomized study compare the reduced-intensity conditioning regimen with standard conditioning in patients with sAML/MDS with an HLA-identical sibling or unrelated donors. From the current phase II study, we conclude that treosulfan/fludarabine is an active preparative conditioning regimen followed by allogeneic stem cell transplantation for elder patients with secondary AML or MDS. This regimen approach should be further tested in standard-risk patients with less advanced disease.

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