

ORIGINAL ARTICLE

Therapy for severe refractory acute graft-versus-host disease with basiliximab, a selective interleukin-2 receptor antagonist

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Basiliximab is a chimeric monoclonal antibody that binds to the α chain of IL-2R on activated cytotoxic T-cells, inhibiting lymphocyte proliferation. We report 34 patients with refractory acute GVHD (grade III–IV) who received basiliximab from December 1998 to October 2003. Adults received 40 mg weekly (2–3 doses) and children received half of this dose. Median age was 13 years. Twenty-five donors were unrelated. The stem cell source was bone marrow in 30 and cord blood in four. Complete responses were seen in 27/32 patients (84%) with skin, 12/25 (48%) with gut and 6/23 (26%) with liver GVHD. Median duration of response was 38 days (5–1103). Overall survival at 5 years was 20%. Eleven patients (32%) are alive. The main causes of death were CMV ($n=4$), fungus ($n=6$), sepsis ($n=8$), hemorrhage ($n=2$), and relapse ($n=2$). Graft-versus-host disease flares were observed in 14 patients (41%), half being rescued by other therapies. In conclusion, basiliximab was able to induce complete responses in patients with refractory acute GVHD. Prospective studies are necessary to evaluate the optimal treatment schedule.

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Introduction

Acute graft-versus-host disease (a-GVHD) is a serious complication of allogeneic HSCT, and occurs in 30–80% of patients.^{1–8} The most frequent target organs include skin, liver and gut.⁹ Mortality can reach 90% in severe refractory disease.²

The conditioning regimen causes host tissue damage and cell activation. Activated host cells secrete inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1).^{3,10–13} During early activation, T cells

express a variety of surface molecules not found in resting cells, such as high-affinity receptors for interleukin-2 (IL-2), also known as CD25. Binding of IL-2 to its receptor is a major requirement for T-cell clonal expansion.^{10–14}

Anti-CD25 monoclonal antibodies act through a competitive mechanism, specifically binding with high affinity to the α chain of IL-2 receptor and blocking at this point the cascade of events that would result in a-GVHD.^{10,14} Daclizumab, a 144 kDa humanized anti-CD25 monoclonal antibody, has a median half-life of 3–4 days, and has been used as salvage therapy of a-GVHD.¹⁰ However, the frontline use of this drug has been associated with higher overall mortality and higher rates of relapse.¹⁵

Basiliximab is a chimeric murine–human antibody, also selective for interleukin-2 receptor (IL-2R). Owing to its murine portion, basiliximab has less immunogenic properties when compared to daclizumab, allowing a low rate of anti-antibodies and increased half-life of about 7 days. Basiliximab has been used to prevent acute rejection in renal transplantation with great efficacy and with absence of severe adverse events. In this setting, infection and lymphoproliferative disease rates were not different between patients treated with this drug and placebo.^{16–18}

We report our experience with basiliximab as therapy for steroid-refractory a-GVHD.

Patients and methods

Basiliximab was initially chosen owing to non-availability of other drugs at our centre. Based on initial results, it became the therapy of choice for grade III–IV steroid-refractory a-GVHD. Thirty-four patients with grade III–IV refractory a-GVHD (according to Glucksberg criteria) who received basiliximab for salvage therapy from December 1998 to October 2003 were evaluated retrospectively. Two patients were excluded owing to early death. Steroid-refractory a-GVHD was defined as progression of GVHD severity after 3 days of steroid therapy or no improvement after 7 days of steroid therapy.

Drug schedule and dose

Adults received 40 mg per week and children 20 mg per week for 2–3 weeks. All patients had failed previous

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therapy with cyclosporine (adjusted for therapeutic levels at 200–400 ng/dl) and methylprednisolone (2 mg/kg/day). No drugs were used before basiliximab. As recommended for anti-IL2 receptor antibodies, in order to achieve a synergistic action, all patients were on steroids at a dose 2 mg/kg/day and cyclosporine (therapeutic levels) during the 3 weeks of basiliximab therapy. In the third week, if complete response (CR) was achieved, steroids were tapered by 20% per week as tolerated. If there was partial (PR) or no response (NR), patients were treated with other regimens. Complete response required no clinical signs of GVHD, PR required improvement of at least one grade in any involved site, and NR signified no improvement of a-GVHD.

Patients

The first 12 patients were treated later in the course of GVHD, having received more days of steroid therapy, and only two doses of the antibody. Two of these patients had received cyclosporine and methylprednisolone (at 2 mg/kg/day) for 70 days before basiliximab could be available.

The second group was composed of 22 patients. Because of the responses observed in the first group of patients, we decided to give the drug earlier for all patients who matched the refractoriness criteria defined below. Moreover, high GVHD flare rate observed in the first group led us to give a third dose for these patients.

Table 1 shows patient characteristics.

Table 1 Characteristics of study patients

Characteristics	Patients N (%)
Median age (range) (years)	13 (2–38)
<i>Sex</i>	
Male	21 (62)
Female	13 (38)
<i>Stem cell source</i>	
Bone marrow	30 (88)
Cord blood	4 (12)
<i>Donor</i>	
Related	9 (26)
Unrelated	25 (74)
<i>Diagnosis</i>	
Acute leukemia	9 (26)
MDS	4 (12)
CML	9 (26)
FA	9 (26)
SAA	3 (10)
<i>GVHD grade</i>	
III	6 (18)
IV	28 (82)
<i>Organs involved</i>	
Skin	32 (94)
Gut	25 (73)
Liver	23 (68)
Median GVHD duration (range) days	16 (3–70)

Abbreviations: CML = chronic myeloid leukemia; FA = Fanconi anemia; GVHD = graft-versus-host disease; MDS = myelodysplastic syndrome; SAA = severe aplastic anemia.

Statistical analysis

Kruskal–Wallis test was used to compare groups. Fisher's exact test was used to compare categorical variables. Kaplan–Meier analysis was used to evaluate probability of survival.

Results

Response and toxicity

At a median follow-up of 196 days (range: 35–1847), overall response rate was 82% (32% CR and 50% PR). Median time to response was 7 days (range: 1–30); median duration of response was 38 days (range: 5–1103). Response varied according to the organ involved (Figure 1). Table 2 shows characteristics of responses.

Acute GVHD flared in 41% of patients, half of who could be rescued by other regimens (mycophenolate, steroids, azathioprine). One patient was rescued by another basiliximab course of three doses and achieved a second CR. No infusion-related symptoms were observed.

Among evaluable patients, nine of 11 CR, four of nine PR, and zero of four NR patients were able to taper steroid dose to less than 1 mg/kg/day. Of the two CR patients unable to decrease steroids, one developed progressive

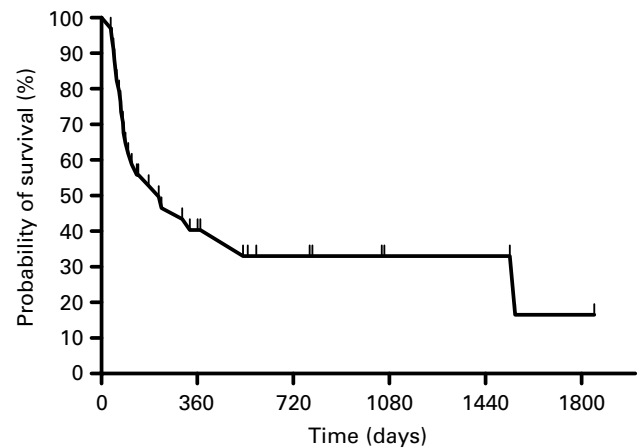


Figure 1 Survival curve.

Table 2 Characteristics of response

	Complete (%)	Partial (%)	NR (%)	NA (%)
Type of response	11 (32)	17 (50)	4 (12)	2 (6)
<i>Response by GVHD site</i>	Complete (%)	Partial (%)	NR (%)	NE (%)
Skin	84	13	3	0
Gut	48	32	16	4
Liver	25	30	35	9
GVHD flare (%)		14 (41)		
Median time (days) to response (range)		7 (1–30)		
Median duration (days) of response (range)		38 (5–1103)		

Table 3 Response × taper of immunosuppression and development of chronic-graft-versus-host disease at evaluable patients

	CR (%)	PR (%)	NR (%)
Refractory acute GVHD ^a	11 (32)	17 (50)	4 (12)
Steroid taper <1 mg/kg/day	9 (26)	4 (12)	0 (0)
Survival ≥100 days	11 (32)	9 (26)	1 (3)
<i>Chronic GVHD</i>			
Quiescent	9 (26)	0 (0)	0 (0)
Progressive	1 (3)	8 (24)	1 (3)
No C-GVHD ^b	1 (3)	0 (0)	0 (0)
<i>Outcome (%)</i>			
Alive	7 (20)	3 (9)	1 (3)
DC IS	5 (15)	0 (0)	0 (0)

^aFailure of steroids 2mg/kg/day and cyclosporine.

^bOne patient died of infection on day +133, with no signs of GVHD.

Abbreviations: CR = complete response; DC IS = discontinued immunosuppression; NR = no response; PR = partial response.

extensive chronic GVHD and the other one died on day +133 from CMV interstitial pneumonia with no signs of GVHD activity.

Of 21 evaluable patients who survived more than 100 days, nine of 11 CRs developed extensive chronic GVHD. Seven of these patients are alive, five of them with no immunosuppression, one continues to taper immunosuppression and one has active extensive chronic GVHD still on therapy. Eight of nine PRs developed chronic GVHD. One died of multiple infections and GVHD at day +232. Four were able to decrease steroid dose to less than 1 mg/kg/day with addition of other drugs; three are alive. One of four patients without response (who had only skin involvement) survived more than 100 days and is still alive on immunosuppression for extensive chronic GVHD of progressive onset (Table 3).

Survival

Eleven patients (32%) are alive at a median follow-up of 196 days (range 45–1847). Eight of 11 (72%) CRs are alive three of 17 (17%) PRs are alive and one of four NRs is alive.

Kaplan–Meier probability of overall survival at 5 years was 20% for the entire group because of a late death from relapsed acute lymphoblastic leukemia (Figure 1). Response correlated well with survival, as demonstrated in Figure 2. Median survival was 1550 days for CR group, 112 days for PR group and 69 days for NR group. We also compared survival for the first 12 patients, who had received two doses of the drug and were included later, to the 22 patients who received three doses and were included according to defined criteria for refractoriness. Survival was not significantly different between these groups (Figure 3).

Infection was the main cause of death in 82% of the patients (Table 4).

Discussion

Therapy for refractory a-GVHD is still a major challenge. Anti-thymocyte globulin (ATG) and several monoclonal

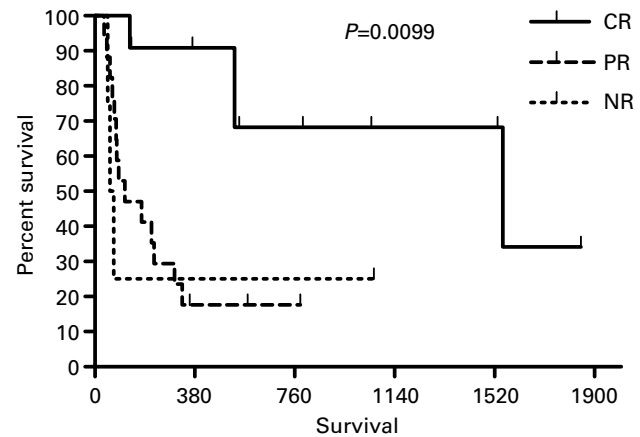


Figure 2 Survival rate × response.

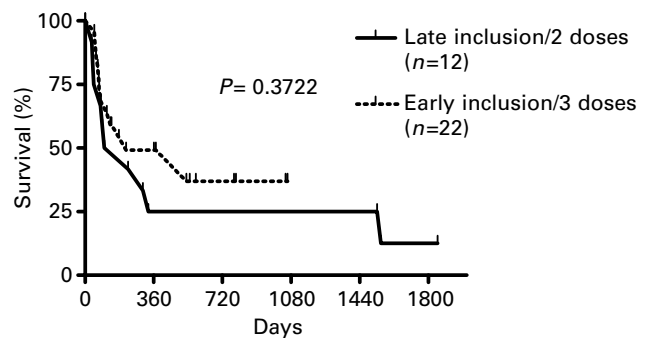


Figure 3 Survival according to timing of inclusion and number of doses received.

Table 4 Causes of death

Causes of death	N (%)
<i>Infection</i>	
Bacteria	8 (35)
Fungus	6 (26)
CMV	4 (17)
Toxoplasma	1 (4)
Disease relapse (ALL)	2 (9)
Bleeding	2 (9)

Abbreviations: ALL = acute lymphoblastic leukemia; CMV = cytomegalovirus.

antibodies against T-lymphocytes (visilizumab), CD25-positive cells (daclizumab, denileukin diftitox) or cytokines (anti-TNF-α and anti interleukin-2 (anti IL-2)) have been used, with CRs being observed in 30–50% of the cases, but with poor overall outcomes.^{5,10,14,19–22}

The high toxicity of ATG together with previous results from anti-IL2R drugs led us to conduct a clinical study of basiliximab as therapy for grade III–IV severe refractory a-GVHD. In the present study, weekly dose schedule was decided based on renal transplantation experience, along with the information of the median half-life of the drug, which is 6–7 days.

We found an overall response rate of 82% with 32% CR. The rate of response varied according to the organ involved. Skin a-GVHD showed the best response (82%),

followed by gut (32%) and liver (26%). Graft-versus-host disease flares were frequently seen (41%), but use of other agents could rescue half of the patients.

Some studies have demonstrated that regulatory CD4+CD25+ T cells have an important role in the achievement of tolerance and preservation of GVL effect after stem cell transplantation.^{23,24} It was also recently shown that denileukin difitox was able to kill these regulatory cells.²⁵ As basiliximab is an anti-CD25 antibody, undesirable inhibition of T-regulatory cells can be responsible for the high rates of GVHD flares and progression to chronic GVHD observed. This mechanism was also hypothesized by Lee *et al.* to explain the bad results achieved with frontline use of daclizumab. A second consideration is that the competitive mechanism of action of these drugs probably also contributes to the high incidence of flares observed. Infection remained a major concern during this study, being the main cause of death in up to 80% of the patients. Massenkeil *et al.* have recently published their experience with basiliximab in 17 patients with steroid refractory a-GVHD, with an overall response rate of 71% and a complete response rate of 51%. A very low rate of infection was seen in these patients.²⁶ These response rates are very similar to those presented in our study; however, severe infection was frequently observed in our data and resulted in death. Cicceri *et al.*¹⁶ have evaluated efficacy of basiliximab in 8 patients following allogeneic non-myeloablative bone marrow transplantation. Despite an overall response rate of 50%, with complete response of 37%, all patients died due to infectious complications. To some extent, high infection rate would be expected as the majority of our patients had grade IV a-GVHD, which results in the delay of immunologic recovery, and persistent severe immunoincompetence.⁹

High levels of IL-2 can overcome the competitive binding to IL-2R and decrease drug efficacy. For this reason, therapeutic doses of cyclosporine and steroids at a dose of 2 mg/kg/day were used with basiliximab therapy in this study, – and this could have also contributed to the higher rate of infection. Indeed, among responding patients, deaths owing to infection were significantly less frequent.

We conclude that basiliximab is a useful drug to treat severe refractory a-GVHD. However, there is a high incidence of a-GVHD flares, progression to chronic GVHD and life-threatening infections. Randomized prospective trials are necessary to address optimal dose, schedule and management of infections.

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