ASBMT
American Society for Blood and Marrow Transplantation

TNF-Inhibition with Etanercept for Graft-versus-Host Disease Prevention in High-Risk HCT: Lower TNFR1 Levels Correlate with Better Outcomes

Sung W. Choi,[1] Patrick Stiff,[2] Kenneth Cooke,[3] James L. M. Ferrara,[1] Thomas Braun,[4] Carrie Kitko,[1] Pavan Reddy,[1] Gregory Yanik,[1] Shin Mineishi,[1] Sophie Paczesny,[1] David Hanauer,[5] Attaphol Pawarode,[1] Edward Peres,[1] Tulio Rodriguez,[2] Scott Smith,[2] John E. Levine[1]

Graft-versus-host disease (GVHD) causes most non-relapse mortality (NRM) after alternative donor (unrelated and mismatched related) hematopoietic cell transplant (HCT). We previously showed that increases in day +7 TNF-receptor-1 (TNFR1) ratios (posttransplantation day +7/pretransplantation baseline) after myeloablative HCT correlate with outcomes including GVHD, NRM, and survival. Therefore, we conducted a phase II trial at 2 centers, testing whether the addition of the TNF-inhibitor etanercept (25 mg twice weekly from start of conditioning to day +56) to standard GVHD prophylaxis would lower TNFR1 levels, reduce GVHD rates, and improve NRM and survival. Patients underwent myeloablative HCT from a matched unrelated donor (URD; N = 71), 1-antigen mismatched URD (N = 26), or 1-antigen mismatched related donor (N = 3) using either total body irradiation (TBI)-based conditioning (N = 29) or non-TBI-based conditioning (N = 71). Compared to historical controls, the increase in posttransplantation day +7 TNFR1 ratios was not altered in patients who received TBI-based conditioning, but was 40% lower in patients receiving non-TBI-based conditioning. The latter group experienced relatively low rates of severe grade 3 to 4 GVHD (14%), 1-year NRM (16%), and high 1-year survival (69%). These findings suggest that (1) the effectiveness of TNF-inhibition with etanercept may depend on the conditioning regimen, and (2) attenuating the expected rise in TNFR1 levels early posttransplantation correlates with good outcomes.

Biol Blood Marrow Transplant 18: 1525-1532 (2012) © *2012 American Society for Blood and Marrow Transplantation*

KEY WORDS: GVHD, Hematopoietic cell transplantation, TNF-α, TNFR1

INTRODUCTION

Allogeneic hematopoietic cell transplant (allo-HCT) is an important therapeutic option for a variety of malignant and nonmalignant conditions. One barrier to increased utilization of allo-HCT is the inferior outcomes when donors other than HLA-matched siblings are used [1,2]. Compared with matched related donors, recipients of matched or single antigen mismatched unrelated donor (URD) and mismatched related donor transplantations are at a significantly increased risk of non-relapse mortality (NRM) [3,4]. The major contributor to NRM is acute graft-versus-host disease (aGVHD), which develops in 50% to 70% of recipients receiving these type of grafts despite standard immunosuppressive prophylaxis [5-8]. Thus, novel GVHD prophylaxis strategies that successfully attenuate aGVHD-related mortality without increasing other causes of NRM or relapse are needed.

TNF-alpha (TNF-α) plays an important role in the inflammatory cascade that ultimately evolves to aGVHD [9], and thus represents a potential target for preemptive treatment in the control of GVHD. We have previously shown that in the first week after myeloablative hematopoietic cell transplant (HCT), the magnitude of change in the TNF-receptor-1 (TNFR1) ratio (posttransplantation day +7/pretransplantation

From the [1]Blood and Marrow Transplantation Program, University of Michigan, Ann Arbor, Michigan; [2]Blood and Marrow Transplantation Program, Loyola University Medical Center, Maywood, Illinois; [3]Blood and Marrow Transplantation Program, Case Western Reserve University School of Medicine, Cleveland, Ohio; [4]Department of Biostatistics, University of Michigan, Ann Arbor, Michigan; and [5]Comprehensive Cancer Center, University of Michigan, Ann Arbor, Michigan.

Financial disclosure: See Acknowledgments on page 1531.

Correspondence and reprint requests: John E. Levine, MD, MS, Blood and Marrow Transplantation Program, University of Michigan, 1500 E. Medical Center Drive, 5303 Cancer Center, Ann Arbor, MI 48109-5942 (e-mail: jelevine@med.umich.edu).

Received February 6, 2012; accepted March 25, 2012

1083-8791/$36.00
doi:10.1016/j.bbmt.2012.03.013

baseline), a surrogate for TNF-α, strongly correlates with important transplantation outcomes such as GVHD, NRM, and overall survival (OS) [10]. We reported that 161 patients who underwent myeloablative URD HCT followed by GVHD prophylaxis, consisting of the widely utilized regimen of a calcineurin inhibitor and mini-methotrexate, experienced a near doubling of TNFR1 levels at day +7 posttransplantation (median 1.84 × baseline, mean 2.4 × baseline). This increase likely reflected the combination of conditioning-induced tissue damage together with release of TNF-α by activated components of the immune system [5,11].

Given that TNF-α amplifies the early alloreactive response in allo-HCT [12], we investigated whether TNF-inhibition could attenuate this pathway. Etanercept consists of 2 recombinant human TNFR (p75) monomers fused to the Fc portion of human Ig G1, binds to TNF-α, and renders it inactive [13]. We tested the hypothesis that addition of etanercept to standard GVHD prophylaxis would lower TNFR1 ratios and thereby reduce severe GVHD and subsequent NRM, improving OS in patients after transplantations from unrelated and partially matched related donor transplantations.

METHODS

Study Cohort

Patients >1 year of age who were candidates for a myeloablative allo-HCT were eligible for inclusion. Donors and recipients were required to match for 7/8 or 8/8 HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci. Patients with an 8/8 HLA-matched related donor were not eligible. Mid-resolution DNA typing was performed for all class I loci. Allelic typing by high-resolution DNA sequencing defined a match at DRB1. Patients with progressive malignancies were ineligible. Patients with uncontrolled infection despite treatment with appropriate anti-infectives were also ineligible. The protocol and informed consents were approved by the Institutional Review Board at the University of Michigan and Loyola University Medical Center. All patients or their legal guardians signed informed consents in accordance with the Declaration of Helsinki.

Cytokine Analysis

Plasma samples collected pretransplantation (baseline) and day +7 after allo-HCT were frozen for later analysis. TNFR1 levels were measured in duplicate using an enzyme-linked immunosorbent assay according to the manufacturer's protocol (R&D Systems, Minneapolis, MN). Enzyme-linked immunosorbent assay plates were read by a microplate reader (Bio-Rad, Hercules, CA).

Table 1. Patient Characteristics for Patients Who Underwent Myeloablative Allogeneic Transplantation and Received Etanercept in Addition to Standard GVHD Prophylaxis from 2005 to 2009

Characteristics	No. of Patients
Sample size	100
Age	
Median, yr	47
Range	2-61
Gender	
Male	57
Female	43
Disease status at transplantation*	
Low risk	39
Intermediate risk	33
High risk	28
Donor source	
8/8 matched unrelated	71
7/8 mismatched unrelated	26
7/8 mismatched related	3
Stem cell source	
Peripheral blood	96
Bone marrow	4
Conditioning regimen†	
Non-TBI	71
Busulfan-based	
FluBu4	37
BuCy ± cytarabine	26
BCNU-based	
CVB	7
BEAM	1
TBI	29
Diagnosis	
Malignant	97
Acute myelogenous leukemia	38
Myelodysplastic syndrome	9
Acute lymphoblastic leukemia	19
Myeloproliferative disease‡	7
Non-Hodgkin lymphoma	20
Myelofibrosis	2
Multiple myeloma	2
Nonmalignant	3

TBI indicates total body irradiation; FluBu4, fludarabine and busulfan; BuCy, busulfan and cyclophosphamide; BCNU, carmustine.

*Disease classifications correspond to the Center for International Blood and Marrow Transplant Research Request for Information Disease Classifications Form (www.cibmtr.org).

†"FluBu4," fludarabine 40 mg/m^2 i.v. daily for 4 days and busulfan 3.2 mg/kg i.v. daily for 4 days; "BuCy," busulfan 3.2 mg/kg i.v. daily for 4 days and cyclophosphamide (either 60 mg/kg i.v. daily for 2 days or in children, 50 mg/kg i.v. daily for 4 days) ± cytarabine 2000 mg/m^2 i.v. twice daily for 2 days; "CVB," cyclophosphamide 1800 mg/m^2 i.v. daily for 4 days, etoposide (VP-16) 200 mg/m^2 i.v. every 12 hours for 4 days, and carmustine (BCNU) 450 mg/m^2 i.v. daily for 1 day; and "BEAM," BCNU 300 mg/m^2 i.v. for 1 day, etoposide 100 mg/m^2 i.v. twice daily together with cytarabine 200 mg/m^2 i.v. twice daily for 4 days and melphalan 140 mg/m^2 i.v. for 1 day; "CyTBI," cyclophosphamide 60 mg/kg i.v. daily for 2 days and total body irradiation (TBI) 200 cGy twice daily for 3 days.

‡Myeloproliferative diseases: chronic myelogenous leukemia (N = 5); chronic myelomonocytic leukemia (N = 1); juvenile myelomonocytic leukemia (N = 1).

Study Design

The study was conducted at 2 institutions as an open-label phase II clinical trial. All patients received a myeloablative conditioning regimen (Table 1) selected by the treating physician on the basis of underlying disease, age, degree of donor match, and disease status at the time of transplantation.

GVHD prophylaxis consisted of tacrolimus initiated on day −3 (titrated to a goal level of 8-12 ng/mL) and mini-methotrexate administered at a dose of 5 mg/m^2 i.v. on days +1, +3, +6, and +11. In the absence of aGVHD, tacrolimus was tapered starting on day +56 posttransplantation by 25% per month so that it was discontinued by day +180 posttransplantation. The first dose of study drug, etanercept (0.4 mg/kg, maximum dose 25 mg), was administered subcutaneously within 24 hours of initiation of the conditioning regimen. The subsequent doses were administered twice weekly (at least 72 hours apart) through day +56 for a total of 18 to 19 doses depending on the length of conditioning regimen. Doses were not modified based upon hepatic or renal function. However, doses were held for persistent bacteremia, hemodynamic instability, or fever above 100.5°F for more than 5 days and not restarted until resolution. Additionally, if the cytomegalovirus (CMV) viral load increased after 72 hours of anti-CMV treatment, etanercept was held until improvement in the viral load was documented. Additional doses of etanercept were not administered if more than 2 consecutive doses were held for any reason. Etanercept was discontinued and patients were replaced if early relapse of the underlying malignancy occurred within day +21.

Supportive care therapies were administered according to institutional guidelines. Antimicrobial prophylaxis included levofloxacin 500 mg once daily for prevention of bacterial infections, voriconazole 200 mg twice daily, acyclovir 400 mg twice daily, and sulfamethoxazole/trimethoprim or pentamidine for prevention of pneumocystis carinii pneumonia. Pediatric patients received age/weight equivalent dosing of antibiotics. CMV DNA was monitored weekly by quantitative PCR [14] and preemptive therapy with antiviral agents begun in the event of a positive assay. The i.v. Ig 400 mg/kg replacement therapy was given for IgG levels <400 mg/dL. Total body irradiation (TBI)-treated patients at Loyola University Medical Center (N = 9) also received palifermin for mucositis protection according to the institutional guidelines.

Infections through Day 100

An infection was defined using the following criteria: one or more positive blood and/or fluid cultures, or the detection of DNA in the plasma by quantitative PCR. CMV disease was defined as an organ infected by CMV [15]. Proven, probable, and possible invasive fungal infections were classified according to international consensus criteria up to day +180 or relapse [16].

GVHD Scoring

The aGVHD was scored weekly by the modified Glucksberg criteria [17]. Biopsies were obtained of involved target organs to confirm the diagnosis of aGVHD. Clinically significant aGVHD was treated with methylprednisolone 2 mg/kg/day orally or by i.v. Etanercept was continued during therapy for aGVHD. Complete response to therapy was defined as the resolution of all manifestations of GVHD (all target organs stage 0). Chronic GVHD (cGVHD) was evaluated according to the National Institutes of Health consensus criteria [18].

Statistical Analysis

The study was originally designed to enroll 80 patients in order to have sufficient power to detect a 50% decrease in NRM and a 20% decrease in aGVHD compared with historical rates at our centers. After it was recognized that etanercept was not benefiting patients receiving TBI-based conditioning, the protocol was amended to exclude patients receiving TBI-based conditioning. Accrual was extended to 100 patients to provide sufficient power to detect a 50% decrease in NRM in the non-TBI-conditioned patients compared with historical NRM rates for these patients at our centers. Differences in median day +7 TNFR1 ratios between study and control patients were assessed using a Wilcoxon rank sum test. The control patients consisted of 161 previously reported patients who underwent myeloablative URD HCT at the University of Michigan between 2000 and 2005. They received GVHD prophylaxis consisting of the widely utilized regimen of a calcineurin inhibitor and mini-methotrexate [10]. Study patients were significantly older (median age 47 versus 38 years; $P = .004$), but otherwise were not statistically different from the control patients with respect to disease treated, match, and use of TBI. OS was estimated with the methods of Kaplan and Meier [19]. The cumulative incidence of NRM and aGVHD were adjusted for competing risks and estimated using Gray's method [20]. The association of day +7 TNFR1 ratios with clinical outcomes was assessed with Cox regression (OS) and competing risks regression (NRM/aGVHD), with relapse treated as a competing risk. Multivariate models included TNFR1 ratio and age at transplantation as continuous variables and categorical variables for conditioning regimen (non-TBI/TBI), donor type (matched/mismatched), and baseline risk group (high/intermediate/low). Statistical significance was defined as a P value less than .05.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. A total of 100 patients participated and underwent transplantation in this study (April 2005-November 2009). The median age was 47 years (range, 2-61 years),

with 26% of the patients ≥55 years of age at the time of transplantation. Seventy-one patients received a myeloablative non-TBI-containing conditioning regimen. These regimens were either busulfan-based (N = 63) or BCNU-based (N = 8). Twenty-nine patients received a myeloablative TBI-containing conditioning regimen.

Toxicity

There were no injection or allergic reactions related to etanercept. Etanercept was discontinued in 1 patient with primary graft failure after a 7/8-HLA mismatched URD bone marrow transplant. The patient subsequently engrafted after a second transplantation from the same donor. All other patients engrafted neutrophils at a median of 12 days (range, 5-23 days). A total of 16 patients did not receive 1 or 2 doses due to persistent fever, a positive blood culture, or inadvertent missed dose. In all but 3 patients, the missed doses occurred either after GVHD had developed or after day +28, precluding an analysis to determine a minimum number of doses needed to effect outcomes. Etanercept was discontinued early in 5 patients whose treatment was being held according to study design and the underlying reason, such as persistent fever, did not resolve before the third dose was held. Six patients died before receiving all of the planned study doses. All of these patients were included in the analysis.

Infections

Given the importance of TNF-α in the innate immune system [12,21], we monitored infectious complications in this trial. Stopping rules, which were never triggered, were in place in the event that fatal complications exceeded the expected rate observed in historical controls. Bacteremia was the most common type of infection in the first 100 days posttransplantation. Fifty-nine patients developed a total of 79 bacteremia episodes. Gram-positive organisms, primarily coagulase negative staphylococci, accounted for 66 (83%) of all culture results. There were 14 gram-negative organisms isolated in the first 100 days posttransplantation. Bacterial infections accounted for 3 deaths (3%). One patient developed fatal bacterial pneumonia while receiving etanercept. In 2 additional patients, bacterial septic deaths occurred 5 and 6 weeks after completion of etanercept and during treatment with high-dose corticosteroids for aGVHD. To better assess the potential impact of etanercept on infection risk, we compared the post-engraftment infection rates during etanercept administration (engraftment to day +56) and after etanercept discontinuation (day +56 to day +100). After engraftment, the relative risk of infection was 1.3 times higher while receiving etanercept, compared to after discontinuation, but this difference was not statistically significant ($P = .36$).

A total of 24 patients developed 29 viral reactivations, most often during treatment with corticosteroids for aGVHD. The predominant virus was CMV (N = 16). Lethal viral infections developed in 3 patients while receiving corticosteroids (CMV pneumonia N = 2, human herpesvirus-6 encephalitis N = 1).

Invasive fungal infections developed in 3 patients in the first 180 days posttransplantation. Nine study patients had a pretransplantation history of invasive fungal infections (disseminated aspergillus N = 7, liver candidiasis N = 1, liver blastomycosis N = 1). All infections were well-controlled with appropriate antifungal treatment at the time of study entry. Two of these patients developed radiographic evidence of progressive fungal pneumonia and died. One additional patient died of invasive *Rhizopus* infection on day +95 that developed while being treated with high-dose corticosteroids for aGVHD.

Etanercept Effect on Plasma Ratios of TNFR1

In our previous study, the median day +7 TNFR1 level for recipients of myeloablative URD HCT was 1.84 × baseline [10]. Therefore, the significantly lower day +7 TNFR1 ratio of 1.34 ($P < .001$; Figure 1A) that was observed on this clinical trial suggests that the addition of TNF-blockade to the GVHD prophylaxis regimen may have attenuated the expected rise in TNF levels. Unexpectedly, the effectiveness of TNF-blockade was confined to patients who received a non-TBI-containing conditioning regimen (Table 2). These patients experienced a significantly low day +7 TNFR1 ratio of 1.10 compared with 1.89 in patients who received a TBI-based conditioning regimen ($P < .001$; Figure 1B). This finding stands out in contrast to our previous study in which significant differences in TNFR1 ratios were not observed between non-TBI- and TBI-treated patients [10]. Furthermore, these findings cannot be explained on the basis of differences in baseline TNFR1 levels among study patients. The median baseline TNFR1 level in patients who received TBI-based conditioning was 1835 pg/mL, which was not significantly different than the median level of 1880 pg/mL in patients who received non-TBI-based conditioning. Moreover, the administration of palifermin as a radioprotectant to 9 patients resulted in no apparent effect on the day +7 TNFR1 ratios in TBI-treated patients (2.1 versus 1.8; P = NS). Although patients with acute lymphoblastic leukemia were overrepresented and patients with acute myelogenous leukemia/myelodysplastic syndrome were underrepresented in the TBI-treated patients, there were no significant differences in other patient characteristics, including age, gender, disease status at transplantation, degree of HLA-match, or stem cell

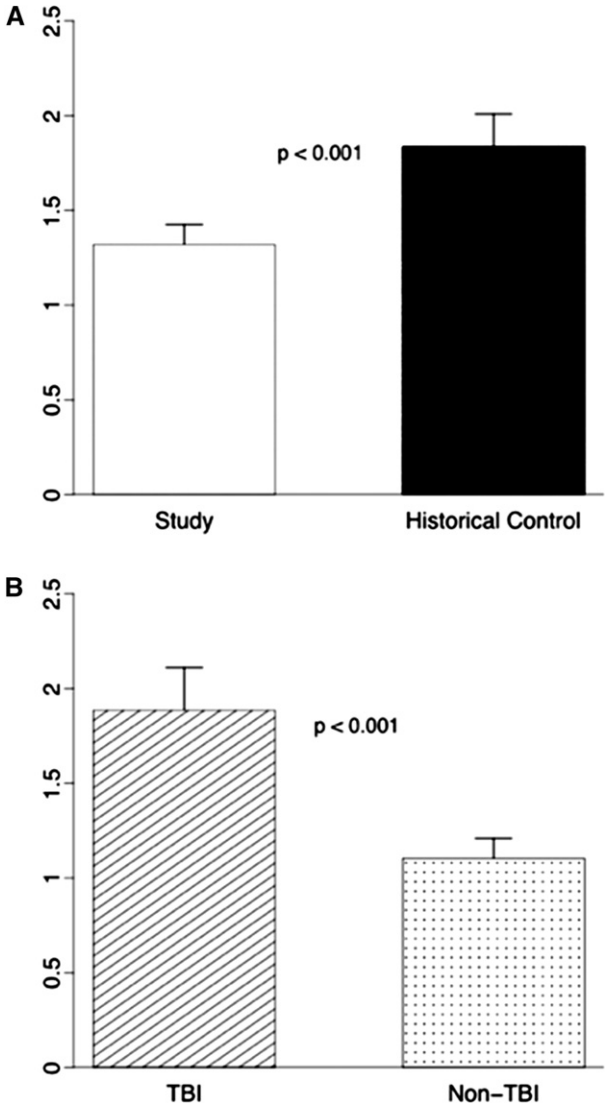

Figure 1. Day +7 tumor necrosis factor receptor 1 (TNFR1) ratio. (A) The median day +7 TNFR1 ratio of 1.34 in our study population (▭, N = 100) was significantly lower than the ratio of 1.84 observed in our previous study (■, N = 161), $P < .001$. (B) Patients who received a non–total body irradiation (non-TBI)-containing regimen (⬚, N = 71) experienced a significantly low day +7 ratio of 1.10 compared to 1.89 in patients who received a TBI-based conditioning regimen (▨, N = 29), $P < .001$.

source between TBI-treated and non-TBI-treated patients in the current study.

TNF-blockade did not alter the prognostic significance of a high day +7 TNFR1 ratio on outcomes. In etanercept-treated patients, the day +7 TNFR1 ratio, when treated as a continuous variable, significantly correlated with an increased risk of NRM within 1 year (hazard ratio [HR], 1.5; $P = .012$) and with a decreased likelihood of survival (HR, 1.5; $P = .027$). Increased day +7 TNFR1 ratios were also associated with increased rates of grades 2 to 4 and 3 to 4 aGVHD (HR, 1.3; $P = .095$; and HR, 1.4; $P = .082$, respectively), although not statistically significant.

Table 2. Day +7 TNFR1 Ratios and Transplantation Outcomes

	Day +7 TNFR1 ratio	GVHD 3-4	1-yr NRM	1-yr OS
Overall (N = 100)	1.34	18%	18%	62%
Non-TBI-treated (N = 71)	1.10	14%	16%	69%
TBI-treated (N = 29)	1.89	28%	50%	45%

TNFR1 indicates tumor necrosis factor receptor-1; GVHD, graft-versus-host disease; NRM, non-relapse mortality; OS, overall survival; TBI, total body irradiation.

aGVHD and NRM

The day +100 cumulative incidents of grades 2 to 4 and grades 3 to 4 aGVHD were 45% and 18%, respectively (Figure 2). GVHD requiring treatment was primarily grade 2 aGVHD (N = 29) involving only the skin (N = 18), upper gastrointestinal (GI; N = 4), lower GI (N = 2), or combined skin and GI (N = 5). These patients had a high rate of complete response to treatment (93%) within a median of 16 days. We expected to find a lower incidence of aGVHD in the non-TBI-treated patients compared to TBI-treated patients based on the lower median day +7 TNFR1 ratio, but the cumulative incidents of grades 2 to 4 aGVHD were the same (45%). There were twice as many cases of severe grade 3 to 4 GVHD in TBI-treated patients (28% versus 14%; $P = .15$) in which TNF blockade was not as effective in attenuating the day +7 TNFR1 ratio, however, this study lacked sufficient power to detect a statistically significant difference for this comparison. Nevertheless, TBI-treated patients were more likely to die from GVHD ($P = .04$) and experienced higher 1-year NRM (50%) compared to non-TBI-treated patients (16%; $P < .001$; Figure 3). All causes of 1-year NRM are provided (Table 3).

Allo-HCT from HLA-mismatched URDs is associated with very high rates of aGVHD and NRM. Given that over 25% of the study population fell into this very high-risk category, we analyzed GVHD and NRM outcomes for this specific population. The grade 3 to 4 GVHD rates for mismatched URD HCT were higher than those seen in the other patients (31% versus 14%; $P = .04$), but did not translate into significant differences in 1-year NRM (35% versus 22%; $P = .24$).

Relapse, cGVHD, and OS

The 1-year cumulative incidence of relapse for the entire study population was 18%, with similar relapse rates by conditioning regimen administered. The cumulative incidence of cGVHD at 1 year was 48% (Supplemental Figure 1). With a median follow-up of 15 months (range, 0.7-63 months), the 1-year OS for the entire study population was 62% (Supplemental Figure 2). There was a trend toward improved survival in patients who received a non-TBI-containing regimen (1-year OS 69% versus 45%; $P = .06$; Figure 3). Notably,

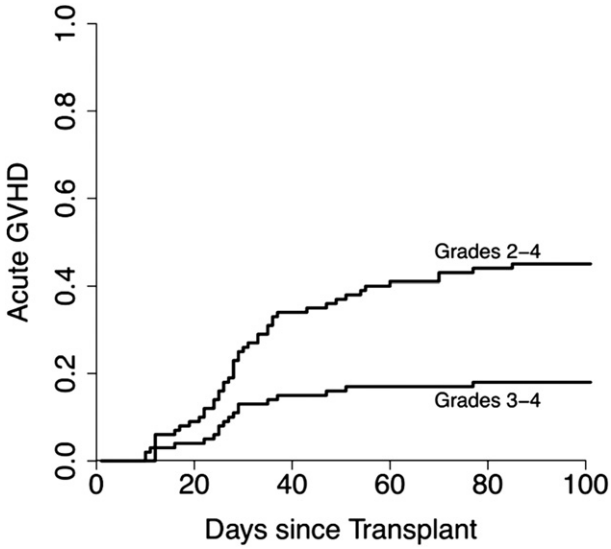

Figure 2. Cumulative incidence of acute graft-versus-host disease (aGVHD).

the 1-year OS for HLA-mismatched, URD HCT recipients was not different from HLA-matched URD/mismatched related donor patients (54% versus 65%; $P = .15$), even when restricting the analysis to non-TBI treated patients only (61% versus 71%; $P = .33$).

DISCUSSION

In this study, the addition of the TNF-inhibitor etanercept to tacrolimus/methotrexate GVHD prophylaxis did not affect the overall risk of grades 2 to 4 aGVHD. However, patients who received a non-TBI-containing myeloablative unrelated or mismatched HCT experienced high rates of steroid-responsiveness in those who developed GVHD, low rates of NRM (16%), and good 1-year survival (69%), and this was associated with lower day +7 TNFR1 ratios. By contrast, there was no apparent effect of etanercept on day +7 TNFR1 ratios, NRM, or survival in patients who received TBI-containing conditioning regimens. Given the nonrandomized study design, we cannot conclude with certainty that etanercept administration was responsible for the favorable outcomes in the non-TBI-conditioned patients. However, when taken in light of recent observations from a large series of URD HCT recipients that showed no significant difference in NRM (31%) and 1-year survival (51%) between TBI and non-TBI-conditioned patients [22], our findings suggest that etanercept may have an overall beneficial impact on the outcome after non-TBI HCT. The reasons for these observations are not clear.

A possible explanation for our findings in the TBI-conditioned patients is that high doses of radiation induced greater tissue injury and inflammatory cytokine

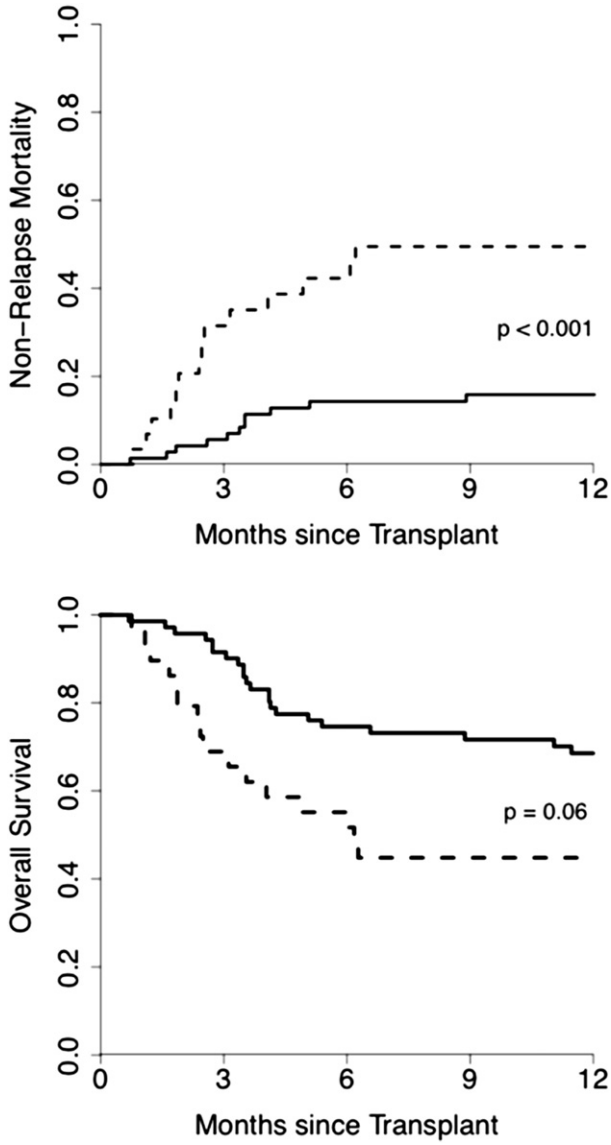

Figure 3. One-year non-relapse mortality and overall survival. Non–total body irradiation (TBI) (▬, N = 71) versus TBI (- - -, N = 29).

release [23,24] than this dose and schedule of etanercept was able to effectively neutralize. If so, a more intensive etanercept dosing regimen may achieve better results, but further studies would be needed to evaluate the merits of such a strategy. Data from animal models demonstrate both TNF-dependent and TNF-independent pathophysiology, whereby TNF inhibition attenuates but does not completely eliminate GVHD [11]. Accordingly, it is also possible that TNF-independent pathways are major contributors to GVHD and NRM in radiation-based conditioning regimens for which even highly effective TNF inhibition will not significantly alter outcomes. Given the large difference in 1-year NRM based on conditioning regimen, we considered the possibility that TBI-conditioned patients experienced excess NRM. The 1-year NRM of 50% we observed in

Table 3. Causes of 1-year Non-Relapse Mortality

Causes	Conditioning Regimen	
	Non-TBI-based N = 71	TBI-based N = 29
aGVHD and therapy-related complications	8	8
cGVHD and therapy-related complications	2	2
Infection	0	3
Others	3*	2†
Total	13	15

TBI indicates total body irradiation; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.
*Veno-occlusive disease (N = 2); myocardial infarction (N = 1).
†Idiopathic pneumonia syndrome (N = 1); central nervous system hemorrhage (N = 1).

TBI-conditioned patients is similar to the 43% to 47% reported in a recently published registry series of over 1200 TBI-conditioned URD recipients [25]. Therefore, although ineffective, compared with results reported in the literature, it does not seem that etanercept administration worsened outcomes in TBI-conditioned patients.

Because TNF-α is an important mediator of both innate and acquired immune responses [12,21], and infections represent an established risk of TNF-inhibitors [26], we paid particular attention to the possibility that etanercept administration could lead to an increased number or severity of infections in this immunocompromised study population. Infections within the first 100 days posttransplantation were common in this study, as expected in patients undergoing high-risk allo-HCT [27]. In the absence of a control population, we are limited in the conclusions we can draw regarding etanercept and bacterial complications. It is encouraging that the rate of bacteremia, as well as bacterial septic death (3%), observed in this study is in line with published rates [28,29]. The incidence and severity of fungal and viral infections were also what would be expected in a high-risk population receiving standard GVHD prophylaxis [30-32]. Importantly, invasive fungal infections were well-controlled in patients with a history of such infections or who were undergoing treatment at the time of HCT. The 3 fungal deaths observed within the first 180 days posttransplantation are in line with published rates of 1% to 5% [33-35]. Nonetheless, further data are needed to fully assess the safety of etanercept in the context of prior invasive fungal infections and allo-HCT, particularly in patients receiving TBI-based conditioning. Furthermore, our trial design, which mitigated the risk of infection by monitoring for pathogens early and initiating preemptive treatments, may have offset any increased risk due to etanercept.

In summary, this study investigated the addition of etanercept given twice weekly for the first 8 weeks posttransplantation to a standard GVHD prophylaxis regimen. Etanercept injections were well-tolerated and we did not identify any excess toxicity either during the 2 months of active therapy or the posttreatment observation period. Based on the lack of efficacy observed in patients receiving TBI-based conditioning, we would caution against the use of etanercept in this context. Conversely, in nonradiation-based conditioning, etanercept seemed to attenuate the expected rise in TNFR1 ratios early posttransplantation, and these patients experienced good outcomes. Future approaches may build upon a TNF inhibition platform by incorporating complementary strategies. One such strategy is to increase the number of regulatory T cells, which inhibit GVHD while preserving graft-versus-lymphoma [28]. An experimental GVHD model has demonstrated that extracorporeal photopheresis induces regulatory T cells [29]. We are, therefore, currently testing this combination approach of TNF inhibition and extracorporeal photopheresis in a prospective clinical trial in URD HCT.

ACKNOWLEDGMENTS

We thank the patients, their families, and the clinical personnel who participated in this study; Jennifer Lay-Luskin and the BMT Program Team at the Clinical Trials Office at the University of Michigan and Loyola University Medical Center for outstanding data collection and management; and the BMT Program research nurses for research support, without which this study would not have been possible.

Financial disclosure: Supported by grants from the National Institutes of Health (2P01CA039542 and 5P30CA046592). Amgen, Inc. supplied the study drug. S.W.C. is the recipient of a St. Baldrick's Scholar Award and the National Institutes of Health K23 Grant AI091623-01.

Conflict of Interest Statement: There are no conflicts of interest to report.

Authorship Statement: J.E.L., K.C., T.B., and J.L.M.F. designed and planned the study. TB was the study statistician. J.W. performed the cytokine assays. S.W.C. and C.K. were in charge of data collection and quality assurance. All authors participated in writing the report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2012.03.013.

REFERENCES

1. Weisdorf DJ, Anasetti C, Antin JH, et al. Allogeneic bone marrow transplantation for chronic myelogenous leukemia: comparative analysis of unrelated versus matched sibling donor transplantation. *Blood*. 2002;99:1971-1977.
2. Arora M, Weisdorf DJ, Spellman SR, et al. HLA-identical sibling compared with 8/8 matched and mismatched unrelated

donor bone marrow transplant for chronic phase chronic myeloid leukemia. *J Clin Oncol*. 2009;27:1644-1652.
3. Ciurea SO, Saliba RM, Rondon G, et al. Outcomes of patients with myeloid malignancies treated with allogeneic hematopoietic stem cell transplantation from matched unrelated donors compared with one human leukocyte antigen mismatched related donors using HLA typing at 10 loci. *Biol Blood Marrow Transplant*. 2011;17:923-929.
4. Walter RB, Pagel JM, Gooley TA, et al. Comparison of matched unrelated and matched related donor myeloablative hematopoietic cell transplantation for adults with acute myeloid leukemia in first remission. *Leukemia*. 2010;24:1276-1282.
5. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
6. Nash R, Piñeiro L, Storb R, et al. FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. *Blood*. 1996;88:3634-3641.
7. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood*. 2007;110:4576-4583.
8. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood*. 2000;96: 2062-2068.
9. Korngold R, Marini JC, de Baca ME, Murphy GF, Giles-Komar J. Role of tumor necrosis factor-alpha in graft-versus-host disease and graft-versus-leukemia responses. *Biol Blood Marrow Transplant*. 2003;9:292-303.
10. Choi SW, Kitko CL, Braun T, et al. Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. *Blood*. 2008;112:1539-1542.
11. Xun CQ, Thompson JS, Jennings CD, Brown SA, Widmer MB. Effect of total body irradiation, busulfan-cyclophosphamide, or cyclophosphamide conditioning on inflammatory cytokine release and development of acute and chronic graft-versus-host disease in H-2-incompatible transplanted SCID mice. *Blood*. 1994;83:2360-2367.
12. Hill GR, Teshima T, Rebel VI, et al. The p55 TNF-alpha receptor plays a critical role in T cell alloreactivity. *J Immunol*. 2000;164:656-663.
13. Korth-Bradley JM, Rubin AS, Hanna RK, Simcoe DK, Lebsack ME. The pharmacokinetics of etanercept in healthy volunteers. *Ann Pharmacother*. 2000;34:161-164.
14. Boeckh M, Huang M, Ferrenberg J, et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. *J Clin Microbiol*. 2004;42:1142-1148.
15. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis*. 2002;34:1094-1097.
16. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis*. 2002;34:7-14.
17. Pzrepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995; 15:825-828.
18. Filipovich AH, Weisdorf D, Pavletic S, 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.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
20. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94:496-509.
21. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology*. 2005;115:1-20.
22. Jagasia M, Arora M, Flowers ME, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood*. 2012;119:296-307.
23. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: The role of gastrointestinal damage and inflammatory cytokines. *Blood*. 1997;90:3204-3213.
24. van der Velden WJ, Herbers AH, Feuth T, Schaap NP, Donnelly JP, Blijlevens NM. Intestinal damage determines the inflammatory response and early complications in patients receiving conditioning for a stem cell transplantation. *PLoS One*. 2010;5:e15156.
25. Uberti JP, Agovi MA, Tarima S, et al. Comparative analysis of BU and CY versus CY and TBI in full intensity unrelated marrow donor transplantation for AML, CML and myelodysplasia. *Bone Marrow Transplant*. 2011;46:34-43.
26. Giles JT, Bathon JM. Serious infections associated with anticytokine therapies in the rheumatic diseases. *J Intensive Care Med*. 2004;19:320-334.
27. Parody R, Martino R, Rovira M, et al. Severe infections after unrelated donor allogeneic hematopoietic stem cell transplantation in adults: comparison of cord blood transplantation with peripheral blood and bone marrow transplantation. *Biol Blood Marrow Transplant*. 2006;12:734-748.
28. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med*. 2010;363:2091-2101.
29. Juvonen E, Aalto S, Tarkkanen J, Volin L, Hedman K, Ruutu T. Retrospective evaluation of serum Epstein Barr virus DNA levels in 406 allogeneic stem cell transplant patients. *Haematologica*. 2007;92:819-825.
30. Zerr DM, Fann JR, Breiger D, et al. HHV-6 reactivation and its effect on delirium and cognitive functioning in hematopoietic cell transplantation recipients. *Blood*. 2011;117:5243-5249.
31. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med*. 2007;356:348-359.
32. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med*. 2007;356:335-347.
33. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood*. 2010;116:5111-5118.
34. Edinger M, Hoffmann P, Ermann J, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med*. 2003;9:1144-1150.
35. Gatza E, Rogers CE, Clouthier SG, et al. Extracorporeal photopheresis reverses experimental graft-versus-host disease through regulatory T cells. *Blood*. 2008;112:1515-1521.