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Etanercept as Treatment of Steroid-Refractory Acute Graft-versus-Host Disease in Pediatric Patients

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A B S T R A C T

Corticosteroids are the standard of care for first-line treatment of patients who develop grade II-IV of acute graft-versus-host disease (aGVHD), but the optimal second-line treatment has not yet been determined. We prospectively evaluated the use of the anti-TNFα monoclonal antibody etanercept (ET) as second-line treatment in children with steroid-refractory (SR) aGVHD. Twenty-five children with either malignant or nonmalignant diseases experiencing grade II-IV SR aGVHD received ET as second-line treatment. ET was administered after a median of 14 days (range, 5 to 135 days) from the onset of aGVHD. Seventeen out of 25 patients (68%) developed a complete response (CR) or partial response (PR) to ET. The overall response rate (CR plus PR) was 78% in patients with cutaneous SR aGVHD, 78% in those with gastrointestinal aGVHD, and 57% in those with hepatic aGVHD. On day +100 after the start of ET, 52% of the children were in CR, 16% were in PR, and the remaining 32% failed to respond. Overall survival was 76.5% in responders and 16.7% in nonresponders ($P = .004$). Transplantation-related mortality at 5 years was 34.1% (95% confidence interval, 18.6% to 57.1%). In our experience, ET has proven to be effective as second-line treatment in children with SR aGVHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established and potentially curative treatment for a variety of malignant and nonmalignant diseases. Graft- versus-host disease (GVHD) is the major complication following HSCT. The introduction of antithymocyte globulin (ATG) as GVHD prophylaxis and of new therapies (eg, mycophenolate mofetil [MMF], budesonide, extracorporeal photopheresis [ECP]) as GVHD treatment has improved the prognosis of this transplantation-related complication, but cases of refractory GVHD still occur.

Even though there is an international consensus on methylprednisolone (MPD) as first-line therapy of acute GVHD (aGVHD) administered at a dose of 2 mg/kg/day [1], suboptimal responses have been reported in 40% to 60% of patients. Several second-line treatments have been attempted in patients with steroid-refractory (SR) aGVHD, including MMF, pentostatin, infliximab, etanercept (ET), daclizumab, alemtuzumab, mesenchymal stromal cells (MSCs), and ECP [1,2]. To date, none of these treatments has proven superior to the others, and the best approach to treating SR aGVHD remains to be defined.

The role of tumor necrosis factor (TNF)-α in the pathophysiology of aGVHD is well known. TNF-α is one of the cytokines that amplifies donor immune response to host tissue, and it has a direct effect on the organs [3,4]. ET is a soluble dimeric TNF-α receptor that binds TNF-α and renders it inactive.

Ferrara et al [5] demonstrated that the combination of ET and steroids for initial treatment of aGVHD resulted in a significantly higher rate of complete remission after 4 weeks of treatment compared with steroids alone. Few studies on the efficacy of ET have been reported in children alone, with the majority of published studies including adults [6,7].

We conducted this prospective Phase II, nonrandomized, single-center study to evaluate the efficacy and toxicity of ET as second-line treatment of SR aGVHD, as well as long-term survival, in children undergoing allogeneic HSCT.

METHODS

Between November 2008 and April 2018, all patients age <18 years who developed grade II-IV SR aGVHD after undergoing allogeneic HSCT for

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malignant or nonmalignant disease were included in this prospective study. GVHD prophylaxis varied according to type of donor and diagnosis (ie, malignant versus nonmalignant disease) and included cyclosporine A (CyA) alone or associated with MMF, short-term methotrexate (MTX), and ATG.

In particular, patients with malignant disease who underwent allogeneic HSCT from a related donor or an alternative donor received CyA (2 mg/kg/day in 2 doses) alone or associated with ATG and short-term MTX (10 mg/m^2 at day +1, 8 mg/m^2 at days +3, +6, +11) respectively. Patients with nonmalignant disease received CyA plus ATG and MMF irrespective of donor type. In patients with nonmalignant disease, CyA plasma levels were monitored twice weekly and then maintained between 100 and 200 ng/dL, whereas in patients with malignant disease, CyA plasma levels remained unchanged in the absence of aGVHD or toxicity.

aGVHD was graded according to the modified Glucksberg criteria [8], and chronic GVHD (cGVHD) was graded according to the National Institutes of Health classification [9].

All children with grade II-IV aGVHD received first-line therapy with MPD 2 mg/kg/day i.v. for 10 consecutive days. The response to steroid therapy was evaluated at 5 to 7 days after the initial dose based on the maximum aGVHD grade evaluated overall and in each organ. Patients were considered not evaluable for response to therapy only in the event of withdrawal of consent or noncompliance.

Complete response (CR) was defined as the complete resolution of aGVHD symptoms in all organs. Partial response (PR) was defined as an improvement in aGVHD grade in all involved organs without complete resolution and without worsening. No response (NR) was defined as stable condition or progression of aGVHD in any organ or patient death. The overall response rate (ORR) was defined as CR plus PR or CR alone. In patients with CR, the steroid dose was tapered by 25% every 7 to 10 days to reach a daily dose of .5 mg/kg. Patients with PR continued to receive steroid therapy and were reevaluated during follow-up.

SR aGVHD was diagnosed when clinical signs worsened or remained stable at 5 to 7 days after initiation of MPD. Biopsy confirmation of aGVHD was not required. In patients with SR aGVHD, second-line treatment with ET was administered in addition to steroid treatment. In patients with severe life-threatening SR aGVHD, the dose of MPD was increased the >2 mg/kg (3.5 to 5 mg/kg), and ECP was added to first-line therapy. ET was administered s.c. at a dose of .4 mg/kg twice weekly for 8 weeks (16 doses total).

Anti-infective prophylaxis included acyclovir (for herpesviruses), fluconazole (for *Candida*), and cotrimoxazole (for *Pneumocystis jirovecii*). Patients were monitored with quantitative polymerase chain reaction for cytomegalovirus, Epstein-Barr virus, adenovirus, human herpesvirus 6, or BK virus reactivation or detection of galactomannan antigen and/or computed tomography scan according to clinical indications [10]. Patients with previous or active tuberculosis, renal or hepatic insufficiency, or previous documented allergic reactions to ET were excluded from the study.

The primary endpoint of the study was the proportion of patients with SR aGVHD who had a PR or CR to ET as second-line treatment. Secondary endpoints were the ORR after 4, 8, and 16 doses of ET corresponding to days +14, +28, and + 56 after ET; the response in each organ affected by SR aGVHD; ORR on days +100 and +180 after initiation of ET and at the last follow-up; the grade of cGVHD; the infectious complications occurring during ET administration; and survival of patients treated with ET.

The study protocol and patient consent forms were approved by the Institutional Ethics Committee in February 2009 (no. 17; Eurotract 2008-006726-34).

Statistical Analysis

A Simon 2-stage optimal design [11] was used to evaluate CR and PR to ET to rule out a 20% response rate (P0 = .20%) and to target the desirable goal of a 40% response rate (P1 = .40%). With α = .10 (probability of accepting a poor treatment) and β = .10 (probability of rejecting a good treatment) in the first stage,17 patients were to be enrolled, and only if at least 4 of these had a PR or CR at the end of treatment would it have been possible to move to the second stage, during which other 20 patients (for a total of 37 patients) could have been enrolled.

ET could be considered effective if ≥11 of 37 patients experienced a PR or CR at the end of therapy.

The data are reported as mean and standard deviation or median and range for continuous variables, and as absolute and relative frequencies for categorical variables. Comparison of frequency distributions was analyzed using the chi-square test. Fisher's exact test was used in case of at least 1 expected frequency <5. The Mann-Whitney *U* test was used to compare continuous variables in 2 independent groups. The log-rank test was used to assess differences between groups.

Overall survival (OS) was evaluated by the Kaplan-Meier method, and the 95% confidence interval (CI) of estimates was calculated. A similar analysis was performed for transplantation-related mortality (TRM), which was defined as death from any cause in the absence of relapse or progression of the primary disease. OS related to ET therapy was defined as the interval from the start of ET to death or the last follow-up. Event-free survival was defined as the interval from the beginning of ET and some events, including relapse, death from GVHD, and presence of GVHD at the last follow-up. A *P* value <.05 was considered statistically significant, and all *P* values were based on 2-tailed tests. Statistical analysis was performed using SPSS for Windows version 18 (SPSS, Chicago, IL).

RESULTS

Twenty-five out of a total of 231 (10.8%) consecutive patients undergoing allogeneic HSCT in our institution developed SR aGVHD and received ET. The incidence of grade III-IV aGVHD was 20%. Patient characteristics with SR aGVHD are reported in Table 1. The median time between HSCT and last follow-up was 15.5 months (range, 1.4 to 100.2 months).

We initially planned to enroll a total of 37 patients according to a Simon optimal 2-stage design and after enrollment of the first 17 patients, the trial was continued because 11 patients (64.7%) developed a PR or CR.

The study was interrupted before we reached the planned sample size, because among the already enrolled 25 patients, 17 (68%) had a PR or CR. In fact, even if the remaining 12 patients needed to reach the planned sample size had shown no response to ET, still we would have observed a response rate of 46%, which exceeds the minimum response rate (40%) necessary to declare the efficacy of the drug.

SR aGVHD was observed in 25 patients involving the skin in 18 (72%), the gastrointestinal tract in 18 (72%), and the liver in 7 (28%). All 25 patients received MPD at a dose of 2 mg/kg/day. This dose remained unchanged in 16 patients (64%) but was increased in the remaining 9 (36%), who developed life-threatening SR aGVHD. Twelve patients (48%) underwent ECP.

Patients received ET at a median of 14 days (range, 5 to 135 days) after the onset of aGVHD. The median number of ET doses was 16; 3 patients did not complete the course of ET and received 8, 9, and 9 doses because they died at days +42, +89, and +96 days after HSCT for severe infectious events—namely cerebral zygomycosis, sepsis due to *Candida parapsilosis*, and severe systemic adenovirus, respectively.

Seventeen patients developed a CR or PR (68%), including 14 with CR (56%) and 3 with PR (12%). On day +28 after ET treatment (ie, after 8 doses), the ORR was 64%. A CR or PR was achieved in 17 patients (68%) after the first 4 doses, in 16 (64%) after 8 doses (ie, day +28), and in 17 (68%) after 16 doses. On completion of the planned treatment (day +56; 16 doses), the ORR was 68%; in particular, the percentage of patients who developed CR to ET increased from 28% to 56%, those with PR decreased from 40% to 16%, and those with NR remained stable after 4 and 8 doses and decreased at the end of the treatment (from 32% to 16%) (Figure 1).

In the 8 patients who did not respond to ET, other therapies were administered. These included MSC infusion in 3 patients, increased steroid dosage in 2 patients, and monoclonal antibody against IL- 6 followed by ruxolitinib, monoclonal antibody against CD25, and imatinib in 1 patient each.

Considering the response of aGVHD in each single organ, we observed that among 18 patients with cutaneous SR aGVHD, CR was achieved in 10 patients (56%) after the first 4 doses of ET, in 13 (72%) after 8 doses, and in 14 (78%) after 16 doses. Among the 18 children with gastrointestinal SR aGVHD, CR developed in 6 patients (33%) after 4 doses, in 9 (50%) after 8 doses, and in 10 (56%) after 16 doses. Among 7 patients with hepatic SR aGVHD, CR was achieved in 3 patients (43%) after 4 and 8 doses and in 4 patients (57%) after 16 doses. The ORR including CR and PR evaluated after 16 doses of ET was 78% (n = 14) for cutaneous SR aGVHD, 78% (n = 14) for gastrointestinal SR aGVHD, and 57% (n = 4) for hepatic SR aGVHD (Figure 2).

Table 1
Patient Characteristics

Characteristic	Total (N = 25)	Responders (N = 17; 68.0%)	Nonresponders (N = 8; 32.0%)	*P* Value
Male/female, n (%)	16 (64.0)/9 (36.0)	10 (58.8)/7 (41.2)	6 (75.0)/2 (25.0)	.66
Malignant disease, n (%)	12 (48.0)	10 (58.8)	2 (25.0)	.20
Nonmalignant disease, n (%)	13 (52.0)	7 (41.2)	6 (75.0)	
Age at HSCT, yr, median (range)	7.8 (.4-15.7)	5.4 (.4-15.3)	9.8 (1.3-15.7)	.22
Interval between onset of aGVHD and ET, d, median (range)	14 (5-135)	14 (5-63)	27.5 (5-135)	.41
Type of HSCT, n (%)				
Alternative donor	18 (72.0)	11 (64.7)	7 (87.5)	.17
Related donor	3 (12.0)	2 (11.8)	1 (12.5)	
Haploidentical donor	4 (16.0)	4 (23.5)	-	
Stem cell source, n (%)				.40
Bone marrow	17 (68.0)	11 (64.7)	6 (75.0)	
Cord blood	5 (20.0)	4 (23.5)	1 (12.5)	
Peripheral blood	3 (12.0)	2 (11.8)	1 (12.5)	
Reduced-intensity conditioning regimen	8 (32.0)	3 (17.6)	5 (62.5)	.06
Myeloablative conditioning regimen	17 (68.0)	14 (82.4)	3 (37.5)	
Busulfan-based	7	7	0	
TBI-based	3	2	1	
Treosulfan-based	7	5	2	
GVHD prophylaxis with ATG, n (%)	21 (84.0)	14 (82.4)	7 (87.5)	
ATG + CyA + MTX	10	7	3	1
ATG + CyA + MPD	4	3	1	
ATG + CyA + MMF	4	3	1	
GVHD prophylaxis without ATG, n (%)	4 (16.0)	3 (17.6)	1 (12.5)	
Campath + CyA + MTX	1	0	1	
CyA + MMF	1	1	0	
CyA + MMF + post-CY	2	2	0	
Grade of aGVHD before ET, n (%)				.78
Grade II	4 (16.0)	3 (17.6)	1 (12.5)	
Grade III	10 (40.0)	6 (35.3)	4 (50.0)	
Grade IV	11 (44.0)	8 (47.1)	3 (37.5)	
Status at last follow-up, n (%)				
Alive	15 (60.0)	13 (76.5)	2 (25.0)	.03
Dead	10 (40.0)	4 (23.5)	6 (75.0)	
TRM, n (%)				
Yes	8 (32.0)	2 (11.8)	6 (75.0)	.004
No	17 (68.0)	15 (88.2)	2 (25.0)	

TBI indicates total body irradiation; post-CY, post-transplantation cyclophosphamide.

The response to ET as GVHD grade in each organ affected by SR aGVHD is shown in Figure 3.

We observed that the increase in the daily dose of MPD did not increase the ORR of SR aGVHD (66.7% with >2 mg/kg MPD versus 68.8% with 2 mg/kg MPD). The median duration of steroid therapy was 49 months (range, 6.17 to 101 months).

ECP was associated with high-dose (>2 mg/kg) steroid therapy in 5 patients with severe SR aGVHD (grade IV) and with standard-dose steroid therapy in 7 patients with grade III (n = 4) or grade II (n = 3) SR aGVHD.

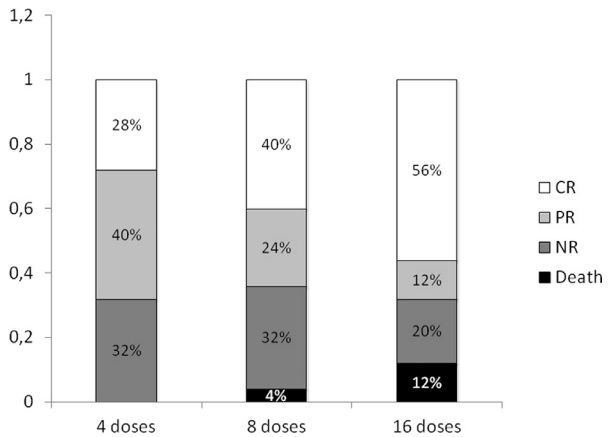

Figure 1. Response to ET including CR, PR, NR, and nonevaluable patients who died before the 16 doses of ET.

Thirteen patients received ECP during ET administration, but in 4 of them ECP was discontinued for severe transplantation-related complications (n = 3) or for its inefficacy (n = 1), whereas the remaining 9 patients (69.2%) reached CR or PR. In the group of 12 patients who did not receive ECP, 7 (58.3%) developed CR or PR. The difference between the groups of patients treated with ECP or without ECP was not statistically significant (*P* = .68). There was statistically significant difference in response between patients treated with ECP plus ET

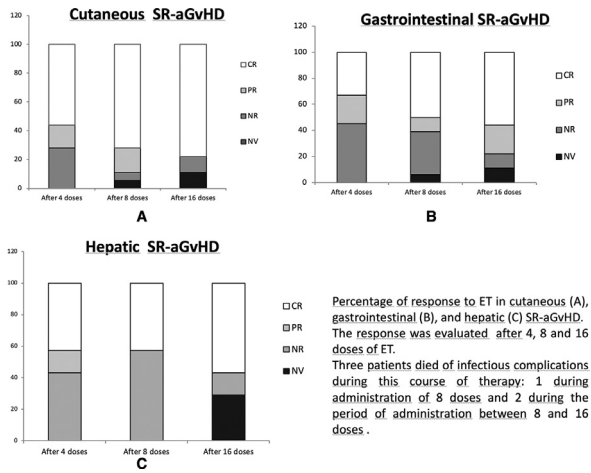

Figure 2. Response to ET including CR, PR, and NR evaluated in each organ affected by SR aGVHD. (A) Cutaneous; (B) Gastrointestinal; (C) Hepatic.

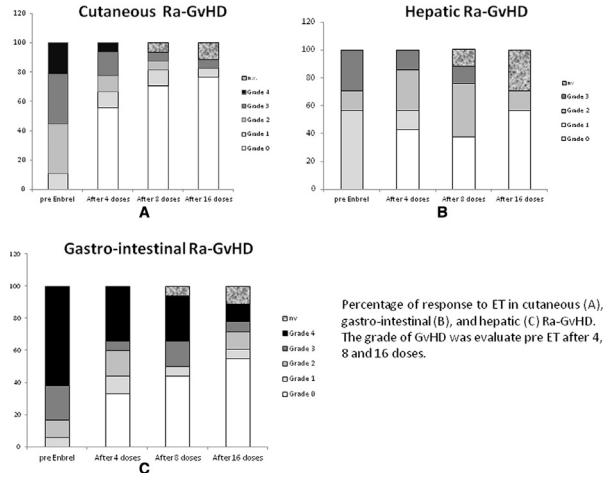

Figure 3. Response to ET by GVHD grade in each organ affected by SR aGVHD. (A) Cutaneous; (B) Gastrointestinal; (C) Hepatic.

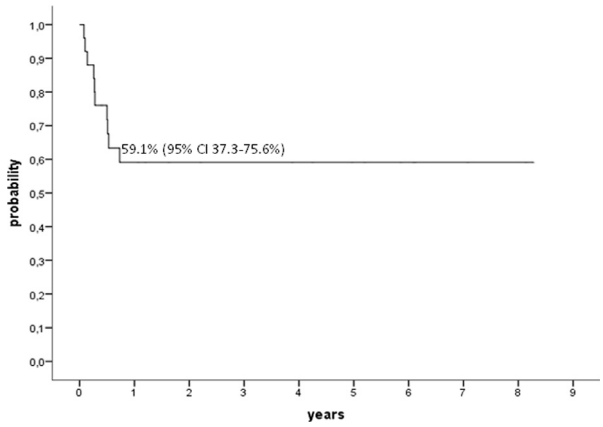

Figure 4. OS.

with no tapering (n = 9) and those in whom ECP was tapered (n = 3) (P = .04).

The ORR evaluated on day +100 after the start of ET was positive in 68% of patients (CR in 13 [52%] and PR in 4 [16%]) and negative (NR) in 8 patients (32%).

Clinically significant infectious complications requiring systemic treatment occurred in 17 patients (68%), and included 9 bacteremias (36%), 19 viral reactivations (76%), and 5 invasive mycoses (20%; zygomycoses in 1, aspergillosis in 2, and candidemias in 2) (Table 2). Ten patients with infectious complications (58%) had a positive overall response after 16 doses and on day +100 (9 viral infections, 1 bacteremia), and 7 (41.2%) did not respond (with 5 viral infections and 3 severe mycoses). One child affected by resistant adenovirus infection died of this complication.

ET was well tolerated in all children, and compliance with s.c. administration was fairly good considering the age of the patients.

cGVHD was observed in 14 of 22 patients (63.6%) evaluable for this complication. cGVHD was grade 1 in 5 patients (22.7%), grade 2 in 4 (18.1%), and grade 3 in 5 (22.7%). On day +180 after HSCT, 19 patients (76%) were alive, 9 (47.4%) of whom had cGVHD.

The median duration of follow-up, calculated as the interval between the start of ET and the last observation, was 14.42 months (range, 1 to 99.9 months). Ten children died, at a median of 175 days after HSCT (range, 42 to 329 days). The causes of death were progressive SR aGVHD in 3 children (30%), cGVHD in 5 (50%; including 3 with severe intractable mycoses), and relapsed acute myelogenous leukemia in 2 (20%).

Among the remaining 15 children alive at the last follow-up evaluation, 2 (13.3%) still had grade 3 cGVHD, 1 at 4.8 months and the other at 59.9 months.

The OS rate at 1 year after ET was 59.1% (95% CI, 37.3% to 75.6%) (Figure 4). The OS rate was 76.5% (95% CI, 48.9% to 90.5%) in responding patients versus 16.7% (95% CI, 1% to 50.8%) in nonresponding patients (P = .004) (Figure 5).

TRM at 5 years was 34.1% (95% CI, 18.6% to 57.1%) (Figure 6). TRM was not statistically significantly different between patients who received MPD >2 mg/kg/day and those whose received MPD ≤2 mg/kg/day (P = .87). Moreover, underlying disease and type of HSCT did not influence TRM. EFS at 3 years was 45.5% (95% CI, 24.4% to 64.4%) (Figure 7).

DISCUSSION

The occurrence of SR aGVHD remains a severe complication, given the very high SR aGVHD-related mortality rate

Table 2
Infectious Events in Patients Treated with ET

Infection	Total (N = 25), n (%)	Responders (N = 17), n (%)	Nonresponders (N = 8), n (%)	*P* Value
Infectious events				
Yes	17 (68.0)	10 (58.8)	7 (87.5)	.20
No	8 (32.0)	7 (41.2)	1 (12.5)	
Sepsis				
No	16 (64.0)	12 (70.6)	4 (50.0)	.39
Yes	9 (36.0)	5 (29.4)	4 (50.0)	
Virus reactivation				
None	11 (44.0)	8 (47.0)	3 (37.5)	.49
One or more reactivation(s)*	14 (66.0)	9 (53.0)	5 (62.5)	
Cytomegalovirus	12	7	5	
BK virus	2	2	—	
Adenovirus	1	—	1	
Human herpesvirus 6	2	1	1	
Epstein-Barr virus	1	1	—	
Varicella zoster virus	1	—	1	
Mycoses				
None	20 (80.0)	16 (94.1)	4 (50.0)	.02
Candidemia	2 (8.0)	1 (5.9)	1 (12.5)	
Aspergillus	2 (8.0)	-	2 (25.0)	
Zygomycoses	1 (4.0)	-	1 (12.5)	

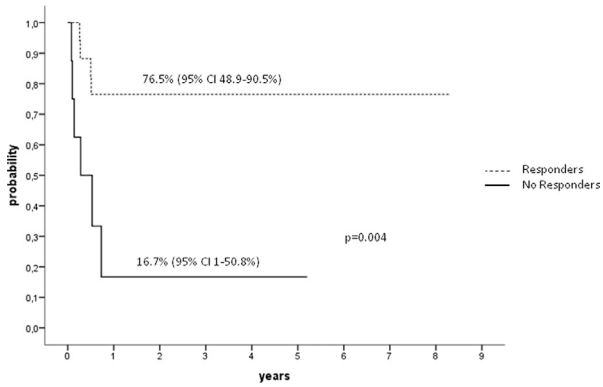

Figure 5. OS evaluated in responders and nonresponders.

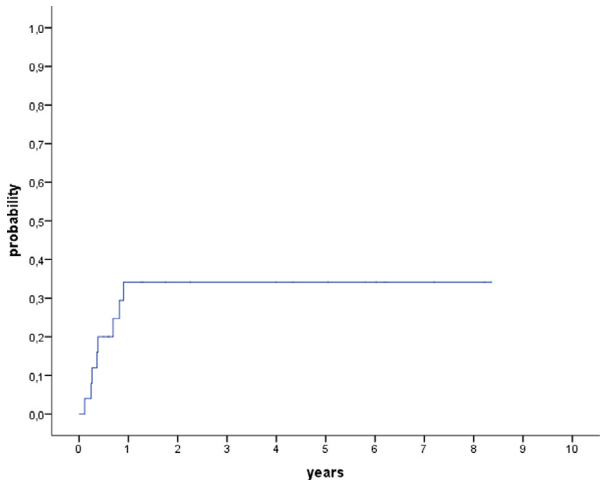

Figure 6. TRM.

reported in the literature [12,13]. In our recently published study [14], 40% of the children who developed SR aGVHD died of disease-related complications.

To our knowledge, this is the largest study on treatment with ET for SR aGVHD in pediatric population [6,15-17]. The majority of studies were conducted in adults, and ET was often associated with other immunosuppressive therapies, such as daclizumab and IL-2 [18], ATG, and tacrolimus [5]. Busca et al [6] reported on 13 patients with SR aGVHD who received ET; in that study, the ORR was 46% (6 patients out of 13), and patients with refractory gut aGVHD (63%) had a higher response rate, followed by patients with cutaneous aGVHD (50%), and hepatic aGVHD (40%).

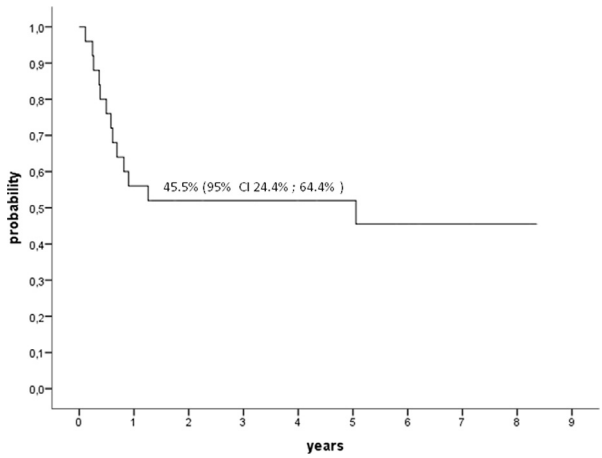

Figure 7. Event-free survival.

In this prospective study, the ORR for ET was higher than that reported in other studies including adult and pediatric patients [19]: 78% in patients with cutaneous SR aGVHD, 78% in those with gastrointestinal SR aGVHD, and 57% in those with hepatic SR aGVHD. These results confirm the efficacy of ET in the treatment of gastrointestinal involvement, supporting the use of TNF as a biomarker in the pathogenesis of gastrointestinal aGVHD [20-22]. The incidence of CR was higher in patients with cutaneous involvement (78% versus 56% in patients with gastrointestinal SR aGVHD and 57% in those with hepatic SR aGVHD), which suggests that the skin is the organ that best responds to immunosuppressive therapy. The addition of high-dose steroids (>2 mg/kg) did not influence the ORR to ET in our patients. The addition of ECP to ET significantly increased the response in these patients, confirming recently published results [23]. The discontinuation or reduction of steroids depended on the occurrence of cGVHD in our series, and for this reason, it was extremely difficult to use this approach as an indicator of response to ET.

The analysis of the response to ET in each involved organ and during the course of ET administration represents a peculiar and important endpoint of this study.

In this study, the highest positive response rate to ET was observed after the first 4 doses in patients with cutaneous and hepatic aGVHD and after the first 8 doses in patients with gastrointestinal SR aGVHD. The response rate in patients with cutaneous and gastrointestinal SR aGVHD increased steadily during the course of treatment, whereas it remained stable in patients with hepatic SR aGVHD. These observations could help predict the time to response to ET in each organ and the prognosis of all treated patients.

The ORR observed in this study (68%) was higher than that reported in other studies that evaluated the results of SR aGVHD treatment with other immunosuppressive agents (46% with low-dose MTX and 67% with alemtuzumab) [24,25]. MSC infusion is a promising alternative therapy for the treatment of SR aGVHD, and Bader et al [26] recently reported an ORR of 83% in adults and children treated with MSCs. It must be noted, however, that the production of MSCs is currently limited to a few laboratories, restricting the procedure to only a minority of centers.

OS at 1 year after ET was 59.1% (95% CI, 37.3% to 75.6%) and remained stable until the last follow-up evaluation (at 8 years), demonstrating that the majority of patients died within the first year after HSCT. There was a statistically significant difference in OS between patients responding to ET and those not responding to ET (P = .004). Moreover, TRM at 5 years was 34.1%. These results support our conclusion that ET is a valid and effective treatment for pediatric patients with SR aGVHD.

The rate of cGVHD evaluated on day +100 in patients previously treated with ET was high (63%), and in 22.7% of them it was grade 3, but at the last observation, 16 patients (72.7%) did not have cGVHD.

In our cohort, invasive mycoses occurred in 20% of the patients with SR aGVHD and was the cause of death in 3 patients (12%), including 1 each with zygomycosis, *Candida* parapsilosis, and aspergillosis. Viral reactivation occurred in 76% of the patients, and bacterial infections occurred in 36%, but none died from these complications. These results are

comparable with the findings of an interim analysis of infectious complications in 11 patients who received ET that demonstrated a high frequency of infectious events, especially invasive mycosis [27], and with the findings confirming the role of GVHD in infectious complications, particularly mycosis [28]. Of course, strict monitoring for viral, bacterial, and fungal infections is mandatory, especially in patients at increased risk because of previous infectious complications.

We conclude that ET represents an effective treatment of SR aGVHD for the high response rate, the few infectious and fatal complications, and the significantly improved OS. In particular, the high response rate obtained in patients with gastrointestinal aGVHD is remarkable. Time to response to ET represents a useful tool for evaluating the response to this drug to predict prognosis in these patients.

The discovery of new drugs and their use also in children are opening up new horizons for the treatment of SR aGVHD, but currently at our transplantation center, ET remains the best therapy for SR aGVHD.

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