Dexamethasone plus rituximab yields higher sustained response rates than dexamethasone monotherapy in adults with primary immune thrombocytopenia

Running Title: Rituximab and dexamethasone in ITP

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ABSTRACT

Previous observational studies suggest that rituximab may be useful in the treatment of primary immune thrombocytopenia (ITP). This randomized trial investigated rituximab efficacy in previously untreated adult ITP patients with a platelet count ≤ 20 x 10⁹/L. One hundred and three patients were randomly assigned to receive 40 mg/day dexamethasone for 4 days with or without 375 mg/m² rituximab weekly for 4 weeks. Patients refractory to dexamethasone alone received salvage therapy with dexamethasone plus rituximab. Sustained response (i.e. platelet count ≥ 50 x 10⁹/L at Month 6 after treatment initiation), evaluable in 101 patients, was higher in patients treated with dexamethasone plus rituximab (n=49) than in those treated with dexamethasone alone (n=52) (63% vs. 36%, P= 0.004, 95% C.I.: [0.079-0.455]). Patients in the experimental arm showed increased incidences of grade 3-4 adverse events (10% vs. 2%, P=0.082, 95% C.I.: [-0.010-0.175]), but incidences of serious adverse events were similar in both arms (6% vs. 2%, P=0.284, 95% C.I.: [-0.035-0.119]). Dexamethasone plus rituximab was an effective salvage therapy in 56% of patients refractory to dexamethasone. The combination of dexamethasone and rituximab improved platelet counts compared to dexamethasone alone. Thus, combination therapy may represent an effective treatment option before splenectomy. This study was registered at http://clinicaltrials.gov as NCT00770562.
INTRODUCTION

Adult primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by increased platelet destruction, impaired megakaryocyte maturation with reduced platelet production and possible hemorrhagic complications. The incidence is nearly 30 new cases per million annually.\(^1\) ITP is a chronic condition presenting most commonly in females and elderly patients, often with an insidious onset and varying severities of thrombocytopenia.

Glucocorticoids, the standard first line of treatment for patients with symptomatic disease,\(^2\) enable the recovery of platelet count in 70-80% of cases; however, in most cases, steroid tapering or withdrawal is followed by a drop in platelet count and the need for additional treatment. Splenectomy still represents the standard salvage therapy, with the caveat that ~40% of patients either do not respond or relapse after surgery,\(^3\) ~10% experience perioperative or delayed infections and some develop surgical complications, though rarely fatal.\(^3\) Recently, romiplostim and eltrombopag, two new thrombopoietin receptor agonists, have shown potent activity in ITP;\(^4,5\) however, these agents do not act on the underlying disease mechanism and therapeutic efficacy is dependent upon continual administration. Recently, the use of high-dose dexamethasone given in a single 4-day course (40 mg/d orally) as first-line treatment for adult ITP patients resulted in an 85% initial response and more importantly, a 42% sustained response (SR) rate (i.e. platelet count > 50 x 10\(^9\)/L six months after initial treatment).\(^6\)

Rituximab (MabThera, Roche) is a chimeric monoclonal antibody\(^7\) highly specific for the CD20 antigen, which is expressed only by mature B-cells. Its administration is accompanied by marked, though transient, B-cell depletion\(^8\) and this effect has been exploited for the treatment of several autoimmune disorders with encouraging results.\(^9-15\) Various uncontrolled reports have described the activity of rituximab administered as a weekly infusion of 375 mg/m\(^2\) for 4 consecutive weeks as a single agent salvage treatment in ITP.\(^16-25\) Despite differences in patient characteristics, results from these studies showed 40% - 70% overall early response rates with 20% - 50% complete response rates, 20% - 40% medium-term relapse rates, nearly 40% SR rates and splenectomy-sparing...
capacity in a substantial number of patients. These studies reported also possible significant toxicities including death (not necessarily attributable to rituximab) in nearly 3% of cases.

The principal mechanism of rituximab action in ITP appears strictly related to B-cell depletion and the consequent inhibition of several B-cell pathologic activities, such as the production of autoantibodies specific for platelet and megakaryocyte glycoproteins (GPIIb/IIIa and Ib/IX), cytokine secretion, and antigen-presenting cell activity. These effects may explain the rapid kinetics of platelet recovery observed in the majority of responders. Recent observations of the reversion of several baseline T-cell abnormalities in ITP patients who responded to rituximab suggest that rituximab may have an indirect regulatory effect which may help explain its long-term therapeutic effect.

To date, no prospective randomized studies evaluating rituximab efficacy in ITP have been performed. We report the results of the first prospective phase III study comparing the efficacy of dexamethasone plus rituximab vs. dexamethasone alone in treatment-naïve adult ITP patients.

METHODS

Study design

This randomized, open-label, phase III, multicenter study began in June 2005; enrollment was stopped in June 2007. The study consisted of two phases: the treatment phase (ML18542 study) lasting from the time of enrollment up to Month 6, and an observational follow-up phase which lasted from Months 6 - 36 (Figure 1).

Patients

Twenty-two Italian academic centers or hospitals participated in this study. The study protocol was approved by the Institutional Review Boards of each participating center and written informed consent was obtained from all patients prior to study entry, in accordance with the Declaration of Helsinki. We enrolled treatment-naïve patients (any prior dose of corticosteroids or other immune-suppressive agents was an exclusion criteria), ≥ 18 years old, with a diagnosis of ITP according to
the guidelines of the American Society of Hematology\textsuperscript{2} and a platelet count of $\leq 20 \times 10^9$/L. No patient stratification was performed. All patients underwent baseline immunophenotypic assessments of peripheral blood lymphocytes (CD3, CD4, CD8, CD19, CD20), serum levels of IgA, IgG, IgM, and HIV, HCV and HBV evaluation.

Exclusion criteria included: positive pregnancy test, congenital or acquired cell or humoral immunological deficit, positive HIV, HBV or HCV tests, history of malignancies within 3 years prior to study entry, concomitant immunosuppressive or cytotoxic treatment, active infection requiring systemic therapy, and non-ITP immune thrombocytopenias. Positive tests for antibodies to nuclear DNA, cardiolipin, or ENA, direct Coomb’s test (DAT), lupus anticoagulant not associated with a specific clinical history or the presence of signs and symptoms of other autoimmune disorders were not criteria for exclusion. Treatment-naïve patients with previously asymptomatic, chronic ITP which subsequently worsened were included in the study.

The study population consisted mainly of patients with newly-diagnosed ITP (85 patients; Table 1) assessed according to recently-published criteria\textsuperscript{32}, but also included treatment-naïve patients with persistent or chronic disease (16 patients; Table 1).

**Treatment**

Patients were randomized 1:1 to receive dexamethasone with or without rituximab. A daily dose of 40 mg oral dexamethasone was administered to both arms for 4 consecutive days (Days 1-4). Patients in the experimental arm received 375 mg/m\textsuperscript{2} intravenous rituximab (regardless of the initial response to dexamethasone) on Days 7, 14, 21 and 28. In the case of lack of response (i.e., platelet count $\leq 20 \times 10^9$/L or bleeding during the first 28 days after the start of treatment) patients in both arms could receive rescue therapy with corticosteroids at the minimum effective dose and/or a single course of 2 g/kg intravenous immunoglobulin (IVIg) over two days according to protocol. When rescue therapy was administered beyond Day 28, this was considered failure of response to study treatment. Salvage therapy with dexamethasone plus rituximab (using the regimen described above) was administered to patients initially assigned to the dexamethasone arm exhibiting platelet
counts ≤ 20 x 10^9/L at any timepoint from Day 28 up to the end of Month 6. The necessity for salvage therapy was a criteria for non-response to the study treatment. Patients in the dexamethasone plus rituximab arm who failed to achieve SR were treated according to the standard practice of the study center.

Assessments and outcome measures during the treatment phase

The primary objective of the study was to compare the rates of SR (defined as platelet count ≥ 50 x 10^9/L at Month 6). We selected platelet count instead of clinical signs of bleeding as the main efficacy parameter in order to eliminate any potential bias in assigning the grade of bleeding. Platelet count was evaluated weekly during the first month and subsequently at bi-weekly intervals until the end of Month 6.

Patients who failed therapy, requiring further treatments in the interval between Day 28 and Month 6, were considered failures. The same efficacy parameters were also adopted to evaluate clinical efficacy in those patients who underwent dexamethasone plus rituximab salvage therapy. In this group, the onset of salvage therapy was considered as Day 1.

The secondary objectives of the study were the following: hematological response (platelet count ≥ 100 x 10^9/L [SR 100] and ≥ 150 x 10^9/L [SR 150]); safety profile (adverse event [AE] incidence up to six months from the beginning of therapy, according to NCI-CTC version 3.0); early response (platelet count ≥ 50 x 10^9/L at Day 28); the efficacy of dexamethasone plus rituximab salvage therapy in patients not responding to dexamethasone monotherapy; the effect of treatment on cellular and humoral immune response, and the identification of clinical and laboratory parameters predictive of SR.

In order to evaluate the levels of B-cell depletion and changes in immunoglobulin levels during the study period, the following analyses were performed at baseline and at monthly intervals: peripheral blood immune-phenotypic analysis of the CD20 lymphoid marker, and serum levels of IgG, IgA and IgM.
Assessments and outcome measures during the observational follow-up phase

In order to monitor the effect of treatment beyond Month 6, patients were monitored at least every 4 months up to the end of Year 3 from the time of enrollment, documenting the occurrence of any delayed side effects, response status and need for further treatment. During this period of observation, treatment efficacy parameters included the duration of response (RD) i.e. the time interval between achievement of SR and relapse, the relapse rate (number of patients who lost SR status) and the need of additional ITP therapy beyond Month 6.

Statistical analysis

Based on the results from Cheng et al., this study was designed for an 80% probability to detect an improvement in SR from 40% of patients treated with dexamethasone and up to 60% of patients treated with dexamethasone plus rituximab, with a one-sided overall significance level of 5%. To account for the evaluation of toxicity, the sample size was adjusted from 154 to an overall size of 198 patients, assuming a 10% dropout rate, to capture > 11% grade 3-5 AEs in the experimental arm in comparison with an expected 2% in the dexamethasone arm. Three interim analyses were performed with every 50 enrolled patients to assess the necessity for early stopping for safety and efficacy reasons. The study would be stopped when a 50% relative difference in SR was demonstrated (α 0.017; Bonferroni adjustment) or if a greater than expected toxicity was observed in the experimental arm (>26%, > 16%, >12.5% at each safety checkpoint, respectively). Results were analyzed according to the intention to treat (ITT) population, which included all randomized patients who received the first dose of dexamethasone. In the cases of missing platelet count for premature discontinuations, the last observation carried forward approach was adopted. Patients who switched to salvage therapy in the dexamethasone arm and any patient requiring steroids or IVIg therapy after Day 28 in both arms were counted as SR failures. Differences in SR and toxicity rates were assessed with the χ2 or Fisher test, depending on the assumption check. An exploratory logistic regression model was applied to the SR data, including the effect of treatment and rescue therapy as covariates; results were expressed as odds ratios and 95% confidence intervals (C.I.).
Analysis of potential predictive factors was performed using logistic regression and results were expressed as odds ratios and corresponding 95% C.I. The following clinical and laboratory factors were considered: age, sex, baseline platelet count (< or ≥ 10 x 10⁹/L), total and CD20-positive lymphocyte counts and IgG serum levels, Baseline vs. Week 24 changes in the levels of IgG, IgA, IgM and CD20-positive lymphocytes.

The early response assessment was performed on the ITT population. Those patients requiring additional treatment with steroids or IVIg during the first month were considered as early response failures, independent of the platelet count achieved at the Day 28 visit.

Safety, response to salvage therapy, and cellular and humoral immune responses were analyzed using descriptive statistics. The Kaplan-Meier method and Log-rank test were used to evaluate the RD during the observational follow-up phase. The relapse rate and the need for additional ITP therapy after Month 6 were assessed using the χ² or Fisher test, depending on the assumption check.

RESULTS

Treatment phase

Accrual and clinical characteristics

Enrollment was stopped in advance in June 2007 after 103 patients had been accrued, when results of the first interim analysis indicated that the primary efficacy goal had already been achieved (SR advantage for the experimental arm: 81% vs. 29%, Δ=52%, P<0.001, 98.3% C.I.:[0.228-0.804]).³³ One-hundred and one patients (52 in the dexamethasone arm and 49 in the experimental arm) comprised the safety and ITT populations. Two patients could not be assessed because they did not receive any study drug after randomization (1 patient in the experimental arm because of no ITP diagnosis, and 1 patient in the dexamethasone arm because of previous treatment received). Both arms were balanced with respect to baseline characteristics (Table 1).

Efficacy

Compliance and additional therapy
Mean compliance to dexamethasone was 100% in both arms. Five patients in the experimental arm discontinued the study before taking the first dose of rituximab. For the remaining 44 patients, mean and median compliance was 95% and 100%, respectively (range: 13-110%).

A total of 37 out of 101 patients, 14 (27%) in the dexamethasone arm and 23 (47%) in the experimental arm received further steroids and/or IVIg therapy up to Day 28. Nine of 14 patients in the dexamethasone arm and 8 out of 23 patients in the experimental arm needed to continue the therapy beyond Day 28 and were therefore considered failures for SR. One additional failure occurred in the experimental arm (patient who began additional therapy after Day 28).

Both groups exhibited differences in mean platelet counts at Day 7 (98 x 10^9/L (± 87) in the dexamethasone arm and 74 x 10^9/L (± 61) in the combination arm).

Sustained response (SR)

Analysis of the ITT population indicated that SR was higher amongst patients in the experimental arm compared to those in the dexamethasone arm (63% vs. 36%, \( P = 0.004 \), difference=27%, 95% C.I.: 7.9-45.5) (Table 2A). A similar pattern was observed for SR 100 and SR 150 (Table 2A). The SR rate was 29 out of 44 (66%, vs. 36% for the dexamethasone arm; \( P = 0.004 \)) when the 5 patients who did not receive rituximab were eliminated. The administration of rescue therapy during the first 28 day period was not associated with the SR rate (odds ratio 0.48; 95% C.I.: 0.20-1.18), thus confirming the expected short-term effects of low-dose steroids and IVIg therapy. The effect of treatment was significant (\( P < 0.004 \)) and the odds ratio of the experimental vs. dexamethasone arms was 3.58 (95% C.I.: 1.51-8.47). None of the potential predictive clinical or laboratory parameters of SR (baseline platelet count, total and CD20-positive lymphocyte count, and Baseline vs. Week 24 changes in serum IgG levels were found to be significant using the logistic regression model. Treatment was the only predictive factor for better response (\( P = 0.004 \)). In both arms, the response rate was similar amongst patients with newly diagnosed or persistent/chronic ITP (dexamethasone arm: 3/8 (37.5%) vs. 16/44 (36%), \( P = 0.951 \); dexamethasone plus rituximab arm: 4/8 (50%) vs. 27/41 (66%), \( P = 0.395 \), respectively).
Early response

During the early response assessment (ITT population), 28 out of 52 patients (54%) in the dexamethasone arm were considered non-responders (11 with platelet count < 50 x 10^9/L, 17 receiving salvage therapy or taking concomitant steroids and/or IVIg); 31 out of 49 patients (63%) were non-responders in the experimental arm (8 with platelet count < 50 x 10^9/L, 23 taking concomitant steroids and/or IVIg).

Salvage therapy with dexamethasone plus rituximab

Twenty-seven patients (52%) initially allocated to receive dexamethasone monotherapy who failed to achieve SR received salvage treatment with dexamethasone plus rituximab, 18 in Month 2, 7 in Month 3 and 2 in Month 5 from the start of treatment. In this subgroup, compliance to dexamethasone and rituximab was 100%. The rates of SR, SR 100 and SR 150 were 56%, 44% and 37%, respectively (Table 2B).

Safety

Due to the early stopping of the trial, the safety evaluation was performed over a shorter period than initially planned. Table 3 summarizes the AE occurrence in both treatment arms. Overall, study treatments were well tolerated and no Grade 5 toxicity, hemorrhaging or deaths occurred. Patients in the experimental arm exhibited a higher incidence of grade 3-4 AEs (10% vs 2%, P= 0.082, 95% C.I.: [-0.010-0.175]) and drug-related AEs (4% vs 0%, P= 0.411, 95% C.I.: [-0.061-0.150]) but no significant increase in serious adverse events (SAEs) (6% vs 2%, P= 0.284, 95% C.I.: [-0.035-0.119]) (Table 3). In the experimental arm, one patient required hospitalization because of platelet decrease and/or bleeding unrelated to study drug during salvage therapy; another patient required hospitalization one month after the end of rituximab treatment for interstitial pneumonia with a probable relation to study drug. No specific microbiological agents were detected and the patient recovered after treatment with levofloxacin plus ceftriaxone and azithromycin. One patient experienced supra-ventricular tachycardia during the first administration of rituximab and one patient experienced seizure during the salvage treatment; both were probably related to study drug.
and both patients were discontinued from the study. One patient with a previous history of vascular disease had a transitory ischemic attack not related to study drug during salvage therapy. All AEs were resolved during the study period.

**Immunologic assessment**

The baseline levels of IgG, IgA, IgM and CD20-positive lymphocytes were similar in both arms. After therapy, a slight reduction in mean IgG was observed in the dexamethasone arm (Baseline vs. Week 24: 13.29 g/L ± 5.12 vs. 9.33 g/L ± 1.96, \( P= 0.0772 \)); however, there were no significant changes in IgA, IgM or CD20-positive lymphocytes. Patients in the experimental arm experienced a significant reduction in IgG, IgA, and IgM levels (IgG Baseline vs. Week 24: 12.04 g/L ± 9.92 vs. 9.72 g/L ± 3.56, \( P< 0.0001 \); IgA Baseline vs. Week 24: 2.23 g/L ± 0.91 vs. 1.51 g/L ± 1.01, \( P< 0.0001 \); IgM Baseline vs. Week 24: 1.16 g/L ± 0.78 vs. 0.82 g/L ± 0.58 \( P< 0.0001 \)). As expected, B-cell depletion in this group was observed from the very first control time point 4 weeks after the start of therapy and persisted until the last scheduled visit at Week 24 (mean CD20+ lymphocyte level: 0.00 x 10^9/L ± 0.01).

**Observational follow-up phase**

Eighty patients (15 in the dexamethasone arm, 41 in the experimental arm and 24 in the dexamethasone plus rituximab salvage therapy group) were followed-up beyond Month 6 for a median observation period of 20 months (range: 4-40 months). Twenty-one patients were not evaluable for this part of the study, as 9 were lost to follow-up during the study period.

**Safety**

One case of Herpes zoster reactivation was documented at Month 22 in a patient initially allocated to the dexamethasone arm who then received dexamethasone plus rituximab salvage therapy. No other infectious, delayed toxic events or deaths were registered.

**Efficacy**

Fifty-four out of 80 patients achieved a SR and could be evaluated for relapse rate and RD beyond Month 6. This group included 13 patients from the dexamethasone arm who were observed after
Month 6 for a median period of 20 months (range: 4-28 months), 27 from the experimental arm observed for a median period of 20 months (range: 4-40 months), and 14 from the dexamethasone plus rituximab salvage therapy group observed for a median period of 20 months (range: 8-34). The rate of relapse (i.e. platelets < 50 x 10^9/L) in the three groups was 23% (3/13), 26% (7/27), and 14% (2/14), respectively (Fisher test; \( P = 0.837 \)). The time to loss of SR was Month 4, 8, 16 in the dexamethasone arm, Month 4, 4, 5, 12, 20, 28 in the experimental arms, Month 8, 12 in the dexamethasone plus rituximab salvage therapy. All relapsed patients underwent additional specific ITP therapy. The 30-month estimated probability of RD for each of the above treatment groups was 77%, 69%, and 85% (Log-rank test, \( P = 0.783 \)), respectively. During the period of observation, 4 out 80 (5%) of the evaluable patients underwent splenectomy.

**DISCUSSION**

We report results from the first randomized clinical trial evaluating the efficacy of rituximab in ITP. Our results suggest that the addition of rituximab to a single course of dexamethasone therapy improves outcomes in treatment-naïve patients with ITP, when used as first-line or salvage therapy. The inclusion of rituximab to the dexamethasone-based treatment regimen resulted in improved SR rates compared to dexamethasone alone (63% vs. 36%). Furthermore, the dexamethasone-rituximab regimen was effective as salvage therapy (SR 56%) in the subgroup of patients refractory to dexamethasone monotherapy.

We chose to assess the efficacy of rituximab in treatment-naïve patients in order to minimize any potential bias in patient baseline characteristics with respect to prior therapies. A single course of dexamethasone treatment (40 mg daily for 4 days) was selected as the comparator treatment based on the data of Cheng et al.\(^6\) Indeed, comparison of the data obtained from our dexamethasone arm with those of Cheng et al. revealed similar SR rates (36% vs. 42%, respectively). A phase II study run by the Italian Group for Hematological Diseases (GIMEMA) performed in treatment-naïve adult ITP patients and published in 2007 (our study began in 2005) suggested that four courses of
four-day, bi-weekly administrations of 40 mg dexamethasone may yield better results than a single course, with response rates of 85%, 60% relapse-free survival at 15 months and good safety profile.\textsuperscript{34} However, these findings need to be confirmed.

We administered rituximab at the standard dosage of 375 mg/m\textsuperscript{2} weekly for 4 weeks, according to the regimen adopted for non-Hodgkin’s lymphoma (NHL), previous findings in ITP and other autoimmune disorders. The first rituximab infusion was administered on Day +7 in order to allow study physicians to confirm the diagnosis and establish platelet count increase or stabilization upon dexamethasone treatment.

Previous studies have demonstrated the therapeutic activity of rituximab and its long-term effects in patients with relapsed/refractory ITP.\textsuperscript{16-26} However, it is difficult to compare these results with those from our study, due to differences in study methodology including patient selection criteria and the parameters used to assess response. Stasi et al.\textsuperscript{17} showed that 10 out of 25 patients responded to rituximab and this response was maintained for 6 months or longer in 7 (28%) patients. Garcia-Chavez et al.\textsuperscript{23} reported a 67% sustained response rate in 18 pretreated ITP patients; Godeau et al.\textsuperscript{26} reported promising 1-year responses in 40% of patients. With one exception,\textsuperscript{23} our data compare well with other studies in terms of long-term response (63% vs. nearly 40%), corroborating the hypothesis that better long-term effects of rituximab can be achieved when administered earlier in the course of the disease.\textsuperscript{21-22} In contrast to published findings,\textsuperscript{20,26} we could not confirm the prognostic significance of age or any other clinical or biological factors including gender, platelet count, lymphocyte count, or immunoglobulin level.

Of the 52 patients initially treated with dexamethasone alone, 27 received dexamethasone plus rituximab salvage therapy due to non-responsiveness and of these, 15 (56%) achieved SR. This proportion of responders is similar to that of the dexamethasone + rituximab arm (63%). This suggests that a short course of dexamethasone therapy does not negate the effects of subsequent rituximab treatment; on the contrary, it could be useful for identifying patients who may not require further treatment with rituximab.
A greater proportion of patients in the experimental arm received rescue therapy up to Day 28 after the start of treatment. This discrepancy may be related to the open-label nature of the study. Nevertheless, this variable did not confound the significance of the primary end-point, as evidenced by the finding that low-dose steroids or a single course of IVIg had only transient and no long-term effects on response outcome.35

No significant differences in the relapse rate, RD and the need for additional ITP therapy were observed in patients who achieved SR following dexamethasone monotherapy or dexamethasone plus rituximab (either front-line or salvage therapy). The risk of relapse after SR was low (~20%), in agreement with the observations of Cheng et al.6 and the relapse-free survival data of Mazzucconi et al.34 Therefore, relapse rate appears to be a potential surrogate of SR, rather than of the treatment regimen.

This study has several limitations. The follow-up analysis is brief in comparison with those following splenectomies3, thus limiting the evaluation of the long-term effects of rituximab in disease stabilization and its long-term splenectomy-sparing potential. The early interruption of patient accrual resulted in insufficient power for detecting subtle differences in clinical events between the groups, including safety outcomes. With this in mind, the experimental arm was associated with an increased incidence of any and grade 3-4 adverse events but not of SAEs. Most of the grade 3-4 AEs were drug-related, all AEs were resolved and no deaths were recorded. Long-term follow-up revealed only one delayed toxic event (a case of Herpes zoster), confirming the long-term safety findings for rituximab previously reported in NHL and ITP.36

Overall, little is known regarding the long-term effects of rituximab in ITP. In our study, one early serious infectious event (interstitial pneumonia) was recorded, which developed following the end of rituximab treatment. The single case of Herpes zoster reactivation developed at Month 22 after dexamethasone plus rituximab salvage therapy. The collective experience from this and other studies shows no evidence of increased risk for infectious complications with rituximab treatment.
multifocal leukoencephalopathy (PML) in rituximab-treated patients highlighted the development of PML in one ITP patient treated with rituximab\textsuperscript{37}. The long-term effects of rituximab treatment in ITP need to be confirmed in further clinical studies in this patient cohort.

In addition to safety, little is known of how rituximab acts in ameliorating the symptoms of ITP. Better characterization of the immune pathways involved in the pathogenesis of ITP and the corresponding patient pharmacogenetic profiles will aid in the selection of candidates for rituximab therapy. Further studies are warranted in order to confirm the effects of low-dose rituximab\textsuperscript{38} and the clinical activity of novel anti-CD20 antibodies that will be available in the near future.

In conclusion, results from this study suggest that the combination of dexamethasone and rituximab improves patient outcomes without compromising the safety profile. This outcome does not appear to be affected by a previous single course of dexamethasone monotherapy. The relatively sustained duration of response observed in some patients suggests a beneficial effect on the underlying disease. This treatment regimen provides an option for second-line therapy, particularly in patients not responding to steroids and as an alternative to splenectomy.
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In addition to the authors, other participants in this trial were as follows: M. De Simone-Department of Hematology, Ospedale Cardarelli, Napoli; A.L. Molinari-Department of Hematology, Ospedale Santa Maria delle Croci, Ravenna; A. Gallamini-Department of Hematology, Cuneo; M.G. Mazzucconi-Department of Hematology, La Sapienza, Roma; S. Mirto and S. Magrin-Department of Hematology, Palermo; D. Veneri-Department of Hematology, University of Verona; G. Semenzato-Department of Hematology, University of Padova; G. Rossi and C. Carbone-Department of Hematology, Brescia; Enrica Morra-Department of Hematology, Niguarda, Milano; G. Fioritoni-Department of Hematology, Pescara; V. Liso-Department of Hematology, University of Bari; G. Leone-Department of Hematology, Catholic University, Roma.

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CONFLICT OF INTEREST DISCLOSURE

Francesco Zaja has received honoraria and financial support for the costs of travel to ASH annual meetings from Roche.

Enrica Gamba is a Roche employee.

All other authors declare no competing financial interests.
REFERENCES


TABLE LEGENDS

**Table 1:** Baseline demographic and disease characteristics in the ITT and safety population (101 patients).

§ Some patients had a prior diagnosis of ITP initially not requiring treatment.

^ Three patients, 1 in the dexamethasone arm and 2 in the dexamethasone plus rituximab arm, were randomized despite a platelet count higher than 20 x 10^9/L.

**Table 2A:** Effects of treatment with dexamethasone or dexamethasone plus rituximab on the rates of sustained response (overall SR: platelet count ≥ 50 x 10^9/L; SR 100: platelet count ≥ 100 x 10^9/L; SR 150: platelet count ≥ 150 x 10^9/L).

**Table 2B:** Effects of treatment with dexamethasone plus rituximab salvage therapy in 27 patients previously allocated to dexamethasone monotherapy who failed to achieve sustained response (overall SR: platelet count ≥ 50 x 10^9/L; SR 100: platelet count ≥ 100 x 10^9/L; SR 150: platelet count ≥ 150 x 10^9/L).

**Table 3:** Most frequent AEs occurring in patients treated with dexamethasone alone, dexamethasone plus rituximab or dexamethasone plus rituximab salvage therapy.
### Tables

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dexamethasone</th>
<th>Dexamethasone plus rituximab</th>
<th>P value</th>
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<tr>
<td>Number of patients</td>
<td>52</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>19/33 (37%/63%)</td>
<td>22/27 (45%/55%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td>98% Caucasian</td>
<td>100% Caucasian</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>2% Asian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>47 ± 19</td>
<td>49 ± 16</td>
<td>0.60</td>
</tr>
<tr>
<td>Months from diagnosis, median (range)§</td>
<td>0 (0-103)</td>
<td>0 (0-100)</td>
<td>0.14</td>
</tr>
<tr>
<td>Patients with newly diagnosed ITP</td>
<td>44 (85%)</td>
<td>41 (84%)</td>
<td></td>
</tr>
<tr>
<td>Patients with persistent or chronic ITP</td>
<td>8 (15%)</td>
<td>8 (16%)</td>
<td></td>
</tr>
<tr>
<td>Platelet 0-10 x 10^9/L</td>
<td>24 (46%)</td>
<td>22 (45%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Platelet 10-20 x 10^9/L</td>
<td>27 (52%)</td>
<td>25 (51%)</td>
<td></td>
</tr>
<tr>
<td>Platelet &gt; 20 x 10^9/L^</td>
<td>1 (2%)*</td>
<td>2 (4%)**</td>
<td></td>
</tr>
</tbody>
</table>

* platelet count 21 x 10^9/L

** platelet count 21 x 10^9/L and 22 x 10^9/L
### Table 2A

<table>
<thead>
<tr>
<th>Sustained response</th>
<th>SR Platelets $\geq 50 \times 10^9$/L</th>
<th>SR 100 Platelets $\geq 100 \times 10^9$/L</th>
<th>SR 150 Platelets $\geq 150 \times 10^9$/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Dexamethasone</td>
<td>Dexamethasone plus rituximab</td>
<td>Dexamethasone plus rituximab</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td>Evaluable</td>
<td>52</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>0.004</td>
<td>0.019</td>
<td>0.029</td>
</tr>
<tr>
<td>Responders</td>
<td>19 (36%)</td>
<td>31 (63%)</td>
<td>17 (33%)</td>
</tr>
<tr>
<td></td>
<td>13 (25%)</td>
<td>26 (53%)</td>
<td>21 (43%)</td>
</tr>
</tbody>
</table>

### Table 2B

<table>
<thead>
<tr>
<th>Sustained response</th>
<th>SR Platelets $\geq 50 \times 10^9$/L</th>
<th>SR 100 Platelets $\geq 100 \times 10^9$/L</th>
<th>SR 150 Platelets $\geq 150 \times 10^9$/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Responders</td>
<td>15 (56%)</td>
<td>12 (44%)</td>
<td>10 (37%)</td>
</tr>
</tbody>
</table>
Table 3

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Dexamethasone</th>
<th>Dexamethasone + rituximab</th>
<th>Dexamethasone + rituximab salvage therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety population</td>
<td>N= 52</td>
<td>N= 49</td>
<td>N= 27</td>
</tr>
<tr>
<td>Patients with any adverse event</td>
<td>26 (50%)</td>
<td>37 (76%)</td>
<td>18 (67%)</td>
</tr>
<tr>
<td>Patients with serious adverse events:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>List of serious adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic disorder</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Supraventricular tachycardia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rib fracture</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Convulsion</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Transitory ischemic attach</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patients with grade 3-4 adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>List of adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic disorder</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Platelet count decreased</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Supra-ventricular tachycardia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Transaminases increased</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Convulsion</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nephrolithiasis</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Patients with grade 3-4 adverse events related to study drug:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>List of adverse events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supra-ventricular tachycardia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Transaminases increased</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Convulsion</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1: CONSORT flow diagram of progress through the study phases.

Figure 2: Kaplan-Meier response duration curves after Month 6 in patients who achieved sustained response after dexamethasone monotherapy, dexamethasone plus rituximab, and dexamethasone plus rituximab salvage therapy.
Patients randomized: 103

2 patients excluded (i.e. did not receive any study medication) due to:
- no diagnosis of ITP
- treatment with other therapy before randomization

Patients randomized and evaluable: 101

Treatment phase
Patients allocated to dexamethasone plus rituximab: 49

Patients evaluated for sustained response: 49
14/49 patients (28%) discontinued the study due to:
- insufficient therapeutic response: 4 (8%)
- protocol violation: 3 (6 %)
- lost to follow-up: 1 (2 %)
- consent withdrawal: 4 (8%)
- adverse event: 2 (4%)

Patients allocated to dexamethasone monotherapy: 52

Patients evaluated for sustained response: 52
39/52 patients (74%) discontinued the study due to:
- insufficient therapeutic response: 20 (38%)
- protocol violation: 13 (25%)
- lost to follow-up: 1 (2%)
- consent withdrawal: 5 (9%)

Treatment phase
Patients allocated to dexamethasone plus rituximab: 49

Observational follow-up phase
Patients evaluable for safety: 15/52
Patients non evaluable: 37/52
- salvage therapy with dexamethasone plus rituximab: 27/37
- protocol violation: 5/37
- lost to follow-up: 3/37
- consent withdrawal: 2/37

Patients evaluable for response duration: 13/19
Patients non evaluable: 6/19
- protocol violation: 2/6
- lost to follow-up: 4/6

Observational follow-up phase
Patients evaluable for safety: 24/27
Patients non evaluable: 3/27
- lost to follow-up: 3/3

Patients evaluable for response duration: 14/15
Patients non evaluable: 1/15
- lost to follow-up: 1/1

Patients evaluable for response duration: 27/31
Patients non evaluable: 4/31
- protocol violation: 1/4
- lost to follow-up: 1/4
- consent withdrawal: 2/4
Figure 2

- Dexamethasone
- Dexamethasone plus Rituximab
- Salvage Therapy
Dexamethasone plus rituximab yields higher sustained response rates than dexamethasone monotherapy in adults with primary immune thrombocytopenia

Francesco Zaja, Michele Baccarani, Patrizio Mazza, Monica Bocchia, Luigi Gugliotta, Alfonso Zaccaria, Nicola Vianelli, Marzia Defina, Alessia Tieghi, Sergio Amadori, Selenia Campagna, Felicetto Ferrara, Emanuele Angelucci, Emilio Usala, Silvia Cantoni, Giuseppe Visani, Antonella Fornaro, Rita Rizzi, Valerio De Stefano, Francesco Casulli, Marta Lisa Battista, Miriam Isola, Franca Soldano, Enrica Gamba and Renato Fanin