Weekly rituximab followed by monthly rituximab treatment for steroid-refractory chronic graft-*versus*-host disease: results from a prospective, multicenter, phase II study

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ABSTRACT

Background

Since it was suggested that B cells play a role in the pathogenesis of chronic graft-*versus*-host disease, rituximab, an anti-CD20 monoclonal antibody targeting B cells, has been shown to be effective in steroid-refractory, chronic graft-*versus*-host disease. However, most of the data were from small numbers of patients or retrospective analyses. We, therefore, conducted a multicenter phase II study to confirm the efficacy of this treatment strategy that targets B cells.

Design and Methods

We diagnosed and evaluated chronic graft-*versus*-host disease according to the National Institute of Health criteria for clinical trials on this condition. The treatment consisted of weekly intravenous infusions of rituximab for 4 weeks followed by monthly rituximab for 4 months. We evaluated the patients' responses and monitored their disease activity until their final visit, which was on day 365. We also assessed the patients' subsequent quality of life and serum levels of B-cell-activating factor of the tumor necrosis factor family.

Results

Among 37 patients enrolled (median age, 29 years; range 8-57 years), 32 patients responded to rituximab with 8 complete and 24 partial responses. Twenty-one patients maintained their response for 1 year, so their steroid treatment was discontinued or its dose reduced (21/37, or 56.8%), and their scores representing quality of life were improved although these changes were not statistically significant. The responses were better for clinical manifestations of the skin, oral cavity and musculoskeletal system (response rate, 71.4-100%) than for other organs. However, infectious complications and primary disease relapse accounted for the majority of treatment failure. The pre-treatment serum level of B cell-activating factor of the tumor necrosis factor family was not associated with better treatment outcome (P=0.147).

Conclusions

Rituximab could improve clinical responses and quality of life of patients with steroid-refractory chronic graft-*versus*-host disease, although such patients may need active prophylaxis against infection. (*ClinicalTrials.gov Identifier: #NCT00472225*)

Key words: rituximab, chronic graft-*versus*-host disease, steroid, allogeneic stem cell transplantation, quality of life.

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Introduction

Chronic graft-*versus*-host disease (GVHD) is a major cause of morbidity and mortality in survivors of allogeneic stem cell transplantation.[1] Chronic GVHD has variable clinical manifestations, similar to those in autoimmune disorders, and may involve diverse tissues, including the skin, eyes, oral mucosa, liver, lungs, and gut.[2] Primary treatment consists of steroids alone or in combination with calcineurin inhibitors such as cyclosporine or tacrolimus, extending for several months and often years.[3,4] Many patients do, however, respond poorly to treatment, resulting in late mortality.[4,5] Furthermore, chronic GVHD and its prolonged treatment may seriously impair a patient's quality of life (QOL).[6] There are few treatments available and there is currently no consensus on the best treatment for steroid-refractory chronic GVHD. There is an urgent need for new options of effective therapy.

Although we do not fully understand the pathogenesis of chronic GVHD, studies suggest that B cells, as well as donor-derived T cells, may be involved. Thus, alloantibodies to Y chromosome-associated minor histocompatibility antigens correlate with chronic GVHD in sex-mismatched allogeneic stem cell transplants, and donor-derived B cells have been shown to be required to induce chronic GVHD in a murine model.[7,8] Elevated levels of B cell-activating factor of the tumor necrosis factor family (BAFF) are also correlated with the occurrence and severity of chronic GVHD.[9,10] Thus, B cells may present a therapeutic target in the treatment of chronic GVHD, especially in steroid-refractory cases.

Rituximab is a chimeric monoclonal antibody that binds to CD20 antigens on the surface of B cells. Although widely used to treat B-cell lymphomas, it has recently been shown to be effective in steroid-refractory chronic GVHD; a meta-analysis found a response rate of up to 70%.[11] Most previous reports, however, concerned single cases or retrospective analyses of small series of patients.[12-15] All but one of four prospective studies included only three to seven patients.[16-19] Furthermore, most previous studies classified chronic GVHD as limited or extensive disease according to a historical definition, and response criteria varied among the studies. As a result, prospective and retrospective studies have produced variable response rates and organ-specific responses.[11] To standardize the criteria for the diagnosis of chronic GVHD and its response to treatment, the National Institutes of Health (NIH) Consensus Development Project released new criteria for clinical trials in this disease.[20,21] Up to now, only one small prospective study of seven patients has used these NIH criteria,[19] and none has addressed patients' QOL. We, therefore, performed this phase II study to evaluate both the effectiveness of rituximab (i.e., the clinical response) and QOL in patients with steroid-refractory chronic GVHD, based on the NIH criteria for clinical trials.[20,21]

Design and Methods

Study design

This study was an open-label, multicenter, prospective, phase II study to evaluate the efficacy of rituximab in terms of response to treatment, changes in QOL and discontinuation of steroids. Eligible subjects were patients with steroid-refractory chronic GVHD who required treatment. Thus, all patients had at least one diagnostic sign of chronic GVHD or at least one distinctive manifestation confirmed by biopsy or other laboratory tests according to the criteria for clinical trials in chronic GVHD released by the NIH Consensus Development Project.[20] All patients also had at least a moderate score for chronic GVHD, defined as a maximum score of at least two in any affected organ or a score of one in all affected organs in the global scoring system for chronic GVHD.[20] Steroid-refractory chronic GVHD was defined as chronic GVHD of sustained severity during the last full month during which the patients had received the equivalent of prednisone 0.5 mg/kg or more per day or 1 mg/kg or more every other day. Concomitant use of other immunosuppressive agents was permitted except for extracorporeal phototherapy or other experimental treatments. We excluded patients who had received donor lymphocyte infusions within 100 days preceding the trial. Patients who had a serious comorbid condition, such as an active infection, or a life expectancy of less than 1 month were also not eligible to participate. The institutional review board at each participating center approved this study, and all patients provided written informed consent. This trial was registered at *www.clinicaltrials.gov* as #NCT00472225.

Treatment and response evaluation

The treatment consisted of weekly intravenous infusions of rituximab 375 mg/m^2 for 4 weeks followed by monthly rituximab for 4 months (Figure 1A). Baseline evaluations were performed within 7 days before enrollment, and the first treatment response was evaluated after completion of the weekly rituximab infusions (day 29, Figure 1A). Four weeks later (day 57), we evaluated all patients' responses again, after which the patients were entered into the maintenance phase, regardless of their response, except in the case of a serious adverse event or refusal to receive further treatment. We evaluated the responses during the maintenance phase (days 85, 113, and 141), and monitored patients three times before their final visit of the study period (day 365). At each visit, routine blood tests, including complete blood cell counts and serum biochemistry, and imaging studies, including chest X-rays, were performed. Tests such as Schirmer's test were used to evaluate organ-specific responses. Patients could receive prophylaxis with acyclovir and trimethoprim-sulfamethoxazole for viral and fungal infections if this was decided to be appropriate by each investigator on the basis of each patient's clinical context.

Response definition and steroid tapering

We used the criteria from the NIH Consensus Development Project to define a response.[21] Complete response was defined as the resolution of all signs and symptoms associated with chronic GVHD. Partial response was defined as a clinical score reduction of at least one point in one or more affected organs, with no evidence of deterioration in any organ. Objective responses therefore included both complete and partial responses. Progressive disease was defined as a clinical score increase of at least one point in one or more organs or occurrence of any new symptoms or signs of chronic GVHD. We defined a lack of response without the requirement for additional immunosuppressive therapy as no response. Based on the objective response, investigators could reduce the steroid dosage. Subjects with no response or progressive disease received a fixed or increased dose of steroid until the next response evaluation. Regimens for immunosuppressants other than steroids were similarly modified.

Quality of life measurement

The Short Form-36 (SF-36) questionnaire, version 2.0 (QualityMetric, RI, USA), was used to evaluate QOL at baseline,

on day 57, and on day 365. The eight domains explored by the SF-36 are general health perceptions, physical function, general mental health, role function limitation due to physical problems, role function limitation due to emotional problems, bodily pain, vitality, and social function. These data were then used to compute physical component summary and mental component summary scores using the "SF-36 Physical and Mental Health Summary Scales".[22] The score was normalized to that of healthy people, set at 50 (±10).

Sample collection and measurement of serum B-cell-activating factor of the tumor necrosis factor family

Serum samples were obtained during the study period (at baseline and on days 57 and 365) and were stored at –80 °C until tested with an enzyme-linked immunosorbent assay (ELISA). To measure serum BAFF, samples were thawed and 50 μL were placed in each of the wells of an ELISA plate coated with a mouse monoclonal antibody against human BAFF (Quantikine Human BAFF Immunoassay®, R&D Systems, Minneapolis, MN, USA). The ELISA was performed according to the manufacturer's manual, and the absorbance at 450 nm was measured. Serum BAFF (pg/mL) was calculated from a standard curve produced with 40,000 pg/mL of recombinant human BAFF. To compare BAFF levels with immune globulin (Ig) levels, serum IgG, IgA, and IgM were measured in the same samples.

Sample size calculation and statistical analysis

A previous study with weekly administration of rituximab showed a 70% overall response rate in steroid-refractory chronic GVHD.[18] Thus, if our treatment regimen of weekly rituximab and monthly rituximab maintenance failed to show more than a 50% overall response, the treatment was to be deemed ineffective. A response rate greater than 70%, however, could indicate effectiveness in the treatment of steroid-refractory chronic GVHD. Based on the above assumption, we designed this trial using Simon's minimax two-stage testing procedure.[23] Assuming a target level of interest, p1=0.70, and a lower activity level of p0=0.50, 23 patients needed to be accrued; if 13 or more objective responses were observed, the trial was to be continued to include 37 patients. This design provided a probability of 0.05 or less of accepting a treatment worse than p0 and a probability of 0.20 or less for rejecting a treatment better than p1.

We used the χ^2 test to evaluate the relationships between the responses to the clinical variables. Improvement in QOL was determined by intent-to-treat (ITT) and per protocol (PP) analyses. The ITT analysis set included the data of all patients with at least one QOL measurement after treatment. Missing values in the ITT set were imputed with the 'last observations carried forward' method. The PP analysis set included only the patients with complete QOL observations. A two-sided *P* value of less than 0.05 was considered to be statistically significant.

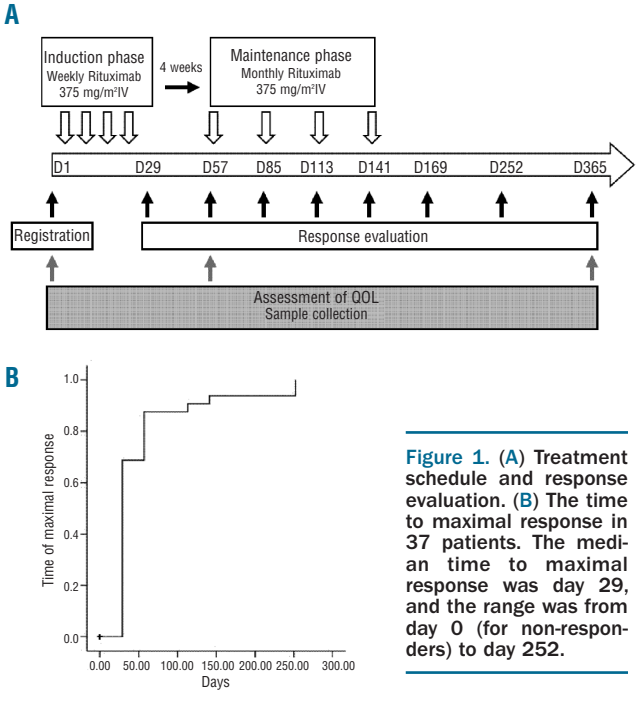

Figure 1. (A) Treatment schedule and response evaluation. (B) The time to maximal response in 37 patients. The median time to maximal response was day 29, and the range was from day 0 (for non-responders) to day 252.

Results

Characteristics of patients and allogeneic stem cell transplants

This study included 37 patients ranging in age from 8 to 57 years (median, 29 years) (Table 1). Approximately one-third of these patients (32.4%) had a poor performance status (ECOG grade 2/3) at the time of entry. Although we excluded patients with serious co-morbidities, we included those with diabetes mellitus and previous tuberculosis. There were no patients with hepatitis B virus antigen, antibody against hepatitis C virus, or human immunodeficiency virus. All patients underwent allogeneic stem cell transplantation and two patients received a second allogeneic stem cell transplant. Myeloablative conditioning was the dominant procedure (78.4%, Table 1), and cells from unrelated donors were more commonly used than cells from sibling donors. Bone marrow was the main source of stem cells, and mismatches of ABO type and sex occurred more frequently than did matched cases.

Characteristics of steroid-refractory, chronic graft-versus-host disease

All patients had at least moderate chronic GVHD, with a median duration of 5.2 months (range, 2.2-25.5 months) (Table 1) before entry into the study. More than one-half of patients had a previous history of acute GVHD (56.8%). The disease involved diverse organs from skin to gut (Table 1). Three patients had other immune-mediated disorders; two had hemolytic anemia and one had proteinuria (Table 1). The majority of patients had multiple-organ involvement, with a median of 2.78 (range, 1-5) organs per person. At the time of enrollment, all patients received a steroid with or without other immunosuppressive agents, and the median duration of steroid treatment was 1.8 months (range, 1-28 months).

Response and steroid discontinuation

We assessed response to treatment at designated time points during the 1-year study period (Figure 1A). Some patients maintained their initial response, while in others, the initial response diminished during follow-up. In the calculation of maximum response for the study period, eight patients had a complete response and 24 patients had a partial response (Table 2). The median time to maximal response was on day 29 (Figure 1B; range, day 0 to day 252). Overall, 21 patients maintained their response until the final visit. In these patients (21/37, or 56.8%) the

steroid treatment was then discontinued or its dose reduced (Table 2). Because the median number of involved organs was 2.78 (range, 1-5) organs per person, the number of evaluable organs was larger than the number of patients. Evaluation of response according to the organs involved showed that lesions in the musculoskeletal system, skin and oral cavity responded better than those in the eyes, liver and gut (Table 3). Only one patient showed a partial response (9.1%, 1/11, Table 3) in the lung, the organ least responsive to treatment. Immune-mediated manifestations such as hemolytic anemia responded well to rituximab (Table 3). The baseline characteristics of patients, including duration of chronic GVHD, did not influence the response to rituximab (P>0.05, *data not shown*). However, all patients up to 15 years old (n=8) had an objective response, and seven of these maintained their responses. Specific features of the allogeneic stem cell transplants, such as stem cell source, HLA matching, or the type of conditioning, were not associated with response (P>0.05, *data not shown*).

Table 1. Baseline characteristics of the patients with chronic GVHD.

Characteristic		Number (n = 37)	%
Demographic			
Age (years)	≤ 15	8	21.6
Median 29 (range, 8-57)	> 15	29	78.4
Sex	Male	20	54.1
	Female	17	45.9
Performance status	ECOG 0/1	25	67.6
	ECOG 2/3	12	32.4
Primary disorder	Acute myeloid leukemia	17	45.9
	Acute lymphoblastic leukemia	8	21.6
	Multiple myeloma	4	10.8
	Chronic myeloid leukemia	3	8.1
	Myelodysplastic syndrome	2	5.4
	Aplastic anemia	1	2.7
	Others	2	5.4
Combined disorder	Diabetes mellitus	7	18.9
	Past history of tuberculosis	4	10.8
	Past history of toxic hepatitis	1	2.7
	None	25	67.6
Stem cell transplantation			
Conditioning regimen	Myeloablative	29	78.4
	Reduced intensity	8	21.6
Stem cell donors	HLA-matched sibling	16	43.3
	HLA-matched unrelated	17	45.9
	HLA-incomplete matched unrelated	4	10.8
Stem cell source	Peripheral blood stem cells	8	21.6
	Bone marrow	27	73.0
	Cord blood	2	5.4
ABO matching	Matched	15	40.5
	Mismatched	22	59.5
Sex matching	Complete	13	35.1
	From male (D) to female (R)	15	40.5
	From female (D) to male (R)	9	24.3
Chronic GVHD			
Duration of disease	< 6 months	27	73.0
Median 5.2 months (range, 2.2-25.5)	≥ 6 months	10	27.0
Previous acute GVHD	Absence	16	43.2
	Presence	21	56.8
Previous CMV antigenemia	Absence	24	64.9
	Presence	13	35.1
Involved organs*	Oral cavity	28	75.7
	Skin	22	59.5
	Eyes	21	56.8
	Lung	10	27.0
	Liver	9	24.3
	Musculoskeletal system	7	18.9
	Gut	3	8.1
	Others	3	8.1
Immunosuppression at enrollment	Steroid alone	6	16.2
	Steroid + cyclosporine	11	29.7
	Steroid + tacrolimus	10	27.0
	Steroid + calcineurin inhibitor and/or others	10	27.0

*ECOG: Eastern Cooperative Oncology Group; D: donor; R: recipient; CMV: cytomegalovirus; *Median number of involved organs per person: 2.78 (range, 1-5).*

Mortality and drop-out

Five patients did not respond to treatment, so they dropped out from the study (#18, 21, 24, 29, 32). Of these, two patients (#29, 32) dropped out after day 29 because of suspected pulmonary tuberculosis and infectious colitis, respectively. Tests showed, however, that they did not have those complications, and they both recovered. Another patient (#18) with no response withdrew his consent to continued treatment after day 85. The other two patients (#21, 24) died of pneumonia (Table 2). On the other hand, six patients showed disease progression after they had achieved a partial response (#6, 9, 10, 13, 15, 36, Table 2). Among these six patients, two patients (#6, 9) died of pneumonia and sepsis during follow-up, and one patient with lung involvement (bronchiolitis obliterans) died of pulmonary hemorrhage (#36). Another patient (#10) showed disease progression late in follow-up (day 252). The other two patients dropped out when their disease progressed (#13, 15). The hematologic toxicity associated with the rituximab infusions was acceptable because only one patient (#4) showed grade III neutropenia on day 113. However, this patient died due to infection after neutropenia. Two patients who maintained their response (partial response) died of infectious complications without any evidence of myelosuppression (#14, 30). Another patient with a partial response (#20) dropped out because her primary disease relapsed. The other patient (#3) died of complications from a fall, a death not related to treatment or chronic GVHD (Table 2). Considering these infectious complications or relapses during the study, we performed competing risk analysis for response to estimate the cumulative incidence rates. The cumulative incidence rate of response was 89.2% at 254 days while that of events including infection-related death and relapse was 10.8% at 254 days.

Quality of life

We assessed the QOL at three designated points (at baseline and on days 56 and 365). With a score of 50 assumed to represent that of a normal healthy person, the baseline scores in all domains were below 50 at study entry in both the ITT and PP analyses (Figure 2). However, patients reported increased scores in six domains (role physical, bodily pain, general health perception, role emotional and general mental health), although the changes were statistically significant only for the role physical and bodily pain domains (Figure 2A, P<0.05). This finding became more prominent in the PP analysis, which includ-

ed only patients with complete follow-up through to day 365 (Figure 2B). Because all of these patients showed an objective response, their final score at day 365 approached 50 in several domains, although these changes were not significant. However, scores in some domains, especially the physical domains, including physical functioning, role physical and bodily pain, decreased. Thus, the physical component summary score decreased as the study progressed, while the mental component summary score did not (Figure 2).

Table 2. Summary of treatment outcomes in the 37 patients studied.

Baseline characteristic at enrollment					Response evaluation (Day)								Follow-up		
N.*	Sex/ /age	Time after chronic GVHD	Steroid dosage (mg)	Immuno-suppressant	29	57	85	113	141	169	252	365	Severe adverse events (onset)	Maximum response	Status after enrollment	Steroid administration (final dose of steroid)
2	F/19	5.4	40		NR	PR	PR	PR	CR	CR	CR	CR		CR	Alive	Stop
11	M/40	24.0	30	F 1mg, M 250mg	CR	CR	CR	CR	CR	CR	CR	CR		CR	Alive	Reduction (7.5mg)
17	M/8	13.6	50		PR	CR	CR	CR	CR	CR	CR	CR		CR	Alive	Stop
37	F/44	2.4	30		PR	PR	PR	PR	CR	CR	CR	CR		CR	Alive	Stop
7	M/26	12.1	20	F 1mg, M 250mg	CR	CR	CR	CR	CR	PR	PR	PR		CR	Alive	Reduction (5mg)
12	F/52	6.3	30	F 2mg, M 250mg	CR	CR	CR	CR	CR	PR	PR	PR		CR	Alive	Reduction (10mg)
16	F/15	13.6	50	C 250mg	PR	CR	CR	CR	CR	PR	PR	PR		CR	Alive	Reduction (10mg)
25	F/29	2.3	50		PR	CR	CR	CR	CR	CR	CR	PR		CR	Alive	Reduction (20mg)
1	M/45	26.4	30	C 125mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (20mg)
5	F/11	2.2	30	F 2mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (10mg)
8	F/17	6.0	40	F 2mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (10mg)
19	M/9	25.9	27	F 1mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (5mg)
26	F/51	12.3	30	C 50mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (5mg)
27	F/44	37.5	30	C 200mg, A 100mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Stop
28	M/41	4.5	40	A 100mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (5mg)
31	F/34	23.0	80	C 250mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Stop
33	M/49	2.3	30	F 2mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Reduction (7.5mg)
34	F/15	2.4	30	C 200mg	PR	PR	PR	PR	PR	PR	PR	PR		PR	Alive	Stop
22	M/8**	33.3	18	C 100mg	NR	NR	NR	PR	PR	PR	PR	PR		PR	Alive	Reduction (18mg)
23	F/11	8.8	25	C 100mg	NR	NR	NR	NR	NR	NR	PR	PR		PR	Alive	Reduction (10mg)
35	M/19	4.2	30		NR	NR	NR	NR	NR	NR	PR	PR		PR	Alive	Reduction (5mg)
3	M/47	23.8	30		PR	PR	PR	PR	PR	PR			Fall (D205)	PR	Died at 6.8 months	
4	M/16	4.2	20	F 4mg	PR	PR	PR	PR					Sepsis (D115)	PR	Died at 4.8 months	
20	F/45	3.1	30	F 2mg	PR	PR	PR	PR	PR	PR			Primary disease relapse (D173)	PR	Dropped out	
30	M/8	2.9	10	F 1mg, M 150mg	PR	PR	PR	PR	PR	PR			Pneumonia (D169)	PR	Died at 7.7 months	
14	M/17	10.9	30	F 2mg	PR	PR							Pneumonia (D57)	PR	Died at 3.1 months	
36	F/24	3.4	60	C 150mg, Ms 750mg	PR	PR	PD						Pulmonary hemorrhage (D106)	PR	Died at 3.5 months	
10	F/25	9.4	30	C 150mg	NR	PR	PR	PR	PR	PR	PD	PD		PR	Alive at 16.6 months	
6	M/51	3.5	30	C 200mg	PR	PR	PR	PR	PD	PD			Sepsis (D203)	PR	Died at 6.9 months	
13	M/27**	2.2	85	F 2mg	PR	PD	PD							PR	Dropped out	
15	M/18	2.4	80	F 6mg	PR	PR	PD	PD						PR	Dropped out	
9	M/52	4.6	30	C 100mg	NR	PR	PR	PR	PD	PD			Pneumonia (D169)	PR	Died at 6.2 months	
24	F/38	6.7	24	F 3mg, Ms 720mg	NR	NR	NR						Pneumonia (D83)	NR	Died at 3 months	
18	M/35	2.2	40	C 250mg, M 150mg	NR	NR	NR							NR	Dropped out	
21	M/57	37.2	32	C 600mg	NR	NR							Pneumonia (D57)	NR	Died at 2.1 months	
29	M/40	2.3	85	C 200mg, Ms 720mg	NR								R/O Tuberculosis (D29)	NR	Dropped out	
32	F/43	17.0	30	F 3mg	NR								R/O Colitis (D33)	NR	Dropped out	

*The patients are listed in the order of their maximum response. F: tacrolimus; M: mycophenolate mofetil; C: cyclosporine; A: azathiopurine; Ms: mycophenolate sodium; D: day. * This number represents the order of registration. ** These two patients received second allogeneic stem cell transplants. NR: no response; PR: partial response; CR: complete response; PD: progressive disease.*

Serum B-cell-activating factor of the tumor necrosis factor family and immune globulins

The median baseline level of serum BAFF was 1225 pg/mL (range, 129-8188pg/mL), higher than the median reported for normal healthy Asian people (range 780-861 pg/mL).[24,25] Generally, we found that patients with a serum BAFF lower than 1000 pg/mL had better outcomes. Thus, 78.5% (11/14) of those with lower levels maintained their response at day 365, while 50% (9/18) of those with higher levels had progressive disease or complications, although this effect was not statistically significant (P=0.147). Serum BAFF increased continuously, with an inverse correlation to the sustained treatment-related decrease in immune globulins (Figure 3). The mean levels of immune globulins decreased from baseline to 1 year as follows: IgG, from 773.6 to 528.2 mg/mL (normal range, 700-1600); IgA, from 105.8 to 63.9 mg/mL (normal range, 70-400); and IgM, from 106.2 to 40.2 mg/mL (normal range, 40-230).

Discussion

Chronic GVHD has increased with the use of unrelated or HLA-mismatched donors and peripheral blood stem cells for allogeneic stem cell transplantation.[26,27] The most commonly used treatment for chronic GVHD is systemic steroid administration; however, many patients are either steroid-refractory or have a steroid-dependent response. Thus, chronic GVHD may require long-term use of steroids and other immunosuppressive agents, although this treatment is still not satisfactory. Rituximab emerged as a treatment for chronic GVHD when a patient who had discontinued steroids incidentally showed improvement during treatment with rituximab for immune thrombocytopenia.[15] Results of subsequent studies supported the effectiveness of rituximab in steroid-refractory chronic GVHD.[12-14,16-19] Four of these studies were prospective and the overall response rate ranged from 43% to 83%.[16-19] However, three pilot studies evaluated only three to seven patients.[16,17,19] One study alone was designed as a phase I/II study;[18] in that study of 21 patients the overall response rate was greater than 70% and two patients achieved a complete response at 1 year. However, the study did not use the criteria for diagnosis of chronic GVHD and response in clinical trials recommended by the NIH Consensus Development Project.[20,21]

Our study is currently the largest one to evaluate the response of steroid-refractory chronic GVHD to rituximab

Table 3. Rituximab responses according to manifestation or organ involved.

Involved organ or manifestation	Total number	Complete response (n)	Partial response (n)	No response (n)	Overall response rate *(%)
Skin	22	5	12	5	77.3
Oral cavity	28	4	16	8	71.4
Musculoskeletal system	7	0	7	0	100.0
Eye	21	0	9	12	42.9
Liver	9	0	4	5	44.4
Gut	3	0	1	2	33.3
Lung	11	0	1	10	9.1
Hemolytic anemia	2	2	0	0	100.0
Proteinuria	1	1	0	0	100.0

**Overall response includes complete and partial responses.*

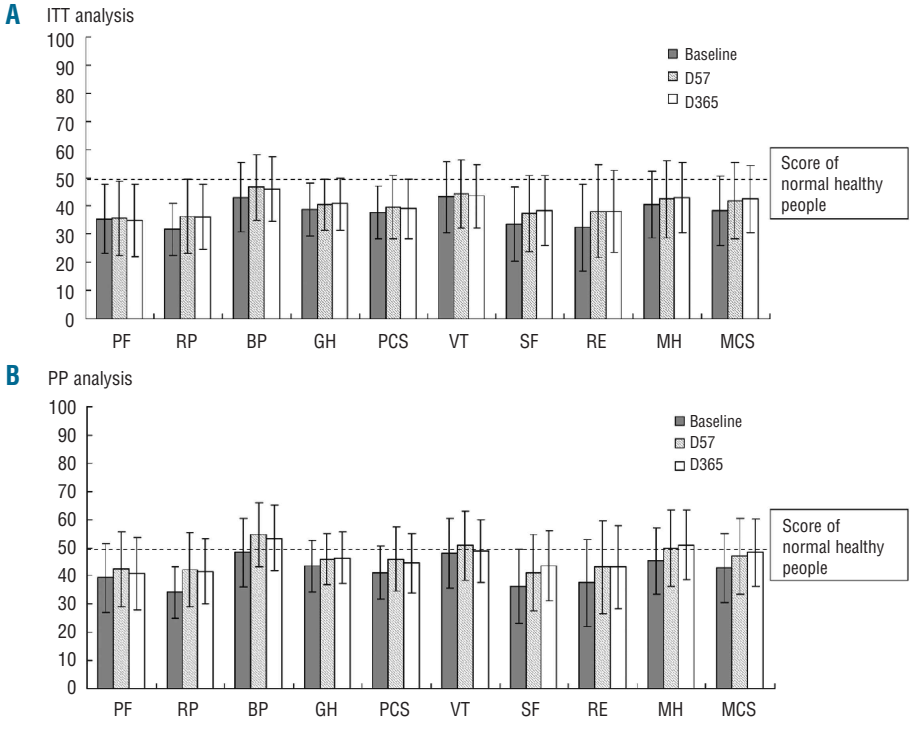

Figure 2. (A) In the ITT analysis of quality of life, patients reported increased scores in six domains (RP, BP, GH, SF, RE and MH) although only the effects on RP and BP were statistically significant (P<0.05). (B) PP analysis, including only patients with complete follow-up through day 365 showed a tendency toward improvement in all domains of the SF36 quality of life questionnaire although not statistically significant. GH, general health perceptions; PF, physical function; MH, general mental health; RP, role function limitation due to physical problems; RE, role function limitation due to emotional problems; BP, bodily pain; VT, vitality; SF, social function; PCS, physical component summary; MCS, mental component summary

using the NIH criteria. Unlike previous prospective studies which recruited only adults, our group of 37 patients included eight patients who were 15 years old or younger. The previous studies used a uniform treatment schedule consisting of a weekly rituximab infusion for 4 weeks, with an additional 4 weeks of this treatment for patients with no response or incomplete response at the first evaluation.[16,18] We treated our group initially with the same schedule, and at the end of an induction phase (day 29), observed an objective response in 26 patients (70.3%, 26/37). After that, we proceeded to the monthly administration of rituximab for 4 months as a maintenance treatment. During or after this phase, six patients who did not respond to the weekly rituximab showed a delayed response (Table 2). Thus, the objective response rate based on the maximum response of each patient was 86.5% (32/37, Table 2). However, only 22 patients completed the study as planned; 15 patients dropped out when their disease progressed or when they developed an infection. The final overall response rate at 1 year was, therefore, 56.8% (21/37), and these patients were able to discontinue steroids or reduce their dosage (Table 2). When infection-related deaths and relapses were considered as competing risks, the cumulative incidence rate of response was 89.2% at 254 days.

The musculoskeletal and dermal manifestations of GVHD responded better than did manifestations in the eyes, liver and gut (Table 3), consistent with results of previous studies.[13,14,17,18] In our study, however, oral cavity lesions showed better responses than those previously reported.[11] This may reflect the shorter duration of chronic GVHD in our study (median, 5.2 months) than in the previous ones (medians, 14-37 months), because long-standing disease might result in destruction of target tissues such as the salivary glands.[14,16,18] As in previous studies, the lungs showed the least responsiveness, while immune-mediated manifestations such as hemolytic anemia responded well to rituximab. Although we found no clinical parameters that predicted the response to rituximab, patients 15 years old or younger responded better than those over 15 years old; seven young patients maintained their response until their last visit (7/8, 87.5%) while 14 older patients maintained their response through to the end of the planned study period (14/29, 48.3%).

In a recent review of QOL assessments, the majority of studies suggested that more than 60% of patients reported good to excellent QOL in the 1 to 4 years after allogeneic stem cell transplantation.[28] All of our patients, however, showed scores below the average for normal healthy people in all domains of the baseline QOL evaluation. Moreover, despite clinical improvement in the majority of patients after treatment, the QOL did not increase significantly, although it showed a positive trend (Figure 2). This finding suggests that steroid-refractory chronic GVHD may seriously impair QOL, even after the resolution of symptoms. Active behavioral interventions as well as medical treatments may be required to improve and maintain QOL.

Although we observed a delayed response during or after the maintenance phase, disease progression also occurred in this later time. Moreover, eight patients died of infectious complications, including pneumonia and sepsis (Table 2). Because all of these patients received both steroids and other immunosuppressive agents throughout the study, we could not demonstrate a causal relationship between rituximab and infection. However, serum immunoglobulin levels remained suppressed below the normal range until the final visit (day 365, Figure 3), which may have increased susceptibility to infection. While our monthly rituximab regimen may have helped responders to maintain their response, it may also have resulted in a higher rate of infectious complications than those reported in previous rituximab trials. Because most of the infections occurred during the maintenance phase and our treatment protocol did not include the prophylactic administration of immunoglobulin, our high incidence of infectious complications might be related to our maintenance rituximab infusion-associated prolonged B-cell depletion. Thus, the patients should have received prophylactic immunoglobulin and careful monitoring for infection. A recent study found a positive effect of low-dose rituximab (50 mg/m^2) on steroid-refractory chronic GVHD,[29] which suggests that dosage reduction may sustain response without increasing the risk of infection.

In our study, patients with a serum BAFF higher than 1000 pg/mL showed treatment failure (e.g. disease progression) more frequently than did those with lower levels. Although this effect was not statistically significant (P=0.147), it is consistent with a role of BAFF in the occurrence and severity of chronic GVHD.[9,10] However, serum BAFF increased during the study period, with an inverse correlation to the decrease in immune globulins (Figure 3).

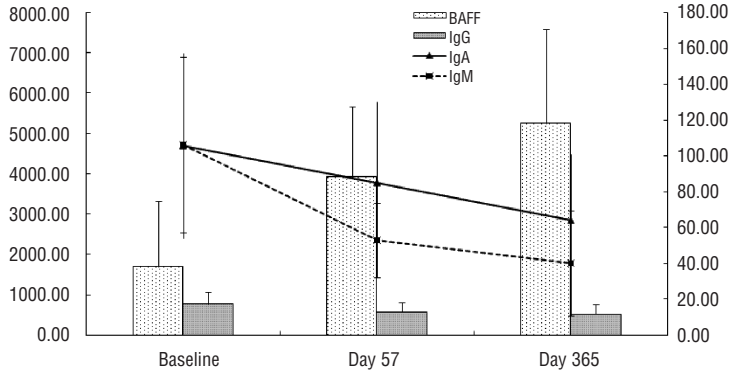

Figure 3. Serial changes in serum BAFF and immunoglobulins. Serum BAFF increased continuously, but was inversely correlated with the sustained decrease in immune globulins.

This decrease in immune globulins and increase in the level of serum BAFF may be related to the depletion of B cells, because rituximab can deplete normal B cells. The serum level of BAFF during treatment may, therefore, not be of any additional value for predicting the response or activity of chronic GVHD although a pre-treatment level of BAFF lower than 1000 pg/mL seemed to be associated with the likelihood of response to rituximab. However, this association of pre-treatment level of serum BAFF with the response to rituximab was not statistically significant and might be related to the number of patients in our study. Thus, it may be worth extending the study to include more patients to confirm the association of serum BAFF with treatment response and disease activity of chronic GVHD.

In summary, we report here the largest multicenter study to date evaluating the efficacy of rituximab using the NIH criteria for diagnosis and response measurement for clinical trials in steroid-refractory chronic GVHD.[20,21] We found that rituximab may reduce clinical manifestations, permit steroid discontinuation, and improve QOL in patients with this disease. However, patients may require prophylactic treatment for infections related to B-cell depletion.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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