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ORIGINAL PAPER

Immunomodulation with romiplostim as a second-line strategy in primary immune thrombocytopenia: The iROM study

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Summary

Thrombopoietin receptor agonists (TPO-RAs) stimulate platelet production, which might restore immunological tolerance in primary immune thrombocytopenia (ITP). The iROM study investigated romiplostim's immunomodulatory effects. Thirteen patients (median age, 31 years) who previously received first-line treatment received romiplostim for 22 weeks, followed by monitoring until week 52. In addition to immunological data, secondary end-points included the sustained remission off-treatment (SROT) rate at 1 year, romiplostim dose, platelet count and bleedings. Scheduled discontinuation of romiplostim and SROT were achieved in six patients with newly diagnosed ITP, whereas the remaining seven patients relapsed. Romiplostim dose titration was lower and platelet count response was stronger in patients with SROT than in relapsed patients. In all patients, regulatory T lymphocyte (Treg) counts increased until study completion and the counts were higher in patients with SROT. Interleukin (IL)-4, IL-9 and IL-17F levels decreased significantly in all patients. FOXP3 (Treg), GATA3 (Th2) mRNA expression and transforming growth factor-β levels increased in patients with SROT. Treatment with romiplostim modulates the immune system and possibly influences ITP prognosis. A rapid increase in platelet counts is likely important for inducing immune tolerance. Better outcomes might be achieved at an early stage of autoimmunity, but clinical studies are needed for confirmation.

KEYWORDS
immunomodulation, ITP, sustained response, TPO-RA, treatment-free remission

INTRODUCTION

Primary immune thrombocytopenia (ITP) is a rare autoimmune-mediated condition of unknown origin presenting with isolated peripheral thrombocytopenia (platelet count, $<100\times10^9$/L).[1] ITP results from immune system disruption, causing a short platelet lifespan and production failure.[2] Immune system alterations induce autoantibody formation,

shift the Th1/Th2 ratio towards Th1, increase Th17 cell counts, reduce regulatory T lymphocyte (Treg) counts and change cytokine levels.[2–5]

Most treatment strategies aim to control bleeding and focus on preventing premature platelet destruction (immunosuppression, splenectomy) and increasing platelet production (thrombopoietin receptor agonists [TPO-RAs]). First-line treatments for ITP include corticosteroids and/or intravenous immunoglobulin (IVIG).[6] However, when tapering first-line steroids, 70%–90% of patients experience relapse.[7]

For >10 years, thrombopoietin receptor agonists (TPO-RAs) have been used as a platelet-enhancing therapy for ITP patients.[8,9] In many countries, TPO-RAs are approved as second-line treatments. Despite excellent platelet responses, continuous TPO-RA treatment is required for most patients to maintain efficacy, requiring durable treatment adherence and high costs.[10] However, several clinical reports indicate sustained remission despite treatment interruption or discontinuation.[11–22] Sustained remission after TPO-RA treatment appears independent of previous treatments, splenectomy status or disease duration.[11] This was reported in a case study of romiplostim.[23] However, a later pooled analysis recorded treatment-free remission (TFR) rates of 16% and 6% in patients with ITP for ≤1 year ($n = 277$) and >1 year ($n = 634$) respectively.[24] Few trials have prospectively investigated sustained remission after TPO-RA treatment, mostly in patients with short disease durations.[13,22,25,26] The remission rates off-treatment were surprisingly much higher than those in the initial retrospective reports. However, the data cannot be accurately compared because of inconsistent definitions of remission in the literature.

Bao et al. suggested that TPO-RAs have additional immunomodulatory activity and indicated their possible protolerogenic effect involving Treg activation.[27] Subsequently, various theories have been discussed,[12,18,28] and an overview was published in preparation of this trial.[29] Our hypothesis is a combination of two mechanisms: (1) immune tolerance induction via high-dose antigen exposure and (2) innate immune activity of platelets, particularly the release of transforming growth factor (TGF)-β,[30] which might stimulate or restore Tregs.

The iROM trial prospectively investigated the effects of romiplostim on the immune system based on cellular, cytokines and genetic assessments. We assumed that romiplostim has a favourable immunomodulatory effect that prevents chronic disease progression, particularly in young and middle-aged patients during the initial disease phase, because of limited immunosenescence and autoimmune expansion or epitope spreading.

METHODS

Ethics statement

The iROM study is a multicentre, open-label, single-arm trial designed as a proof-of-concept study. Patients were recruited from five study centres in Switzerland (University Hospitals of Basel and Bern, and Cantonal Hospitals of Aarau, Liestal and Lucerne). The study was approved by the ethical committee of Northwest and Central Switzerland and conducted in accordance with the Declaration of Helsinki. All patients provided informed consent and met the inclusion criteria.

Patients

The patients were young adults (age, 18–45 years) with ITP and platelet counts $<30 \times 10^9$/L or those at high risk of falling under this threshold, who experienced failure, relapse or significant side effects following first-line treatment. The exclusion criteria were previous second-line treatment, secondary ITP suspicion, thromboembolic disease history and concomitant use of drugs with known immune system effects. Owing to recruitment difficulty, 1 year after study initiation, we increased the maximum age of inclusion to 60 years.

Patients with complete and sustained remission (platelet count, $>100 \times 10^9$/L) off-treatment (SROT) over weeks 22–52 comprised the 'SROT' group. Patients requiring platelet-enhancing treatment after week 22 comprised the 'relapse' group. There was no patient with stable platelet counts between 30×10^9/L and 100×10^9/L, and who did not require subsequent treatment.

Study plan

Romiplostim subcutaneous injection was administered weekly for 22 weeks (starting dose, 1 μg/kg body weight [bw]). The dose was adjusted weekly based on the platelet response according to the product information (target platelet count, 50×10^9 to 200×10^9/L). Alternatively, these same dose adjustment rules allowed dose reduction or treatment discontinuation at any point. We permitted all first-line treatments to increase platelet counts starting before enrolment and all rescue medications until week 3. From this time to week 22, only corticosteroids were allowed, and other treatments necessitated withdrawal.

We collected participant data, including clinical features at initial ITP diagnosis, ITP course to registration date, drug history (last 28 days), significant medical history (last 5 years), and physical and laboratory data at baseline. Patients were observed weekly for 22 weeks and subsequently followed up (weeks 24, 30, 35, 40 and 52). Bleeding, hospitalisation, concomitant medication use and rescue treatments were recorded. We categorised bleeding severity (none, mild, moderate and severe) using the Bolton–Maggs scale.[31] Severe adverse events were continuously monitored. Figure 1 presents the study overview.

Immunological samples

Immunological studies were performed at weeks 1, 6, 12, 22 and 52 to investigate the balance among Th1, Th17, Th2 and

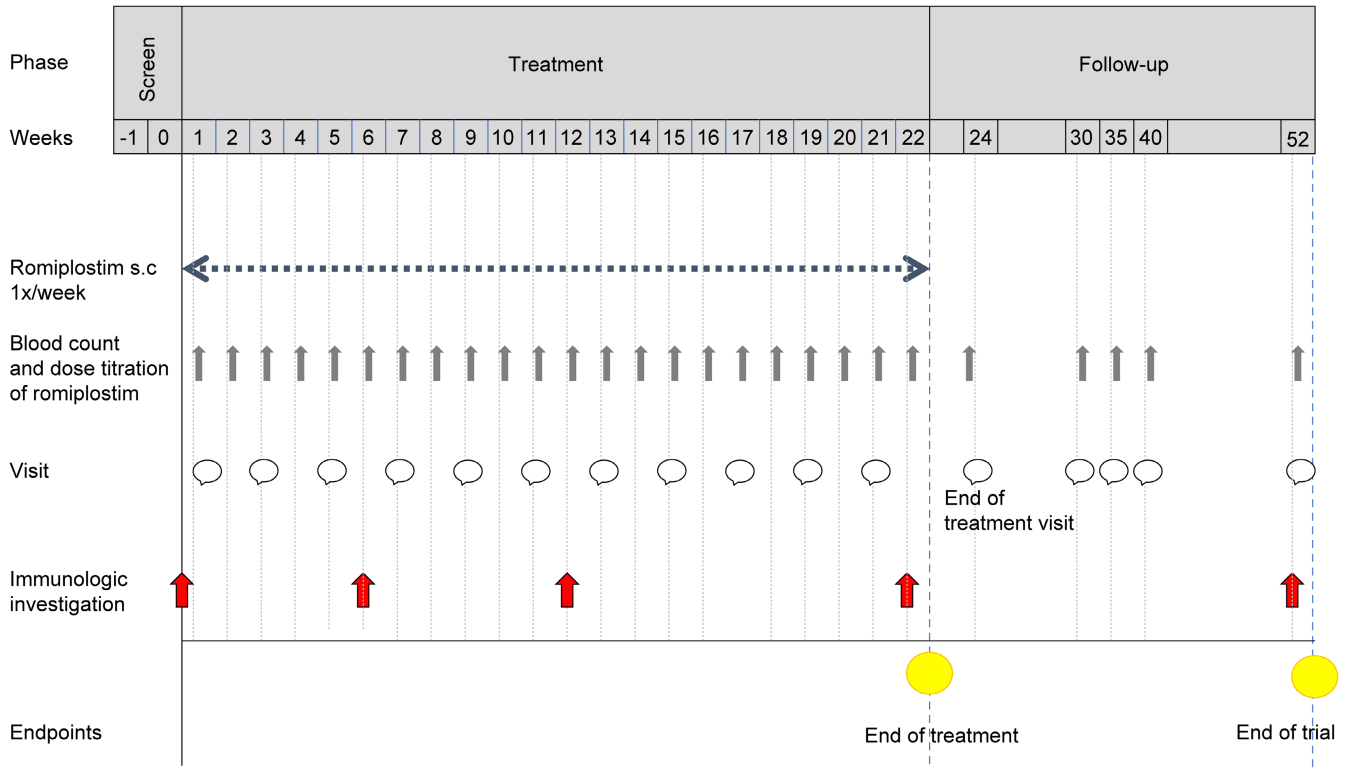


FIGURE 1 Overview of the iROM trial. Patients were treated for 22 weeks with subcutaneous romiplostim. The starting dose was 1 μg/kg body weight, and dose titration was performed according to the product information. The last dose of romiplostim was given at week 22 (end of treatment), and patients were monitored for relapse until week 52 (end of the trial). The immunological profile was assessed at weeks 1 (before treatment initiation), 6, 12, 22 and 52 (red arrows).

Treg activity profiles. Immunological pattern was evaluated by fluorescence-activated cell scanning (FACS), cytokine levels and mRNA expressions. See Supplement for supporting content.

Statistical analysis

The sample size was calculated to ensure significant differences between pre- and post-treatment IL-4 levels in >90% of hypothetical repetitions. We assumed a temporal correlation of 0.6 and a difference between pre- and post-treatment IL-4 levels of 1 pg/mL (log scale). Power analysis was based on two previous studies.[32,33]

Immunological end-points were reported as the median and interquartile range. The statistical tests of pre–post differences were accompanied by the estimated median change over treatment and 95% confidence interval (CI). For cytokine levels below the specified threshold, we set the values to zero. IL-4 levels (primary end-point) were studied using a null hypothesis. We tested the difference between the defined time points using a paired-sample Wilcoxon test (two-sided $\alpha = 5\%$). We used Pratt's method to handle the zeros in the Wilcoxon test when present. Because we performed multiple tests for all secondary end-points, the p-values were adjusted using the Holm method. A value of $p < 0.05$ was considered significant.

Considering comedication use and delayed cellular response, we calculated Treg count changes between weeks 6 (baseline) and 22 or 52. Conversely, the prompt changes in cytokine levels upon treatment initiation allowed us to compare changes between weeks 1 and 6, as well as later time points.

We calculated the correlation between TGF-β levels and platelet counts using a mixed linear regression model by regressing the platelet count on TGF-β and time point, generating a random intercept for patients. The time point was included as a categorical predictor in the model.

The Th1/Th2/Treg and Th17 profiles were analysed between week 1 and all later time points. To analyse the relative changes in gene expression from real-time PCR experiments, we used the $2^{-\Delta\Delta Ct}$ method. Fold change >1 indicated upregulation, and fold change <1 indicated downregulation.

Clinical data were extracted from the secuTrial® database and descriptively analysed. We conducted subgroup analyses for the SROT and relapse groups.

RESULTS

Clinical end-points

Overall, 15 patients (7 women and 8 men) were recruited from December 2016 to February 2020. Two patients were excluded because of an insufficient response or loss of response to romiplostim. The remaining 13 patients were analysed, including

TABLE 1 Patient characteristics before enrolment for the entire cohort and the patient groups (patients with sustained remission off-treatment [SROT] or relapse after week 22 or the end of treatment).

	All patients	Patients with SROT	Patients with relapse
All patients[a]	13	6	7
Male	6	5	1
Female	7	1	6
Newly diagnosed ITP at study enrolment (median ITP duration until the day of enrolment: 4 weeks)	9	6	3
Chronic disease at study enrolment (median ITP duration until the day of enrolment: 87 months)	4	0	4
Median age (range)	31 (19–51)	33 (19–51)	31 (19–51)
Number of patients with comedication in the last 7 days prior to enrolment			
Steroids	10	5	5
IVIG	5	3	2
Onset of ITP (at first diagnosis)			
Acute	12	6	6
Insidious (over weeks)	1	0	1
Lowest platelet count since the ITP diagnosis			
$<10\times10^9$/L	11	6	5
10×10^9–20×10^9/L	2	0	2
Bleeding location before enrolment			
Cutaneous	12	5	7
Oral cavity	5	4	1
Epistaxis	8	3	5
Haematuria	1	1	0
Muscle	1	1	0
Menorrhagia	1	0	1
Treatment and response before enrolment			
IVIG	11	5	6
No response (platelets $<30\times10^9$/L)	2	2	0
Partial response (platelets = 30×10^9–100×10^9/L)	4	1	3
Good response (platelets $>100\times10^9$/L)	5	2	3
Prednisone	11	4	7
No response	4	3	1
Partial response	3	0	3
Good response	4	1	3
Dexamethasone pulse	6	4	2
No response	2	2	0
Partial response	1	0	1
Good response	3	2	1
Transfusion	1	1	0

Abbreviations: ITP, primary immune thrombocytopenia; IVIG, intravenous immunoglobulin.

[a]Following comorbidities were recorded: neoplasm ($n=1$); infectious and parasitic diseases ($n=1$); genitourinary disease ($n=1$); endocrine, nutritional and metabolic diseases ($n=1$); mental and behavioural disorders ($n=3$); cardiovascular diseases ($n=1$); musculoskeletal and connective tissue diseases ($n=1$) and nervous system diseases ($n=1$).

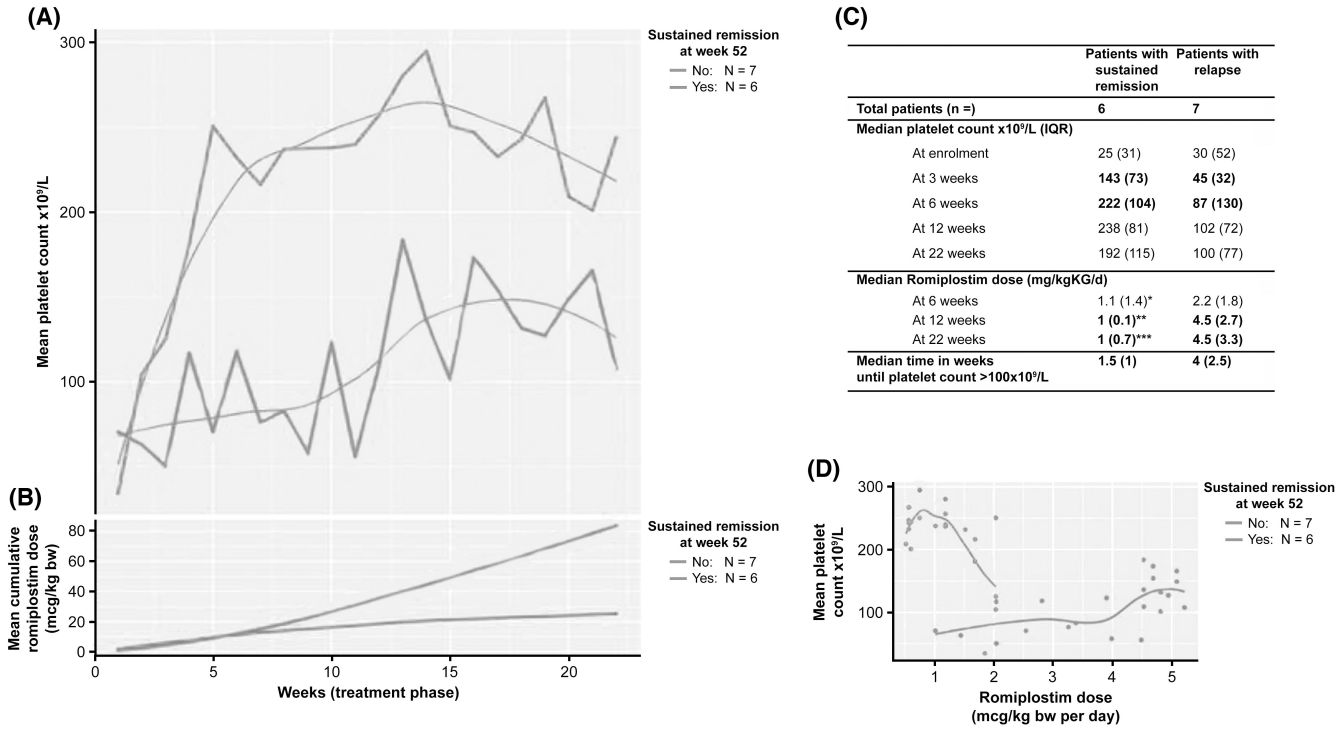
3 women and 6 men with newly diagnosed ITP and 4 women with chronic ITP. The median patient age was 31 (range, 19–51) years, and only two patients were >45 years. The median initial platelet count was 29×10^9/L (interquartile range = 45). Table 1 describes the patient characteristics before enrolment. Treatments received within 1 week of study initiation included corticosteroids ($n=10$) and IVIG ($n=5$). Figure 2 presents the median platelet count, romiplostim response and cumulative romiplostim dose. The romiplostim dose titration was lower and the platelet count response was faster and stronger in SROT patients than in relapsed patients.

Rescue medications included corticosteroids in six patients, IVIG in two patients and tranexamic acid in one patient. Corticosteroids were mostly initiated before enrolment and tapered over the following weeks. Only one patient was hospitalised because of bleeding during the first 3 weeks of the study. At week 22, one patient required additional prednisone. At the end of treatment, six patients with newly diagnosed ITP (67%) remained in complete and sustained remission without further drug therapy until week 52, and seven patients relapsed (three with newly diagnosed ITP and four with chronic disease). Overall, 6 of 13 patients (46%) were in SROT at study closure. No patients with initial chronic ITP exhibited SROT. Patients who relapsed after week 22 were subsequently rescued with (mostly as combinations) romiplostim ($n=5$), eltrombopag ($n=5$), IVIG ($n=3$), prednisone ($n=2$), dexamethasone ($n=1$) and tranexamic acid ($n=1$). One patient underwent splenectomy (week 45). At week 52, six patients received ongoing ITP treatment with romiplostim ($n=2$), eltrombopag ($n=3$) or eltrombopag+prednisone ($n=1$).

Immunological end-points

FACS analysis of cell counts

Treg counts increased between weeks 6 and 22 and between weeks 6 and 52 in the entire cohort, with median changes of 0.49 (95% CI = −0.09, 0.91; $p=0.5$, Holm-corrected) and 0.62 (95% CI = 0.14, 1.26; $p=0.017$, Holm-corrected) respectively.



(C)

	Patients with sustained remission	Patients with relapse
Total patients (n =)	**6**	**7**
Median platelet count x10⁹/L (IQR)		
At enrolment	25 (31)	30 (52)
At 3 weeks	**143 (73)**	**45 (32)**
At 6 weeks	**222 (104)**	**87 (130)**
At 12 weeks	238 (81)	102 (72)
At 22 weeks	192 (115)	100 (77)
Median Romiplostim dose (mg/kgKG/d)		
At 6 weeks	1.1 (1.4)*	2.2 (1.8)
At 12 weeks	**1 (0.1)****	**4.5 (2.7)**
At 22 weeks	**1 (0.7)*****	**4.5 (3.3)**
Median time in weeks until platelet count >100x10⁹/L	**1.5 (1)**	**4 (2.5)**

FIGURE 2 Platelet count and romiplostim dose during the treatment phase. (A) Time course of the mean platelet count per week (thick line) and smoothed line (thin line) over 22 weeks within the groups of patients who did or did not achieve sustained treatment-free remission (SROT). (B) Time course of the mean cumulative romiplostim dose per week within the groups over 22 weeks. (C) Platelet count, romiplostim dose and time to response during the treatment phase within the groups. Three patients could terminate romiplostim before week 22: */**Calculated without one patient (dose, 0 mg/kg); ***Calculated without three patients (dose, 0 mg/kg). (D) Association between the individual dose and platelet counts in the SROT and relapse groups with smoothed mean platelet counts.

Figure 3 presents the individual Treg variation and median change in Treg counts in the SROT and relapse groups.

Cytokine level changes

IL-4 levels decreased rapidly and significantly in all patients during the first 6 weeks ($p = 0.001$), and the median change was similar between groups. However, initial IL-4 levels were higher in relapsed patients than SROT patients. Similar patterns were observed for IL-22, IL-13, IL-10, IL-9, IL-6, TNF-α, IL-17A and IL-17F. Using Holm correction, significance was detected for IL-17F and IL-9. IL-5, IL-2 and IFN-γ levels decreased during this period, but the initial levels were similar between groups. Conversely, TGF-β and IL-35 levels increased during the first 6 weeks, and both were initially higher in SROT patients than in relapsed patients. The initial median TGF-β level was threefold higher in the SROT group and twofold higher in the relapse group after 6 weeks; however, the median change was negative in the relapse group, as only one of seven patients exhibited an evident increase. The platelet count increased by approximately 0.5 with every unit increase in the TGF-β level ($p < 0.001$). The median change in IL-35 levels was higher in SROT patients than in relapsed patients, albeit without significance. Table 2 presents cytokine changes between weeks 1 and 6. The median values at all time points are reported in Table S1.

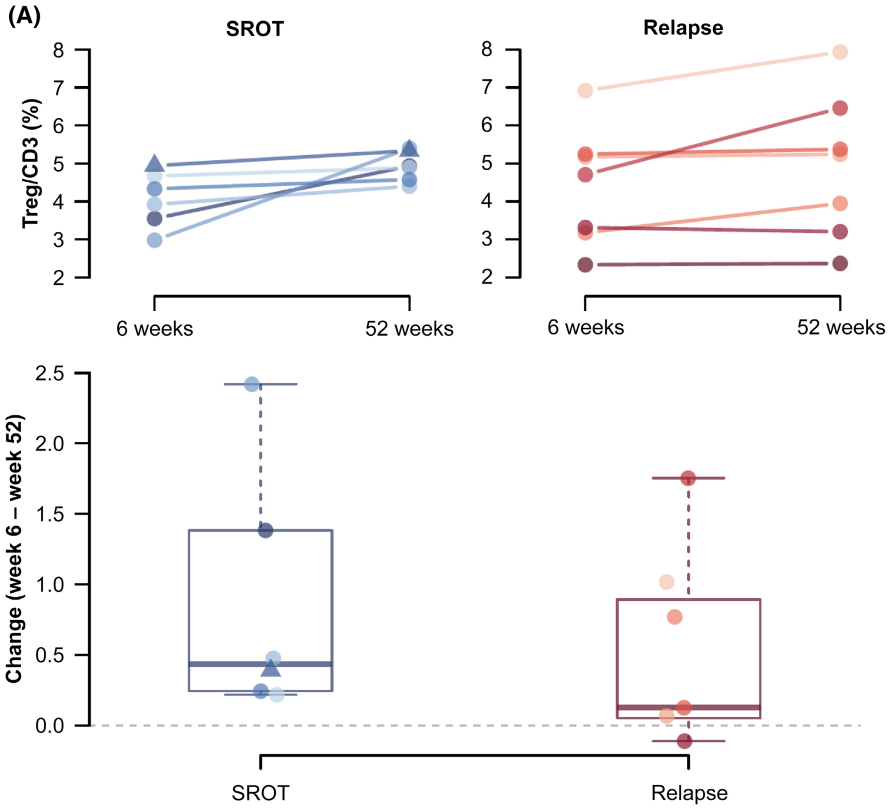
mRNA expression changes

FOXP3 expression (Treg profile) increased gradually, peaking at week 22 (Figure 4); however, the increase was restricted to the SROT group (median increase, 2.9-fold). Patients with later relapse exhibited no changes in FOXP3 expression at week 22 or 52. GATA3 expression (Th2 profile) displayed little variation in the entire cohort. However, an increase was observed at every time point in the SROT group, with stable up-regulation at 22 and 52 weeks (1.7-fold). Conversely, relapsed patients exhibited slightly decreased or unchanged expression. The Th1 profile (TBX21) mostly displayed no differences over time or between groups. The Th17 profile (ROR) exhibited small increases at weeks 22 and 52 in the entire cohort.

IL-4 and TGF-β mRNA expressions increased slightly over time (TGF-β median increase, 1.35-fold [52 weeks vs. baseline]). IL-6 and HEBI3 (a subunit of IL-35 and IL-27) were down-regulated (0.79- and 0.68-fold respectively), whereas TNF-α was up-regulated (1.42-fold at week 52). Table S2 presents the variation in cytokine mRNA expression for all patients and groups.

DISCUSSION

Accumulating data suggest that the TPO-RAs can induce tolerogenic mechanisms in ITP patients.[11–26] Few prospective



(B)

	Patients with SROT	Patients with relapse
Treg/CD3 [25% and 75% quantiles]		
Δ from nadir to week 22*	0.7 [0.1, 0.71]	0.57 [0.06, 0.8]
Δ from nadir to week 52*	0.48 [0.24, 1.38]	0.13 [0.05, 0.89]

FIGURE 3 Regulatory T lymphocytes during the iROM study. (A) Individual course and change in the regulatory T lymphocyte (Treg)/CD3 ratio (%) between weeks 6 and 52 (end of the trial) for patients with sustained remission off-treatment (SROT) or relapse. (B) Median change (%) between weeks 6 and 22 (end of treatment). Because of comedication (e.g. steroids), shortly before study initiation, week 6 was defined as the nadir for the cell subset analysis.

trials have investigated this phenomenon, and the inclusion criteria and end-points vary. Factors predictive of SROT remain unclear. In our trial, six of nine patients with newly diagnosed ITP achieved complete sustained remission at 1 year without the need for additional treatments beyond 22 weeks, versus zero of four patients with chronic ITP. Interestingly, in SROT patients, the romiplostim dose titration was lower and the platelet count response was faster, stronger and more stable. Platelet counts in relapsed patients displayed a jagged pattern. A previous prospective trial recorded similar platelet curves and dose titration.[13] Patients with early ITP (<6 months) had a remission rate of 32% (platelet count, $\geq 50 \times 10^9$/L over 24 weeks) after romiplostim treatment (maximum, 12 months). No clinical predictors of remission were identified excluding a higher mean platelet count in the first 2 months among remission patients than among non-remission patients. Additionally, the average weekly romiplostim dose was lower among remission patients (not significant).[13] Comparable assumptions were described in case series[34] and retrospective trials.[11] Indeed, patients requiring lower TPO-RA doses were more likely to achieve durable remission after discontinuing treatment. In an observational retrospective study involving 50 patients with newly diagnosed ITP, 27 discontinued TPO-RAs because of an enduring complete response.[17] The estimated 2-year TFR rate was 66% (median follow-up, 24 months), similar to our results. No predictive factors of remission based on the initial patient characteristics were detected. However, patients achieving a platelet response within 14 days after treatment initiation exhibited a high 2-year TFR rate.[17]

The immunological profile was partly unexpected and probably the result of simultaneous immunological stimuli (Figure 5). The decreased levels of most cytokines and increased TGF-β levels and Treg counts could be associated with rapid platelet mass augmentation within the first 6 weeks of treatment. Platelets have the highest cellular TGF-β concentration, with 45% of the basal plasma level believed to be derived from platelets.[30] In our study, the platelet mass and

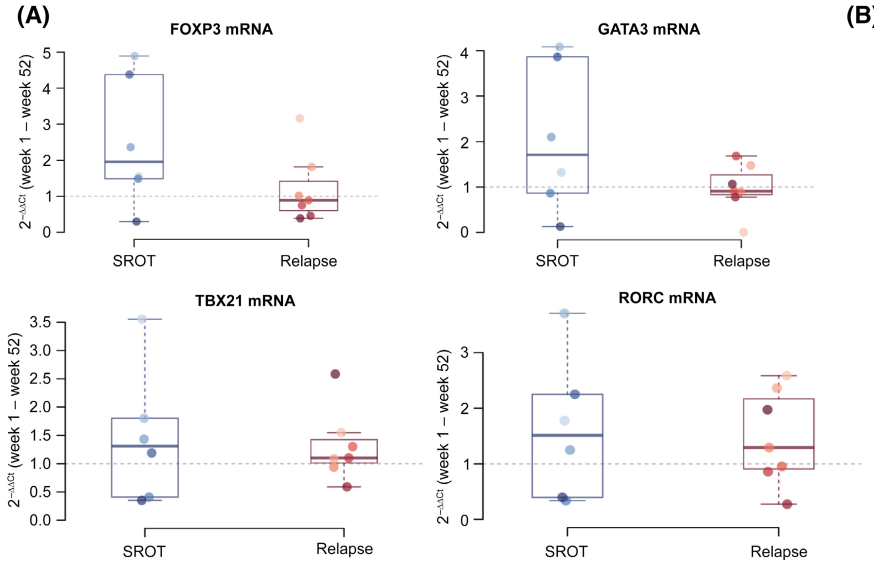
TABLE 2 Cytokine (pg/mL) levels and changes between weeks 1 and 6 in the entire cohort and the sustained remission off-treatment (SROT) and relapse groups.

	Median concentration at week 1 (pretreatment) (interquartile range)	Median concentration at week 6 (interquartile range)	Δ median change (95% confidence interval)	*p* (Holm-corrected *p*)
IL-4 (all patients)	56.5 (33.9, 145.3)	16.6 (0, 30.7)	−39.9 (−121.1, −23.4)	0.001*
Patients with SROT	52.5 (37.5, 103.9)	12.9 (2.3, 27.2)	−40.6 (−78.6, −32.3)	
Patients with relapse	140.5 (27.5, 179)	17.3 (0.3, 31.7)	−39.9 (−115.9, −14.2)	
IL-6	44.2 (27.1, 105.3)	15.1 (0, 22.3)	−41.7 (−107.9, −13.6)	0.007 (0.08)
Patients with SROT	42.9 (30.8, 73.5)	0 (0, 11.3)	−42.9 (−62.2, −30.8)	
Patients with relapse	71.1 (19.6, 110.2)	19.9 (7.5, 23.5)	−39.3 (−87.9, 6.1)	
IL-17F	38.8 (28.7, 94.1)	7.0 (0, 20.5)	−31 (−107.4, −18.2)	0.001 (0.01*)
Patients with SROT	37.6 (30.64, 63)	3.5 (0, 10.3)	−34.2 (−54.5, −28.9)	
Patients with relapse	71.1 (19.3, 122)	14.1 (2.4, 22)	−31 (−104.7, −10.8)	
IL-9	63.1 (40, 155.8)	18.6 (11, 24.8)	−36.3 (−109, −13.6)	0.003 (0.04*)
Patients with SROT	53.2 (40.9, 81)	14.3 (9.4, 23.2)	−38.5 (−68.8, −22.1)	
Patients with relapse	106.44 (35.9, 216.9)	18.61 (14.7, 35.8)	−36.3 (−99.4, −7.1)	
IL-22	14.3 (8.6, 28.7)	4 (0, 8.3)	−8.6 (−23, −0.9)	0.025 (0.25)
Patients with SROT	13.5 (3.2, 14.5)	0 (0, 0)	−13.5 (−14.5, −3.2)	
Patients with relapse	18.1 (9, 35.7)	5.4 (4.7, 21.4)	−6.9 (−16.7, −2)	
TNF-α	32.3 (10.2, 86.7)	18.9 (0, 30.2)	−26 (−59.7, 0)	0.043 (0.39)
Patients with SROT	29.12 (14.1, 51.6)	9.44 (0, 25)	−21.2 (−37.5, −2.6)	
Patients with relapse	80.1 (18.1, 117.7)	26 (0, 64)	−26 (−68.3, −1.1)	
IL-10	15.4 (9.3, 31.6)	3.1 (0, 10.5)	−10.5 (−22.6, 0)	0.052 (0.42)
Patients with SROT	12.4 (6.9, 15.4)	1.3 (0, 4.4)	−9.4 (−14.7, −4.8)	
Patients with relapse	20.6 (9.9, 41)	8.8 (2.7, 23.4)	−10.5 (−20.2, 4.1)	
IL-5	15.8 (6.3, 26.6)	0 (0, 8.5)	−7.4 (−23.8, 0.4)	0.057 (0.42)
Patients with SROT	17.9 (8.6, 26.9)	0 (0, 0)	−9.7 (−20.9, −3.2)	
Patients with relapse	15.8 (5.8, 23.2)	6.3 (1.3, 9.7)	−6.3 (−15.7, −4)	
IFN-γ	31.4 (0, 61.3)	0 (0, 20.2)	−16.5 (−52.2, 0)	0.102 (0.48)
Patients with SROT	27.7 (4.1, 54.3)	0 (0, 0)	−8.2 (−33.3, 0)	
Patients with relapse	31.4 (10.1, 70.5)	0 (0, 22)	−31.4 (−60.4, 0)	
IL-17A	0.67 (0.5, 1)	0.48 (0, 0.6)	−0.37 (−0.6, 0.2)	0.163 (0.48)
Patients with SROT	0.59 (0.46, 0.7)	0.33 (0.1, 0.5)	−0.3 (−0.4, −0.2)	
Patients with relapse	0.97 (0.63, 1.4)	0.59 (0, 1.45)	−0.4 (−0.6, 0.2)	
IL-2	3.1 (2.6, 4.1)	0 (0, 3.1)	−1.0 (−3.6, 0.5)	0.072 (0.43)
Patients with SROT	2.88 (2.6, 3.5)	0 (0, 1.6)	−1.8 (−2.6, −1)	
Patients with relapse	3.1 (2.6, 5.2)	0 (0, 4.7)	−0.5 (−3.9, 1)	
IL-13	19.17 (0, 47.5)	0 (0, 0)	0.0 (−40, 2.1)	0.164 (0.48)
Patients with SROT	9.6 (0, 25.8)	0 (0, 0)	−5.7 (−17.2, 0)	
Patients with relapse	40 (0, 60)	0 (0, 26.6)	0 (−43.8, 7.8)	
TGF-β	6633 (5106, 11 084)	20 479 (9005, 23 495)	4996 (−1850, 18 260)	0.127 (0.48)
Patients with SROT	7880.5 (6172.5, 10 046)	22 146 (20 729, 22 324)	15 820 (9041, 18 818)	
Patients with relapse	5825 (5059, 13 536)	10 821 (8219, 23 075)	−23 (−3947, 4494)	
IL-35	29 (0, 56)	61 (31, 86)	3 (−1, 40.5)	0.097 (0.48)
Patients with SROT	52.5 (34, 67.3)	89.5 (49, 101.5)	35 (10, 58.5)	
Patients with relapse	13 (0, 31.5)	44 (0, 64)	0 (−2, 25)	

Note: Concentrations below the threshold were set at zero (no *p*-value changes if set at threshold).

Abbreviations: IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor.

*Significant difference.



(B)

	Δ Median change (quartiles) week 22	Δ Median change (quartiles) week 52
FOXP3 (Treg)	1.56 (0.85, 3.71)	1.48 (0.75, 2.36)
- For patients with SROT	**2.9 (2.08, 3.78)**	**1.95 (1.5, 3.87)**
- For patients with relapse	**0.99 (0.57, 1.43)**	**0.89 (0.6, 1.41)**
GATA3 (Th2)	1.16 (0.89, 2.33)	1.06 (0.86, 1.68)
- For patients with SROT	**1.73 (0.99, 2.82)**	**1.71 (0.98, 3.42)**
- For patients with relapse	**1.16 (0.86, 1.5)**	**0.91 (0.83, 1.27)**
RORC (Th17)	1.39 (0.91, 1.83)	1.29 (0.86, 2.25)
- For patients with SROT	**1.83 (1.58, 2.03)**	1.51 (0.61, 2.13)
- For patients with relapse	**1.17 (0.85, 1.37)**	1.29 (0.91, 2.17)
TBX21 (Th1)	1.20 (1.05, 1.3)	1.19 (0.94, 1.55)
- For patients with SROT	1.18 (0.81, 1.68)	1.31 (0.6, 1.71)
- For patients with relapse	1.2 (1.09, 1.28)	1.1 (1.01, 1.42)

Quotient	Week 22	Week 52
Th17 / Treg		
- For patients with SROT	0.63	0.77
- For patients with relapse	1.18	1.44
Th1 / Th2		
- For patients with SROT	0.68	0.76
- For patients with relapse	1.03	1.2

FIGURE 4 mRNA profiles of Th1, Th2, Th17 and regulatory T lymphocytes during the iROM study. (A) Individual course and change in the mRNA Th profiles ($2^{-\Delta\Delta Ct}$) between weeks 1 and 52 for patients with sustained remission off-treatment (SROT) or relapse. FOXP3 represents Treg, GATA3 represents Th2, TBX21 represents Th1 and RORC represents Th17. Value >1 denotes activation (x-fold), and value <1 means suppression (x-fold). (B) mRNA expression changes ($2^{-\Delta\Delta Ct}$) between week 1 and week 22 or 52 in all patients and the SROT and relapse groups.

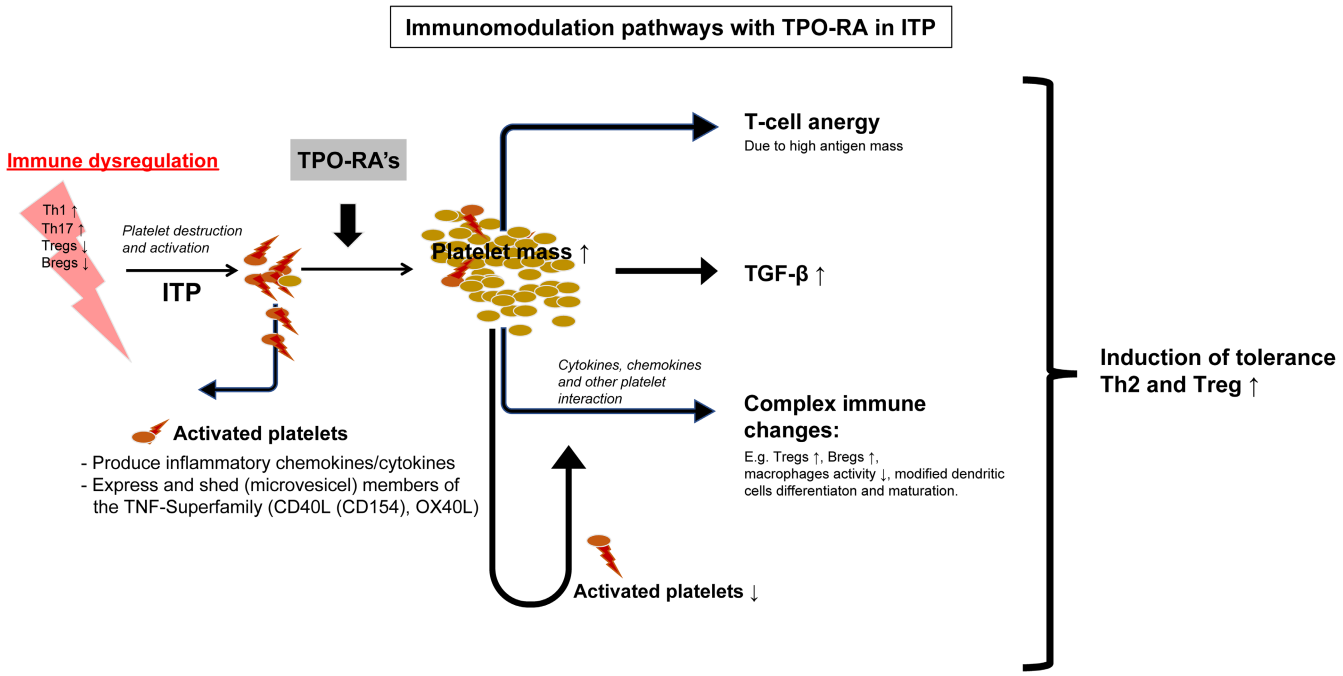


FIGURE 5 Hypothesis of immunomodulation with thrombopoietin receptor agonists (TPO-RAs) in patients with primary immune thrombocytopenia (ITP). The platelet mass seems to be a central pathway leading to an increase of Tregs and a recovery of the Th1/Th2 disbalance. Bregs, regulatory B lymphocytes; IL, interleukin; TGF-β, transforming growth factor-β; TNF, tumour necrosis factor; Tregs, regulatory T lymphocytes.

TGF-β level were related, as previously reported.[27,35] The TGF-β mRNA expression increased only slightly, supporting the assumption that an increased TGF-β level is caused by an increased platelet mass as opposed to mRNA up-regulation. However, TGF-β reservoir augmentation could be a reliable explanation for the abrupt decreases in all Th1 and Th2 cytokine levels.[36] Indeed, TGF-β is a pleiotropic cytokine that can inhibit cytotoxic T lymphocytes, Th1 cells and Th2 cells while promoting Tregs.[36] The significant decrease in IL-9 (inflammatory cytokine) levels might have served as an additional trigger or positive loop in the suppression of the inflammatory state.[37] Apart from TGF-β, only IL-35 plasma

levels increased over the first 6 weeks. IL-35 plays immunosuppressive roles in autoimmune diseases by inhibiting Th1 and Th17 cells and promoting Treg differentiation. Although the fundamental mechanisms of IL-35 function remain unclear, its serum levels are higher in healthy controls than in ITP patients and IL-35 levels are positively correlated with platelet counts.[38–41]

As a second mechanism, we hypothesised that platelet count augmentation in ITP patients stabilises homeostasis and therefore reduces the number of activated and stressed platelets.[42–46] Platelets release various inflammatory chemokines upon activation. Moreover, activated platelets carry CD40L on their membrane and release a soluble form in extracellular vesicles and also contain OX40L, which both are potent pro-inflammatory members of the TNF superfamily.[47–50] A decrease in the number of activated platelets could reduce the inflammatory state and explain the cytokine shut down promptly after treatment initiation, but this is controversial.[35,44,45,51] In a recent study analysing platelet function in 151 children with ITP, 22 were treated with romiplostim. TPO-RA treatment was associated with decreased platelet size and reduced preactivation.[52] Integrin (PAC1) activation in the resting state was increased in the untreated group, whereas there was no difference between romiplostim-treated patients and healthy controls. Procoagulant platelets and P-selectin (CD62p) were increased in all ITP patients regardless of therapy. However, in response to stimulation, romiplostim-treated patients exhibited decreased activation profiles for PAC1 and CD62p.[52]

A third pathway could involve high-dose antigen stimulation with consecutive T lymphocyte anergy, which can modulate the inflammatory state. Recently, TPO-RA-treated mice exhibited reduced immunoglobulin G anti-platelet antibody levels despite increased platelet counts.[53] High-dose antigen administration can paradoxically suppress the immune response to the same antigen. This antigen-specific unresponsiveness induces anergy or apoptosis.[54–56] This pathway could explain the increased mRNA expressions of FOXP3 (Tregs) and GATA3 (Th2), which are present at low levels in ITP patients.[2–5]

The increase in TNF-α mRNA levels without changes in plasma TNF-α levels remains unclear. Romiplostim might have a direct cellular effect by stimulating the c-MPL–JAK2 pathway.[57]

In this study, patients were strictly selected according to age, comorbidities and previous ITP treatment exposure, minimising several risks that could negatively affect the outcome. This selection might have led to higher treatment success rates and a distinct immune pattern. Treatment at a young age and early stage of autoimmunity might contribute to more successful tolerance induction. Autoimmunity is known to develop and expand, becoming resistant to treatment over time.[58–60] In older adults, immune dysregulation probably cannot be corrected easily or rapidly because of immune system senescence. New immunomodulatory approaches with different upfront treatment combinations are being evaluated, such as eltrombopag+high-dose dexamethasone (HD-DXM),[25,26] rituximab+HD-DXM,[61–64] rituximab+eltrombopag+HD-DXM[65] and mycophenolate mofetil+short-term corticosteroids.[66] Early multimodal therapy is a well-known oncologic strategy that may be of interest in autoimmune conditions. Consequently, the implementation of early immunomodulation might prove beneficial in averting treatment-refractory ITP.[67] Promising results have been described in this context.[26,68,69] Hence, a subsequent study—the iROM2 trial—will investigate the potential of randomly adding eltrombopag to HD-DXM as a first-line therapy in patients aged ≤55 years with newly diagnosed ITP (clinicaltrials.gov: NCT04346654).

The main study limitations were the lack of a comparable standard treatment arm and the use of various ITP medications at study initiation. The immunological effect of concomitant platelet-enhancing treatment at the beginning cannot be excluded; however, exposure was similar between groups. Notably, our study group was small because sample size analysis was conducted according to laboratory end-points. Laboratory costs prohibit us from extending the study to a larger patient group. Thus, additional clinical trials are warranted to confirm the findings.

AUTHOR CONTRIBUTIONS

Alexandra Schifferli: Conceptualisation, methodology, formal analysis, investigation, data curation, writing original draft, visualisation, supervision, project administration and funding acquisition. **Axel Rüfer**: Methodology, investigation, resources, review and editing. **Alicia Rovo**: Methodology, investigation, resources, review and editing. **Falk Nimmerjahn**: Conceptualisation, methodology, investigation, resources, review and editing. **Nathan Cantoni**: Methodology, investigation, resources, review and editing. **Andreas Holbro**: Methodology, investigation, resources, review and editing. **Geneviève Favre**: Methodology, investigation, resources, review and editing. **Jan Dirks**: Methodology, investigation, resources, review and editing. **Anna Wieland**: Investigation, review and editing. **Heike Faeth**: Data curation and formal analysis. **Renata Pereira**: Formal analysis. **Thomas Kühne**: Conceptualisation, methodology, review, editing, supervision, project administration and funding acquisition.

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CONFLICT OF INTEREST STATEMENT

Schifferli: *Sobi*: Honoraria; *Novartis*: Honoraria, research funding, *Amgen*: research funding. Rovo: *Novartis*: research funding; *AG Alexion*: Honoraria; *BMS*: Honoraria; *OrPhaSwiss GmbH*: Honoraria; *Swedish Orphan Biovitrum AG*: Honoraria; *Amgen*: Other: financial support for congresses and conference travel; *AstraZeneca*: Other; *BMS*: Other; *Sanofi*: Other; *Roche*: Other; *AstraZeneca*: Honoraria; *Novartis*: Honoraria; *CSL Behring*: research funding; *AG Alexion*: research funding. Kühne: *Novartis*: research funding; *UCB*: Honoraria; *SOBI*: Honoraria; *Amgen*: research funding. All other authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All immunological end-points were included in the Shiny app. The app allows exploration of the change in each parameter over time and offers the ability to track the relationship of two selected concentrations over time. Password access to this app and protocol can be requested from the authors via email.

ETHICS STATEMENT

The study was approved by the ethical committee of Northwest and Central Switzerland and conducted in accordance with the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All patients provided informed consent and met the inclusion criteria.

CLINICAL TRIALS REGISTRATION

clinicaltrials.gov: NCT02760251.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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