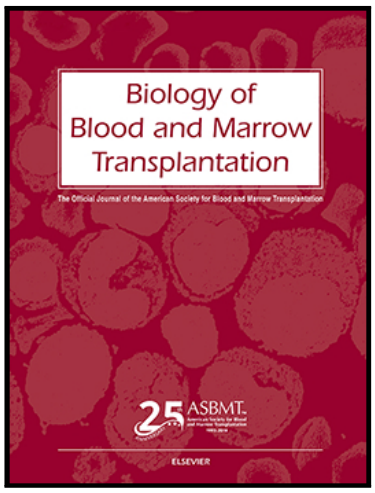

ETANERCEPT AS TREATMENT OF STEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE IN PEDIATRIC PATIENTS

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Highlights

- The overall response rate to Etanercept in children with steroid-refractory acute GvHD was 68%
- The better response was obtained in cutaneous and gastro-intestinal SR-a-GvHD.
- Overall Survival (OS) in responders was 76.5% (p=0.004)
- .Etanercept represents an effective treatment of SR-a-GvHD for the high response rate

ETANERCEPT AS TREATMENT OF STEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE IN PEDIATRIC PATIENTS

Running title: Etanercept in refractory a-GvHD in children

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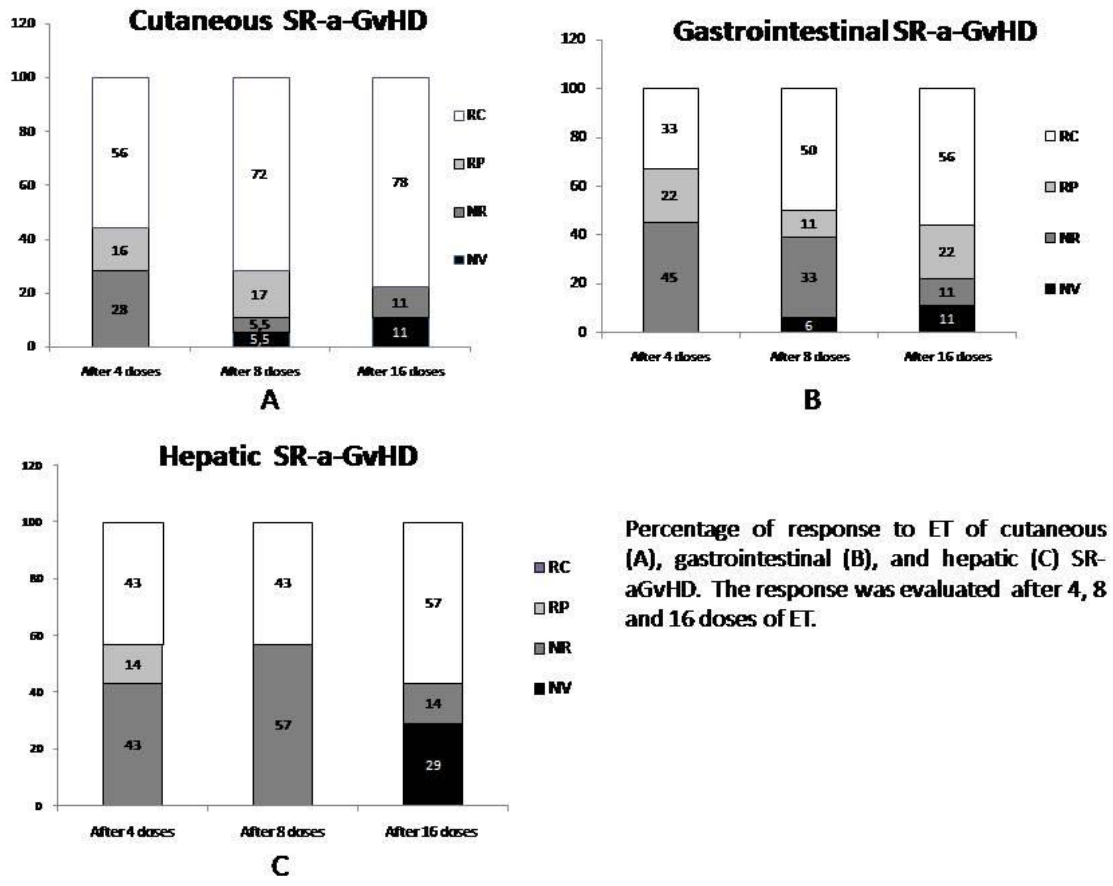
Conflict of interest statement: the authors Maura Faraci, Maria Grazia Calevo, Stefano Giardino, Massimiliano Leoni, Erica Ricci, Elio Castagnola, Edoardo Lanino declare that there are no competing financial interests in relation to the work described herein.

Graphical abstract



Percentage of response to ET of cutaneous (A), gastrointestinal (B), and hepatic (C) SR-aGvHD. The response was evaluated after 4, 8 and 16 doses of ET.

ABSTRACT

Introduction.Corticosteroids are the standard of care for first line treatment of patients who develop grades II-IV of acute Graft-versus-Host Disease (a-GvHD), but the optimal second-line treatment has not been determined yet. We prospectively evaluated the use of anti-TNFα monoclonal antibody Etanercept (ET) as second line treatment in children with steroid-refractory a-GvHD.

Materials and Methods Twenty-five children with either malignant or non malignant diseases experiencing grade II-IV steroid-refractory (SR)-a-GvHD received ET as second line treatment.

ET was administered after a median of 14 days (5-135 days) from onset of a-GvHD.

Results. Seventeen out of 25 patients (68%) developed complete or partial response (CR or PR) to ET. Overall response rate (ORR) (CR or PR) was 78% of patients with cutaneous SR-a-GvHD, 78% with gastro-intestinal a-GvHD, and 57% with hepatic a-GvHD. On day +100 after the start of ET, 52% of children were in CR, 16% in PR, while the remaining 32% failed to respond. Overall Survival (OS) in responders was 76.5% and 16.7% in non- responders (p=0.004). Transplant-related mortality (TRM) at 5 years was 34.1% (95% CI; 18.6%- 57.1%).

Conclusion. In our experience, ET proved to be effective as second line treatment in children with SR-a-GvHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established and potentially curative treatment for a variety of malignant and non-malignant diseases. Graft versus Host Disease (GvHD) represents the main complication following HSCT. The introduction of anti-thymocyte globulin (ATG) as GvHD prophylaxis and of new therapies (e.g. mycophenolate mofetil, budesonide or extracorporeal photophoresis) as GvHD treatment has improved the prognosis of this transplant-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 (a-GvHD) administered at the dose of 2 mg/Kg/day (1), suboptimal responses have been reported in 40-60% of patients. Several second-line treatments have been attempted in patients with steroid-refractory a-GvHD (SR-a-GvHD), including mycophenolate mofetil (MMF), pentostatin, infliximab, etanercept, daclizumab, alemtuzumab, mesenchymal stromal cells (MSC), and extracorporeal photophoresis (ECP) (1-2). To date, none of these treatments proved to be superior to the others, and the best approach to SR-a-GvHD remains to be defined.

The role of Tumor Necrosis Factor (TNF)-α in the pathophysiology of a-GvHD 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). Etanercept (ET) is a soluble dimeric TNF α receptor binding TNF α and rendering it inactive.

J. Ferrara et al (5) demonstrated that the combination of ET and steroids as initial treatment of a-GvHD resulted in significantly higher complete remission rate after 4 weeks of treatment compared to steroids alone. Few studies on the efficacy of ET were reported in children alone, while the majority included adults (6-7).

We performed a prospective, phase 2, non-randomized, single-center study to evaluate the efficacy and toxicity of ET as second-line treatment of SR-a-GvHD in children receiving allogeneic HSCT, as well as their long-term survival.

PATIENTS AND METHODS

From November 2008 to April 2018,all the patients aged <18 years who developed grade II-IV SR-a-GvHD after allogeneic HSCT for malignant or non malignant diseases were included in this prospective study. GvHD prophylaxis varied according to type of donor and diagnosis (malignant *vs* non-malignant disease) and included Cyclosporin A (CyA) alone or associated with MMF, short-term Methotrexate, and ATG.

In particular, patients with malignant disease who underwent allogeneic HSCT from related donor (RD) or alternative donor (AD) received CyA (2 mg/kg/day in two doses) alone or associated with ATG and short term Methotrexate (10 mg/m^2 at day +1, 8 mg/m^2 at day +3, +6, +11) respectively. Patients with non-malignant disease received CyA plus ATG and MMF irrespective of donor type. CyA plasma levels of patients with non-malignant disease were monitored twice a week and maintained between 100 and 200 ng/dL, while CyA plasma levels of patients with malignant disease, in the absence of a-GvHD or toxicity, were not changed.

Acute GvHD was graded according to the modified Glucksberg criteria (8), while chronic GvHD was graded according to the National Institutes of Health (NIH) classification (9).

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

Complete response (CR) was defined as the complete resolution of a-GvHD symptoms in all organs; partial response (PR) was defined as an improvement of a-GvHD grade in all involved organs without complete resolution and without worsening; no response (NR) was defined as stable condition or progression of a-GvHD in any organ or patient death. The overall response rate (ORR) was defined as CR + PR or CR alone.

In case of CR, steroid dose was tapered by 25% every 7-10 days to reach a daily dose of 0.5 mg/kg.

Patients with PR continued to receive steroid therapy and were re-evaluated during follow-up.

SR-a-GvHD was diagnosed when the clinical signs worsened or remained stable 5–7 days after initiation of MPD. Biopsy confirmation of a-GvHD was not required. In cases with SR-a-GvHD, second line treatment with ET was administered in addition to steroid treatment. In patients with severe life threatening SR-a-GvHD, the dosage of MPD was increased above 2 mg/Kg (3.5-5 mg/Kg) and ECP was added to first line therapy. ET was administered subcutaneously at the dose of 0.4 mg/Kg twice weekly for a period of eight weeks (16 doses).

Anti-infective prophylaxis included acyclovir (*herpes viruses*), fluconazole *(Candida*), and cotrimoxazole (*Pneumocystis jirovecii*). Patients were monitored with quantitative polymerase chain reaction (PCR) for cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus, HHV6, and BK reactivations and with detection of galactomannan antigen and/or CT scan according to clinical indications (10). Patients with previous or active tuberculosis, renal or hepatic insufficiency, and previous documented allergic reactions to ET were excluded from the study.

The primary endpoint of this study was to evaluate the proportion of SR-a-GvHD patients with complete or partial response to ET as second line treatment. Secondary endpoints were: i) to report the overall response rate (ORR) after 4, 8 and 16 doses of ET that correspond on day +14, +28 and + 56 days after ET;, the response in each organ affected by SR-a-GvHD, and the ORR on day +100 and +180 after start of ET, and at the last follow-up; the grade of chronic GvHD; the infectious complications occurred during ET administration; ii) to analyze the survival of patients treated with ET.

The study protocol and patient consent forms were approved by the Institutional Ethics Committee in February 2009 (number 17; Eurotract 2008-006726-34).

STATISTICAL ANALYSIS

Simon two-stage optimal design (11) was used to evaluate complete response (CR) and partial response (PR) to ET in order to rule out 20% response rate (p0 = 0.20%) and to target the desirable goal of 40% response rate (p1 = 0.40%). With alpha = 0.10 (probability of accepting a poor treatment) and beta = 0.10 (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 complete or partial response at the end of treatment, it would 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 PR or CR after the end of therapy.
The data were described as mean and standard deviation (SD) or median and range for continuous variables, and as absolute and relative frequencies for categorical variables. Comparison of frequency distributions was analyzed by the chi-square test. Fisher's exact test was used in case of at least one expected frequency <5. The Mann-Whitney U test was used to compare continuous variables in two independent groups. The log-rank test was used to assess differences between groups .
The 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 transplant-related mortality (TRM), 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 time interval from beginning of ET to death or the last follow-up. Event free survival (EFS) 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 less than 0.05 was considered statistically significant, and all p-values were based upon two-tailed tests. Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, Illinois USA).

RESULTS

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

We initially planned to enrol a total of 37 patients according to Simon optimal two-stage design and after enrolment of the first 17 patients the trial was continued since 11 of them (64.7%) developed CR or PR. The study was interrupted before reaching the planned sample size because, among the already enrolled 25 patients, 17 (68%) had partial or complete response. In fact, even if the remaining 12 patients necessary to reach the planned sample size had shown no response to ET, still we would have observed a response rate of 46%, which is higher of the minimum response rate (40%) necessary to declare the efficacy of the drug.

SR-a-GvHD observed in 25 patients involved the skin in 18 of them (72%), the gastro-intestinal tract in others 18 (72%), and the liver in 7 children (28%). All of them received MPD at the dosage of 2 mg/Kg/day. This dosage remained unchanged in 16 patients (64%), while it was increased in the remaining 9 (36%) who developed life-threatening SR-a-GvHD. Twelve patients (48%) underwent ECP.

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

Seventeen patients developed a complete or partial response (68%), in particular 14 had CR (56%) and 3 PR (12%). On day 28 after ET treatment (i.e. after 8 doses), the ORR was 64%. Seventeen patients (68%) developed a complete or partial response after the first 4 doses, 16 (64%) after 8 doses (i.e. day 28 after), and 17 (68%) after 16 doses. At the completion of the planned treatment (day +56; 16 doses), the ORR was 68% and 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 8 patients who did not respond to ET other therapies were administered, namely: MSC infusion in 3 patients, increased steroid dosage in 2, rmonoclonal antibody against IL 6 followed by ruxolitinib in 1, monoclonal antibody against CD25 in 1, and imatinib in 1.

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

SR-a-GvHD (n=14), and 57% (n=4) for hepatic SR-a-GvHD (**Figure 2. A-B-C).** The response to ET as GvHD grade in each organ affected by SR-a-GvHD was shown in **Figure 3. A-B-C.**

We observed that the increase in the daily dose of MPD did not increase the ORR of SR-a-GvHD (66.7% with >2 mg/Kg MPD *vs* 68.8% with 2mg/Kg MPD). The median duration of steroid therapy was 49 months (range 6.17 to 101 months).

ECP was associated with high dose steroids (>2mg/Kg) in 5 patients affected by severe SR-a-GvHD (grade 4) and with standard dose steroids in 7 patients with grade 3 (n=4) and grade 2 (n=3) SR-a-GvHD.

Thirteen patients received ECP during ET administration, but in 4 of them ECP was discontinued for severe transplant related complications (n=3) or for its inefficacy, while the remaining 9 (69.2%) patients reached CR or PR. In the group of 12 patients who did not receive ECP, 7 of them (58.3%) developed CR or PR. The difference between the groups of patients treated or not with ECP was not statistically significant (p=0.68). There was statistically significant difference in response between patients treated with ECP + ET with no tapering (n=9) and those in which ECP was tapered (n=3) (p=0.04).

The ORR evaluated on day +100 after 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 candidiemias in 2) **(Table 2)** . Ten patients with infectious complications (58%) had positive overall response after 16 doses and on day + 100 (9 viral infections, 1 bacteremia) , while 7 (41.2%) did not respond (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 subcutaneous administration was fairly good considering the age of these patients.

Chronic GvHD (c-GvHD) was observed in 14 of 22 (63.6%) patients evaluable for this complication; it 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 were alive (76%) and c-GvHD was present in 9 of them (47.4%).

The follow-up, calculated as the time interval between start of ET and last observation was 14.42 months (range 1-99.9 months). Ten children died after a median of 175 days from HSCT (range 42-329 days); the causes of death were progressive SR-a-GvHD in 3 children (30%), c-GvHD in 5 (50%) (3 of them with severe intractable mycoses), and relapse of acute myeloid leukemia in 2 (20%).

Among the remaining 15 children alive at the last follow-up evaluation, 2 of them (13.3%) maintained grade 3 c-GvHD at 4.8 and 59.9 months, respectively.

The OS rate at 1 year after ET was 59.1% (95% CI; 37.3%- 75.6%) (**Figure 4**). The OS rate in responding patients was 76.5% (95% CI; 48.9%- 90.5%) *vs* 16.7% (95% CI; 1%- 50.8%) in non responding patients (p=0.004) (**Figure 5**).

TRM at 5 years was 34.1% (95% CI; 18.6%- 57.1%) **(Figure 6).** The TRM in patients who received MPD > 2mg/Kg/day *vs* ≤ 2mg/Kg/day was not statistically significant (p=0.87). Moreover, underlying disease and type of HSCT did not influence TRM. EFS at 3 years was 45.5% % (95% CI; 24.4%- 64.4%) **(Figure 7**).

DISCUSSION

The occurrence of SR-a-GvHD remains a severe complication since the SR-a-GvHD-related mortality rate reported in the literature is very high (12-13). In our recently published experience (14), 40% of children who developed SR-a-GvHD died of disease-related complications.

To our knowledge, this is the largest study on treatment with ET for SR-a-GvHD in pediatric population (6, 15, 16, 17). The majority of studies were conducted on adults, and ET was often associated with other immunosuppressive therapies as daclizumab and IL2 (18), ATG, and tacrolimus (5). *Busca et al.* (6) reported on 13 patients with SR-a-GvHD who received ET; in this study, the ORR was 46% (6 patients out of 13), and patients with refractory gut a-GvHD (63%) had a higher response rate, followed by patients with cutaneous a-GvHD (50%), and hepatic a-GvHD (40%).

In this prospective study, the ORR to ET was higher than that reported in other studies including adult and pediatric patients (19). It was 78% in patients with cutaneous SR-a-GvHD, 78% in those with gastro-intestinal SR-a-GvHD, and 57% in those with hepatic SR-a-GvHD. This result confirms the efficacy of ET in the treatment of gastro-intestinal involvement, due to the implication of TNF as a biomarker in the pathogenesis of gastro-intestinal a-GvHD (20- 21-22). The percentage of CR was higher in patients with cutaneous involvement (78% *vs*. 56% and 57% in patients with gastro-intestinal and hepatic SR-a-GvHD, respectively), which suggests that the skin remains the organ best responding to immunosuppressive therapies. The addition of high dose steroids (> 2mg/Kg) did not influence the overall response to ET in our patients. The addition of ECP to ET significantly increased the response of these patients confirming recently published results (23). The discontinuation or reduction of steroids depended on the occurrence of c-GvHD 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 end point 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 a-GvHD and after the first 8 doses in patients with gastro-intestinal SR- a-GvHD. The response rate in patients with cutaneous and gastro-intestinal SR- a-GvHD increased steadily during the course of treatment, while in patients with hepatic SR- a-GvHD it remained stable. These observations could help predict time to response to ET in each organ and the prognosis of all treated patients.

The ORR observed in this study (68%) was higher to the responses described in other studies that reported the results of SR-a-GvHD treatment with other immunosuppressive agents (46% with low dose of MTX , and 67% with alentuzumab) (24-25). The infusion of MSCs represents a promising alternative therapy for the treatment of SR-a-GvHD, and recently *P. Bader et al* (26) reported an overall response rate of 83% in adults and children treated with MSC. It must be noted, however, that the production of MSCs is currently limited to few laboratories, thus making the procedure still to the benefit of a minority of centers.

The OS rate at 1 year after ET was 59.1% (95% CI; 37.3%- 75.6%) and remained stable until the last follow-up evaluation (8 years), which shows that the majority of patients died within the first year after HSCT. There is a statistically significant difference between the OS of patients responding to ET and of those not responding to ET (p=0.004). Moreover, in our experience TRM at 5 years was 34.1%. These results support our conclusion that ET represents a valid and effective treatment for pediatric patients with SR-a-GvHD.

The percentage of c-GvHD 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 show c-GvHD.

In our study, invasive mycoses occurred in 20% of SR-a-GvHD patients and represented the cause of death in 3 (12%) (one with zygomycosis, one with candida parapsilosis, and one with aspergillosis). Viral reactivations occurred in 76% of patients and bacterial infections occurred in 36% of patients but none died of these complications. These results are comparable with an interim analysis conducted on infectious complications occurred in 11 patients who received ET that demonstrated a high frequency of infectious events, especially invasive mycosis (27), and with the observation that confirms the role of GvHD in the occurrence of infectious complications and, in particular, in the development of mycosis (28). Of course, a strict monitoring of viral, bacterial and fungal infections is mandatory, in particular in patients at higher risk because of previous infectious complications.

We conclude that ET represents an effective treatment of SR-a-GvHD for the high response rate, the few infectious and fatal complications, and the significant improvement of overall survival rate. In particular, the high response rate obtained in patients with gastrointestinal a-GvHD is remarkable. Time to response to ET represents a useful tool to evaluate response to this drug to predict the prognosis of these patients.
The discovery of new drugs and their use also in children open up new horizons for the treatment of SR-a-GvHD but, until now, in our transplant centre ET represented the best therapy for SR-a-GvHD.

REFERENCES

1. Bacigalupo A. Management of acute graft-versus-host disease. *Br J Haematol* 2007;137:87-98.
2. Martin PJ, Rizzo JD, Wingard JR, Ballen K, Curtin PT, Cutler C, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2012; 18(8):1150±63.
3. Ferrara JL. Novel strategies for the treatment and diagnosis of graft-versus-host-disease. Best Pract Res Clin Haematol. 2007 Mar;20(1):91-7.
4. Carrie L. Kitko, Sophie Paczesny, Gregory Yanik, Thomas Braun, Dawn Jones, Joel Whitfield et al Plasma Elevations of Tumor Necrosis Factor-Receptor-1 at Day 7 Post Allogeneic Transplant Correlate with Graft Versus Host Disease Severity and Overall Survival in Pediatric Patients. *Biol Blood Marrow Transplant*. 2008 Jul; 14(7): 759–765.
5. Levine JE, Paczesny S, Mineishi S, Braun T, Choi SW, Hutchinson RJ. Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease. Blood. 2008 Feb 15;111(4):2470-5.
6. Busca A, Locatelli F, Marmont F, Ceretto C, Falda M. Recombinant human soluble tumor necrosis factor receptor fusion protein as treatment for steroid refractory graft-versus-host disease following allogeneic hematopoietic stem cell transplantation. *Am J Hematol*. 2007 Jan;82(1):45-52.
7. Kennedy GA, Butler J, Western R, Morton J, Durrant S, Hill GR. Combination antithymocyte globulin and soluble TNFalpha inhibitor (etanercept) +/- mycophenolate mofetil for treatment of steroid refractory acute graft-versus-host disease. *Bone Marrow Transplant*. 2006 Jun;37(12):1143-7.
8. 6.Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED. et al. 1994 Consensus Conference on acute GVHD grading. *Bone Marrow Transplant* 1995;15:825-828.

9. Filipovich AH, Weisdorf D, Pavletic SSocie G, Wingard JR, Lee SJ et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005;11:945-956.
10. Lehrnbecher T, Robinson PD, Fisher BT, Castagnola E, Groll AH, Steinbach WJ et al. Galactomannan, β-D-Glucan, and Polymerase Chain Reaction-Based Assays for the Diagnosis of Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation: A Systematic Review and Meta-Analysis. *Clin Infect Dis*. 2016 Nov 15;63(10):1340-1348.
11. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials, 1989 (10); 1-10
12. Westin JR, Saliba RM, De Lima M, Alousi A, Hosing C, Qazilbash MH et al.Steroid-Refractory Acute GVHD: Predictors and Outcomes. *Adv Hematol*. 2011:601953.
13. S. Arai, J. Margolis, M. Zahurak, V. Anders, and G. B. Vogelsang, "Poor outcome in steroid-refractory graft-versus host disease with antithymocyte globulin treatment," *Biology of Blood and Marrow Transplantation*, vol. 8, no. 3, pp. 155–160, 2002.
14. Faraci M, Caviglia I, Biral E, Morreale G, Giardino S, Garbarino L et al . Acute graft-versus-host disease in pediatric allogeneic hematopoietic stem cell transplantation. Single-center experience during 10 yr. *Pediatr Transplant.* 2012 Dec;16(8):887-93.
15. Andolina M, Rabusin M, Maximova N, Di Leo G. Etanercept in graft-versus-host disease. *Bone Marrow Transplant.* 2000 Oct;26(8):929.
16. Park JH, Lee HJ, Kim SR, Song GW, Lee SK, Park SY et al . Etanercept for steroid-refractory acute graft versus host disease following allogeneic hematopoietic stem cell transplantation. *Korean J Intern Med*. 2014 Sep;29(5):630-6.
17. De Jong CN, Saes L, Klerk CPW, Van der Klift M, Cornelissen JJ, Broers AEC. Etanercept for steroid-refractory acute graft-versus-host disease: A single center experience. PLoS One. 2017 Oct 26;12(10).
18. Wolff D, Roessler V, Steiner B, et al. Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept. *Bone Marrow Transplant* 2005;35:1003-1010.
19. Ma CKK, García-Cadenas I, Fox ML, Ai S, Nivison-Smith I, Milliken ST, Dodds A,Fay K et al . Poor prognosis in patients with steroid refractory acute graft versus host disease treated with etanercept: a multi-centre analysis. *Bone Marrow Transplant*. 2018 May 24.
20. Bosani M, Ardizzone S, Porro GB. Biologic targeting in the treatment of inflammatory bowel diseases. *Biologics.* 2009;3:77-97.

21. Brown GR, Lee E, Thiele DL. TNF-TNFR2 interactions are critical for the development of intestinal graft-versus-host disease in MHC class II-disparate (C57BL/6J-->C57BL/6J x bm12)F1 mice. *J Immunol.* 2002 Mar 15;168(6):3065-71.
22. Piguet PF, Grau GE, Allet B, Vassalli P. Tumor necrosis factor/cachectin is an effector of skin and gut lesions of the acute phase of graft-vs.-host disease. *J Exp Med*. 1987 Nov 1;166(5):1280-9.
23. Kitko CL, Braun T, Couriel DR, Choi SW et al. Combination Therapy for Graft-versus-Host Disease Prophylaxis with Etanercept and Extracorporeal Photopheresis: Results of a Phase II Clinical Trial. Biol Blood Marrow Transplant. 2016 May;22(5):862-8.
24. Inagaki J, Fukano R, Kodama Y et al. Safety and efficacy of low-dose methotrexate for pediatric patients with steroid-refractory acute graft-versus-host disease after hematopoietic stem cell transplantation. Ann Hematol. 2014 Apr;93(4):645-51.
25. Khandelwal P, Emoto C, Fukuda T et al. A Prospective Study of Alemtuzumab as a Second-Line Agent for Steroid-Refractory Acute Graft-versus-Host Disease in Pediatric and Young Adult Allogeneic Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2016 Dec;22(12):2220-2225.
26. Bader P, Kuçi Z, Bakhtiar S et al. Effective treatment of steroid and therapy-refractory acute graft-versus-host disease with a novel mesenchymal stromal cell product (MSC-FFM). Bone Marrow Transplant. 2018 Jul;53(7):852-862.
27. Faraci M, Lanino E, Morreale G, Giardino S, Fossati M, Moroni C et al . Bacteremias and invasive fungal diseases in children receiving etanercept for steroid-resistant acute GVHD. *Bone Marrow Transplant*. 2011 Jan;46(1):159-60.
28. Castagnola E, Bagnasco F, Bandettini R, Caviglia I, Morreale G, Lanino E et al . Role of acute graft-versus-host disease in the risk of bacteremia and invasive fungal disease after allogeneic hemopoietic stem cell transplantation in children. Results from a single-center observational study. *Biol Blood Marrow Transplant*. 2014 Jul;20(7):1068-73.

Table 1. Characteristics of patients

	Total n=25	**Responders n=17 (68.0%)**	**Non Responders 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)	0.66
Malignant disease, n(%)	12 (48.0)	10 (58.8)	2 (25.0)	0.20
Non Malignant disease, n(%)	13 (52.0)	7 (41.2)	6 (75.0)	
Median age at HSCT (yrs, range)	7.8 (0.4-15.7)	5.4 (0.4-15.3)	9.8 (1.3-15.7)	0.22
Interval between onset of a-GVHD and ET (days, range)	14 (5-135)	14 (5-63)	27.5 (5-135)	0.41
Type of HSCT, n (%)				
AD	18 (72.0)	11 (64.7)	7 (87.5)	0.17
RD	3 (12.0)	2 (11.8)	1 (12.5)	
Haplo	4 (16.0)	4 (23.5)	-	
SC Source, n (%)				0.40
HPC-BM	17 (68.0)	11 (64.7)	6 (75.0)	
HPC-CB	5 (20.0)	4 (23.5)	1 (12.5)	
HPC-PB	3 (12.0)	2 (11.8)	1 (12.5)	
Conditioning regimen RIC	8 (32.0)	3 (17.6)	5 (62.5)	0.06
Conditioning regimen MAC	17 (68.0)	14 (82.4)	3 (37.5)	
• *Bus based* • *TBI based* • *Treo based*	*7* *3* *7*	*7* *2* *5*	*0* *1* *2*	
GvHD prophylaxis ATG, n(%)	21 (84.0)	14 (82.4)	7 (87.5)	
• *ATG + CSA+MTX* • *ATG +CSA+MPD* • *ATG+ CSA+MMF* • *ATG+CSA* • *ATG + T depletion*	*10* *4* *4* *2* *1*	*7* *3* *3* *1* *0*	*3* *1* *1* *1* *1*	1
GvHD prophylaxis No ATG, n(%)	4 (16.0)	3 (17.6)	1 (12.5)	
• *Campath + CSA + MTX* • *CSA+ MMF* • *CSA+ MMF+ CY post*	*1* *1* *2*	*0* *1* *2*	*1* *0* *0*	
Grade of a-GvHD before ET n, (%)				0.78
Grade 2	4 (16.0)	3 (17.6)	1 (12.5)	
Grade 3	10 (40.0)	6 (35.3)	4 (50.0)	
Grade 4	11 (44.0)	8(47.1)	3 (37.5)	
Status at last FU, n(%)				
Alive	15 (60.0)	13 (76.5)	2 (25.0)	0.03
Dead	10 (40.0)	4 (23.5)	6 (75.0)	
TRM ,n (%)				
Yes	8 (32.0)	2 (11.8)	6 (75.0)	0.004
No	17 (68.0)	15 (88.2)	2 (25.0)	

Legend : HSCT= hematopoietic stem cell transplantation; yrs= years; ET= Etanercept; AD= alternative donor; RD= related donor; haplo= haploidentical donor; BM= bone marrow; PBSC= peripheral blood stem cell; CB= cord blood; MAC= myeloablative conditioning regimen; RIC= reduced intensity conditioning regimen; Bus= Busulfan; TBI= total body irradiation; Treo=treosulfan; GvHD= graft versus host disease; CSA= cyclosporine; MMF= mycophenolic acid; MTX = methotrexate; post CY = post transplant cyclophosphamide; FU= follow-up; TRM =transplant related mortality.

Table 2. Infectious events in patients treated with Etanercept

		Total n=25	**Responders n=17**	**Non Responders n=8**	**P value**
		N (%)	N (%)	N (%)	
Infectious Events					
Yes		17 (68.0)	10 (58.8)	7 (87.5)	0.20
No		8 (32.0)	7 (41.2)	1 (12.5)	
Sepsis:					
	No	16 (64.0)	12 (70.6)	4 (50.0)	0.39
	Yes	9 (36.0)	5 (29.4)	4 (50.0)	
Virus Reactivation:					
	None	11 (44.0)	8 (47.0)	3 (37.5)	0.49
	One or more Reactivation *	14 (66.0)	9 (53.0)	5 (62.5)	
	CMV	*12*	*7*	*5*	
	BK	*2*	*2*	-	
	Adenovirus	*1*	-	*1*	
	HHV6	*2*	*1*	*1*	
	EBV	*1*	*1*	-	
	Varicella Zoster	*1*		*1*	
Mycoses:					
None		20 (80.0)	16 (94.1)	4 (50.0)	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 1 . Response to ET included complete response, partial response, no response, and non evaluable patients because they died before the 16 doses of ET

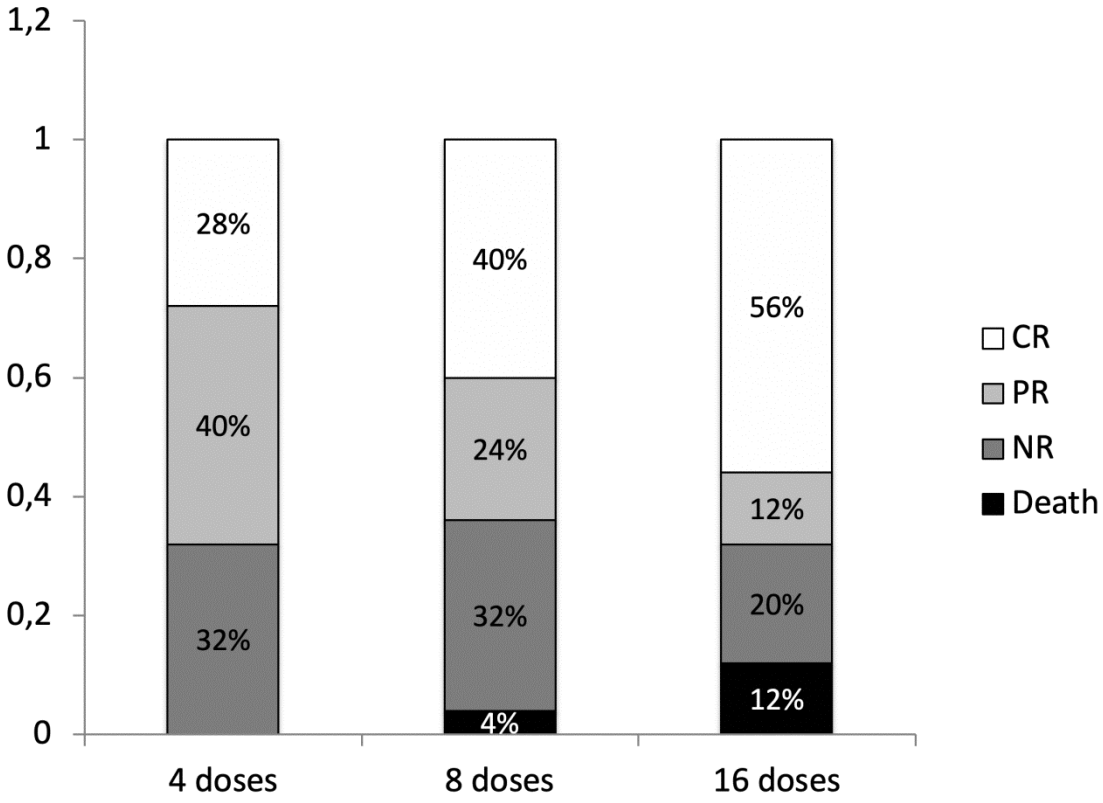
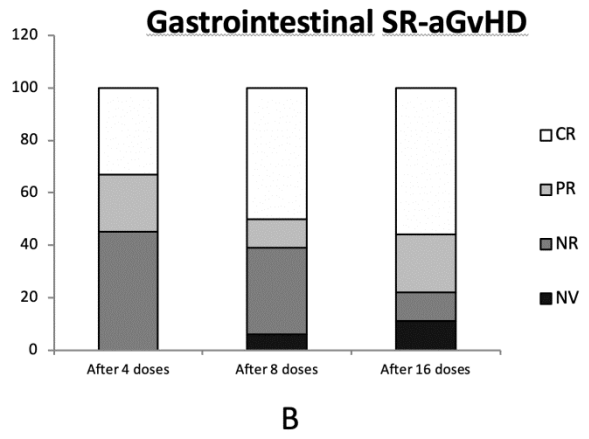


Figure 2 A-B-C: Response to ET including complete, partial and no response evaluated in each organ affected by SR-a-GvHD.



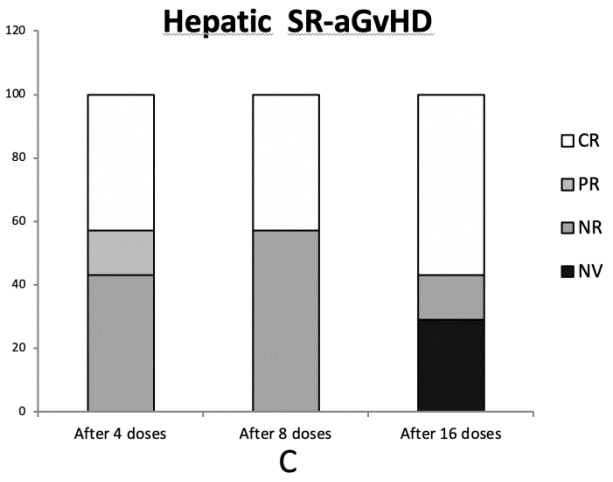


Figure 2 (A,B,C)

Percentage of response to ET in cutaneous (A), gastrointestinal (B), and hepatic (C) SR-aGvHD. The response was evaluated after 4, 8 and 16 doses of ET.
Three patients died of infectious complications during this course of therapy: 1 during administration of 8 doses and 2 during the period of administration between 8 and 16 doses .

Figure 3 A-B-C. Response to ET as GvHD grade in each organ affected by SR-a-GvHD

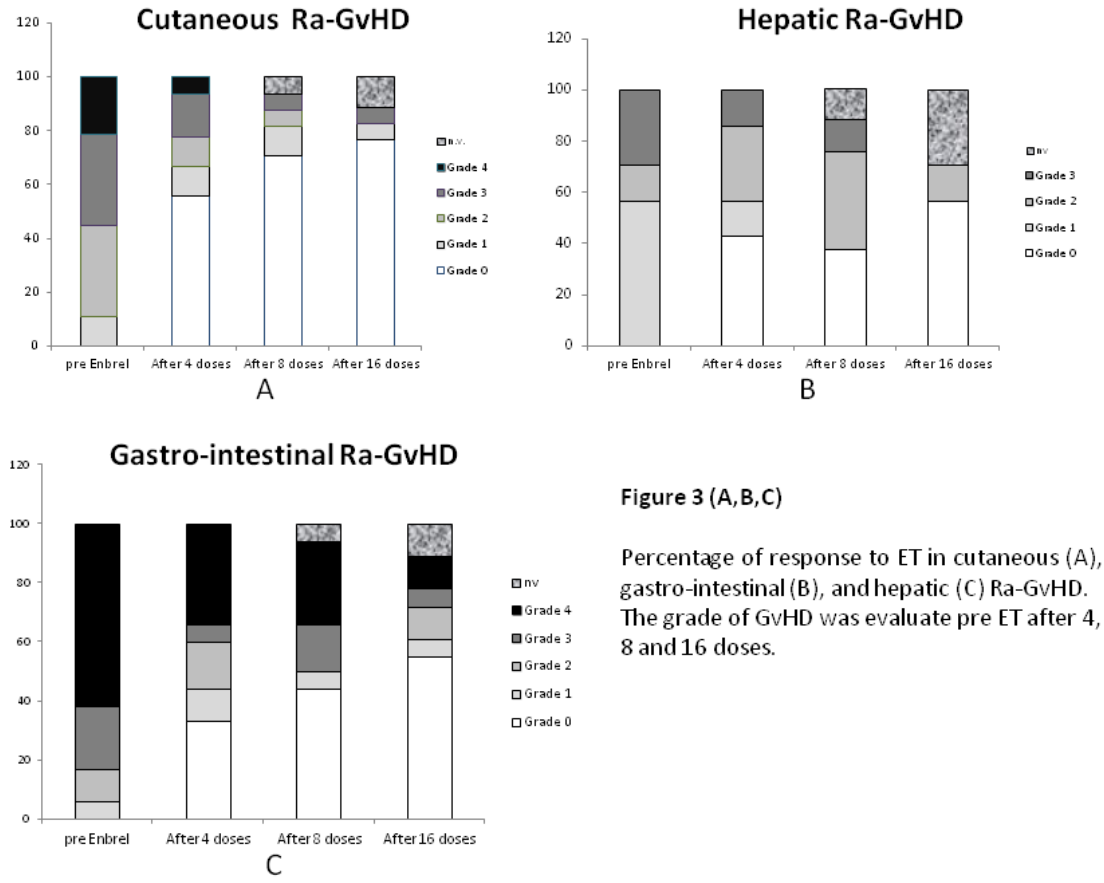


Figure 3 (A,B,C)

Percentage of response to ET in cutaneous (A), gastro-intestinal (B), and hepatic (C) Ra-GvHD. The grade of GvHD was evaluate pre ET after 4, 8 and 16 doses.

Figure 4. Overall Survival

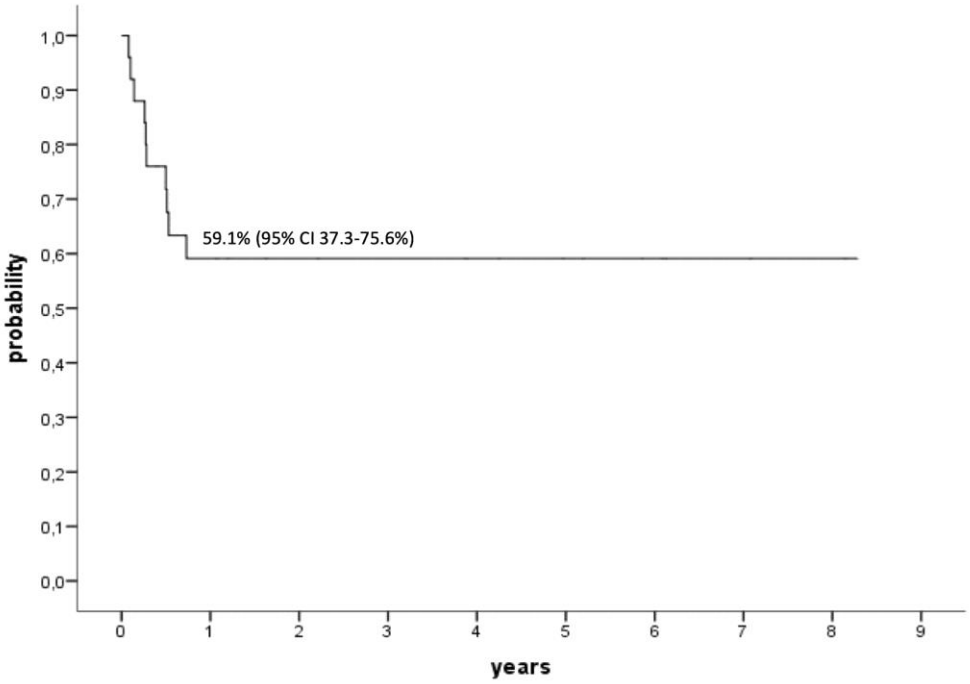


Figure 5. Overall Survival evaluated in responders and non responders

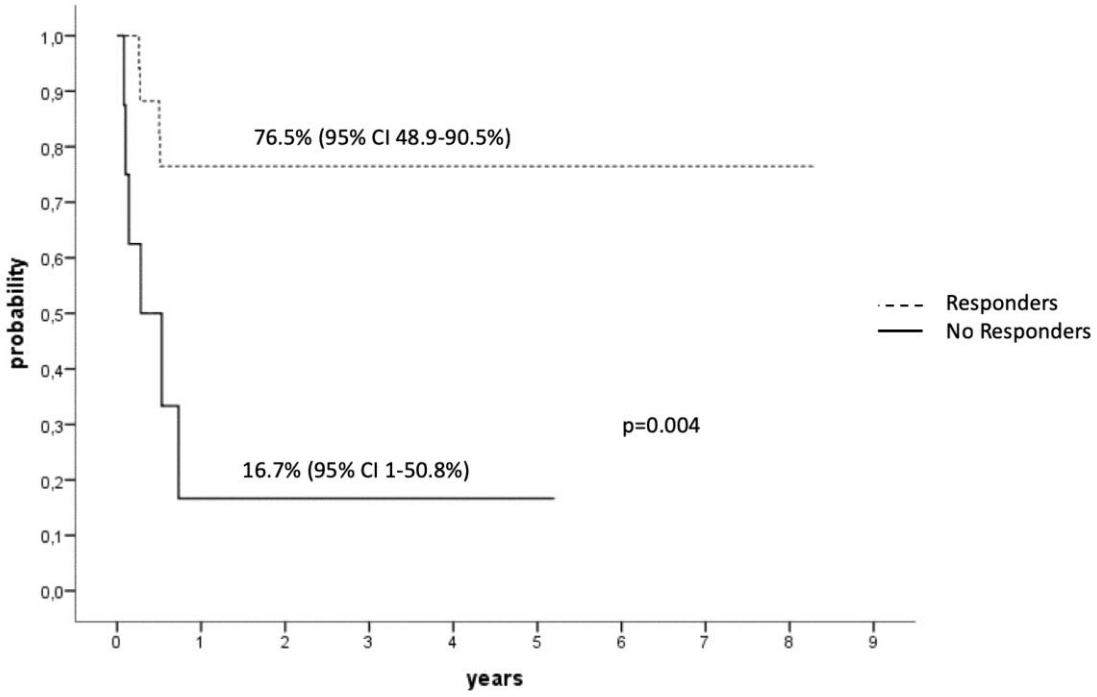


Figure 6. Transplant related mortality

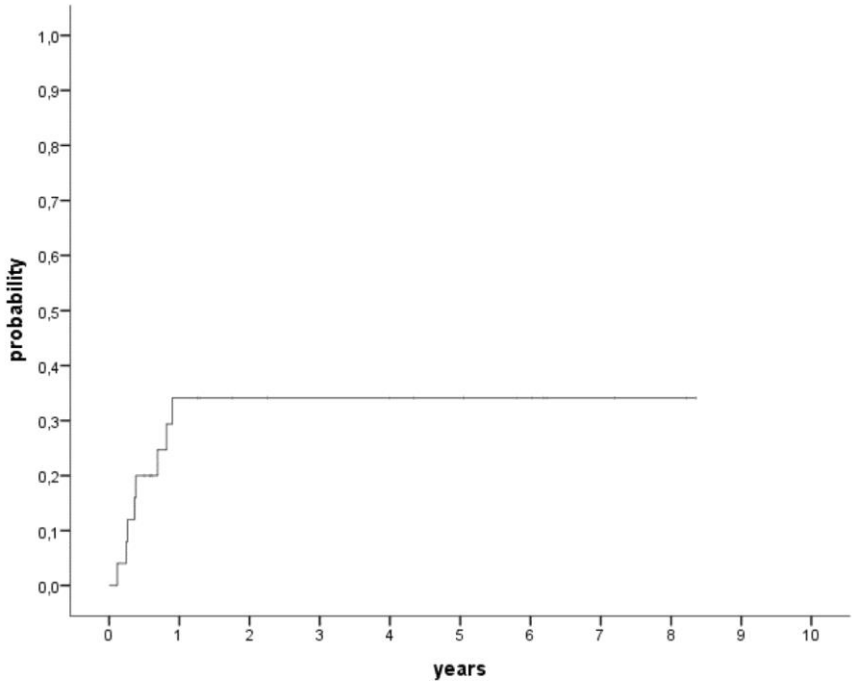


Figure 7. Event free survival