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ORIGINAL ARTICLE

Rituximab therapy for refractory autoimmune bullous diseases: A multicenter, open-label, single-arm, phase 1/2 study on 10 Japanese patients

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ABSTRACT

This was a multicenter study of rituximab, a chimeric monoclonal immunoglobulin G antibody directed against CD20, for the treatment of refractory autoimmune bullous diseases (pemphigus and pemphigoid). Ten patients (three with pemphigus vulgaris, six with pemphigus foliaceus and one with bullous pemphigoid) were treated with a single cycle of rituximab (four weekly infusions at a dose of 375 mg/m^2 of body surface area). The primary endpoints were the number of serious adverse events and rate of complete remission at 40 weeks. Five patients (50%) achieved complete remission with minimal therapy (defined as no active lesions with lower doses of systemic corticosteroids compared to that with prednisolone 10 mg/day). Improvements in clinical scores (Pemphigus Disease Area Index) and decreases in autoantibody titers in the sera were observed in the four pemphigus patients who failed to achieve complete remission. This suggests that rituximab was effective in nine of 10 cases. Two serious adverse events (*Pneumocystis carinii* pneumonia and septic shock due to infectious arthritis) were observed and adequately treated with hospitalization. CD19-positive B lymphocytes in the peripheral blood decreased on day 29 following rituximab treatment, and remained at low levels throughout the observation period (280 days). Our results confirmed the efficacy of rituximab therapy for refractory autoimmune bullous diseases in Japan.

Key words: CD20 antigens, immunotherapy, pemphigoid, pemphigus, rituximab.

INTRODUCTION

Autoimmune bullous diseases, including pemphigus and pemphigoid, are potentially life-threatening due to symptoms of widespread blisters and erosions on the skin and mucosa. In pemphigus, the blisters and erosions are caused by circulating immunoglobulin (Ig)G autoantibodies against desmoglein (Dsg) 1 and Dsg3, adhesion molecules in desmosomes that have a crucial role in the cohesion between keratinocytes.[1,2] In pemphigoid, the formation of erythemas and blisters is mediated by autoantibodies against basement membrane proteins such as BP180 (type XVII collagen) and BP230.[3] Currently, the first line of treatment for patients with severe pemphigus and pemphigoid is systemic corticosteroids often administrated in combination with other immunosuppressants to reduce autoantibody production. In most cases, this course of therapy is effective, resulting in successful remission of pemphigus and pemphigoid. However, most dermatologists do encounter intractable cases, where clinical remission is difficult to achieve. Additionally, long-term therapy with corticosteroids and immunosuppressants is associated with adverse effects, such as diabetes mellitus, osteoporosis and serious infections, which are potentially fatal.[4,5] More targeted therapies against autoantibody production are needed in the treatment of autoimmune blistering diseases.

Rituximab (RTX) is a chimeric human–mouse IgG monoclonal antibody that binds specifically to the transmembrane antigen CD20 expressed on B lymphocytes from the pre-B-cell stage to the pre-plasma cell stage.[6] The binding of RTX to CD20 leads to B-cell depletion through various mechanisms, including complement-mediated lysis and induced apoptosis.[7] In many countries, RTX is used as the first-line treatment for B-cell non-Hodgkin lymphomas at a dose of 375 mg/m^2 with

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four weekly infusions.[8] RTX is also approved as a treatment for rheumatoid arthritis at a dose of 2 × 1000 mg at 2-week intervals.[9,10] In addition, RTX has been used as an off-label drug for different autoimmune diseases, such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus, myasthenia gravis and dermatomyositis. Since 2001, a number of case reports and case series have reported remarkable therapeutic effects of RTX in patients with autoimmune bullous diseases, particularly pemphigus. In addition, the US Food and Drug Administration approved RTX for treatment of pemphigus in the USA in 2018. However, it is difficult to prescribe RTX for autoimmune bullous diseases in Japan because all treatments are required to be covered by basic health insurance.

Here, we report a multicenter study of 10 patients with refractory pemphigus and pemphigoid treated with a single cycle of RTX. This is the first prospective study of RTX therapy for autoimmune blistering diseases in Japan.

METHODS

This study was approved by the institutional review board of Keio University (no. 20140238), Hokkaido University, Okayama University and Kurume University, and registered in the University Hospital Medical Information Network (no. 000015451).

Patients

Four institutes in Japan (Keio University, Hokkaido University, Okayama University and Kurume University) participated in this study. The study was designed as a single-arm, prospective, open-label, interventional trial and was approved by the institutional review boards of each institute. In all patients, the diagnosis of pemphigus vulgaris (PV), pemphigus foliaceus (PF) or bullous pemphigoid (BP) was confirmed by clinical, histopathological and immunological findings in direct immunofluorescence and serological analysis. Written informed consent was obtained from each patient.

The inclusion criteria were as follows: group 1, insufficient response to initial treatment with prednisolone (PSL) 1 mg/kg per day; and group 2, relapse of disease during the period when PSL doses were decreased to 10 mg/day (definition of remission) or relapse with PSL doses greater than 10 mg/day (Fig. 1). Lesions in each patient were assessed based on Pemphigus Disease Area Index (PDAI), a clinical severity score for pemphigus.[11] We excluded patients with consolidation therapy, dose increases of corticosteroid or immunosuppressive agents within 2 weeks, administration of i.v. high-dose Ig within 2 weeks or plasmapheresis within 4 weeks. For safety reasons, our exclusion criteria also included the following: pregnancy; past history of allergic reaction against chimeric human–mouse IgG monoclonal antibody; severe organ disorder (such as chronic obstructive pulmonary disease, asthma, heart disease and hypertension); active or chronic infection; deep-seated infection (such as abscess, fasciitis and osteomyelitis) within 1 year; past history of malignant tumor, surgery within 4 weeks; and administration of antibiotics within 8 weeks.

Treatment

Figure 1 depicts the study protocol. Patients were treated with one cycle of four weekly infusions of RTX at a dose of 375 mg/m^2 of body surface area, which is the standard protocol for B-cell lymphoma. PSL was administrated using dose regimens of 0.5 or 1.0 mg/kg per day, based on the PDAI at study initiation (Fig. 1). Immunosuppressive agents were continued unless a dose increase was necessary. PSL doses were reduced in all patients following the predefined schedule to ensure that PSL doses would reach 10 mg/day between 21 and 34 weeks. Patients were clinically evaluated every month during the first year of follow up and every other month during their second year on the RTX regimen.

End-points

The primary end-points were the number and the rate of severe adverse events (grade 3–5) and the number and rate of complete remission (CR) at 40 weeks following the first RTX infusion. CR was defined as the absence of new or established lesions for at least 2 months while the patient was receiving minimal therapy (PSL $\leq$10 mg/day and/or minimal adjuvant therapy).[12]

The secondary end-points were efficacy assessed by PDAI, titers of autoantibodies and all adverse events. In this study, we defined "clinical improvement" as a reduction in PDAI on the therapy with lower PSL doses compared with before initiation of RTX treatment. All adverse events were recorded according to version 4.0 of the Common Terminology Criteria for Adverse Events.

Immunological evaluation

The titers of IgG autoantibodies against Dsg1 for PF, Dsg1 and Dsg3 for PV, and BP180 for BP were measured using enzyme-linked immunoassay (ELISA; Medical Biological Laboratories, Nagoya, Japan). The phenotype of peripheral blood mononuclear cells was determined by flow cytometry with antibodies against CD3 (T cells) and CD19 (B cells) on day 0, 29, 50, 162 and 274. Human anti-chimeric antibodies (HACA), known to be antibodies against RTX, were examined on day 0, 29, 162 and 274.

Statistical analysis

This trial was designed for a two-sided hypothesis and *P*-values of less than 0.05 indicated statistical significance. The sample size calculation was based on CR ratios at 40 weeks. Previous studies showed that the total CR ratio was 58–86% using different study designs and definitions of CR.[13–16] We set the threshold objective response rate to 50% and expected the objective response rate to be 80%. To detect clinically significant differences in CR ratios between with/without rituximab with 80% power, 20 patients were needed.

RESULTS

Patient characteristics

Ten patients (five men and five women; three PV, six PF and one BP) as group 2 and no patient as group 1 were enrolled in this study. The initial design of this trial included 20 patients,

but only 10 patients were enrolled from 2010 to 2013, and enrollment was terminated after this period. The clinical characteristics of patients are shown in Table 1. Their median age was 49 years (range, 32–73 years). The duration of disease before the study ranged 7–118 months (median, 22 months). There were no reductions in the dose of PSL to 10 mg/day due to relapse during the course of treatment. At baseline, eight of 10 patients were taking immunosuppressive agents (one for mycophenolate mofetil, five for azathioprine and two for cyclosporin) in addition to corticosteroids. The median PDAI was 15 (range, 6–24) and the median ELISA titer index for autoantibodies against Dsg and BP180 was 199 (range, 42–625).

Response to rituximab

Following 40 weeks of rituximab infusions on minimal therapy, five of 10 patients (50%; 95% confidence interval, 19–81%) achieved CR, including two of four patients with PV and three of five patients with PF (Table 1). In the five patients who achieved CR, PDAI decreased to 0 in 4–24 weeks (median, 7 weeks) and autoantibody titers decreased to approximately 25% from baseline in 6 weeks. No new skin lesions and increases in autoantibody titers were observed after CR was achieved.

The other five patients (two PV, two PF and one BP) did not achieve CR, but four (excluding the BP case) showed clinical improvement in PDAI and decreases in autoantibody titers, resulting from reduced doses of PSL. The PDAI of two patients decreased to 0 at 42 days (PSL 45 mg/day, patient 7) and 133 days (PSL 15 mg/day, patient 4), but new lesions emerged and PDAI increased to 1 and 4, respectively, when PSL was reduced to 10 mg/day (Fig. 2). Decreases in PDAI were observed in the other three patients with PV, PF and BP, but lesions remained throughout the trial. In four out of five patients with clinical improvement, autoantibody titers

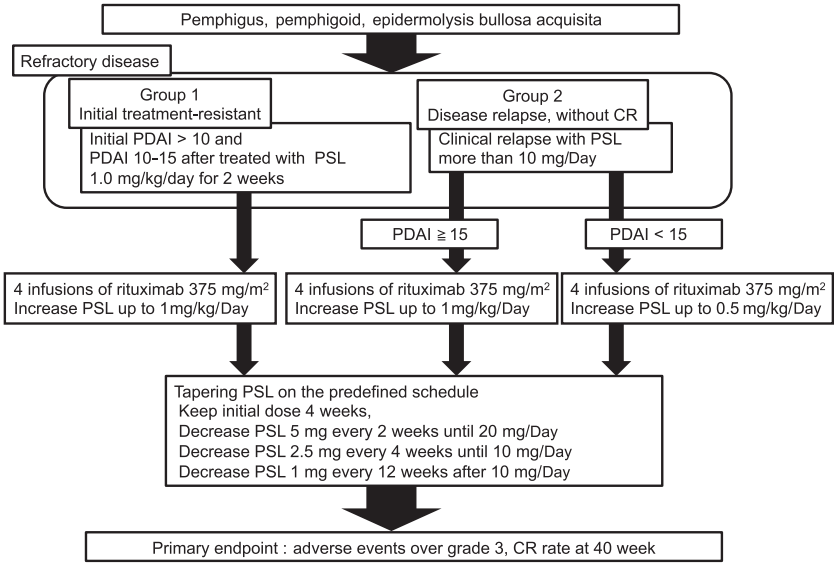

Figure 1. Study protocol. CR, complete remission on therapy; PDAI, Pemphigus Disease Area Index; PSL, prednisolone.

Table 1. Characteristics of patients treated with rituximab

Patients	Diagnosis	Sex	Age (years)	Disease duration from the onset (months)	PSL dose at baseline (mg/day)	Immunosuppressive agents	PDAI at baseline	Antibody titer at baseline	Response to RTX	SAE
1	PV	M	47	118	20	MMF	8	397.7	CI	–
2	PV	M	43	9	14	AZA	15	182.4	CR	–
3	PV	F	60	8	30	–	17	96.9	CI	–
4	PF	M	58	9	12.5	AZA	8	79	CR	SS
5	PF	F	32	17	10	AZA	21	42	CR	–
6	PF	F	40	36	15	AZA	13	301.3	CI	–
7	PF	M	48	27	20	CyA	24	215.5	CI	–
8	PF	F	64	33	14	–	6	102.01	CR	–
9	PF	M	73	12	15	AZA	14	625.8	CR	PCP
10	BP	F	49	27	15	CyA	22	592	PO	–

AZA, azathioprine; BP, bullous pemphigoid; CI, clinical improvement; CR, complete remission on minimal therapy; CyA, cyclosporin A; MMF, mycophenolate mofetil; PDAI, Pemphigus Disease Area Index; PF, pemphigus foliaceus; PO, protocol off; PCP, *Pneumocystis carinii* pneumonia; PV, pemphigus vulgaris; RTX, rituximab; SAE, serious adverse event; SS, septic shock.

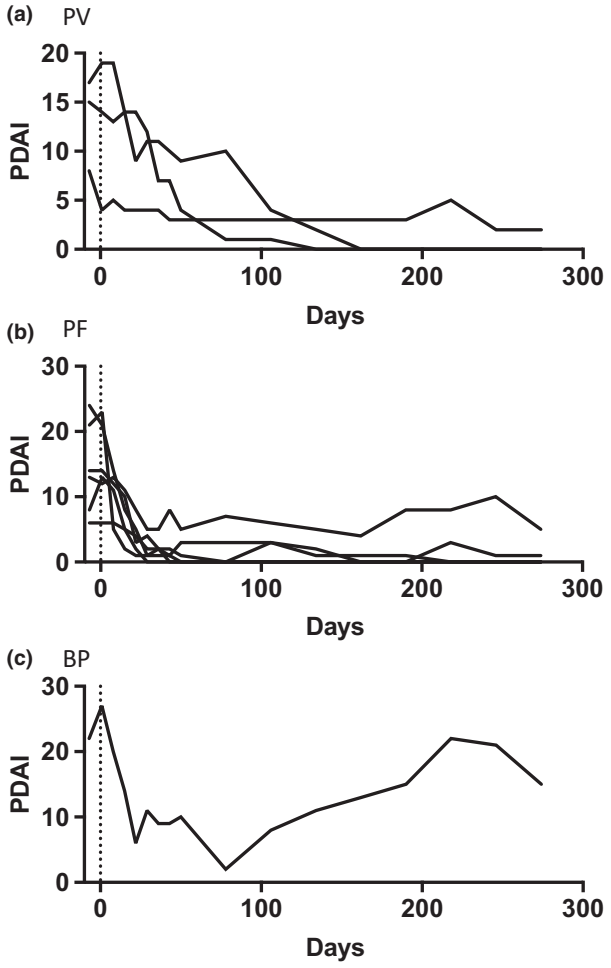

Figure 2. Changes in PDAI, the clinical severity score, of patients treated with rituximab. All patients improved and five of 10 patients achieved complete remission with minimal therapy. (a) patients with PV. (b) patients with PV. (c) patient with BP. PV, pemphigus vulgaris; PF, pemphigus foliaceus; BP, bullous pemphigoid; PDAI, Pemphigus Disease Area Index.

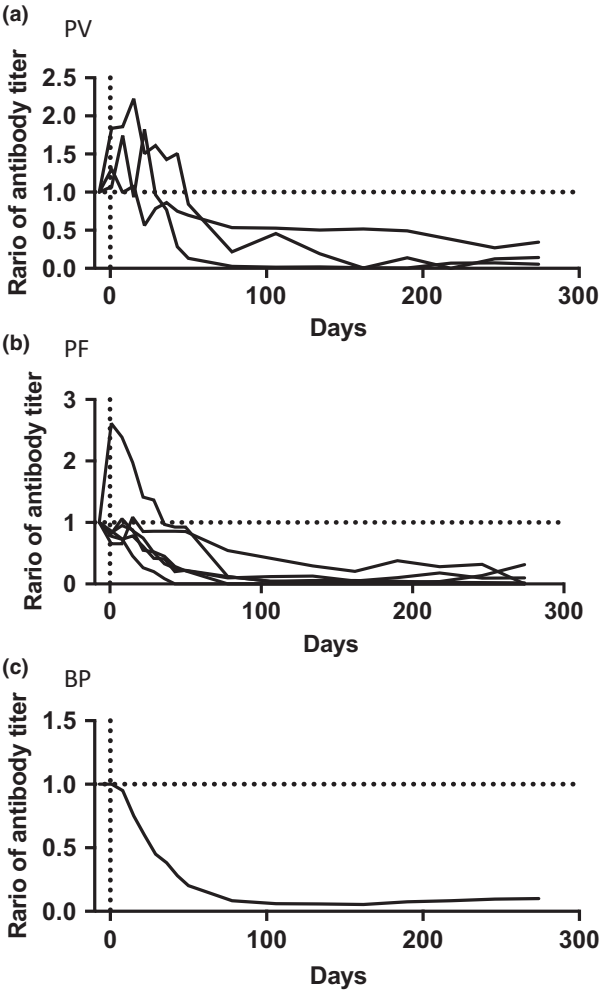

Figure 3. Changes in ratio of autoantibodies from baseline (enrollment) of patients treated with rituximab. All patients demonstrated improvement. (a) patients with PV. (b) patients with PV. (c) patient with BP. PV, pemphigus vulgaris; PF, pemphigus foliaceus; BP, bullous pemphigoid.

remained above 60% from baseline at 6 weeks, and remained above the titer levels of patients with CR throughout the trial.

One BP patient (patient 7) was taken off the protocol at 36 weeks due to use of roxithromycin (macrolide antibiotic), which was added as additional treatment for BP. The results are presented in Table 1, and the changes in PDAI and autoantibody titers are shown in Figures 2 and 3.

Adverse events

A total of 58 adverse events were reported including 30 infections (bacterial, herpes and fungal), eight gastrointestinal disorders and four abnormal laboratory results (two cases of hypogamma-globulinemia, one case of hypergamma-glutamyltransferase and one case of high low-density lipoprotein level). All patients had minor and transient adverse effects, but no infusion reactions were observed. Six adverse events were considered greater than grade 3 and two serious adverse events (SAE) were recorded, including a case of *Pneumocystis carinii* pneumonia (PCP) and a case of joint infection and septic shock (Table 2). Although patients with SAE needed to be hospitalized for treatment, all infections were cured with appropriate therapy. One patient with hypoglobulinemia (patient 4) required sustained and constant Ig replacement treatments until the end of the trial.

Immunological outcome

The number of CD19-positive peripheral blood B lymphocytes was evaluated in eight patients. In all patients, it decreased to 0.3–9 counts/μL at day 29 from 0.2 to 646 counts/μL and remained at this level throughout the trial (Fig. 4). In contrast, the number of CD3-positive peripheral blood T lymphocytes in the same patients did not change during the trial (data not

Table 2. Adverse events of grade 3 or greater

Patients	Event	Onset time (weeks)	Maximum grade	SAE	Outcome
4	Hypogamma-globulinemia	6	3	–	Continued
4	Joint infection/septic shock	2	4	+	Remission
5	Hypergamma-glutamyltransferase	5	3	–	Remission
6	Dental caries	16	3	–	Cured
9	Skin infection (furuncle)	12	3	–	Remission
9	Lung infection (PCP)	24	3	+	Remission

Overall, four of 5 events were in the infection category. PCP, *Pneumocystis carinii* pneumonia; SAE, serious adverse event.

shown). HACA were not detected in any of the patients throughout the entire course of the study.

DISCUSSION

This was the first study to demonstrate the efficacy of RTX for refractory autoimmune bullous diseases in Japan. At the primary end-point, five of 10 patients (50%) achieved CR with the therapy. This suggests that RTX contributed to the clinical and serological improvements as well as reductions in corticosteroids, because reduction in the dose of PSL to 10 mg/day with effective control of disease had not been achieved in these patients. In fact, the ratio of CR in our study (50%) was lower than that in previous studies (86%) reporting RTX efficacy in pemphigus (Table 3).[13–16] This may be due to our strict definition and accurate determination of CR based on PDAI, a clinically validated measure used in this study. In order to increase objectivity, we adopted a previously proposed definition of CR: the absence of new or established lesions for at least 2 months while the patient is receiving minimal therapy (PSL ≤10 mg/day and/or minimal adjuvant therapy).[12] A PDAI of 0 was considered CR and the use of this index could allow the evaluation of clinical symptoms to be more accurate and quantitative as it provides fewer opportunities to overlook lesions by scoring for each body part (e.g. ears, nose, chest, arms).[11] Our study demonstrated the utility of PDAI as an objective evaluation of clinical symptoms. Although we used PDAI in both pemphigus and pemphigoid in this study, the Bullous Pemphigoid Disease Area Index (BPDAI) was recently developed as an analogous clinical score for BP. Thus, PDAI and BPDAI should be used in future clinical trials for autoimmune bullous diseases. Additionally, as one BP patient was taken off the protocol in this trial, BP is thought to be more difficult to treat with rituximab, and pemphigus and pemphigoid should be separated in the future clinical trials.

To correctly estimate RTX efficacy for the treatment of autoimmune bullous diseases, we needed to consider the combination use of corticosteroids and relapse in RTX treatment. Because the dose of PSL was increased to 0.5 or 1.0 mg/kg per day at the same point of administration of RTX, it was impossible to exclude the possibility that increased PSL affected the results in this study. We incorporated this PSL

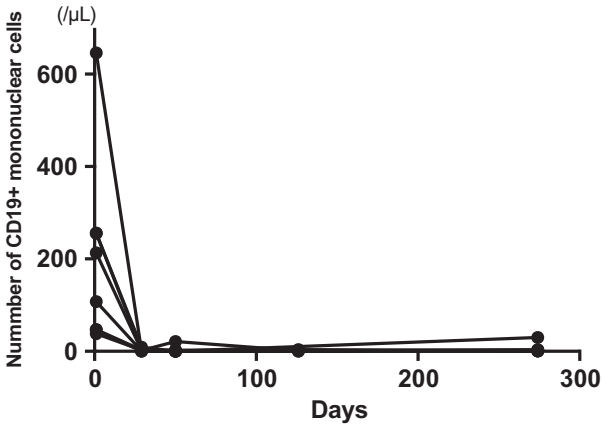

Figure 4. Changes in CD19-positive peripheral blood B lymphocytes. Levels decreased below 10 counts/μL at day 29 and were maintained throughout the study period.

increase because RTX may require several weeks to exert its effects and, thus, patients would need support from corticosteroids to recover from acute erosion and blister formations. The results of this study suggest that RTX could be effective with increasing corticosteroid doses. Protocols for future clinical trials with RTX should circumspectly consider not increasing corticosteroids. The ratio of relapse following RTX treatment was not carefully observed in this study. Previous studies have reported the ratio of relapse after achieved transient remission to be 27–48% following 6 months to several years from the first infusion of RTX.[13–16] One of our PF patients relapsed 11 months after RTX administration, but the other cases remained in CR on the therapy (data not shown). The observation period of this study was 40 weeks to evaluate the efficacy and safety of RTX over a relatively short time, but we need to know if the length of follow up would affect the rate of CR and relapse. Previous reports have suggested that four cycles of RTX treatment would prevent relapse in most cases of pemphigus.[16] Therefore, determining the most effective regimen of RTX in autoimmune bullous diseases is an important issue that requires further investigation.

With regards to safety, all adverse events were adequately managed, with the exception of two SAE (PCP and septic

Table 3. Comparison between previous studies and the present study

	Country	Cases	No. of CR	Ratio of CR	Ratio of relapse	SAE
Joly *et al.*[13]	France	21	18	86%	43%	2
Muller *et al.*[14]	Germany	22	– (CI: 22)	– (CI: 100%)	27%	0
Cianchini *et al.*[15]	Italy	42	36	86%	48%	0
Lunardon *et al.*[16]	USA	31	18	58%	44%	2
This trial	Japan	10	5	50%	–	2

The definitions of "CR" were different among each study. All studies suggested the efficacy of rituximab treatment. CI, clinical improvement; CR, complete remission; SAE, serious adverse event.

shock due to infectious arthritis), both of which were categorized as infections. Possible inducible factors of these SAE include frequent joint injections for omalgia in patient 4 and discontinuation of trimethoprim-sulfamethoxazole, a preventive agent for PCP, due to renal dysfunction in patient 9. These results suggest that although it was not possible to determine whether these SAE were caused by RTX, continued use of preventive agents for PCP and avoiding unnecessary surgical interventions in patients on RTX would be highly recommended. To reduce the risk of infectious adverse events, stopping combination of the immunosuppressive agents (e.g. cyclosporin A, mycophenolate mofetil and azathioprine) during the RTX treatment may be an important issue in further investigation.

CD20, the target of RTX, is expressed on the surface of B cells, beginning from the pre-B-lymphocyte stage through maturity to plasmacytoid B lymphocyte. The short-term efficacy of RTX depends on B-cell depletion and decrease of autoantibodies. We observed that the decreased number of CD19 B lymphocytes remained at the same level in all study patients until 10 months following initial RTX administration. Previous studies have suggested that reconstruction and repopulation of the B-lymphocyte repertoire, induced by RTX treatment, can contribute to long-term efficacy and, thus, patients with refractory diseases may be effectively treated with multiple cycles of RTX.[13,17,18]

Our study suggests that RTX is a promising option for refractory autoimmune bullous diseases, provided that risk of serious infection as an anticipated adverse event can be managed. Larger and long-term follow-up studies are necessary to precisely assess the efficacy and safety of RTX treatment in Japanese patients with autoimmune bullous diseases.

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CONFLICT OF INTEREST: Rituximab used in this trial was provided, free of charge, by Zenyaku Kogyo (Tokyo, Japan). The company did not have any other participation and did not provide other funding for the trial.

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