complications (such as thrombosis). Oral warfarin potassium was discontinued 6 weeks after delivery.

Second pregnancy

The patient had a second spontaneous pregnancy at the age of 33. Laboratory data at 9 weeks of gestation were as follows: Hb levels 6.2 g/dl, WBC count 4.20×10^9/l, platelet count 153×10^9/l, LDH levels 1130 IU/l, D-dimer levels 0.84 μg/ml, CRP levels 0.07 mg/dl, BUN levels 10 mg/dl and creatinine levels 0.41 mg/dl. Similar to her first pregnancy, no thrombosis was detected by ultrasonography. She received anticoagulant therapy starting at 11 weeks of gestation. During her second pregnancy, the patient was put on a higher dose of UFH than during her first pregnancy for two reasons. First, a larger population of CD55(−) CD59(−) erythrocytes was seen during her second pregnancy (43.7 vs. 36.8% during the second and first pregnancies, respectively). Second, her D-dimer levels were unstable during her first pregnancy. Subcutaneous administration of UFH (7500 units twice daily) was started. The subcutaneous UFH dose was controlled with the aim of achieving an aPTT of 50–60 s (Fig. 1). A total of 24 units of washed RBCs were administered before the delivery. Laboratory data immediately before delivery were as follows: Hb levels 9.3 g/dl, WBC count 5.36×10^9/l, platelet count 93×10^9/l, LDH levels 1083 IU/l, D-dimer levels 1.34 μg/ml, BUN levels 9 mg/dl and creatinine levels 0.56 mg/dl. At 37 weeks and 5 days of gestation, labour was induced, and a female infant was delivered vaginally without complications. The infant weighted 2824 g (+0.2 SD) and had Apgar scores of 8 at 1 min and 9 at 5 min. The mother and her baby were discharged on day 20 postpartum without complications. In contrast to the patient's first pregnancy, her D-dimer and LDH levels were stable throughout this pregnancy. Similar to the treatment strategy used during her first pregnancy, UFH was switched to warfarin potassium, which was discontinued 12 weeks postpartum.

Discussion

We report the case of a woman with PNH who was treated with anticoagulant therapy and whose two pregnancies resulted in two successful vaginal deliveries without significant complications. The clinical courses of the patient's pregnancies suggest that UFH therapy with a therapeutic dose might contribute to a greater decrease in not only thrombogenesis but also haemolysis in pregnant patients with PNH.

We performed anticoagulation using a prophylactic dose of UFH during the patient's first pregnancy and a therapeutic dose during the second pregnancy. The aPTT was 30–40 s during the first pregnancy and 40–60 s during the second pregnancy (Fig. 1). Although the PNH clone size slightly increased during the patient's second pregnancy, her LDH levels before the first and second pregnancies

were similar, indicating that the PNH status before the patient's second pregnancy was stable or slightly advanced compared with her first pregnancy. On the basis of the lower D-dimer levels during her second pregnancy (when compared with those during her first pregnancy), the patient's thrombotic risk might have been lower during her second pregnancy (Fig. 2a). Furthermore, the PNH condition might have been more stable during the second pregnancy than the first pregnancy, as the patient's LDH (haemolytic marker) levels were lower (Fig. 2b). Although the cause of the lower LDH levels is not clear, it is possible that heparin treatment helped suppress haemolysis (Fig. 2b). The suppressive effect of heparin on complement activation has been reported previously [13–21]. Heparin inhibits the formation of the classic and alternative pathway C3 convertases [13–16] and suppresses complement factor H, which is the major regulator of the central complement protein C3b in the alternative pathway of complement activation [17]. Furthermore, heparin interferes with the assembly of the C5b-9 complex [18]. In PNH, the absence of cell surface complement regulatory proteins leads to complement-mediated haemolysis. *In vitro*, heparin inhibits the complement-mediated lysis of PNH RBCs by suppressing the insertion of C5b-7 in RBCs [19]. Therefore, heparin might help suppress haemolysis via complement inactivation in pregnant patients with PNH. However, a clear limitation of this case report is that a direct association between the heparin dose and the clinical benefit could not be established.

In summary, we successfully treated a pregnant patient with PNH over the course of her pregnancies, which both resulted in normal vaginal deliveries. No lethal complications, such as thrombosis during the perinatal period, occurred. This case suggests that UFH therapy using a therapeutic dose (as opposed to a prophylactic dose) might contribute to a significant decrease in haemolysis in pregnant patients with PNH. Currently, no standardized criteria for determining the appropriate dose for anticoagulant therapy in pregnant patients with PNH exist. PNH patients treated with the anti-C5 antibody 'eculizumab' therapy have less symptoms and disease complications as well as longer survival [22]. Eculizumab improves haemolysis, transfusion requirements and smooth muscle dystonia and reduces the incidence of thrombotic events [22–25]. Successful deliveries without serious complications were achieved in several pregnant women with PNH who were treated with eculizumab therapy [26–31]. It has also been suggested that eculizumab does not cross the placenta [28,32] and does not affect complement system activity in the newborn [31]. We therefore expect that eculizumab therapy will be used to treat pregnant women with PNH. However, it will take time to establish the efficacy and safety of eculizumab, as no clinical trials during pregnancy have been conducted yet.

In conclusion, we believe that our data will be important for the use of anticoagulant therapy in pregnant patients with PNH.

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

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ORIGINAL ARTICLE

Efficacy and safety of rituximab in Japanese patients with relapsed chronic immune thrombocytopenia refractory to conventional therapy

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Abstract Primary immune thrombocytopenia (ITP) is an autoimmune disease mediated by the production of auto-antibody against platelets. Rituximab, an anti-CD20 antibody, is reported to be useful for treatment of ITP. In Japan, however, robust evidence on this treatment has not been accumulated. Hence, we conducted this open-label phase III clinical trial to confirm the efficacy and safety of rituximab, administered at 375 mg/m^2 once per week at weekly intervals for 4 consecutive weeks in Japanese patients with chronic ITP, who had relapsed and were refractory to conventional therapy. The primary endpoint was defined as the percentage of patients with a platelet count above 50×10^9/L at week 24 after the first dose of rituximab, which was 30.8 % of 26 patients (95 % confidence interval 14.3–51.8 %). Although the lower confidence limit of primary endpoint failed to meet the pre-specified threshold of 20 %, the clinical efficacy of rituximab is substantial in consideration of the 2 % response rate in the placebo arm in other clinical studies in patients with chronic ITP. We conclude that rituximab is clinically useful and safe in the treatment of Japanese patients with chronic ITP, achieving the goal of maintaining platelet count and reducing risk of bleeding while minimizing treatment-related toxicity.

Keywords Immune thrombocytopenia · Platelets · Rituximab

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Introduction

Primary immune thrombocytopenia (ITP) is a thrombocytopenia-causing autoimmune disease. Approximately 20,000 patients are suffered from this disease in Japan, and about 3000 new cases occur per year [1]. The causes of decrease in the platelet count are known to be destruction of platelets, to which auto-antibody is attached, in the spleen, and defects of proliferation and maturation of megakaryocyte due to the relative shortage of thrombopoietin (TPO). The major clinical symptoms and signs of ITP are petechiae and mucosal hemorrhage. ITP is classified by duration into newly diagnosed (within 3 months of onset), persistent (3–12 months' duration) and chronic (12 months or more in duration). Of ITP with onset in adult age, about 90 % of the patients are chronic and its male-to-female ratio is 1:2. Massive hemorrhage is relatively uncommonly seen in chronic ITP, whereas seen in the other types of ITP.

Patients who show no effects on standard therapy and have platelet count $\leq 30 \times 10^9$/L are diagnosed with refractory ITP, and those are about 10 % of total patients with ITP. Such patients have a 4.2-fold higher risk of death than healthy population [2]. In Japan, nearly half of patients with ITP are infected with *Helicobacter pylori*, and *H. pylori* eradication therapy is effective for restoring the platelet count in 60 % of patients [3].

The first-line treatment of standard therapy is corticosteroid administration. Corticosteroid administration can only be discontinued in about 10–20 % of the patients. For most of the patients, the steroid therapy is continued especially in Japan. Splenectomy is chosen as a second-line treatment for patients who fail to respond or have poor tolerability to corticosteroids. A radical cure can be expected from splenectomy in about 70 % of patients, while for the remaining 30 % of patients this surgical intervention makes to be ineffective. Splenectomy has several concerns such as complications in the perioperative phase (mortality rate at 0.1 % and complication rate at 10 %), postoperative depression of immune functions, and relapses in about 20 % of patients [4]. Since it is difficult to predict the efficacy of surgical removal of the spleen in patients with ITP, both patients and physicians tend to avoid splenectomy in Japan, as well as in Europe and the United States. Non-responders to splenectomy die at about 10 % from serious hemorrhage such as cerebral hemorrhage. Therefore, the goal of ITP treatment is to increase the platelet count to $\geq 30 \times 10^9$/L to avoid such fatal bleeding [5, 6].

Immunosuppressants such as azathioprine and cyclosporine, and anticancer agents such as cyclophosphamide and vincristine, in off-label use, are empirically prescribed for patients who are non-responding or ineligible for splenectomy. With these drugs, response rates are as low as about 30 %, and adverse events are rather frequently observed. Recently, thrombopoietin receptor agonists (romiplostim and eltrombopag) have been approved for refractory ITP and showed efficacy in nearly 60 % of patients. However, some concerns arise from these agonists, including offset of drug effect back to baseline level in about 2 weeks after discontinuation of the medication, a high cost of drug expense as high as 2–3 million yen per year, a high incidence of thrombotic complications in some of patients with a certain background, and disease progression to myelofibrosis or acute leukemia after long-term treatment.

Rituximab is a chimeric monoclonal antibody against the CD20 antigen and prepared by recombinant DNA technology. Rituximab was approved in Japan for the treatment of B cell non-Hodgkin's lymphoma, microscopic polyangiitis (MPA), and granulomatous polyangiitis (GPA; Wegener granulomatosis). In addition to these indications, this drug was approved for the treatment of chronic lymphoid leukemia and rheumatoid arthritis in the United States and Europe. Rituximab specifically eliminates CD20-positive B lymphocytes; therefore, its efficacy for various disorders relevant to B cell abnormalities is anticipated [7, 8]. Recent studies have demonstrated that B cells are involved in the onset and maintenance of autoimmune diseases, and the efficacy of treatment with rituximab has been reported in autoimmune disorders such as systemic lupus erythematosus (SLE), multiple sclerosis, nephrotic syndrome, and thrombotic thrombocytopenic purpura (TTP). Outside of Japan, rituximab is extensively prescribed and accepted as the second-line treatment for refractory ITP [5, 9–11]. A systematic review on the efficacy and safety of rituximab in approximately 300 patients with ITP by Arnold et al. [11] showed that the response rate was 62.5 %, and the time to therapeutic response was 5.5 weeks. A phase II clinical trial of rituximab in 60 patients with refractory ITP in France showed that the response rate was 40 % after 1 year of the treatment [12]. As seen in a number of reports outside Japan, the efficacy and safety of rituximab in patients with refractory ITP have been extensively evaluated and the effectiveness of the drug has been demonstrated.

In Japan, there are several case reports to indicate the efficacy of rituximab in the treatment of refractory ITP, but robust evidence on this treatment has not yet been accumulated such as from clinical studies. Rituximab is then prescribed off-label for rescuing patients with refractory ITP, but the medication cost of rituximab is not reimbursed under the Japanese National Health Insurance program.

Hence, we conducted this open-label phase III clinical trial to confirm the efficacy and safety of rituximab, administered at 375 mg/m^2 (body surface area) once a week, at weekly intervals for 4 consecutive weeks in Japanese patients with chronic refractory ITP. This study was implemented in accordance with the International

Consensus Guidelines for Diagnosis and Treatment of ITP [10], and the Japanese Guidelines for Treatment of ITP. This study was registered with the Japan Medical Association Center for Clinical Trials (JMACTR; CTR Number: JMA-IIA00070, https://dbcentre3.jmacct.med.or.jp/jmactr/default.aspx?JMACCTID=JMA-IIA00070).

Materials and methods

Patient population

Patients included in this study were: Japanese, aged ≥20 years, and diagnosed with chronic refractory ITP at least 12 months before the enrollment of this study. The definition of the term *refractory* in this study was as follows: platelet counts ≤30 × 10^9/L (measured at weeks 4 and 2 before enrollment), ineffective or intolerable for steroids, ineffective or judged as inappropriate by investigators for splenectomy, and ineffective, intolerable or judged as inappropriate by investigators for thrombopoietin receptor agonists.

Study design

This study was an open-label multicenter phase III clinical trial conducted between October 2011 and July 2013 in ten clinical institutions in Japan. The study consisted of screening (4 weeks), treatment (4 weeks), and follow-up periods (20 weeks).

Rituximab was administered at 375 mg/m² in once a week consecutively for 4 weeks (weeks 0, 1, 2, and 3). To prevent infusion reactions associated with rituximab infusion, patients received pre-medications of oral antipyretic–analgesics, oral antihistamines, and intravenous hydrocortisone at 30 min before each administration of rituximab.

During the study period, the following concomitants or therapies were prohibited: immunoglobulin preparations, drugs which stimulate platelet production, splenectomy, *H. pylori* eradication therapy, hematopoietic factors, antineoplastics and platelet transfusion.

Efficacy and safety analysis

The primary efficacy endpoint was a response rate: the percentage of patient with the platelet count ≥50 × 10^9/L at 24 weeks after the first administration of the study drug.

The major secondary efficacy endpoints included the percentage of patients with the platelet count ≥100 × 10^9/L and who did not have bleeding at week 24, the percentage of patients with the platelet count ≥30 × 10^9/L and ≥two-fold higher than the baseline value and who did not have bleeding at week 24, and the improvement rate of bleeding symptoms [World Health Organization (WHO) bleeding scale]. In addition, the changes of peripheral blood B cells (CD19 and CD20) and T cells (CD3), and changes of serum IgG, IgM, and IgA levels were evaluated as exploratory endpoints. Safety parameters (adverse events and clinical laboratory data) were also assessed.

Statistical considerations

Sample size and its rationale were pre-specified in the study protocol. Response rate in the primary endpoint was assumed to be 50 % based on results of clinical studies [11, 12]. Twenty-four patients were needed to have ≥80 % power to show that the lower limit of 95 % confidence interval (CI) for the response rate is greater than a threshold (20 %). The threshold was determined conservatively taking account of a response rate, 2 % (1/42 patients, 95 % CI 0–12.6 %) in placebo group in the phase III clinical trial of romiplostim in patients with refractory ITP [13]. All enrolled patients were included in the primary efficacy analysis population (full analysis set, FAS). Demographic factors and baseline characteristics were summarized with mean ± standard deviation (SD) or median (interquartile range, IQR) depending on distributions. Exact 95 % CIs for proportions were calculated with the Clopper–Pearson method. Paired proportions were compared with the exact McNemar's test. For continuous valuables, values at each time point were compared with baseline values by signed rank sum test. Significance level was a two-sided 5 % for all tests. All data were analyzed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Ethical considerations

This clinical trial was conducted in compliance with the ethical principles of the Declaration of Helsinki, the Japanese Guidelines for Good Clinical Practice, and other relevant regulatory requirements. The investigator or co-investigator gave a full explanation of the clinical trial to patients prior to participation in the study and, upon confirming that the patients gained a good understanding of the nature of the study, obtained written informed consent for voluntary participation in the study. Prior to conduct of this clinical trial, the institutional review board (IRB) of each participating medical facility reviewed the ethical, scientific, and medical propriety of this clinical trial and approved this study.

Table 1 Patient demography and disease characteristics

	Analysis set: $n = 26$
Sex (females)[a]	23 (88.5 %)
Age (years)[b]	39.7 ± 13.0 (23, 69)
Body weight (kg)[b]	56.5 ± 9.7 (37.9, 73.1)
Duration of ITP (years)[c]	5.9 (1.9, 11.2)
Hemorrhagic symptoms (WHO bleeding scale)	
Grade 0[a]	11 (42.3 %)
Grade 1[a]	14 (53.8 %)
Grade 2[a]	1 (3.8 %)
Baseline platelet count (10^9/L)[c]	22 (17, 24)
Baseline CD3 cells (/µL)[c]	1035.5 (798, 1588)
Baseline CD19 cells (/µL)[c]	97 (63, 147)
Baseline CD20 cells (/µL)[c]	91.5 (59, 145)
Had splenectomy (yes)[a]	4 (15.4 %)
Had *H. pylori* eradication (yes)[a]	9 (34.6 %)
Had complications (yes)[a]	21 (80.8 %)
Previous therapy for ITP	
Had corticosteroids (yes)[a]	18 (69.2 %)
Had high-dose immunoglobulin therapy (yes)[a]	10 (38.5 %)
Had thrombopoietin receptor agonists (yes)[a]	7 (26.9 %)
Number of previous therapies for ITP[c]	2 (1, 3)

[a] Number of patients (%)

[b] Mean ± standard deviation (range)

[c] Median (25 % point, 75 % point)

Results

Patient characteristics

Written informed consent was obtained from 49 patients in this clinical trial. Of them, 26 patients who met the inclusion criteria were enrolled. All the 26 patients completed a total of four doses of rituximab infusion and were included in the FAS. None of the patients discontinued the study treatment. The following measured values of the platelet count were partially excluded from the FAS: the platelet counts of one patient at week 4 and week 0 when platelet aggregation was seen in the sample of the patient, and the platelet count of other patient at the time when the platelet count was increased due to emergency treatment (high-dose immunoglobulin therapy plus platelet transfusion) at week 2.

Although protocol deviations were seen in 12 patients, none were relevant to patient eligibility or discontinuation criteria. None of these patients were excluded from the efficacy and/or safety analysis, as the deviations in this study were examined at the case-conference meeting and judged not to significantly influence on the evaluation of the study.

Most of the patients with refractory ITP enrolled in this study were female (88.5 %). The mean age was 39.7 ± 13.0 years (Table 1). The median duration of ITP was 5.9 years (IQR 1.9–11.2), and Grade 0, 1, and 2 hemorrhagic symptoms in severity at baseline were 42.3, 53.8, and 3.8 % of patients, respectively. The median platelet count at baseline was 22×10^9/L (IQR 17–24). Of the enrolled patients, 15.4 % had previously received splenectomy and 34.6 % underwent *H. pylori* eradication. The percentages of patients who had previously received corticosteroids, high-dose immunoglobulin therapy, and thrombopoietin receptor agonists, as prior therapy for ITP, were 69.2, 38.5, and 26.9 %, respectively.

Primary efficacy endpoint

The percentage of patients who had achieved the platelet count $\geq 50 \times 10^9$/L at week 24 was 30.8 % (8/26 patients). The 95 % CI of the response rate was 14.3–51.8 %, and the lower limit of CI did not exceed the threshold of 20 %. However, in comparison with the response rate of 0–2 % in placebo group reported in other clinical studies with a similar target population to this study, it was suggested that the efficacy of rituximab observed in this study is substantial. The number of patients who achieved the platelet count $\geq 50 \times 10^9$/L at each time point for the assessment is shown in Table 2. Box plots of the trajectory of the platelet count over 24 weeks in a subgroup which consists of eight responders is shown in Fig. 1. In these eight responders, mean platelet count reached $\geq 50 \times 10^9$/L at week 4 and continued to increase throughout the follow-up period (Fig. 1).

Secondary efficacy endpoints

The percentage of patients with the platelet count $\geq 100 \times 10^9$/L and who did not have bleeding at week 24 was 15.4 % (4/26 patients; 95 % CI 4.4, 34.9 %). The percentage of patients with the platelet count $\geq 30 \times 10^9$/L and $\geq$twofold higher than the baseline value and who did not have bleeding at week 24 was 26.9 % (7/26 patients; 95 % CI 11.6, 47.8 %).

As for the percent changes of the platelet count, the median platelet count was significantly increased compared with the baseline at every time point from week 1 to week 24 ($P < 0.01$, signed rank sum test; Fig. 2). The median platelet count exceeded $\geq 30 \times 10^9$/L at week 8 and remained stable throughout the follow-up period.

The percentage of patients with Grade ≥ 1 hemorrhage in the WHO bleeding scale was numerically decreased at every time point compared with the baseline. Statistically significant decrease was observed at week 8 compared with the baseline (26.9 vs 57.7 %, $P = 0.0215$; exact

Table 2 Percentages of patients who had achieved the platelet count $\geq 50 \times 10^9$/L at each time point

Time points	n	No. of patients who achieved	%	Two-sided 95 % CI of the percentage
Week 1	26	3	11.5	(2.4, 30.2)
Week 2	25	2	8.0	(1.0, 26.0)
Week 3	26	4	15.4	(4.4, 34.9)
Week 4	26	3	11.5	(2.4, 30.2)
Week 8	26	7	26.9	(11.6, 47.8)
Week 12	26	6	23.1	(9.0, 43.6)
Week 16	26	8	30.8	(14.3, 51.8)
Week 20	26	8	30.8	(14.3, 51.8)
Week 24	26	8	30.8	(14.3, 51.8)
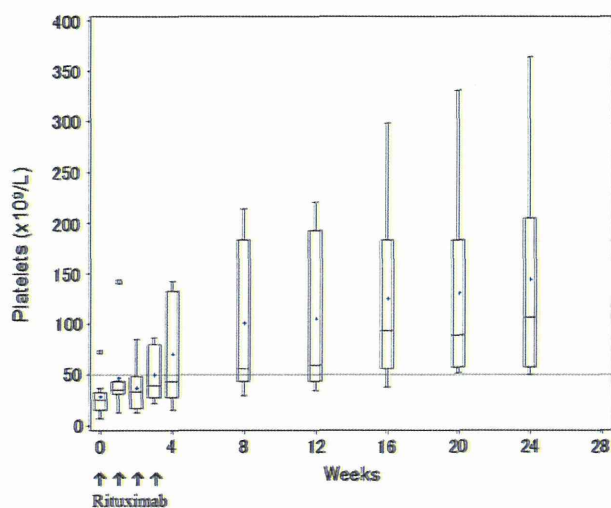
Fig. 1 *Box plots* of platelet counts of the eight patients who met the primary response (platelet count >50 × 10^9/L at week 24). *Central horizontal bold line* is the median: the *lower* and *upper box* limits are the 1st and 3rd quartiles, respectively: and the *whiskers* extended to the most extreme data points, which do not exceed the 1.5 × the interquartile of the box. *Plus symbol* represents the mean value
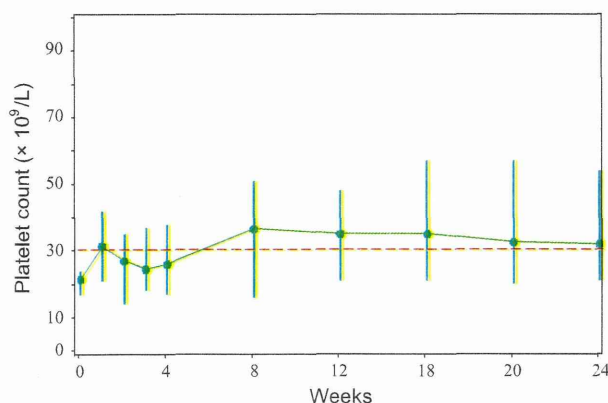
Fig. 2 Time course of median platelet count (FAS). *Black circles* are median values of platelet count. The *lower* and *upper ends* of *vertical lines* are the 1st and 3rd quartiles, respectively

McNemar's test), whereas no significant differences were observed at other time points.

Subgroup analysis

In Table 3, the results of subgroup analyses of the percentage of patients with the platelet count $\geq 50 \times 10^9$/L at week 24 were summarized. Subgroups with higher response rate were patients with duration of ITP shorter than the median duration 5.9 years (46.2 %, 6/13 patients), patients who underwent splenectomy (50.0 %, 2/4 patients), patients who did not have concomitants for ITP at baseline (60.0 %, 3/5 patients), patients who did not previously receive thrombopoietin receptor agonists (36.8 %, 7/19 patients), and patients with previous therapies for ITP less than 3 (41.2 %, 7/17 patients). While factors predictive of response to rituximab have not been consistently identified across studies, shorter duration of ITP was reported to be associated with good response from several studies [14, 15] as we found in this study.

Exploratory efficacy endpoints

Peripheral blood B cells (CD20-positive cells and CD19-positive cells) were significantly decreased at week 2 and subsequent time points compared with the baseline ($P < 0.001$ for both parameters: signed rank sum test). The median absolute B cell count at week 2 was <5 cells/μL and persisted in low during the study. Transient but significant decrease of peripheral blood T cells (CD3-positive cells) was observed at weeks 2, 4, and 12, compared with the baseline ($P < 0.05$, signed rank sum test); however, the median value of absolute cell count of CD3-positive T cells remain >790 cells/μL throughout the study.

Serum IgG levels were significantly increased at week 4 compared with the baseline ($P = 0.023$, signed rank sum test), whereas IgM levels were significantly decreased at weeks 12 and 24, compared with the baseline ($P < 0.001$, signed rank sum test). These changes of serum IgG and IgM, however, were within the normal range. Serum IgA

Table 3 Subgroup analysis of the percentage of patients who had achieved the platelet count $\geq 50 \times 10^9$/L at week 24 after administration of the study drug

Subgroups	n	No. of patients who achieved	%	Two-sided 95 % CI of the percentage
Duration of ITP (median: 5.9 years) (years)				
<5.9	13	6	46.2	(19.2, 74.9)
≥5.9	13	2	15.4	(1.9, 45.4)
Had splenectomy				
No	22	6	27.3	(10.7, 50.2)
Yes	4	2	50.0	(6.8, 93.2)
Had concomitant drugs for ITP at baseline				
None	5	3	60.0	(14.7, 94.7)
Yes	21	5	23.8	(8.2, 47.2)
Baseline platelet count (/L)				
<15 × 10⁹	6	2	33.3	(4.3, 77.7)
≥15 × 10⁹	20	6	30.0	(11.9, 54.3)
Previously received thrombopoietin receptor agonists				
No	19	7	36.8	(16.3, 61.6)
Yes	7	1	14.3	(0.4, 57.9)
Number of previous therapies for ITP				
<3	17	7	41.2	(18.4, 67.1)
≥3	9	1	11.1	(0.3, 48.2)

levels were not significantly changed from the baseline over 24-week study period.

Safety

Three serious adverse events required inpatient hospitalization were reported in three patients: one patient with grade 3 viral infection, one with grade 2 viral infection and one with grade 2 hypermenorrhea. All these three events recovered by supportive treatment and the patients discharged from the hospital in a week. The causal relationship of all the serious adverse events with rituximab was not completely ruled out.

The other adverse drug reactions (ADRs) that occurred in two or more patients were upper respiratory tract infection and headache in three patients each, and diarrhea, abdominal pain, malaise, and cough in two patients each. All these ADRs were grade 1 or 2 in severity.

Infusion related reactions were observed in eight patients and those that occured in two or more patients were fever, oropharyngeal pain, headache, pruritus, urticaria, and hypersensitivity, all of which were grade 1 or 2 in severity. Infusion related reactions were most frequently observed at the initial administration of rituximab (at week 0) among the injection-time points in the 4-dose study drug regimen. None of patients had adverse events led to discontinuation of the study drug, and no deaths were reported in this study.

Discussion

The response rate of the primary efficacy endpoint in this study, the percentage of patients with the platelet count $\geq 50 \times 10^9$/L at week 24 after the first administration of rituximab, was 30.8 % (95 % CI 14.3–51.8 %), and failed to meet the pre-determined statistical criteria of the lower confidence limit of 20 %.

However, the efficacy of rituximab in patients with chronic refractory ITP in this study is substantial when compared with the modest response rate of 2 % (1/42 patients; 95 % CI 0, 12.6 %) in placebo group reported in other clinical studies in patients with refractory ITP [13]. Also, as seen in the subgroup analysis, even heavily treated patients with chronic refractory ITP in this study exhibited moderate efficacy, with the platelet count $\geq 50 \times 10^9$/L at week 24, to rituximab as shown below: 50.0 % (2/4 patients) of patients who underwent splenectomy and 14.3 % (1/7 patients) of patients who previously received thrombopoietin receptor agonists. This trend becomes much clearer in this study when considering clinical benefit to patients who are at risk of fatal bleeding (i.e., the platelet count $\leq 30 \times 10^9$/L). As additional analysis, the percentages of patients with the platelet count $\geq 30 \times 10^9$/L at week 24 after administration of the study drug were evaluated, and rituximab then showed considerably high effectiveness in a total of patients (57.7 %, 15/26 patients) as well as patients previously heavily treated, who underwent splenectomy (75.0 %, 3/4 patients) and who received

thrombopoietin receptor agonists (71.4 %, 5/7 patients). These lines of evidence suggest that rituximab can clinically useful for the treatment of Japanese patients with chronic refractory ITP.

The goal of treatment in ITP is to maintain the platelet count which reduces the risk of bleeding while minimizing treatment-related toxicity. To accomplish this goal, thrombopoietin receptor agonists are recently used. However, thrombocytopenia usually recurring shortly after the drug withdrawal is known as one of the drawbacks of these agonists. Thus, these agents are indefinitely used to maintain the platelet count to minimize bleeding [16]. In the sense, this study showed that rituximab's effect lasted longer after completion of treatment; the platelet counts at all the time points exceeded 30×10^9/L until week 24 after the last dose of the study drug at week 3.

Clinical significance of rituximab in patients with ITP is still being investigated extensively outside Japan to position the therapy at an alternative treatment for ITP prior to splenectomy [15–18]. Although the details of study design was varied from study to study in terms of target population (e.g., newly diagnosed or relapsed ITP), concomitant therapy (e.g., with or without steroids), dosage and administration of rituximab (e.g., 4-weekly 375 mg/m^2 or 2-times 1000 mg 2 weeks apart), and endpoints of efficacy analysis, rituximab commonly showed a clinically substantial efficacy and well tolerability in patients with ITP. Especially, several studies showed a clinically meaningful sustained response to have a chance for sparing splenectomy [12, 18–23], supporting our results in this study. Regarding the safety of rituximab, we observed severe adverse events in three patients. Two had viral infection of unknown etiology and one had hypermenorrhea. All three events were resolved by supportive treatment and all patients were discharged from hospital within a week of admission.

We, thus, conclude from the above-mentioned results that rituximab is clinically useful and involves no particular safety concerns in the treatment of Japanese patients with chronic refractory ITP.

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