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ORIGINAL ARTICLE

Combination antithymocyte globulin and soluble TNFα inhibitor (etanercept) +/− mycophenolate mofetil for treatment of steroid refractory acute graft-versus-host disease

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Antitumor necrosis factor-α antibodies are increasingly being used for the treatment of steroid-refractory acute graft-versus-host disease (GVHD) complicating allogeneic stem cell transplantation. We retrospectively reviewed the outcomes of 16 patients with refractory acute predominantly visceral GVHD treated with combination antithymocyte globulin (ATG), tacrolimus and etanercept +/− mycophenolate mofetil (MMF) at our institution. Overall response rate (CR + PR) was 81%, with median survival post commencing salvage immunosuppression 224 days (range 20–1216 days). In total, eight patients (50%) died, including from progressive GVHD in two cases (13%), infection in five (31%) and relapse of underlying malignancy in one (6%). In comparison to our previous experience of ATG + tacrolimus as treatment for refractory visceral GVHD, both response rate and overall survival were improved with addition of etanercept, with no apparent increase in infectious complications. As such, use of etanercept in combination with ATG +/− MMF for treatment of steroid refractory acute GVHD appears to be associated with high response rates, significant survival and no unexpected toxicity. Further study of this immunosuppression combination in a larger cohort of patients in this setting is indicated.
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Introduction

Graft-versus-host disease (GVHD) remains a significant complication of allogeneic stem cell transplantation (SCT). Although methylprednisolone (MP) is recommended as initial therapy of moderate to severe acute GVHD,[1] for patients refractory to corticosteroids, no specific salvage therapy has gained widespread acceptance. Two therapeutic approaches to steroid refractory acute GVHD are generally available. The first involves use of cytotoxic agents directed towards effector (T) cells, with agents such as antithymocyte globulin (ATG), OKT3 or mycophenolate mofetil (MMF).[2–6] The second approach is based upon blockage of cytokine pathways involved in the pathogenesis of acute GVHD, by use of antibodies inhibiting especially tumor necrosis factor-α (TNFα) (etanercept, infliximab) and interleukin-2 (dacluzimab, basiliximab) pathways.[7–17]

We have previously reported on use of tacrolimus and ATG for steroid refractory acute GVHD.[2] In brief, although this combination appeared efficacious in patients with refractory cutaneous GVHD (100% response rate; 80% survival), for patients with refractory visceral GVHD, outcome was significantly inferior (60% response rate; 20% survival). In an attempt to improve these relatively poor results in patients with refractory visceral acute GVHD, we subsequently added etanercept (Enbrel®, Wyeth, Australia) +/− MMF to our standard salvage regimen of tacrolimus and ATG. Here, we report our experience with this immunosuppressive combination for the treatment of steroid refractory acute GVHD.

Methods

Outcomes of all patients at the Royal Brisbane and Women's Hospital who had received combination tacrolimus, ATG and etanercept as salvage therapy for steroid refractory acute GVHD prior to November 2005 were retrospectively reviewed. GVHD was required to be biopsy proven and was graded according to the Glucksberg criteria.[18] A complete response (CR) was defined as resolution of all manifestations of GVHD (i.e. all organs stage 0), partial response (PR) as reduction in severity of GVHD by at least 1 overall grade, and progressive disease (PD) as any new organ involvement and/or worsening of $\geqslant$1 grade of GVHD. Steroid refractoriness was defined as either PD after 3 days of MP at $\geqslant$2 mg/kg/day, no change in GVHD grade after 7 days of MP, $<$CR after 14 days of MP, or recurrence of GVHD on steroid taper while receiving $>$50 mg of prednisolone per day.

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Salvage therapy initially consisted of MP at $\geqslant$2 mg/kg/day in combination with five consecutive days of ATG at 15 mg/kg/day. Tacrolimus was substituted for cyclosporin, with trough levels (measured by high performance liquid chromatography on whole blood samples) aimed to be maintained between 10 and 20 ng/ml. Etanercept was planned to commence within 2 weeks of starting ATG, at a dose of 0.4 mg/kg (to maximum 25 mg) s.c. twice per week for 4 weeks, then once per week for 4 weeks. For patients with refractory cutaneous GVHD, MMF 15 mg/kg BD was also allowed at individual physician discretion.

All patients also received valaciclovir and cotrimoxazole for *Herpes simplex* and *Pneumocyctis carinii* prophylaxis, respectively. Although fluconazole 200 mg o.d. was the standard antifungal prophylaxis recommended within the unit, in individual cases itraconazole or voriconazole were also used. Cytomegalovirus (CMV) reactivation was monitored for at least weekly via quantitative polymerase chain reaction performed on plasma samples.

Study end points were GVHD response (CR + PR) at end of etanercept therapy and overall survival (OS). Overall survival was calculated from time from commencing salvage immunosuppressive therapy, and curves produced using the Kaplan–Meier method. In the comparison with our previously published cohort, categorical data were compared using the two-tailed Fisher's exact test and nonparametric data via the long-rank test.

Results

In total, 16 patients with refractory GVHD had been treated with ATG plus etanercept. Patient characteristics and outcomes are shown in Tables 1 and 2, respectively. Although etanercept was planned to commence within 2 weeks of starting ATG, due to delays in drug approval, this was not achieved in all cases. Median time from start of ATG to commencing etanercept was 10 days (range 5–20 days). In five cases (all subsequently CR), MMF was also commenced with salvage ATG + etanercept (Table 2).

Overall response rate was 81% (95% confidence interval 60–100%), with 11 CR (69%) and two PR (12%) observed. In four cases an initial PR occurred during administration of ATG; all four of these patients subsequently achieved CR after commencing etanercept. In all other cases responses occurred only after commencement of etanercept.

Responses in GVHD were maintained in all CR patients. Progressive disease occurred in one PR patient after tacrolimus was ceased at 62 days post commencing salvage therapy owing to development of thrombotic thrombocytopenic purpura (TTP). This patient subsequently achieved CR after re-treatment with etanercept in combination with high dose prednisolone and MMF, and remains alive on reducing dose steroids. The second PR patient suffered a fatal relapse of their underlying malignancy (myeloma) at approximately 2 weeks post commencing salvage immunosuppression.

Thrombotic thrombocytopenic purpura occurred in a total of five patients, of whom four subsequently died.

Significant infectious complications occurred in 13 patients (81%), including bacterial sepsis in five (31%), viral infections in nine (64%; including CMV reactivation requiring pre-emptive antiviral therapy in six cases, CMV pneumonitis in one, BK virus cystitis in one and both CMV and HBV reactivation in one), and invasive fungal infection in five (36%; including new proven fungal infections in three cases and progression of previously documented fungal infection in two). Note that one case of new fungal infection occurred only after further immunosuppressive therapy was commenced for extensive stage chronic GVHD (see Table 2).

For the entire cohort, median survival was 224 days (range 20–1216 days; see Figure 1). To date, eight patients (50%) have died at a median of 50 days (range 20–224 days) post commencing salvage immunosuppressive ther-

Table 1 Patient characteristics

Case	*Sex*	*Age*	*Disease*	*Status at SCT*	*Conditioning*	*Donor source*	*Stem cell source*	*1° GVHD prophylaxis*
1	M	49	AML	CR1	Cy TBI	Sib	PBPC	CsA MTX
2	M	47	MM	PR1	Flu TBI	Sib	PBPC	CsA MMF
3	M	18	ALL	CR2	Cy TBI	UD	BM	CsA MTX
4	F	57	MDS	Untreated	Cy TBI	UD	BM	CsA MTX
5	M	57	CML	CP2	Cy TBI	UD	PBPC	CsA MTX
6	M	50	CML	CP1	Cy TBI	UD	BM	CsA MTX
7	M	45	FL	Refractory	Flu TBI	Sib	PBPC	CsA MMF
8	M	53	AML	CR1	Cy TBI	Sib	G-BM	CsA MTX
9	M	37	ALL	CR1	Cy TBI	UD	PBPC	CsA MTX
10	F	34	CML	Accelerated	Cy TBI	UD	PBPC	CsA MTX
11	M	52	FL	Refractory	Flu TBI	UD	PBPC	CsA MMF
12	M	38	MM	CR1	Flu TBI	Sib	PBPC	CsA MMF
13	M	26	ALL	CR1	Cy TBI	UD	BM	CsA MTX
14	M	58	AML	CR2	Cy TBI	UD	PBPC	CsA MTX
15	F	21	AML	CR2	Cy TBI	UD	BM	CsA MTX
16	M	16	ALL	CR1	Cy TBI	UD	PBPC	CsA MTX

Abbreviations: BM = bone marrow; CsA MTX = cyclosporine + day 1–11 MTX; CsA MMF = cyclosporine + mycophenolate mofetil; Cy TBI = cyclophosphamide/total body irradiation; FL = follicular lymphoma; Flu TBI = fludarabine/total body irradiation; G-BM = G-CSF stimulated bone marrow; GVHD = graft-versus-host disease; PBPC = peripheral blood progenitor cells; SCT = stem cell transplantation; UD = unrelated donor; Sib = sibling donor.

Table 2 Patient and GVHD outcomes

Case	*GVHD onset*[a]	*GVHD grade (S/G/L)*	*MMF*	*Response*	*Survival*	*Follow-up*[b]	*Complications*
1	30	4 (1/4/0)	No	PD	Dead	20	TTP; *Enterococcal* sepsis
2	32	4 (3/4/0)	No	PR	Dead	29	Progressive myeloma; *Pseudomonus* line sepsis
3	23	2 (3/1/0)	No	CR	Dead	34	BK virus cystitis; disseminated fusarium[c]
4	15	3 (2/3/0)	No	CR	Dead	45	TTP; CMV pneumonitis
5	20	3 (3/3/0)	No	PD	Dead	55	Pulmonary aspergillosis
6	7	2 (3/0/0)	Yes	CR	Dead	56	TTP; disseminated aspergillosis[c]
7	19	4 (1/4/3)	No	NR	Dead	68	TTP; *Enterococcal* sepsis
8	13	3 (3/2/0)	Yes	CR	Dead	224	Chronic GVHD; *mucormycosis*[d]
9	11	3 (3/2/0)	Yes	CR	Alive	184	Nil
10	13	2 (2/1/0)	Yes	CR	Alive	299	Nil
11	36	4 (0/4/0)	No	PR	Alive	301	TTP; progressive GVHD; *Pseudomonus* line sepsis
12	27	4 (0/4/0)	No	CR	Alive	316	HBV reactivation
13	63	4 (0/4/0)	No	CR	Alive	359	*Enterococcal* line sepsis
14	27	4 (3/4/0)	No	CR	Alive	558	Chronic GVHD
15	25	3 (3/2/0)	No	CR	Alive	945	Chronic GVHD; pulmonary aspergillosis
16	9	3 (3/1/2)	Yes	CR	Alive	1216	Chronic GVHD

[a]Days post-SCT.
[b]Days post commencing salvage immunosuppressive therapy.
[c]Fungal infection initially diagnosed prior to receiving etanercept.
[d]Fungal infection occurred during immunosuppressive therapy for chronic GVHD.
Abbreviations: CMV = cytomegalovirus; GVHD = graft-versus-host disease.

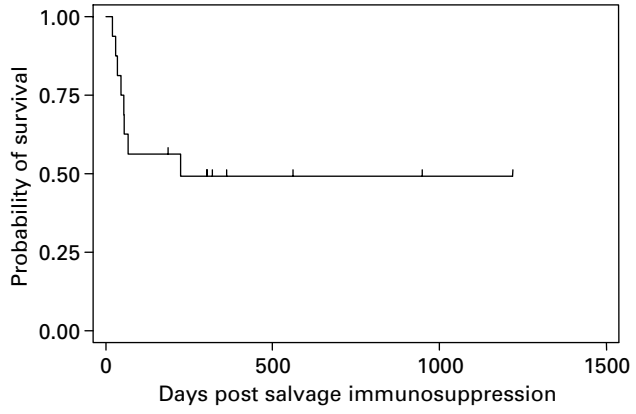

Figure 1 Overall survival (OS).

apy. Causes of death included progressive GVHD in two cases (13%), infection in five (31%) and relapsed malignancy in one (6%). Median follow-up post commencing salvage therapy for the eight surviving patients is 338 days (range 184–1216 days). Of nine patients who survived >4 months post-SCT, four (44%) subsequently developed chronic GVHD, which was extensive stage in all cases. Relapse of underlying malignant disease occurred in one patient.

Discussion

There are increasing numbers of reports of use of anti-TNFα antibodies (etanercept and infliximab) for the treatment of acute GVHD.[7–13,19] Etanercept is a fusion protein consisting of two identical chains of the human TNF-receptor p75 monomer fused with the Fc domain of human IgG1. It binds to soluble TNFα, neutralising its activity. Infliximab is a chimeric anti-TNFα-receptor antibody, which binds with high affinity to both soluble and transmembrane forms of TNFα. Binding of infliximab to transmembrane TNFα results in cell lysis secondary to complement activation and antibody-mediated cellular toxicity. Although there are no comparative studies between the two anti-TNFα agents in the treatment of GVHD, etanercept is arguably the preferred agent in this setting owing to its concomitant inhibition of lymphotoxin α,[20] as well as its lack of cytotoxic effect on phagocytic cells expressing surface TNFα. This latter attribute may be associated with reduced risk of infection in comparison to infliximab.[19]

Similar to our approach, the only previously reported series using etanercept for treatment of refractory acute GVHD used the antibody in combination with other immunosuppressive therapy (dacluzimab).[13] Our overall response rate of 81%, with 50% survival with follow-up of >6 months post commencing salvage therapy, compares extremely well with reported results with both combination etanercept and dacluzimab (67% response rate in 21 patients with 19% survival),[13] as well as infliximab (response rate 59–67% with approximately 40% survival)[8,11] in refractory acute GVHD. In comparison to our previous experience of combination ATG and tacrolimus in refractory visceral GVHD, both overall response rate and survival were improved with the addition of etanercept (Table 3 and Figure 2).[2]

The main toxicities experienced in our cohort were TTP and infection. We believe that the incidence of TTP noted in our cohort (36%) is not out of keeping with that expected for a group of patients with refractory predominantly visceral GVHD also receiving tacrolimus.[21] Similar to the other reported series of etanercept use in acute GVHD, we experienced a high rate of CMV reactivation and invasive fungal infection.[13] However, the incidence of infection in our current cohort was not dissimilar to that experienced in our previously reported series treating refractory acute GVHD without etanercept (Table 3).[2]

Table 3 Comparison of outcomes between previously reported ATG + tacrolimus cohort[2] and current ATG + tacrolimus + etenercept cohort for treatment of steroid refractory GVHD

Cohort	*Visceral GVHD/total no of patients*	*Response rate*[a]	*Median survival (days)*[a]	*Infection*[b]		
				Bacterial	*Viral*[c]	*Fungal*
ATG + tacrolimus	15/20	9 (60%)	42 (range 21–1081)	12 (60%)	8 (40%)	4 (20%)
ATG + tacrolimus + etanercept	15/16	12 (80%)	224 (range 20–1216)	5 (31%)	3 (19%)	5 (31%)
P		0.43	0.06	0.11	0.28	0.47

[a]For response/survival comparison, only patients with visceral GVHD included.
[b]For comparison of infectious complications all patients in both cohorts included.
[c]Excluding CMV reactivation.
Abbreviations: ATG = antithymocyte globulin; CMV = cytomegalovirus; GVHD = graft-versus-host disease.

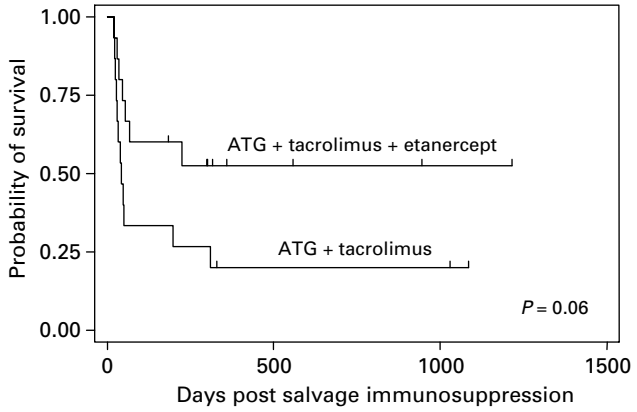

Figure 2 Overall survival (OS) of patients with refractory visceral graft-versus-host disease (GVHD) treated with previously reported antithymocyte globulin (ATG) + tacrolimus protocol[2] versus current ATG + tacrolimus + etenercept regimen.

This suggests that the important risk factor for infection in this setting is the development of refractory GVHD *per se*, rather than the specific immunosuppressive therapy protocol used to treat it.[22]

In our current cohort, four of 13 patients (31%) who responded to salvage immunosuppressive therapy subsequently died from infectious (predominantly fungal) complications. Although there is some evidence that preemptive antifungal therapy can reduce the mortality related to invasive fungal infection in this setting,[16] more studies on the most efficacious approach to both prevention and early detection of fungal infection during treatment of GVHD are required.

In conclusion, combination ATG and etanercept +/− MMF as treatment of refractory acute GVHD appears to be associated with high response rates, significant survival and no unexpected toxicity. Further study of this immunosuppression combination in a larger cohort of patients in this setting is indicated.

References

1 Sullivan K. Graft-versus-host disease. In: Blume K, Forman S, Appelbaum F (eds). *Thomas' Hematopoietic Cell Transplantation*, 3rd edn. Blackwell Publishing Ltd: Massachuscetts, 2004, pp 635–664.
2 Mollee P, Morton A, Irving I, Durrant S. Combination therapy with tacrolimus and anti-thymocyte globulin for the treatment of steroid refractory acute graft-versus-host disease developing during cyclosporin prophylaxis. *Br J Haematol* 2001; **113**: 217–223.
3 MacMillan M, Weisdorf D, Davies S, DeFor T, Burns L, Ramsay N *et al*. Early antithymocyte globulin therapy improves survival in patients with steroid-resistant acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2002; **8**: 40–46.
4 Hebart H, Ehninger G, Schmidt H, Berner B, Reuss-Borst M, Waller H *et al*. Treatment of steroid-resistant graft-versus-host disease after allogeneic bone marrow transplantation with anti-CD3/TCR monoclonal antibodies. *Bone Marrow Transplant* 1995; **15**: 891–894.
5 Basara N, Kiehl M, Blau W, Romer E, Bischoff M, Schmetzer B *et al*. Mycophenolate mofetil in the treatment of acute and chronic GVHD in hematopoietic stem cell transplant patients: four years of experience. *Transplant Proc* 2001; **33**: 2121–2123.
6 Krejci M, Doubek M, Buchler T, Brychtova Y, Vorlicek J, Mayer J. Mycophenolate mofetil for the treatment of acute and chronic steroid-refractory graft-versus-host disease. *Ann Hematol* 2005; **84**: 681–685.
7 Andolina M, Rabusin M, Maximova N, Di Leo G. Etanercept in graft-versus-host disease. *Bone Marrow Transplant* 2000; **26**: 929.
8 Couriel D, Saliba R, Hicks K, Ippoliti C, de Lima M, Hosing C *et al*. Tumor necrosis factor-a blockade for the treatment of acute GVHD. *Blood* 2004; **104**: 649–654.
9 Jacobsohn D, Hallick J, Anders V, McMillan S, Morris L, Vogelsang GB. Infliximab for steroid-refractory acute GVHD: a case series. *Am J Hematol* 2003; **74**: 119–124.
10 Kobbe G, Schneider P, Rohr U, Fenk R, Neumann F, Aivado M *et al*. Treatment of severe steroid refractory acute graft-versus-host disease with infliximab, a chimeric human/mouse anti-TNFα antibody. *Bone Marrow Transplant* 2001; **28**: 47–49.
11 Patriarca F, Sperotto A, Damiani D, Morreale G, Bonifazi F, Olivieri A *et al*. Infliximab treatment for steroid-refractory acute graft-versus-host disease. *Haematologica* 2004; **89**: 1352–1359.
12 Yamane T, Yamamura R, Aoyama Y, Nakamae H, Hasegawa T, Sakamoto C *et al*. Infliximab for the treatment of severe steroid refractory acute graft-versus-host disease in three patients after allogeneic hematopoietic transplantation. *Leuk Lymphoma* 2003; **44**: 2095–2097.
13 Wolff D, Roessler V, Steiner B, Wilhelm S, Weirich V, Brenmoehl J *et al*. Treatment of steroid-refractory acute graft-versus-host disease with daclizumab and etanercept. *Bone Marrow Transplant* 2005; **35**: 1003–1010.

14 Pzerpiorka D, Kernan N, Ippoliti C, Papadopoulos E, Giralt S, Khouri I *et al*. Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody for treatment of acute graft-versus-host disease. *Blood* 2000; **95**: 83–89.
15 Willenbacher W, Basara N, Blau I, Fauser A, Kiehl M. Treatment of steroid refractory acute and chronic graft-versus-host disease with daclizumab. *Br J Haematol* 2001; **112**: 820–823.
16 Srinivasan R, Chakrabarti S, Walsh T, Igarashi T, Takahashi Y, Kleiner D *et al*. Improved survival in steroid-refractory acute graft versus host disease after non-myeloablative allogeneic transplantation using a daclizumab-based strategy with comprehensive infection prophylaxis. *Br J Haemat* 2004; **124**: 777–786.
17 Massenkeil G, Rackwitz S, Genvresse I, Rosen B, Dorken B, Arnold R. Basiliximab is well tolerated and effective in the treatment of steroid-refractory acute graft-versus-host disease after allogenic stem cell transplantation. *Bone Marrow Transplant* 2002; **30**: 899–903.
18 Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA *et al*. Clinical manifestations of graft versus host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation* 1974; **18**: 295–304.
19 Uberti J, Ayash L, Ratanatharathorn V, Silver S, Reynolds C, Beker M *et al*. Pilot trial on the use of etanercept and methylprednisolone as primary treatment for acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2005; **11**: 680–687.
20 Bogunia-Kubik K. Poymorphisms within the genes encoding TNF-a and TNF-b associate with the incidence of post-transplant complications in recipients of allogeneic hematopoietic stem cell transplants. *Arch Immunol Ther Exp* 2004; **52**: 240–249.
21 Ho V, Cutler C, Carter S, Martin P, Adams R, Horowitz M *et al*. Blood and Marrow Transplant Clinical Trials Network Toxicity Committee summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2005; **11**: 571–575.
22 Marty F, Lee S, Fahey M, Alyea E, Soiffer R, Antin J *et al*. Infliximab use in patients with severe graft-versus-host disease and other emerging risk factors of non-Candida invasive fungal infections in allogeneic hematopoietic stem cell transplant recipients: a cohort study. *Blood* 2003; **102**: 2768–2776.