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ORIGINAL ARTICLE

Evaluation of bleeding and thrombotic events during long-term use of romiplostim in patients with chronic immune thrombocytopenia (ITP)

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Summary. *Background:* Romiplostim is a peptibody protein that raises platelet counts during long-term treatment of patients with chronic immune thrombocytopenia (ITP). Clinical outcomes related to increased platelet counts include a reduced risk of bleeding and a potential risk of thrombosis. *Objective:* To evaluate bleeding and thrombotic events occurring in chronic ITP patients during two phase 3, randomized, placebo-controlled, 24-week studies of romiplostim and during subsequent treatment in an open-label extension study. *Patients/Methods:* In the phase 3 trials, 125 patients were randomized to romiplostim or placebo; romiplostim dose was adjusted to maintain platelet counts of $50–200 \times 10^9$ L^{-1}. Patients who completed the phase 3 trials could enroll in the extension study in which all patients received romiplostim. *Results:* In the phase 3 trials, a significantly greater percentage of patients treated with placebo (34%) had bleeding adverse events of moderate or greater severity than did patients treated with romiplostim (15%, $P = 0.018$). In the extension study, the incidence of bleeding adverse events of moderate or greater severity decreased from 23% of patients in the first 24 weeks to 12% after 24–48 weeks, remaining ≤ 6% thereafter. The exposure-adjusted incidence of thrombotic events was 0.1 per 100 patient-weeks in the phase 3 studies, and 0.08 per 100 patient-weeks in the extension study where patients received romiplostim for up to 144 additional weeks. *Conclusions:* The incidence and severity of bleeding was decreased in chronic ITP patients treated with romiplostim compared with placebo, and the incidence of thrombotic events was not different between the two groups.

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Introduction

Chronic immune thrombocytopenia (ITP) is an autoimmune disorder that is primarily associated with antibody-mediated platelet destruction [1–6]. Recent evidence, however, suggests that decreased platelet production also plays a role in ITP [7,8].

Clinical studies in patients with ITP have demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction. Romiplostim (Nplate®, AMG 531) is an Fc-peptide fusion protein (peptibody) [9,10] that has been shown to produce a dose-dependent increase in platelet counts in healthy individuals [11] and improve platelet counts during both short- and long-term use in patients with chronic ITP [9,12]. In addition, romiplostim was observed to be well tolerated and to produce sustained increases in platelet counts in splenectomized and non-splenectomized patients with ITP in two, phase 3, randomized, placebo-controlled, studies [13]. In these studies, a durable platelet response (platelet count $\geq 50 \times 10^9$ L^{-1} during ≥ 6 of the last 8 weeks of a 24-week treatment period without use of rescue medications) was achieved by 49% of romiplostim-treated patients but only 2% of placebo-treated patients ($P < 0.0001$) despite the greater use of concurrent ITP medications in the placebo group. Specifically, 60% of patients in the placebo group required ITP rescue medications to treat or prevent bleeding (compared with 22% in the romiplostim group) and only 38% of placebo patients were able to reduce or discontinue their concurrent ITP medications (compared with 87% in the romiplostim group) [13]. These results suggest that stimulation of platelet production may provide a unique and effective approach to ITP treatment.

The long-term safety of romiplostim therapy in ITP is being evaluated in an ongoing long-term, open-label extension study. Several interim analyses of the extension study have been conducted and the overall safety findings for patients treated

for up to 156 weeks as of July 2007 have been published [14]. Because romiplostim exhibits its biological effect on platelet production, adverse events of particular interest are those that might result from platelet counts significantly below or above the target platelet range ($50–200 \times 10^9$ L^{-1}); specifically, bleeding and thrombotic events.

Summaries of the incidence of bleeding and thrombotic events in the phase 3 studies and in the extension study have been published previously [13,14]; however, these events were not prespecified endpoints of the studies. We have conducted a detailed *post hoc* analysis to: (i) evaluate effects of romiplostim treatment on the incidence and severity of bleeding, and the relationship between platelet counts and bleeding events; and (ii) more fully describe the thrombotic events that occurred in these studies and the associated risk factors, and determine the effect of long-term romiplostim treatment on the rate of thrombotic events. The incidence of bleeding and thrombotic events among ITP patients who participated in the phase 3 romiplostim studies was analyzed during their treatment with romiplostim both in the phase 3 studies and in the open-label extension study. At this time, the long-term extension study is still ongoing and this analysis represents an interim analysis of the data as of July 2008.

Materials and methods

Study design

Two phase 3, randomized, prospective, multicenter, double-blind, placebo-controlled, 6-month clinical studies (one with splenectomized patients and one with non-splenectomized patients) were conducted to evaluate the efficacy and safety of romiplostim in patients with ITP. These phase 3 studies have been described in detail elsewhere [13], and their protocols are summarized briefly below. Upon completion of either phase 3 study, patients were eligible for enrollment in a long-term, open-label, extension study in which all patients were treated with romiplostim.

Patients

This report presents an analysis of bleeding and thrombotic events that occurred in patients during their participation in either of the phase 3 studies and from their time in the long-term open-label extension study (Fig. 1). Patients were eligible for the phase 3 studies if they were adults with a diagnosis of chronic ITP (according to American Society of Hematology guidelines) [4], and had a mean platelet count $< 30 \times 10^9$ L^{-1}, no active malignancy or history of stem cell disorder, creatinine ≤ 2 mg dL^{-1}, bilirubin ≤ 1.5 times the upper limit of normal, and hemoglobin ≥ 9.0 g dL^{-1}. Patients > 60 years of age were required to have a bone marrow examination consistent with the diagnosis of ITP. Unlike other ITP clinical studies, there were no exclusions based on thrombotic risk factors or previous thrombotic events and patients' history of thrombotic risk factors was not collected routinely. There was no upper age limit for patient inclusion. On completion of the phase 3 study, patients were eligible to enroll in the open-label extension study. Patients were excluded from enrollment in the extension study if any bone marrow stem cell disorders or new active malignancies had been diagnosed since enrollment in the phase 3 study. Patients could not have been treated with any alkylating agents within 4 weeks before the screening visit for the extension study. Institutional Review Boards at each center approved the phase 3 and extension study protocols. All patients gave written informed consent prior to participation in each study in which they participated.

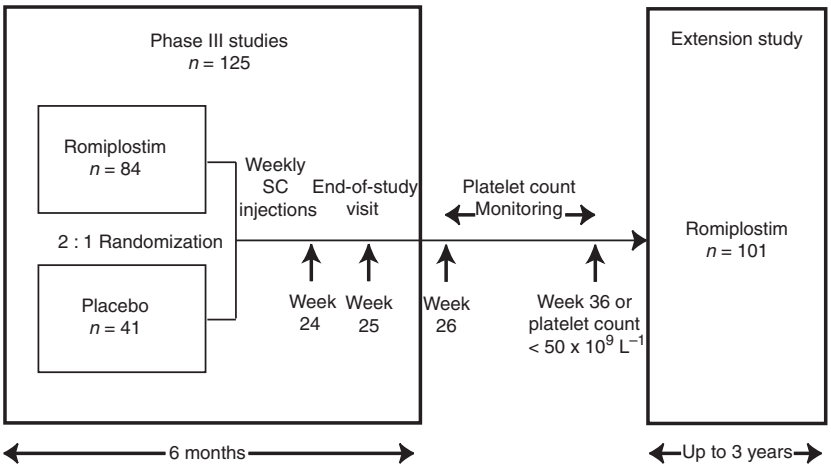

Fig. 1. Study design. Bleeding and thrombotic events reported in patients who participated in one of two phase 3 clinical studies were analyzed in this report; additional data from their participation in an open-label extension study are presented. In the phase 3 studies, patients were randomized 2:1 to receive romiplostim or placebo for 24 weeks. Patients who completed the phase 3 studies were eligible to enroll in the extension study when their platelet counts were $< 50 \times 10^9$ L^{-1}. All patients in the extension study were administered romiplostim. Primary results from the phase 3 and extension studies, including platelet responses and summaries of adverse events, have been described elsewhere [13,14].

Treatment protocol

During the phase 3 studies, patients were randomized 2:1 to receive weekly subcutaneous injections of romiplostim or placebo for 24 weeks. The dose was adjusted to achieve a target platelet count of $50–200 \times 10^9$ L^{-1}. Study treatment was discontinued at 24 weeks, and all patients who completed week 24 had an end-of-study visit at week 25. Platelet counts were monitored starting at week 26 and continuing through week 36 or until platelet counts were $< 50 \times 10^9$ L^{-1}, whichever came first. Patients who completed the phase 3 studies were eligible for entry into the open-label extension study.

Upon entering the extension study, patients who had been treated with romiplostim during the phase 3 study started with the same weekly dose they received at the end of that study unless > 24 weeks has passed since the last romiplostim injection. These patients and those who had received placebo during the phase 3 study started the extension study with 1 mcg kg^{-1} per week. Dose adjustments were made based on platelet counts. Additional ITP therapy necessary for patient safety (ITP rescue medication) could be administered at any time during the study.

Outcome measures

The evaluations of bleeding and thrombotic adverse events reported here were performed as *ad hoc* analyses and were not predefined endpoints. These analyses included incidence and severity of bleeding and thrombotic adverse events as well as a review of the patient's available medical history and platelet count at or near the time of the event.

During the phase 3 studies, patients were evaluated during weekly visits to the clinical study site. During the extension study, all patients returned to the clinic weekly through to week 4. Thereafter, patients who met criteria for self-administration and elected to self-administer were required to return for ongoing evaluation every 4 weeks; all other patients were required to return weekly. Assessments of platelet counts and adverse events were made at each visit and reports of adverse events were collected continuously. All adverse events were rated by the clinical investigator on a scale of 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, and 5 = fatal. An event was defined as: mild if the patient was aware of the sign or symptom, but tolerated it easily; moderate if it caused enough discomfort to interfere with usual activity; and severe if it was incapacitating, making it impossible to work or engage in usual activities. A rating of life-threatening referred to an event in which the patient was at risk of death at the time of the event. An adverse event was considered serious if it was fatal, life-threatening, required hospitalization or prolongation of existing hospitalization, or resulted in persistent or significant disability/incapacity. Bleeding adverse events were considered clinically significant if they met the Amgen adverse event grading scale of grade 3 or higher.

Statistical analysis

The analysis of bleeding and thrombotic adverse events during the phase 3 studies included data from all patients who received at least one dose of study medication (either romiplostim or placebo) in either phase 3 study. There was no difference in the adverse event profile between splenectomized and non-splenectomized patients[13], and therefore data from the two phase 3 studies were pooled for all analyses. Analyses of bleeding and thrombotic adverse events that occurred during the extension study are reported up to the time of the latest data analysis cut-off date (July 2008).

The patient incidence of a particular adverse event was defined as the number of patients experiencing the event divided by the total number of patients. The adverse event incidence per 100 patient weeks was calculated from the total number of reports of an adverse event, divided by the total number of patient weeks on study, multiplied by 100.

Changes in patient and event incidence over time were summarized for each 24-week period during the extension study. Data from patients still enrolled at the beginning of each period were included in the assessment of the rates for that period.

The *P*-value for the between-group (romiplostim vs. placebo) comparison of patient incidence of bleeding adverse events by maximum severity distribution was determined using the Cochran–Mantel–Haenszel (CMH) test based on a grouping of the patients into three categories: (i) patients with no bleeding adverse events; (ii) those with highest bleeding adverse event severity of grade 1 (mild); and (iii) those with highest bleeding adverse event severity of grade 2 or above (moderate, severe, life-threatening, or fatal). The CMH test was stratified by splenectomy status. Other *P*-values for the between-group (romiplostim vs. placebo) comparison of bleeding adverse events were from CMH test stratified by splenectomy status.

Results

Study population

A total of 125 patients (41 in the placebo groups, 84 in the romiplostim groups) were enrolled in the two phase 3 studies and all received at least one dose of study medication. Of the 115 patients who completed the phase 3 studies (35 in the placebo groups, 80 in the romiplostim groups), 101 (33 from the placebo groups, 68 from the romiplostim groups) enrolled in the extension study. Eight placebo-treated and 16 romiplostim-treated patients from the phase 3 studies did not participate in the extension study. At the time that the database was locked for the present analysis, phase 3 study patients had been treated in the extension study for up to 144 weeks. Patient disposition, including the numbers of patients who discontinued and reasons for doing so, has been described elsewhere [13,14].

Baseline characteristics and patient demographics for the phase 3 studies have been reported previously and are

summarized in Table 1. All characteristics were similar in the placebo and romiplostim treatment groups. Thirty-five per cent of the population was at least 60 years old and more than half of participants (60%) had been diagnosed with ITP at least 3 years prior to study entry. The median platelet count was 16×10^9 L^{-1}.

Patient-reported prior history of selected bleeding symptoms is shown in Table 2. At the time of enrollment in the phase 3 studies, approximately half of all women reported current or past menorrhagia. Approximately 54% of all patients reported current or past oral bleeding, 20% reported current or past gastrointestinal bleeding, and 6% had a history of intracranial bleeding.

Bleeding adverse events

Bleeding adverse events during the phase 3 studies In the phase 3 studies, 25 patients (61%, 25/41) experienced 77 bleeding adverse events in the placebo group, and 48 patients (57%, 48/84) experienced 172 bleeding adverse events in the romiplostim group ($P = 0.68$; Table 3). The number of bleeding adverse events per 100 patient-weeks was very similar between the placebo (7.9) and romiplostim (7.8) groups. The median number of events per patient was one in both groups, and 24.4% of patients in the placebo group had four or more bleeding adverse events compared with 15.5% of patients in the romiplostim group ($P = 0.23$).

In the romiplostim group, the majority of patients with bleeding adverse events had events rated as mild (grade 1) in severity, while in the placebo group the majority of patients with bleeding adverse events had events rated as at least grade 2 in severity (i.e. at least moderate) (Fig. 2). Overall, a significantly greater percentage of patients in the placebo group had bleeding adverse events of at least grade 2 severity than did patients treated with romiplostim (placebo, 14/41, 34%; romiplostim, 13/84, 15%; $P = 0.018$). A consistent trend was observed in both the splenectomized and non-splenectomized populations: the proportion of splenectomized patients with bleeding events grade 2 and above was 38% (8/20) in the placebo group and 21% (9/42) in the romiplostim group; the proportion of non-splenectomized patients with bleeding events grade 2 and above was 30% (6/20) in the placebo group and 10% (4/42) in the romiplostim group. The percentage of patients with bleeding events of at least grade 3 severity (i.e. severe, life-threatening, or fatal) was 12% (5/41) in the placebo group and 7% (6/84) in the romiplostim group ($P = 0.36$). None of the patients with bleeding events of at least grade 3 severity achieved a durable platelet response (platelet count $\geq 50 \times 10^9$ L^{-1} during ≥ 6 of the last 8 weeks of treatment) during the phase 3 study.

Nine patients experienced bleeding events considered serious (placebo, 4/41, 9.8%; romiplostim, 5/84, 6.0%). Two patients died as a result of a bleeding event (both from intracranial hemorrhage). One patient who received placebo for 9 weeks experienced an intracranial hemorrhage when their platelet count was 19×10^9 L^{-1}. One romiplostim-treated patient experienced a cerebral vascular accident 3 days after their last romiplostim dose. Romiplostim was discontinued and the patient treated with antiplatelet and antihypertensive medications. The intracranial hemorrhage occurred 7 days after the cerebrovascular accident (10 days after discontinuation of romiplostim and 1 day after study completion) at a platelet count of 5×10^9 L^{-1}.

In the phase 3 studies, the most common bleeding adverse events were epistaxis, petechiae, gingival bleeding and injection site bruising (Table 3). Intracranial and gastrointestinal bleeding were rare, occurring in five patients (Table 3). All patients exhibiting such events had a past history of bleeding, which included intracranial and gastrointestinal bleeding in all but one patient.

Table 1 Patient demographics and baseline characteristics

	Placebo ($n = 41$)	Romiplostim ($n = 84$)	Total ($n = 125$)
Age (years)			
Median (min, max)	52 (23, 88)	52 (21, 88)	52 (21, 88)
≥ 60, *n* (%)	18 (44)	26 (31)	44 (35)
Women, *n* (%)	26 (63)	55 (66)	81 (65)
Race, *n* (%)			
White or Caucasian	37 (90)	65 (77)	102 (82)
African American	3 (7)	6 (7)	9 (7)
Other*	1 (2)	13 (15)	14 (11)
Weight (kg)			
Median (min, max)	81 (52, 169)	78 (44, 138)	79 (44, 169)
Years since ITP diagnosis			
Median (min, max)	4.3 (0.1, 31.4)	4.5 (0.1, 44.8)	4.3 (0.1, 44.8)
≥ 3 years, *n* (%)	23 (56)	52 (62)	75 (60)
Baseline platelet count (10^9 L^{-1})†			
Median (min, max)	18 (2, 31)	16 (2, 29)	16 (2, 31)
Receiving concurrent ITP therapy at baseline, *n* (%)	15 (37)	24 (29)	39 (31)

*Includes: Hispanic or Latino, Asian, and Native Hawaiian or Other Pacific Islander. †Baseline platelet count = mean of platelet counts from 1, 2 and 8 days before study start.

Table 2 Patient-reported history of bleeding symptoms

	Placebo (n = 41) n (%)	Romiplostim (n = 84) n (%)	Total (n = 125) n (%)*
Epistaxis			
Past	19 (46)	42 (50)	61 (49)
Current (ongoing at screening)	5 (12)	7 (8)	12 (10)
Purpura/petechiae			
Past	14 (34)	31 (37)	45 (36)
Current (ongoing at screening)	17 (42)	40 (48)	57 (46)
Oral bleeding			
Past	17 (41)	32 (38)	49 (39)
Current (ongoing at screening)	7 (17)	11 (13)	18 (14)
Bruising			
Past	10 (24)	27 (32)	37 (30)
Current (ongoing at screening)	25 (61)	49 (58)	74 (59)
Intracranial bleeding			
Past	3 (7)	4 (5)	7 (6)
Current (ongoing at screening)	0 (0)	0 (0)	0 (0)
Gastrointestinal bleeding			
Past	7 (17)	16 (19)	23 (18)
Current (ongoing at screening)	0 (0)	2 (2)	2 (2)
Menorrhagia	(n = 26 females)	(n = 55 females)	(n = 81 females)
Past	8 (31)	19 (35)	27 (33)
Current (ongoing at screening)	5 (19)	9 (16)	14 (17)

*Patients not included here had no history of the bleeding event listed.

Relationship between platelet counts and bleeding adverse events Across both treatment groups in the phase 3 studies, most bleeding adverse events (of any severity) occurred at platelet counts at or below 20×10^9 L^{-1} (Fig. 3). All but one grade 2 event occurred at counts at or below 20×10^9 L^{-1}, and no events of grade 3 or higher severity occurred at platelet counts above 20×10^9 L^{-1} (Fig. 3). No bleeding adverse events of grade 2 or higher severity occurred at platelet counts above 50×10^9 L^{-1}.

In the phase 3 studies, bleeding adverse events of any severity were reported in a lower percentage of romiplostim patients who achieved the primary efficacy endpoint of a durable platelet response than among those not achieving a durable response (responders, 44%; 18/41; non-responders, 63%; 27/43; P = 0.083). Only one patient in the romiplostim group who achieved a durable platelet response experienced a bleeding event of grade 2 or higher severity (2%, 1/41) as compared with 26% (11/43) of those not achieving a durable response. The grade 2 bleeding event in the responding patient occurred before the durable response began. Bleeding adverse events of grade 3 or higher severity were reported for none of the patients in the romiplostim group who achieved a durable platelet response and 14% (6/43) of those not achieving a durable platelet response.

We evaluated the effect of dose-holding on bleeding event incidence. In the phase 3 studies, there were 111 weeks when the dose of romiplostim was withheld for the following reasons: per protocol (platelet counts over 400×10^9 L^{-1}) (n = 72), non-compliance (n = 15), adverse event (n = 8), dose administration error (n = 2), and other (n = 14). The median platelet count within 1 week after romiplostim dose withholding was 82×10^9 L^{-1}, and bleeding events occurred on 12 occasions within 1 week after a withheld romiplostim dose; a rate of 10.8 bleeding events per 100 patient-weeks of dose-withholding. This was similar to the overall rate of bleeding adverse events in the phase 3 romiplostim group (7.8 per 100 patient-weeks), suggesting there was no difference in the incidence of bleeding adverse events occurring within 1 week following a withheld dose of study medication, and the incidence of events occurring at any other time during the study.

Bleeding adverse events during the long-term extension study During the long-term extension study, the percentage of phase 3 study patients experiencing a bleeding event of any severity dropped from 48% (48/101) during weeks 1–24 of the extension study to 30% (28/93) during weeks 24–48 (Fig. 4A). There was a steady decrease in the percentage of patients experiencing bleeding adverse events of grade 2 or higher severity; from 23% (23/101) during weeks 1–24, to 12% (11/93) during weeks 24–48. Eight patients discontinued the study during the first 24 weeks. Reasons for discontinuing were consent withdrawn (five patients), administrative decision (one patient), adverse event (one patient) and death (one patient). Five of these eight patients achieved a platelet count $> 50 \times 10^9$ L^{-1} before discontinuing and six experienced a bleeding event. The proportion of patients who experienced a bleeding event was similar between those who previously received placebo and those who previously received romiplostim (data not shown). The event incidence for all bleeding adverse events decreased from 5.4 per 100 patient-weeks during weeks 1–24, to 2.3 per 100 patient-weeks during

Table 3 Patient incidence of bleeding adverse events. (A) Phase 3 studies. (B) Extension study

(A) Phase 3 studies

	Placebo *n* = 41 *n* (%)	Romiplostim *n* = 84 *n* (%)
Patients reporting bleeding adverse events (all types)*	25 (61)	48 (57%)
Most common bleeding adverse events†		
Epistaxis	10 (24)	27 (32)
Petechiae	9 (22)	14 (17)
Gingival bleeding	5 (12)	9 (11)
Injection site bruising	2 (5)	8 (10)
Oral mucosal blistering	3 (7)	7 (8)
Ecchymosis	6 (15)	6 (7)
Hematoma	1 (2)	6 (7)
Menorrhagia/metrorrhagia‡	1 (4)	4 (7)
Injection site hematoma	3 (7)	1 (1)
Other bleeding adverse events		
Intracranial bleeding/cerebral hemorrhage	2 (5)	1 (1)
Gastrointestinal bleeding	1 (2)	2 (2)

*P = 0.68 for placebo vs. romiplostim. †Those occurring in at least 5% of patients in either treatment group. ‡Percentages were calculated based on the number of female patients (n = 26 for placebo; n = 55 for romiplostim).

(B) Extension study

	≤ 24 weeks *n* = 101 *n* (%)	≤ 24 to ≤ 48 weeks *n* = 93 *n* (%)	≤ 48 to ≤ 72 weeks *n* = 82 *n* (%)	< 72 to ≤ 96 weeks *n* = 78 *n* (%)	< 96 to ≤ 120 weeks *n* = 66 *n* (%)	> 120 weeks *n* = 34 *n* (%)
Any bleeding adverse event	48 (48)	28 (30)	18 (22)	16 (21)	10 (15)	1 (3)
Most common bleeding adverse events*						
Epistaxis	20 (20)	8 (9)	7 (9)	8 (10)	7 (11)	1 (3)
Petechiae	12 (12)	8 (9)	4 (5)	5 (6)	1 (2)	0
Gingival bleeding	9 (9)	3 (3)	3 (4)	2 (3)	0	0
Hematoma	9 (9)	2 (2)	0	1 (1)	0	0
Blood blister	7 (7)	3 (3)	3 (4)	2 (3)	1 (2)	0
Ecchymosis	6 (6)	2 (2)	2 (2)	2 (3)	0	0 (0)
Conjunctival hemorrhage	5 (5)	0	0	0	0	0
Other bleeding events						
Gastrointestinal hemorrhage	1 (1)	1 (1)	0	0	0	0
Upper gastrointestinal hemorrhage	1 (1)	0	0	0	0	0

*Those occurring in at least 5% of patients.

weeks 24–48 (Fig. 4B). The event incidence for bleeding adverse events of grade 2 or higher severity decreased from 2.0 per 100 patient-weeks during weeks 1–24, to 0.5 per 100 patient-weeks during weeks 24–48.

The most common bleeding adverse events among phase 3 study patients in the long-term extension study were epistaxis, petechiae, oral mucosal blistering and hematoma (Table 3B). There were two cases of gastrointestinal bleeding; one during the first 12 weeks of the extension study and one between weeks 36 and 48. There were no cases of intracranial bleeding.

Thrombotic adverse events

Incidence and descriptions of thrombotic events Brief case descriptions for all patients experiencing thrombotic events are provided in Table 4. In the phase 3 studies, thrombotic events occurred in one patient in the placebo group (1/41, 2.4%) and two patients in the romiplostim group (2/84, 2.4%; Table 5A), with an event incidence of 0.1 per 100 patient-weeks in both groups. These events included one case of fatal pulmonary embolism in the placebo group and one case each of cerebrovascular accident (life-threatening) and right popliteal arterial embolism (severe) in the romiplostim group.

During long-term treatment in the extension study, eight additional thrombotic adverse events occurred in four more patients; patient incidence was 4%, with an event incidence similar to that in the phase 3 studies (0.08 per 100 patient-weeks; Table 5B). These included one patient with coronary artery occlusion and one with a superficial vein thrombosis. The other two patients had multiple events; one had two incidents of myocardial infarction and a pulmonary embolism and one experienced septic jugular vein thrombosis and inflammatory venous thrombosis at the site of a suspected catheter-related infection, and a transient cerebral ischemic attack. Except for the coronary artery occlusion, all of these new events were considered serious by the investigator. The

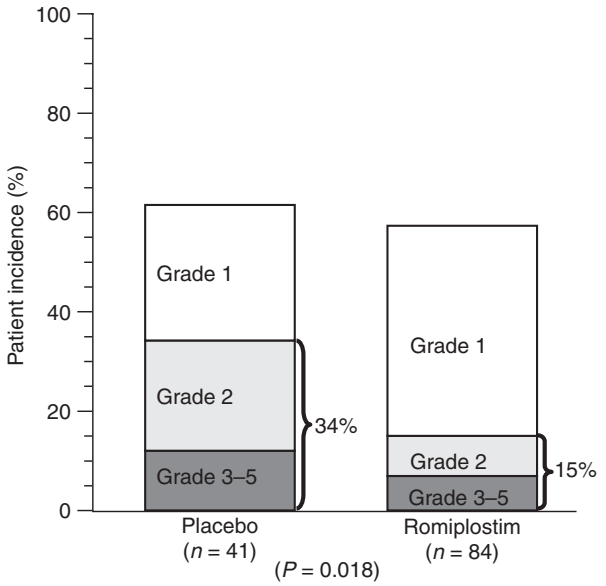

Fig. 2. Patient incidence of bleeding adverse events by maximum severity in the phase 3 studies. Patients treated with romiplostim experienced milder bleeding adverse events than patients in the placebo group. A significantly greater percentage of patients in the placebo group had bleeding adverse events of grade 2 or higher in severity than did patients treated with romiplostim ($P = 0.018$).

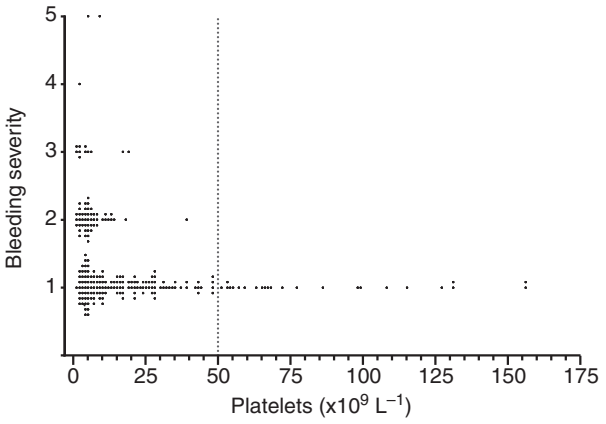

Fig. 3. Distribution of bleeding adverse events by severity and platelet count in both treatment groups in the phase 3 studies. Each point represents one bleeding adverse event. One grade 1 bleeding adverse event that occurred at a platelet count of 505×10^9 L^{-1} is not shown.

coronary artery occlusion was rated by the investigator as mild (responded to medical therapy and did not require hospitalization).

Across both phase 3 studies and the extension study, patients evaluated for thrombotic events had been exposed to a median of 116 weeks of romiplostim therapy (minimum, 5 weeks; maximum, 168 weeks). The thrombotic adverse event rate across all studies was 0.09 events per 100 patient-weeks on romiplostim therapy.

All patients who experienced thrombotic adverse events in either the phase 3 or extension studies had pre-existing risk factors for cardiovascular disease or for thrombosis (Table 4). These included congestive heart failure, antiphospholipid antibodies, coronary artery disease, hypertension, cancer, and/or a history of thrombotic events. Four patients with thrombotic events were post-splenectomy and three patients were not.

One patient in the extension study experienced two events of myocardial infarction, occurring at platelet counts of 230 and 948×10^9 L^{-1}, with a maximum platelet count of 1359×10^9 L^{-1} 8 days prior to the second event (Table 4). The patient had a prior history of coronary and carotid artery disease, peripheral vascular disease, 40 pack-years of smoking and cardiomyopathy and had discontinued aspirin 1 week before the first myocardial infarction. In between the two myocardial infarction events the patient was hospitalized for rhabdomyolysis (day 98) and cardiac failure (day 102); neither event was considered treatment related. Both of the events of myocardial infarction resolved within 1 week and neither resulted in study withdrawal. The romiplostim dose was subsequently reduced from 9 to 5 μg kg^{-1} as per the study protocol; after this time platelet counts remained generally within the target range, with no platelet counts above 400×10^9 L^{-1}. At the time of data cut-off the patient had received romiplostim for 128 weeks in the extension study.

Platelet counts and thrombotic events The rate of thrombotic events per 100 patient-weeks of platelet counts above and below 200 and 500×10^9 L^{-1} was calculated. As expected from the romiplostim dosing algorithm, patients spent less time with platelet counts $> 200 \times 10^9$ L^{-1} (1640 weeks) and $> 500 \times 10^9$ L^{-1} (146 weeks) than with platelet counts $\leq 200 \times 10^9$ L^{-1} (9438 weeks) and $\leq 500 \times 10^9$ L^{-1} (10932 weeks). Three thrombotic events occurred at a platelet count $> 200 \times 10^9$ L^{-1} at a rate of 0.18 per 100 patient-weeks and eight thrombotic events occurred at a platelet count $\leq 200 \times 10^9$ L^{-1} at a rate of 0.08 per 100 patient-weeks. One thrombotic event occurred at a platelet count $> 500 \times 10^9$ L^{-1} at a rate of 0.68 per 100 patient-weeks and 10 thrombotic events occurred at a platelet count $\leq 500 \times 10^9$ L^{-1} at a rate of 0.09 per 100 patient-weeks.

Discussion

Romiplostim has been shown to produce a sustained increase in platelet count in patients with chronic ITP in 2, phase 3, randomized, 6-month, controlled clinical studies [13]. The present analysis demonstrates that patients treated with romiplostim for 6 months experienced significantly fewer bleeding adverse events of grade 2 or higher severity than did patients treated with placebo, while the incidence of thrombotic events remained similar in the two groups. During longer-term treatment of phase 3 patients with romiplostim, there was a steady decrease in severe bleeding and overall bleeding frequency, without any apparent increase in incidence of thrombosis.

During the phase 3 studies, the patient incidence of bleeding events grade 2 or higher severity was significantly lower among

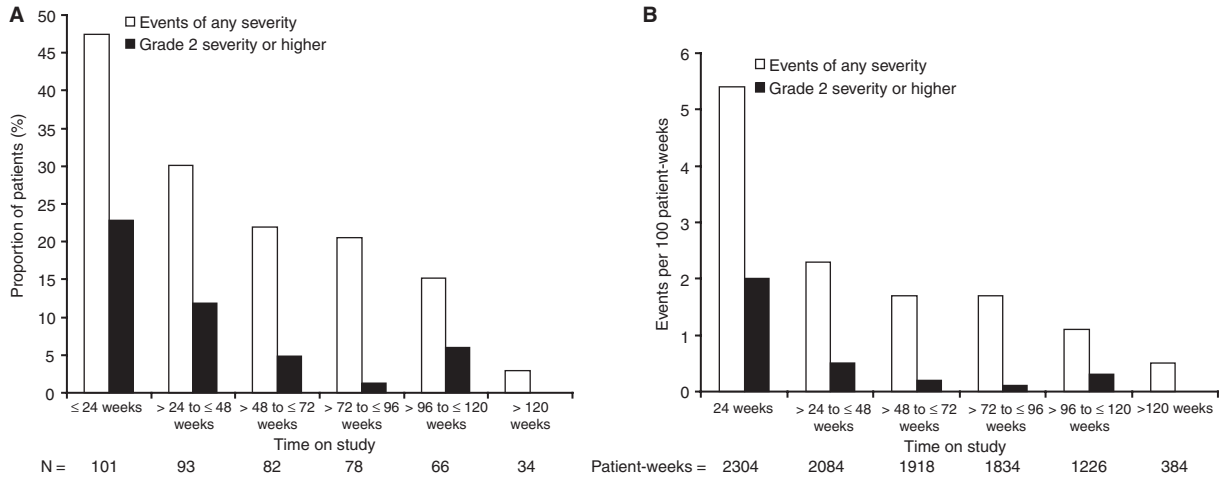

Fig. 4. Bleeding adverse events in each 12-week period during the extension study. (A) Patient incidence. (B) Event incidence.

patients treated with romiplostim than among those treated with placebo, despite the markedly greater use of ITP rescue medications within the placebo group [13]. This suggests the use of rescue therapies such as IVIg or steroids as a management strategy for bleeding or severe thrombocytopenia was not as effective at preventing clinically relevant bleeding as was the durable increase in platelet counts produced by romiplostim.

The present analyses demonstrate an inverse correlation between platelet count and both bleeding adverse event rate and bleeding adverse event severity. In the phase 3 studies, a target platelet count of $\geq 50 \times 10^9$ L^{-1} was achieved by $> 25\%$ of romiplostim-treated patients after just one dose and more than 50% after three doses (week 4) [13]. This proved to be an important target as no bleeding events of grade 2 or higher severity occurred at counts above 50×10^9 L^{-1}. All but one grade 2 event occurred at platelet counts $< 30 \times 10^9$ L^{-1} and no event of grade 3 or higher severity occurred at platelet counts above 20×10^9 L^{-1} (Fig. 3).

In the open label extension study 87% of all patients achieved a platelet response (platelet count $\geq 50 \times 10^9$ L^{-1} and double baseline in the absence of rescue medication within the preceding 8 weeks) [14], and the phase three patients treated in this study had a steady decrease in the number and severity of bleeding adverse events over time. With such a trend, one must consider if the change in clinical outcomes is due to withdrawal of increasing numbers of non-responders as the study continues. However, in the long-term study described here, few patients withdrew from the study, and only a small proportion of patients who withdrew had experienced serious bleeding events. The 24 patients who withdrew are unlikely to account for the marked changes in bleeding adverse event incidence during the extension study because of the large number of patients for whom long-term data were available ($n = 101$ for the first 24 weeks; $n = 93$ for weeks 24–48). More likely, the incidence of bleeding decreased over time as the dose of romiplostim was adjusted to achieve the target platelet count of 50×10^9 L^{-1}.

The combined findings from the phase 3 and extension studies suggest that the majority of patients are protected from serious bleeding events at platelet counts above 30×10^9 L^{-1}, supporting published clinical guidelines [4,15]. Our findings also suggest that achievement of a sustained increase in platelet count above 50×10^9 L^{-1} provides a margin of safety for control of bleeding. There is a potential risk of worsened thrombocytopenia following romiplostim withdrawal, and patients who discontinue romiplostim treatment should be closely monitored for platelet counts and risk of bleeding. The present *post hoc* analysis is currently the largest published study of bleeding events over the longest period of observation and their relation to platelet counts in patients with ITP provides validation of current clinical practice.

In this analysis, the incidence of thrombotic events in ITP patients treated with romiplostim (2.4%) was not different from that seen in patients receiving placebo. The event incidence was 0.1 per 100 patient-weeks in both treatment groups in the phase 3 study, and remained low (0.08 per 100 patients-weeks) in the extension study when all patients were receiving romiplostim. This low incidence of thrombotic events is notable considering that our analysis included patients who had been treated for up to 168 weeks with romiplostim. All patients who had a thrombotic event had pre-existing risk factors for cardiovascular disease, or for thrombosis, and/or a history of a thrombotic event. Regardless of treatment, patients who experienced thrombotic events were not observed to have more marked increases in their platelet count than patients without these events. When we looked at the rate of thrombotic events at platelet counts above the recommended target range ($50–200 \times 10^9$ L^{-1}), there was an apparently higher event rate when platelet counts were above 200×10^9 L^{-1} or above 500×10^9 L^{-1} than when platelet counts were below these thresholds. The number of thrombotic events reported was small, and additional studies will be required to fully determine whether an association between platelet counts and thrombotic events exist. Nonetheless, these results support the use of romiplostim in ITP patients to maintain the lowest platelet

Table 4 Case descriptions for patients experiencing thrombotic events

Study	Age (Years)	Sex (M/F)	Splenectomy (Y/N)	Thrombotic event	Led to study withdrawal (Y/N)	Start (Study day)	Platelet count at time of event* ($\times 10^9$ L^{-1})	Max platelet count prior to event* (days prior)	Max dose romiplostim prior to event (μg kg^{-1})	Known risk factors prior to receiving investigational product†
Phase 3	80	M	N	Cerebrovascular accident	N§	143	107	167 (73)	3	Congestive heart failure Hypertension Rectal cancer Venous stasis
Phase 3	82	M	Y	Popliteal arterial embolism	N	94	11	533 (51)	3	Diastolic dysfunction Coronary artery disease Congestive cardiac failure Atrial fibrillation Thrombolectomy of radial, ulnar and bronchial artery Cardiac pacemaker insertion
Phase 3	51	F	Y	Pulmonary embolism	Y	131	3	69 (89)	0 (placebo group)	Hypertension
Extension	84	F	N	Myocardial infarction	N	60	230	45 (23)	9	Congestive heart failure
				Myocardial infarction	N	136	948	1359 (8)	9	Hypertension
				Pulmonary embolism	N	561	149	1359 (433)	9	Edema Smoking
Extension	67	F	Y	Superficial vein thrombosis	N	156	47	408 (189)	10	DVT Hypertension Hypercholesterolemia Factor V Leiden
Extension	83	F	N	Coronary artery occlusion	N	311	220	301 (73)	2	Hypothyroidism Ankle edema
Extension§	57	F	Y	Septic jugular vein thrombosis	Y	95	40	1084 (74)	3	Varicose vein operation Antiphospholipid antibodies
				Inflammatory venous thrombosis	N	95	40	1084 (74)	3	Indwelling Port-a-Cath**
				Transient cerebral ischemic attack	N	152	49	1084 (131)	3	

*If the platelet count at the time of event was not available, the closest preceding platelet count was used. Platelet counts are $\times 10^9$ L^{-1}. †Includes only risk factors present before initiating treatment with investigational product. ‡Received IV Ig 4 days prior to thrombotic event for the treatment of gastrointestinal hemorrhage. §Led to treatment discontinuation. **The events of septic jugular vein thrombosis and inflammatory venous thrombosis occurred at the site of the Port-a-Cath that had been in place for 13 years.

Table 5 Thrombotic events. (A) Phase 3 studies. (B) Extension study

(A) Phase 3 studies

	Placebo	Romiplostim
Total number of patients	41	84
Patient incidence of thrombotic/thromboembolic adverse events: *n* (%)	1 (2.4%)	2 (2.4%)
Total number of patient-weeks on study	985	2200
Total number and event incidence of thrombotic/thromboembolic adverse events: *n* (*r*)*		
All	1 (0.1)	2 (0.09)
Cerebrovascular accident	0 (0.0)	1 (0.05)
Popliteal arterial embolism	0 (0.0)	1 (0.05)
Pulmonary embolism	1 (0.1)	0 (0.0)

*r = Event incidence = Event rate per 100 patient-weeks = (number of events/Pt-wk) × 100.

(B) Extension study

All patients	
Total number of patients	101
Patient incidence of thrombotic/thromboembolic adverse events: *n* (%)	4 (4%)
Total number of patient-weeks on study	9750
Total number and event incidence of thrombotic/thromboembolic adverse events: *n* (*r*)*	
All	8 (0.08)
Myocardial infarction	2 (0.02)
Thrombosis	2 (0.02)
Coronary artery occlusion	1 (0.01)
Peripheral septic thrombophlebitis	1 (0.01)
Transient ischemic attack	1 (0.01)
Pulmonary embolism	1 (0.01)

*r = Event incidence = Event rate per 100 patient-weeks = (number of events/Pt-wk) × 100.

count that is protective from bleeding rather than the normal count.

Supraphysiologic doses of thrombopoietin have been shown to facilitate platelet adhesion[16] and aggregation induced by shear stress and various agonists [17] *in vitro*, raising concerns that stimulation of the thrombopoietin receptor by thrombopoietic agents might cause increased platelet responsiveness and activation. However, treatment of patients with advanced cancer with thrombopoietic agents increased platelet count without producing any evidence of *in vivo* activation [18]. Moreover, in studies using a thrombopoietic agent in non-human primates, Harker was unable to show a disproportionate increase in ^{111}In platelet or ^{125}I-fibrin deposition [19] on endarterectomized aorta and vascular grafts. Romiplostim did not increase platelet aggregability in 24 healthy individuals who received doses of the drug shown to be clinically active in many ITP patients [20].

The data reported here are consistent with data reported in a prior study of 186 adult, romiplostim naive, ITP patients in which 5% of patients reported a history of thrombotic and/or ischemic events at some time in the past [21]. Among these patients, venous thrombosis was at least as common as arterial thrombosis. Platelets from patients with ITP have been shown to be activated and platelet microparticles to circulate [22,23], and preliminary data from a proteomic analysis comparing platelets from healthy subjects with those from ITP patients treated with romiplostim showed a difference in expression levels for approximately 11% of platelet proteins [24]. Additional data comparing platelets from treated and untreated ITP patients and healthy controls at the same platelet count will be required to fully evaluate effects of romiplostim on platelet function and activity.

An important limitation of this study was that the assessments of bleeding and thrombotic adverse events were performed as *post hoc* analyses and not as prespecified endpoints in either the phase 3 or long-term extension studies. This limited the amount of detailed information available for full analysis of the relationship between these adverse events and all possible contributing factors.

In summary, 6 months of treatment with romiplostim decreased the severity of bleeding events by raising the platelet count above 50×10^9 L^{-1} in a majority of patients, confirming the relationship between platelet count and bleeding outcomes. Treatment for more than 120 additional weeks with romiplostim further decreased episodes of severe bleeding as platelet counts were maintained. Overall bleeding frequency decreased with duration of time on treatment. Our data suggest that the rate of thrombosis, which appears to be increased at baseline [21], did not increase further with extended exposure to romiplostim and does not appear to be related to the absolute platelet count.

Platelet kinetics, reactivity in ITP, and risk of bleeding and thrombosis are influenced by a variety of factors, therapeutic intervention being only one among them. Emerging therapies targeting platelet production present new opportunities for treatment, but careful consideration of the interplay between all factors affecting platelet count and risks of bleeding and thrombosis is required to maintain a safe equilibrium.

Addendum

Contributions: T. B. Gernsheimer performed the research, interpreted the data, and wrote the paper; J. N. George performed the research, interpreted the data, and wrote the paper; L. M. Aledort performed the research, interpreted the data, and wrote the paper; M. D. Tarantino performed the research and reviewed and edited the paper; U. Sunkara performed the research and reviewed and edited the paper; M. Guo collected, analyzed and interpreted the data, and edited the paper; J. L. Nichol designed the trials, analyzed and interpreted the data, and wrote the paper. All authors provided their approval of the final version of the paper.

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Disclosure of Conflict of Interests

L. M. Aledort and J. N. George have received research support from Amgen Inc. T. B. Gernsheimer has received honoraria from Amgen Inc. T. B. Gernsheimer and J. N. George have acted as a consultant to Amgen Inc.; M. D. Tarantino has acted as a consultant to Biovitrum and is a member of a speakers bureau for GlaxoSmithKline. L. B. Aledort has served on a speakers bureau through a third party for Amgen Inc. and GlaxoSmithKline. M. Guo is an employee of Amgen Inc. and J. L. Nichol was an employee of Amgen Inc. at the time of this study.

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