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Graft-versus-Host disease Prophylaxis with Everolimus and Tacrolimus Is Associated with a High Incidence of Sinusoidal Obstruction Syndrome and Microangiopathy: Results of the EVTAC Trial

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A calcineurin inhibitor combined with methotrexate is the standard prophylaxis for graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). Everolimus, a derivative of sirolimus, seems to mediate antileukemia effects. We report on a combination of everolimus and tacrolimus in 24 patients (median age, 62 years) with either myelodysplastic syndrome (MDS; n = 17) or acute myeloid leukemia (AML; n = 7) undergoing intensive conditioning followed by HSCT from related (n = 4) or unrelated (n = 20) donors. All patients engrafted, and only 1 patient experienced grade IV mucositis. Nine patients (37%) developed acute grade II-IV GVHD, and 11 of 17 evaluable patients (64%) developed chronic extensive GVHD. Transplantation-associated microangiopathy (TMA) occurred in 7 patients (29%), with 2 cases of acute renal failure. The study was terminated prematurely because an additional 6 patients (25%) developed sinusoidal obstruction syndrome (SOS), which was fatal in 2 cases. With a median follow-up of 26 months, the 2-year overall survival rate was 47%. Although this new combination appears to be effective as a prophylactic regimen for acute GVHD, the incidence of TMA and SOS is considerably higher than seen with other regimens.

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INTRODUCTION

Beyond disease biology, the success of allogeneic hematopoietic stem cell transplantation (HSCT) in patients with hematologic malignancies is determined mainly by the occurrence and extent of graft-versus-host disease (GVHD) [1]. This is due to the close link between the extent of GVHD and nonrelapse mortality. In fact, patients who experience advanced GVHD have mostly limited survival [2]. Consequently, prevention of GVHD is the major goal and primary challenge in clinical HSCT. Although numerous trials have investigated various immunosuppressive drug combinations for GVHD prophylaxis, cyclosporin A (CsA) and methotrexate has remained the standard combination for more than 20 years [3]. The use of an alternative calcineurin inhibitor, tacrolimus, can significantly reduce acute, but not chronic, GVHD [4,5]. Despite these treatments, however, > 50% of patients who undergo HSCT develop clinically significant GVHD. In addition, methotrexate is highly toxic, inducing mucositis and delayed hematopoietic engraftment. Consequently, alternative immunosuppressive drug combinations have been investigated, including mycophenolate mofetil, but none has produced significantly better results [6,7].

Sirolimus (rapamycin), first found on Easter Island (Rapa Nui) as a naturally occurring compound isolated from a soil saprophyte, belongs to a new generation of immunosuppressive agents that inhibit the mammalian target of rapamycin (mTOR), an essential regulator of cell cycle in proliferating T cells. Sirolimus and tacrolimus (FK-506) act through different binding sites on a transcription factor–binding protein, FKBP-12, producing synergistic effects [8,9]. One advantage of using a combination of sirolimus and tacrolimus

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instead of CsA is the absence of interactions at the cytochrome level. A combination of sirolimus and CsA has been successfully used in patients after organ transplantation [10] and with tacrolimus after allogeneic matched related HSCT [11]. A short course of methotrexate was added in patients receiving grafts from unrelated donors [12], resulting in low rates of acute grade II-IV (26%) and chronic (42%) GVHD. Recently, methotrexate was successfully omitted, with no significantly change in the overall results [13].

Everolimus is a hydroxyethylester derivative of sirolimus that has a shorter half-life (22 vs 72 hours) and thus is more clinically manageable than sirolimus. It has been successfully used in combination with CsA after solid organ transplantation [14,15]. Like sirolimus [16,17], it exerts antiproliferative effects not only in T cells, but also in malignant cells, which theoretically could prevent disease recurrence after allogeneic HSCT [18,19]. Tacrolimus appears to be an ideal partner for everolimus in combination therapy, because it has minimal effects on serum everolimus levels compared with CsA. A pharmacokinetic interaction between CsA and everolimus has been described previously for healthy volunteers after single-dose administration, presumably originating from inhibition of hepatic cytochrome (CYP3A4) or P-glycoprotein efflux transporter. As a result, a higher dose of everolimus is needed in everolimus–tacrolimus combination therapy (EVTAC) than in everolimus–CsA combination therapy to achieve the desired everolimus blood level [20]. Given the potential synergism and favorable toxicity profile of EVTAC after allogeneic HSCT, we sought to investigate the efficacy of this combination in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

METHODS

Study Design

The aim of this prospective pilot Phase II study was to evaluate EVTAC in the setting of allogeneic HSCT after busulfan-based intensive conditioning. All patients provided written informed consent, and the study design was approved by the local institutional review board and the German Federal Administration. Before recruitment, the study was registered at www.clinicaltrials.gov as NCT00117702. The trial's primary endpoint was the incidence and severity of acute GVHD, and secondary endpoints were the safety and incidence of chronic GVHD and infectious complications. A data safety monitoring board (DSMB) was installed to review toxicities. Inclusion criteria were hematologic malignancy, age 18 to 70 years, and adequate liver, renal, cardiac, and pulmonary function conferring eligibility for intensive busulfan-based conditioning. A patient could be included if a peripheral blood stem cell donor (either related or unrelated) with a maximum of 1 allele mismatch (9 out of 10) were available. DNA-based HLA typing of donor and recipient was performed using intermediate resolution for HLA class I (A, B, and C) and under high resolution for HLA class II (DRB1 and DQB1).

Study Therapy

Tacrolimus was administered either i.v. at a dose of 0.03 mg/kg/day or as a bioequivalent oral dose in 2 divided doses starting on the day before HSCT (day -1). The dose of tacrolimus was adjusted to maintain blood levels between 5 and 10 ng/mL. Starting on day 100 after HSCT, oral tacrolimus administration was tapered by 5% each week if GVHD was inactive. Everolimus was given orally starting on day 0 and a starting dose of 1.5 mg/day in 2 divided doses. The dose was subsequently adjusted to achieve a target blood concentration between 3 and 8 ng/mL. Everolimus administration was stopped on day 56 in the absence of uncontrolled GVHD. Serum concentrations of both drugs were obtained at least twice weekly. Acute and chronic GVHD were treated primarily with prednisone.

Tests for cytomegalovirus (CMV) pp65 antigen or polymerase chain reaction (PCR) for CMV DNA were performed weekly in patients at risk for CMV reactivation. In the event of a positive test result, preemptive therapy with valganciclovir was initiated and administered until day 100 or until PCR results were negative, whichever occurred last. Prophylaxis against infectious disease consisted of ciprofloxacin, fluconazole, and acyclovir.

DNA Extraction and Genotyping

To detect single-nucleotide polymorphisms (SNPs), DNA was extracted from whole blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol [21]. SNPs for glutathion-S-transferase (GST), such as GSTP1-Ile105Val, GSTA1*a/b, and Cyp3A4*1B polymorphisms, were detected by PCR–restriction fragment length polymorphism analysis, and null genotypes of GSTM1 and GSTT1 were detected by multiplex PCR as described previously [22,23]. Samples were genotyped for Cyp3A4*3, Cyp3A5*2, and Cyp3A5*3C polymorphisms (Cyp3A4*3: C_27535825_20, rs4986910; Cyp3A5*2: C_30633862_10, rs28365083; Cyp3A5*3C: C_26201809_30, rs776746) using a custom-designed system (Assay-on-Demand; Applied Biosystems, Darmstadt, Germany). In brief, a 10-ng DNA sample was added to a reaction volume of 15 μL containing 7.5 μL of TaqMan Universal PCR Master Mix, No AmpErase UNG, and 0.75 μL of custom-designed probe. Amplifications were performed on an Applied Biosystems 7500 real-time

PCR system: 95°C for 10 minutes, followed by 45 cycles of 93°C for 15 seconds and 60°C for 1 minute. Post-PCR plate reading was used to determine genotypes. For quality control purposes, positive controls of each genotype were used in each genotyping approach.

Statistical Analyses

Overall survival and disease-free survival were obtained by the Kaplan-Meier method, with patients censored at last follow-up if still alive [24]. The incidences of relapse and nonrelapse mortality and GVHD were calculated using cumulative incidence estimates [25]. Regimen-related toxicity was scored using the Common Toxicity Criteria (CTC), version 3.0 (National Cancer Institute; available at (http://ctep.cancer.gov/reporting/ctc.html). Acute and chronic GVHD were diagnosed and graded using established criteria, with a cutoff on day 100 after HSCT [26,27]. Smoothing spline curve estimation techniques were used to fit the trough serum levels of everolimus and tacrolimus measured during the study period.

RESULTS

Patient Characteristics

Between 2005 and 2008, a total of 24 patients with MDS (n = 17, including 1 patient with therapy-related MDS [tMDS] 8 years after therapy for non-Hodgkin lymphoma and 1 with AML evolving from MDS) or de novo AML (n = 7) were included in this trial. The median patient age was 62 years (range, 47 to 70 years). Patient characteristics are summarized in Table 1. In 8 unrelated patient–donor pairs, a single-allele HLA mismatch was accepted. Donors and patients were sex-mismatched in 5 cases, with a female donor for a male recipient in 2 cases. CMV seropositivity of either donor or recipient was documented in 18 patients. All patients received granulocyte colony-stimulating factor– mobilized peripheral blood stem cells from related (n = 4) or unrelated (n = 20) donors as described previously [28]. A median of 7.0×10^6/kg $CD34^+$ cells (range, 3.0 to 9.6×10^6/kg) were transplanted.

Engraftment

Rapid engraftment of neutrophils (median, 17 days; range, 11 to 20 days), defined as the first of 3 consecutive days of a neutrophil count > 0.5 Gpt/L, and platelets (median, 15 days; range, 11 to 139 days), defined as the first of 3 consecutive days of a platelet count > 50 Gpt/L without platelet support, was achieved in all patients. In 2 patients, however, platelet counts dropped by more than half on days 37 and 40 after HSCT. At that time, neither elevated everolimus serum trough levels nor transplantation-associated

Table 1. Patient Characteristics and Outcome

UPN	Age	Disease	Previous Treatment	Karyotype	IPSS	HCT-CI	Conditioning	Defibrotide Prophylaxis	Liver Toxicity	TMA	Status	Follow-Up, Months
1262	58	RCMD	Untreated	Normal	INT-1	0	Bu/Flu	-	SOS	-	Alive	32
1265	62	CMML-1	Untreated	Normal	INT-1	0	Bu/Flu	-	-	-	Alive	32
1267	67	RAEB-2	Untreated	Complex	HIGH	0	Bu/Flu	-	-	-	Relapse	2†
1269	47	RAEB-2	IC/1. PR	Normal	INT-1	0	Bu/Flu	-	-	-	Alive	32
1273	54	RCMD	Untreated	Complex	INT-2	1	Bu/Flu	-	-	Yes	TRM	3†
1283	63	RAEB-1	Decitabine/SD	-7	INT-2	0	Bu/Flu	-	-	-	Alive	31
1301	61	RAEB-2	Untreated	Complex	HIGH	0	Bu/Flu	-	-	Yes	Relapse	3†
1303	55	AML	IC/2. CR	Normal/FLT3+	NA	1	Bu/Flu	-	-	Yes	TRM	15†
1311	49	AML	IC/1. CR	Normal	NA	0	Bu/Flu	-	SOS	-	Relapse	26†
1318	43	RCMD	Untreated	-7	INT-2	0	Bu/Flu	-	-	-	Alive	28
1324	54	RAEB-2	Untreated	+13	HIGH	0	Bu/Flu	-	-	Yes	TRM	7†
1331	62	AML	IC/1. CR	del(20q)	NA	0	Bu/Flu	-	-	-	Alive	27
1333	50	tRAEB-1	Untreated	Normal	NA	3	Bu/Flu	-	SOS	-	TRM	2†
1340	68	RAEB-2	Untreated	Normal	INT-2	1	Bu/Flu	-	-	-	TRM	8†
1350	64	MDS/AML	IC/1. CR	Normal	NA	0	Bu/Flu	-	SOS	-	TRM	1†
1363	63	AML	IC/2. CR	inv16	NA	0	Bu/Flu	-	-	-	Alive	24
1416	69	RAEB-1	ATG/PD	Normal	INT-2	1	Bu/Cy	-	-	-	Died*	19†
1475	66	CMML-2	Lenalidomide/PR	del(5q)	NA	0	Bu/Cy	-	SOS	Yes	Alive	15
1483	70	RAEB-2	Decitabine/SD	t(1;3), 11q23	HIGH	1	Bu/Cy	-	SOS	-	Alive	14
1497	65	CMML-1	Untreated	Normal	NA	0	Bu/Flu	Yes	-	-	Alive	13
1502	67	AML	IC/1. CR	Complex	NA	1	Bu/Flu	Yes	Hyperbilirubinemia	-	Relapse	3†
1519	62	RCMD	Lenalidomide/CR	Complex	INT-2	0	Bu/Flu	Yes	-	-	TRM	5†
1541	64	AML	IC/1. PR	Normal	NA	0	Bu/Flu	Yes	PVT	Yes	Relapse	10
1590	56	AML	IC/1. CR	Normal/FLT3+	NA	0	Bu/Flu	Yes	-	Yes	Relapse	2†

RCMD, refractory cytopenia with multilineage dysplasia; CMML, chronic myelomonocytic leukemia; IPSS, International Prognostic Score; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; t, therapy-related; FLT3+, FLT3 internal tandem duplication positive; HCT-CI, hematopoietic stem cell transplantation comorbidity index [48]; Bu/Flu, busulfan and fludarabine; Bu/Cy, busulfan and cyclophopshamide; IC, induction chemotherapy; NA, not applicable; ATG, antithymocyte globulin; PVT, portal vein thrombosis; INT, intermediate.

*Due to stroke.

†Death

microangiopathy (TMA) was present. In both patients, platelets recovered shortly after everolimus was stopped.

Dosage and Blood Trough Levels

During the first 56 days after HSCT, the median daily dose was 1.5 mg for everolimus and 2 mg for tacrolimus. Of note, all patients were able to take everolimus orally during times of aplasia and rapidly achieved the target blood concentrations of the study drugs. Blood trough levels for both drugs over the first 60 days are provided in Figure 1. As shown, median blood through levels for everolimus were 4.25 ng/mL on day 3 and 5.6 ng/mL on day 9, and those for tacrolimus were 7.65 ng/mL on day 3 and 7.5 ng/mL on day 9. A few patients occasionally had levels below or above the therapeutic serum level during the study phase, but all returned promptly to the target level after dose adjustment. But although everolimus was scheduled to be administered up to day 56 after HSCT, the drug actually was administered for only a median of 44 days (range, 10 to 56 days). The reason for premature discontinuation (required in 50% of the patients) included early-onset (ie, day 6) GVHD-associated hyperbilirubinemia, CTC grade 4 (n = 1); TMA (n = 3); sinusoidal obstructive syndrome (SOS) of the liver (n = 6); and at least a 50% drop in platelet level after engraftment (n = 2).

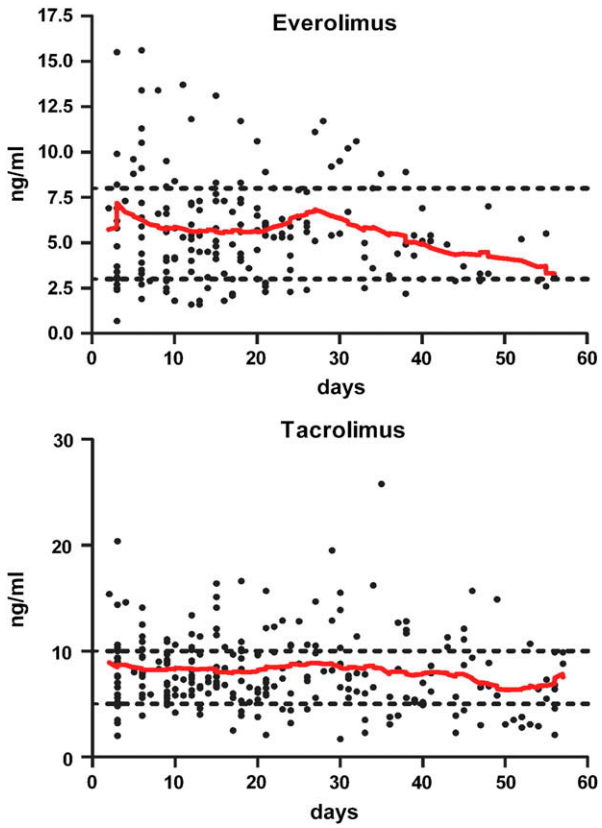

Figure 1. Everolimus and tacrolimus blood levels. The solid line is a spline-smoothing curve. The everolimus and tacrolimus target ranges were 5 to 10 ng/mL and 3 to 8 ng/ml (dashed lines), respectively.

Infectious and Miscellaneous Complications

CMV-DNA or pp65 antigen was detectable in only 9 of the 18 patients at risk (50%). Nonfatal toxicities during aplasia included mucositis, CTC grade I (n = 6), II (n = 7), III (n = 10), or IV (n = 1), and neutropenic fever (n = 13). Ten patients developed diarrhea, CTC grade II (n = 6) or III (n = 4), during conditioning-induced aplasia; 3 of these patients had neutropenic enterocolitis. Two patients developed hyperlipidemia, CTC grade II, not requiring pharmacologic intervention.

Acute and Chronic GVHD

As shown in Figure 2, the cumulative incidences of grade II-IV and grade III-IV acute GVHD were 38% and 18%, respectively. Three of 4 patients with a related donor did not develop acute GVHD. Of 17 evaluable patients, 14 (82%) developed chronic GVHD, 11 (65%) with an extensive form (Figure 3). As of the time of this writing, 2 patients had been able to discontinue immunosuppressive drug therapy.

Transplantation-Associated Microangiopathy

Decreased platelet counts with abnormally elevated schistocyte levels, compatible with the recently published criteria for TMA [29], was documented in 7 patients (29%), 2 of whom subsequently developed acute renal failure. TMA was diagnosed a median of 32 days (range, 8 to 54 days) after HSCT. In 5 of the 7 patients with TMA, either tacrolimus (n = 4) or everolimus (n = 1) blood trough levels were slightly

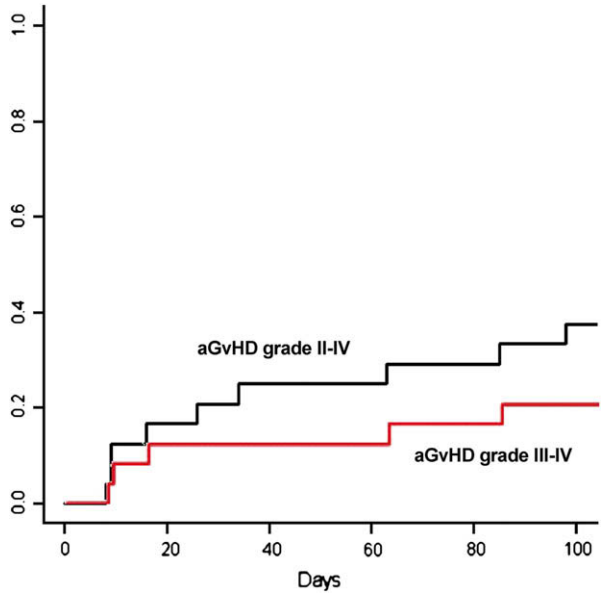

Figure 2. Cumulative incidence of acute GVHD in patients during EVTAC prophylaxis.

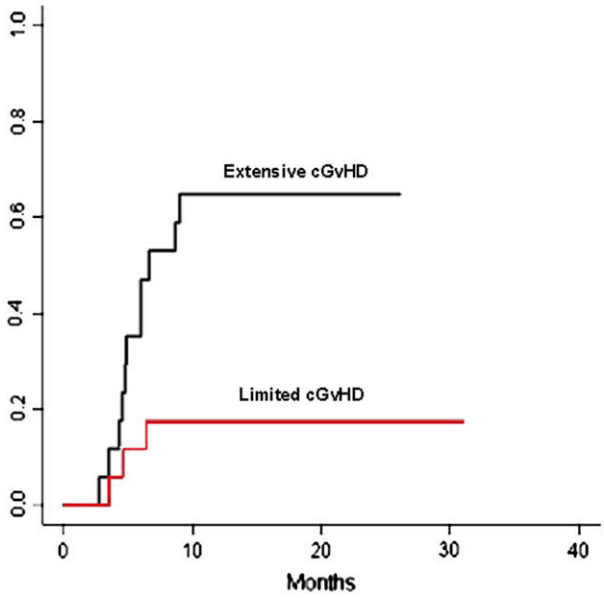

Figure 3. Cumulative incidence of extensive chronic GVHD in 17 evaluable patients.

above the upper target level at the onset of TMA. In the 2 patients with TMA associated with acute renal failure, the EVTAC combination was replaced by prednisone (2 mg/kg) and mycophenolate mofetil. One patient subsequently developed grade IV acute GVHD and died. In the remaining patients, TMA was managed conservatively, including dose reductions of either tacrolimus or everolimus as appropriate.

Liver Toxicity

During aplasia (day 6), patient UPN 1502 developed hyperbilirubinemia, CTC grade IV, without ascites or weight gain (and thus not fulfilling the criteria for SOS). Because this patient also had histologically proven gut GVHD, compatible with early-onset GVHD [30], the treating physicians decided to switch the immunosuppressive therapy to CsA/mycophenolate mofetil plus prednisone (2 mg/kg/day). Subsequently, the patient's bilirubin levels normalized.

SOS of the liver with hyperbilirubinemia, weight gain, ascites, and subsequent increased serum creatinine level was observed in 6 patients (25%) a median of 32 days (range, 10 to 51 days) after HSCT (Table 1). This was not associated with elevated blood through levels of either tacrolimus or everolimus at that time point. After SOS was found in 4 of the first 16 patients evaluated (including 2 who subsequently died and 1 with tMDS), all of whom had been conditioned with busulfan (3.45 mg/kg/day i.v. for 4 days [days -6, -5, -4, and -3]) plus fludarabine (30 mg/m^2 i.v. daily for 4 days [days -5 to -2]), as reported recently [31,32], the DSMB decided to replace fludarabine with cyclophosphamide (60 mg/kg i.v. on days -3 and -2), given the potential effects of fludarabine on liver endothelial cells [33]. But even after this switch, 2 patients of this series (UPN 1475 and UPN 1483) experienced SOS (Table 1). Consequently, subsequent patients were maintained on fludarabine and busulfan; however, during the time of combined EVTAC prophylaxis, defibrotide was administered prophylactically (10 mg/kg/day), because of the protective effect demonstrated in vitro [34] and in vivo [35]. Furthermore, patient UPN 1541 also developed hyperbilirubinemia, CTC grade IV, and complete thrombosis of the left portal vein branch, as well as partial thrombosis of the right branch, on day 34 after HSCT. Consequently, given the occurrence of TMA despite defibrotide prophylaxis in patients UPN 1541 and 1590, the DSMB decided to terminate the trial prematurely, citing safety issues, before reaching the target study population of 30 patients.

Association with Liver Toxicity and Metabolism of Study Drugs

We investigated polymorphisms that could possibly explain the high frequency of SOS seen in our study population (see Methods). Genotyping of all of these polymorphisms was performed successfully in all subjects. Primarily, we explored whether SNPs of cytochrome 3A5 and 3A4, being involved in the metabolism of tacrolimus and everolimus, respectively [20], could be attributed to the toxicity seen with this regimen. All patients exhibited the wild type, however, excluding the presence of "low metabolizers" as a reason for the high incidence of SOS (data not shown). Furthermore, analyses of polymorphisms of various GSTs (GSTP1-Ile105Val, GSTA1*a/b, and null genotypes of GSTM1 and GSTT1), which have been linked to SOS [36], demonstrated no association with the high incidence of SOS (data not shown).

Mortality, Relapse, and Survival

The day 100 and 1 year treatment-related mortality (TRM) rates were 12.5% and 29%, respectively. As shown in Figure 4A, 7 patients died due to nonrelapse mortality after a median of 165 days (range, 45 to 454 days), and 1 patient died due to apoplexia almost 2 years after HSCT. Hematologic relapse occurred in 6 of 24 patients (4 with AML, 2 with refractory anemia with excess blasts (RAEB)-2), a median of 100 days (range, 68 to 796 days) after HSCT (Figure 4B). All but 1 patient (currently receiving reinduction) with relapse died. With a median follow-up of 26 months for surviving patients, the 2-year probabilities of overall survival and disease-free survival were 47% and 37%, respectively (Figure 5). There was no significant correlation between overall or disease-free survival and host CMV seropositivity, CD34$^+$ cell dose, age, or female donor of a male host (data not shown).

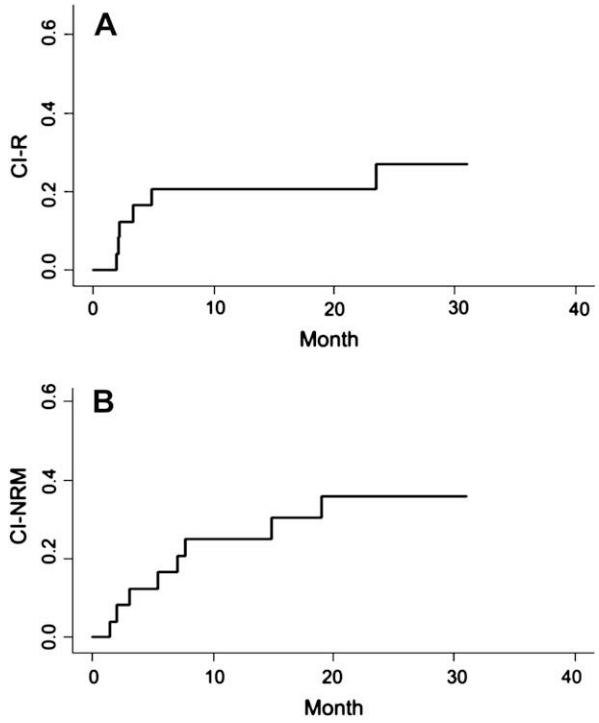

Figure 4. Cumulative incidence of relapse (CI-R) and nonrelapse mortality (CI-NRM) of the entire study cohort.

DISCUSSION

The aim of this prospective trial was to study the efficacy and toxicity of EVTAC as GVHD prophylaxis after allogeneic HSCT. Although this combination has been used successfully after renal transplantation [37], data for HSCT are lacking. Overall, our findings indicate some major problems and argue against the feasibility of this approach in elderly patients receiving intensive busulfan-based conditioning. The use of EVTAC in these patients was associated with a low incidence of severe mucositis, and all of the patients demonstrated rapid and stable engraftment; however, 2 patients had a significant drop in platelets after initial engraftment. This toxicity was not unexpected, given the important function of mTOR in megakaryocytic differentiation [38].

The rate of acute GVHD seen in this study seems low given the high proportion of unrelated and even mismatched donors. It compares quite well with that reported by the Dana-Farber group [13] in their study of tacrolimus and sirolimus combination therapy. But the rate of extensive chronic GVHD appears to be as high as that seen with other regimens, arguing against a general protective effect of everolimus on GVHD. It might be speculated that a longer administration of the study drug could possibly overcome this problem; however, this approach does not seem feasible, given the toxicity profile in our patient cohort. In fact, half of our patients had to stop everolimus before day 56 due to anticipated toxicities. Major concerns included the high incidence of SOS of the liver, which exceeded that generally reported in the literature (~10%) [39], as well as increased risk of toxicity in patients over age 60 receiving ablative conditioning [40]. The reason for these effects remains unclear, given that none of our patients had received gemtuzumab ozogamicin (which has been shown to increase the overall risk of SOS [39]) before HSCT. Sirolimus has been shown to induce thrombogenic alteration of endothelial cells [41,42]. Interestingly, Cutler et al. [43] recently reported an increased incidence of SOS with sirolimus-based GVHD prophylaxis compared with conventional GVHD prophylaxis (15% vs 6%), although this did not translate into worse patient outcomes. Furthermore, SOS occurred later (median, 22 days vs 15 days), in line with our findings. Although no definitive conclusions can be drawn from our small cohort, it seems that the omission of fludarabine (which affects liver sinusoidal and endothelial cells [33]) may abrogate the development of SOS with this regimen. Our use of busulfan without total body irradiation for

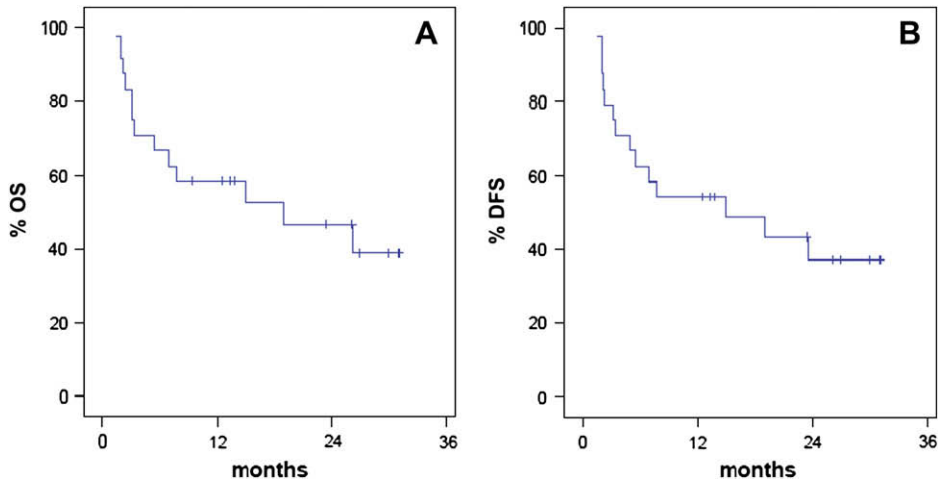

Figure 5. Probability of survival (A) and disease-free survival (B) of 24 patients with MDS or AML in the EVTAC trial.

intensive conditioning also might be a factor in the differences between our findings and those of others [13]. Whether the use of targeted busulfan might have altered the incidence of SOS within our protocol remains to be studied. The presence of "low metabolizers" was not a factor in the high incidence of SOS, because SNPs of cytochrome 3A4 and 3A5 were not present. This is important, because cytochrome 3A4 is involved in the metabolism of everolimus, and cytochrome 3A5 is involved in the metabolism of tacrolimus [44,45]. The null genotype of GSTM1 predisposes children with thalassemia undergoing busulfan-based allogeneic HSCT to SOS [36]; however, analyses of these and further polymorphisms of various GSTs revealed no explanation for the high incidence of SOS observed in our study population.

We are also concerned about the frequency of TMA in our trial, which is higher compared with the Dana-Farber experience with a sirolimus-tacrolimus combination [13]. However, recent data from a Seattle study [46] are compatible with our observations and, together with experience in the treatment of steroid-refractory GVHD [47], suggest that mTOR inhibition might increase the overall risk of TMA. Cutler et al. [13] found that TMA could be overcome by dose reduction or cessation of tacrolimus in all cases. Together with our observation that TMA was associated mainly with slightly increased tacrolimus levels, this suggests that everolimus increases the potential of tacrolimus to induce TMA in transplant recipients.

In summary, use of the EVTAC combination for GVHD prophylaxis is associated with significant toxicity and thus does not seem to improve overall outcome. For this reason, this combination cannot be recommended as a prophylactic regimen after busulfan-based intensive conditioning. Future studies in the context of total body irradiation–based or reduced-intensity conditioning regimens might reach a different conclusion, however.

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