Combination Therapy with Inolimomab and Etanercept for Severe Steroid-Refractory Acute Graft-versus-Host Disease

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A B S T R A C T

Steroid-refractory acute graft-versus-host disease (aGVHD) remains an important cause of morbidity and mortality after allogeneic stem cell transplantation (SCT). A protocol on the management of aGVHD was introduced in our center that incorporated a prospective study on combination therapy with inolimomab (anti-IL-2Rα) and etanercept (anti–tumor necrosis factor-α) for steroid-refractory aGVHD. We evaluated the efficacy and safety in 21 consecutively treated patients. The patients had developed refractory aGVHD after SCT (n = 16) or donor lymphocyte infusion (n = 5), and aGVHD was classified as severe in all patients, mostly due to gastrointestinal involvement stages 2 to 4. No drug-related side effects were observed apart from the infections expected to occur in these severely immunocompromised patients. Overall response at day 28 of second-line therapy was 48% (10/21), with 6 and 4 patients achieving a complete and partial response, respectively. Eventually, 19 patients died (90%), with early mortality (<6 months) predominantly resulting from refractory aGVHD and secondary infections and late mortality resulting from relapse of the underlying disease. With a median follow-up of 55 days, the estimated rates of 6-month and 2-year overall survival were dismal, 29% and 10%, respectively. In conclusion, the combination of inolimomab and etanercept for steroid-refractory aGVHD failed to improve the dismal prognosis of severe steroid-refractory aGVHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (SCT) constitutes a potentially curative approach for hematologic malignancies, but its success can be reduced by the occurrence of acute graft-versus-host disease (aGVHD) [1]. First-line treatment with high-dose corticosteroids (ie, methylprednisolone 2 mg/kg) is currently the standard for patients developing aGVHD grade ≥ II, achieving complete responses (CRs) in 40% to 50% [2,3]. However, the prognosis of patients with aGVHD who are refractory to this therapy is poor, especially when it concerns grades III to IV (severe) aGVHD due to gastrointestinal (GI) GVHD [2,4]. Several treatment modalities have been used for second-line therapy, but the results have generally been disappointing, with long-term overall survival (OS) rates of approximately 20% to 30% at 3 years [5]. Therefore, there is no consensus on what should be considered the most efficacious second-line therapy [3].

In the past our center used high-dose methylprednisolone, 1000 mg for 3 days, for refractory aGVHD with poor results, especially in those with severe aGVHD due to GI involvement [6]. Therefore, we adopted a new protocol that incorporated a prospective study of the combination of inolimomab and etanercept. The choice was based on previous reports on the efficacy of inolimomab and etanercept as single agents and the theoretical advantage of combining 2 drugs that target key pathways involved in GVHD pathogenesis. Etanercept is a tumor necrosis factor-α–receptor fusion protein that scavenges tumor necrosis factor-α and has shown promising results in first- and second-line therapy for aGVHD [7,8]. Inolimomab is a monoclonal antibody targeting the IL-2 receptor subunit α (CD25) that predominantly inhibits activated alloreactive T cells. Several studies have shown encouraging results in the treatment of steroid-refractory aGVHD with inolimomab [9,10]. In this study we evaluated the efficacy and safety of the combination therapy and expected higher response rates.

METHODS

Treatment Protocol

We introduced a protocol in February 2010 for managing aGVHD that incorporated a prospective open-label study on the combination of inolimomab and etanercept for the treatment of aGVHD refractory to corticosteroids. Approval of the local ethics committee (CMO Regio Arnhem-Nijmegen) was obtained before implementation of the protocol and start of the study. Patients signed informed consent before participating in the protocol.

aGVHD was scored according to the consensus criteria of Przepiorka et al. [11], and GVHD was preferably confirmed by histologic examination of biopsies taken from involved sites. The initial treatment of aGVHD grades ≥ II consisted of prednisolone (2 mg/kg) in combination with cyclosporine A or mycophenolate mofetil. Steroid-refractory aGVHD was defined as failure to achieve at least a partial response (PR) 5 days after the initiation of prednisolone, no further improvement on day 10 compared with day 5, or a recurrence of aGVHD after tapering of corticosteroids. Second-line treatment with inolimomab and etanercept started when refractory aGVHD was established.

Etanercept (Enbrel; Wyeth Pharmaceuticals bv, Hoofddorp, The Netherlands) was given subcutaneously twice weekly at a dose of 25 mg for 4 weeks. Inolimomab (Leukotac; EUSA Pharma, Limonest, France) was administered intravenously with a dose of .3 mg/kg daily from days 1 to 8 and a dose of .4 mg/kg every other day from days 9 to 28. Primary endpoints were the overall response rate on day 28 of second-line therapy and the 6-month estimated OS [3,12-14]. Our goal was to achieve an overall response rate of at least 50% and a 6-month OS rate of at least 30%. Secondary endpoints were response on day 56, long-term OS, and safety and tolerability.

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Patients received prophylaxis for *Pneumocystis jirovecii*, herpes viruses, and *Candida* in case of colonization. All patients were monitored twice weekly for Epstein-Barr virus (EBV) and cytomegalovirus (CMV). EBV reactivation, defined as an EBV PCR $\geq$ log 3, was preemptively treated with 4 doses of rituximab (375 mg/m^2). In case of CMV reactivation patients received ganciclovir, valganciclovir, or foscarnet. Galactomannan was tested twice weekly, and a high-resolution computed tomography was ordered when invasive mold disease was suspected followed by bronchoalveolar lavage when applicable. Possible, probable, and proven invasive mold disease were treated with voriconazole.

Definitions of Outcome

CR was defined as resolution of all signs and symptoms of aGVHD. A PR was defined as an improvement of 1 stage in 1 or more organs without progression or newly developed aGVHD in another organ. Mixed response was considered an improvement in at least 1 organ with progression or newly developed aGVHD in another organ. No response was defined as absence of improvement. Progression was defined as worsening by 1 or more stages without improvement in any involved organ [12]. All adverse events observed during the treatment were recorded and scored according the Common Toxicity Criteria version 4.

Statistical Analysis

We used descriptive statistics for the study group characteristics and outcome of therapy. Univariable analyses for risk factors for treatment response and OS at 6 months were performed by Fisher's exact test and chi-square test. Logistic regression analysis was used to perform multivariable analysis for risk factors with $P \leq .2$ in univariable analysis. OS was estimated by the Kaplan-Meier method. SPSS was used for the statistical analysis. $P < .05$ was considered to be statistically significant (SPSS version 22.0.01; IBM, Armonk, NY).

RESULTS

Patient Characteristics

From February 2010 to February 2014, 185 patients received an allogeneic SCT with a follow-up of at least 6 months. Seventy-one patients developed histologically confirmed aGVHD grade $\geq$ II (38%: post-SCT n = 59 [32%], post–donor lymphocyte infusion [DLI] n = 12 [6%]), and severe aGVHD occurred in 39 patients (21%). All patients were treated initially with prednisolone 2 mg/kg, but aGVHD proved to be steroid refractory in 25 of 71 cases (35%), and all these cases concerned grades III to IV aGVHD (25/39 [64%]). Of these, 21 were treated with combination therapy. Four did not receive this treatment, mostly because of a very poor performance status defined as a life expectancy of less than 28 days. Patient characteristics, conditioning regimens, and graft characteristics of these 21 patients are summarized in Table 1.

Table 1
Characteristics of Patients (N = 21) Receiving Second-Line Therapy with Inolimomab and Etanercept

Characteristics	Value
Median age, yr (range)	54 (24-66)
Male/female	11/10
Donor type, n (%)	
MRD	11 (52%)
MUD	7 (33%)
MMUD	3 (15%)
Diagnosis, n (%)	
AML/MDS	12 (57%)
NHL/CLL	4 (19%)
Other	5 (24%)
Conditioning, n (%)	
NMA/RI	8 (38%)
MA	13 (62%)
T cell depletion, n (%)	
Yes	
In vivo (ATG or alemtuzumab)	8 (38%)
Ex vivo	16 (76%)
No	4 (19%)
Stem cell source peripheral blood, n (%)	21 (100%)
GVHD setting, n (%)	
Post-SCT	16 (76%)
Post-DLI	5 (24%)
Dose of DLI (n = 5) CD3$^+$ cells $\times$ 10^8/kg	.01
aGVHD, n (%)	
Skin	17 (81%)
Lower GI	19 (90%)
Upper GI	6 (29%)
Liver	7 (33%)
Acute GI GVHD, n (%) (n = 19)	
Stage 1	1 (5%)
Stage 2-4	18 (95%)

MRD indicates matched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; NMA, nonmyeloablative; RI, reduced intensity; MA, myeloablative; ATG, antithymocyte globulin.

aGVHD and Response to Second-Line Therapy

All 21 patients had severe aGVHD at the start of second-line treatment: 17 with grade III and 4 with grade IV. The median time from SCT or DLI to the diagnosis of aGVHD was 37 days (range, 15 to 263). Seventeen patients had GVHD involving the skin, 19 involving the gut, and 7 involving the liver (Table 1). Hence, most patients had aGVHD involvement of at least 2 organs (17/21 [81%]).

Start of second-line therapy was a median of 8 days (range, 4 to 24) after start of corticosteroid therapy. The median treatment duration was 25 days (range, 6 to 28). Of the 21 patients 6 and 4 patients achieved a CR and PR, respectively, bringing the overall response rate on day 28 to 48% (10/21). Steroids were not tapered at the time of starting inolimomab and etanercept. In case of GVHD improvement, defined as at least a PR, prednisone was tapered in accordance with the Seattle scheme for aGVHD, which means tapering of .2 mg/kg prednisone every 5 days. Patients also continued cyclosporine or mycophenolate mofetil. When cyclosporine toxicity was suspected, the dosage of cyclosporine was decreased on the treating physician's judgment. If there was no toxicity, cyclosporine was continued during the treatment with etanercept and inolimomab. Three patients, who initially had achieved a response, developed a flare-up and received third-line therapy consisting of mesenchymal stem cells, but without effect. Hence, on day 56 the overall response rate was 33%, with 5 patients retaining a CR and 2 a PR.

Univariable analysis revealed several risk factors for treatment response with $P < .2$, namely liver GVHD stages 2 to 4, albumin level < 25 g/L at diagnosis, disease category acute myeloid leukemia, and GVHD setting post-DLI. Logistic regression analysis with these 4 risk factors confirmed a statistically significant impact of GVHD setting post-DLI ($P = .01$) and liver GVHD stages 2 to 4 ($P = .03$) but only a trend for low albumin ($P = .07$). Disease category was no longer statistically significant (Table 2).

aGVHD and DLI

Patients who suffered from steroid-refractory aGVHD that had occurred post-DLI all died of GVHD despite the use of inolimomab/etanercept. DLI had been given in a low dose (range, .5 to 5 $\times$ 10^6 CD3$^+$ T cells/kg). The indication for the preemptive DLI in all these patients who had been transplanted with a partially T cell–depleted graft was realization of full donor chimerism and boosting of the graft-versus-leukemia effect.