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Eltrombopag for the treatment of inherited thrombocytopenias: a phase II clinical trial

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ABSTRACT

Patients with inherited thrombocytopenias often require platelet transfusions to raise their platelet count before surgery or other invasive procedures; moreover, subjects with clinically significant spontaneous bleeding may benefit from an enduring improvement of thrombocytopenia. The hypothesis that thrombopoietin-mimetics can increase platelet count in inherited thrombocytopenias is appealing, but evidence is scarce. We conducted a prospective, phase II clinical trial to investigate the efficacy of the oral thrombopoietin-mimetic eltrombopag in different forms of inherited thrombocytopenia. We enrolled 24 patients affected by *MYH9*-related disease, *ANKRD26*-related thrombocytopenia, X-linked thrombocytopenia/Wiskott-Aldrich syndrome, monoallelic Bernard-Soulier syndrome, or *ITGB3*-related thrombocytopenia. The average pre-treatment platelet count was 40.4 x10^9/L. Patients received a 3- to 6-week course of eltrombopag in a dose-escalated manner. Of 23 patients evaluable for response, 11 (47.8%) achieved a major response (platelet count >100 x10^9/L), ten (43.5%) had a minor response (platelet count at least twice the baseline value), and two patients (8.7%) did not respond. The average increase of platelet count compared to baseline was 64.5 x10^9/L (P<0.001). Four patients with clinically significant spontaneous bleeding entered a program of long-term eltrombopag administration (16 additional weeks): all of them obtained remission of mucosal hemorrhages, with the remission persisting throughout the treatment period. Treatment was globally well tolerated: five patients reported mild adverse events and one patient a moderate adverse event. In conclusion, eltrombopag was safe and effective in increasing platelet count and reducing bleeding symptoms in different forms of inherited thrombocytopenia. Despite these encouraging results, caution is recommended when using thrombopoietin-mimetics in inherited thrombocytopenias predisposing to leukemia. ClinicalTrials.gov identifier: NCT02422394.

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Introduction

Inherited thrombocytopenias are a heterogeneous group of disorders characterized by a reduced number of blood platelets which can result in a bleeding tendency of variable severity. Although inherited thrombocytopenias are rare, recent improvements in the knowledge of these conditions have indicated that, taken together, their prevalence is higher than previously thought. In fact, based on a registry of patients with thrombocytopenia, the prevalence of inherited thrombocytopenias in the Italian population is estimated to be 2.7 cases per 100,000 population.[1]

Most patients with an inherited thrombocytopenia have mild or no spontaneous

bleeding: however, even patients who do not have spontaneous hemorrhages often require platelet transfusions prior to surgery or other invasive procedures because their platelet count is below the safe threshold for the specific procedure.[1-5] Platelet transfusions have several drawbacks, as they expose patients to the risk of acute reactions, transmission of infectious diseases, and alloimmunization with consequent refractoriness to subsequent platelet transfusions.[3,6,7] The last is a particularly critical event in these patients with lifelong thrombocytopenia. Moreover, the availability of platelet units is conditioned by the scarceness of blood donors. Less commonly, some patients with inherited thrombocytopenias have frequent episodes of spontaneous bleeding that affect their quality of life, expose them to the risk of major hemorrhages, and may require frequent hospitalization and/or transfusions. In these subjects, obtaining an enduring increase of platelet count sufficient to stably abolish or reduce spontaneous hemorrhages would be a major achievement.

Thrombopoietin-receptor agonists (TPO-RA) are targeted agents that can stimulate megakaryopoiesis and platelet production through the activation of the thrombopoietin receptor, MPL. These drugs are currently approved for the treatment of a few forms of acquired thrombocytopenia.[8] Although the hypothesis that TPO-RA can increase platelet count also in patients with inherited thrombocytopenias is appealing, the evidence on this topic is very scarce, mainly because of the difficulties in carrying out clinical trials in these orphan diseases. In fact, only one prospective study has been conducted so far: moreover, this study enrolled patients affected with only one of the many forms of inherited thrombocytopenia. In this trial, a short course of the oral TPO-RA eltrombopag was given to 12 patients with thrombocytopenia deriving from mutations in *MYH9* (i.e., *MYH9*-related disease, *MYH9*-RD): 11 patients showed an increase of platelet count.[9] Based on these results, there are anecdotal reports of short-term eltrombopag treatment to prepare *MYH9*-RD patients for major surgery.[10-12] The remaining available clinical information on the effects of TPO-RA in inherited thrombocytopenias derives from single case reports[13-17] and the retrospective investigation of one small case series.[18]

Here we report the results of the second prospective clinical trial on the use of TPO-RA in genetic thrombocytopenias. We investigated the efficacy of eltrombopag in increasing platelet count in patients affected by different forms of inherited thrombocytopenia. Patients received short-term eltrombopag to test whether this treatment can raise platelet count up to safe levels for major surgery. Moreover, in those patients with clinically significant spontaneous bleeding, we also investigated whether prolonged administration of eltrombopag could induce a persistent remission of the spontaneous hemorrhages.

Methods

Patients

Patients were enrolled at five Italian centers (*Online Supplementary Table S1*). The study protocol was approved by the institutional review boards of all centers. Patients or their legal guardians signed written informed consent to participation in the study, which was conducted according to the Declaration of Helsinki. Inclusion and exclusion criteria for this trial are detailed in the *Online Supplementary Methods*.

Study design

This was a phase II, open-label, dose-escalation trial. The study consisted of two parts.

The main aim of part 1 was to test whether, and in which forms of inherited thrombocytopenia, a short-term course of eltrombopag is effective in increasing platelet count above the safe threshold for all types of surgery ($100x10^9$/L).[4,5] All patients eligible for the study entered part 1. Patients received eltrombopag 50 mg/day for 3 weeks. Patients who obtained a platelet count >100 $x10^9$/L by day 21 stopped treatment (as they had achieved the primary endpoint). In the other cases, patients received eltrombopag 75 mg/day for 3 additional weeks.

The main aim of part 2 of the study was to test the efficacy of long-term eltrombopag in achieving an enduring remission of bleeding symptoms in patients with clinically significant spontaneous hemorrhages. Criteria for entering part 2 were the following: patients with spontaneous bleeding at baseline grade ≥2 according to the World Health Organization (WHO) bleeding scale, who completed part 1 without severe side effects and obtained a reduction of bleeding symptoms at the end of part 1. Part 2 consisted of 16 weeks of treatment. Patients were started on eltrombopag 25 mg/day and then re-evaluated every 4 weeks: the eltrombopag dose was adjusted based on bleeding tendency and platelet count according to the schedule reported in *Online Supplementary Figure S1*.

Endpoints and outcome measures

The primary endpoint of part 1 was the achievement of a platelet count >$100x10^9$/L, i.e., a safe level for all types of surgery according to current guidelines.[4,5] Major response was defined as the achievement of the primary endpoint. Minor response was defined as the achievement of a platelet count at least two-fold higher than baseline without reaching the criteria for major response.

The primary endpoint of part 2 was the stable reduction of spontaneous bleeding manifestations according to the WHO bleeding scale during the last 2 weeks of treatment. A major response was defined as a complete remission of hemorrhages. A minor response was defined as a reduction of bleeding according to the WHO bleeding scale without reaching the criteria for a major response.

Secondary endpoints included safety and tolerability of the treatments; dosages of eltrombopag required to achieve the primary endpoints; and improvement of health-related quality of life (HR-QoL) with long-term eltrombopag administration (part 2 only).

Exploratory endpoints included the effects of treatment on serum thrombopoietin levels and on platelet function investigated by light transmission aggregometry and/or flow cytometry.

Investigation of patients

Studies performed to investigate patients at baseline and at each subsequent visit are detailed in the *Online Supplementary Methods*.

Statistical analysis

The statistical analysis was performed as described in the *Online Supplementary Methods*.

Results

Study population

Twenty-four patients were enrolled between April 2015 and May 2017. They consisted of nine patients with *MYH9*-RD; nine with *ANKRD26*-related thrombocytope-

nia (*ANKRD26*-RT);[19] three with thrombocytopenia caused by *WAS* mutations (2 with X-linked thrombocytopenia, XLT, and 1 with Wiskott-Aldrich syndrome, WAS);[20,21] two with monoallelic Bernard-Soulier syndrome (mBSS) caused by the Ala156Val mutation of GPIbα;[22] and one with thrombocytopenia deriving from an *ITGB3* mutation (*ITGB3*-RT).[23] The patients' mean platelet count was 40.4x10^9/L. Table 1 and *Online Supplementary Table S4* describe the features of the study population at baseline.

Part 1 of the study

Twenty-three patients completed part 1 of the study, whereas one patient with *ANKRD26*-RT discontinued treatment early because of an adverse event (see below).

Primary endpoint

Responses in part 1 of the study are summarized in Table 2 and detailed in *Online Supplementary Tables S5* and *S6*. Twenty-one of the 23 evaluable patients [91.3%, 95% confidence interval (95% CI): 72.0-98.9] obtained a response according to the study criteria: 11 patients (47.8%) achieved a major response and ten (43.5%) a minor response. Two patients (8.7%) did not respond (one with *ANKRD26*-RT and the patient with *ITGB3*-RT). Most patients with *MYH9*-RD and the two subjects with mBSS obtained a major response, whereas most patients with *ANKRD26*-RT and the three subjects with XLT/WAS achieved a minor response (Table 2).

The mean platelet count at the end of part 1 of the study was 104.9x10^9/L (P<0.001 compared to baseline). The mean increase in platelet count with respect to baseline

Table 1. Main features of the study population at baseline.

	Overall	*MYH9*-RD	*ANKRD26*-RT	XLT/WAS	mBSS	*ITGB3*-RT
Patients, n.	24	9	9	3	2	1
M/F, n. of patients	14/10	2/7	7/2	3/0	2/0	0/1
Age, years - mean [SD]	41.1 [13.7]	42.9 [14.7]	40.9 [15.1]	29.3 [6.8]	50 [5.7]	45 [-]
Platelet count,[1] x10^9/L - mean [SD]	40.1 [22.4]	38.2 [22.7]	37.4 [22.2]	26.3 [15.8]	70 [1.4]	62 [-]
Spontaneous bleeding,[2] n. of patients	13	3	7	1	1	1
WHO grade = 1, n.	7	0	7	0	0	0
WHO grade = 2, n.	4	1	0	1	1	1
WHO grade = 3, n.	2	2	0	0	0	0
Previous splenectomy,[3] n. of patients	2	2	0	0	0	0

[1]As evaluated by phase-contrast microscopy in a counting chamber. [2]Spontaneous bleeding during the week preceding the baseline evaluation according to the World Health Organization bleeding scale. [3]Previous splenectomy because of a mistaken diagnosis of immune thrombocytopenia. *MYH9*-RD: *MYH9*-related disease; *ANKRD26*-RT: *ANKRD26*-related thrombocytopenia; XLT/WAS: X-linked thrombocytopenia/Wiskott-Aldrich syndrome; mBSS: monoallelic Bernard-Soulier syndrome; *ITGB3*-RT: *ITGB3*-related thrombocytopenia; n: number; M: male; F: female; SD: standard deviation; WHO: World Health Organization.

Table 2. Responses in part 1 of the study (primary endpoint), overall and according to the different forms of inherited thrombocytopenia.

	Overall	*MYH9*-RD	*ANKRD26*-RT	WAS/XLT	mBSS	*ITGB3*-RT
Evaluable patients, n.	23	9	8	3	2	1
Response[1]						
Any response, % [95% CI]	91.3 [72.0-98.9]	100.0 [66.4-100.0]	87.5 [47.3-99.7]	100.0 [29.2-100.0]	100.0 [15.8-100.0]	0 [0-97.5]
Major response, % [95% CI]	47.8 [26.8-69.4]	77.8 [40.0-97.2]	25.0 [3.2-65.1]	0 [0-70.8]	100.0 [15.8-100.0]	0 [0-97.5]
Minor response, % [95% CI]	43.5 [23.2-65.5]	22.2 [2.8-60.0]	62.5 [24.5-91.5]	100.0 [29.2-100.0]	0 [0-84.2]	0 [0-97.5]
Platelet count[2]						
Baseline, x10^9/L, mean [SD]	40.4 [22.8]	38.2 [22.7]	38.0 [23.7]	26.3 [15.8]	70.0 [1.4]	62.0 [-]
End of part 1, x10^9/L, mean [SD]	104.9 [56.7]§	136.3 [68.0]#	75.5 [28.5]§	67.7 [38.4]	150.5 [13.4]	78.0 [-]
Mean increase, x10^9/L [95% CI]	64.5 [43.7-85.3]	98.1 [53.3-142.9]	37.5 [24.1-50.8]	41.4 [22.1-104.8]	80.5 [27.5-188.5]	16.0 [-]
Mean increase in responders, x10^9/L [95%CI]	69.5 [48.0-91.1]	98.1 [53.3-142.9]	41.8 [31.7-52.0]	41.4 [22.1-104.8]	80.5 [27.5-188.5]	-
Spontaneous bleeding[3]						
Patients with SB at baseline, n.	12	3	6	1	1	1
Complete remission of SB at end of part 1, % [95% CI]	83.3 [51.6-97.9]	100 [29.2-100]	83.3 [35.9-99.6]	100 [2.5-100]	100 [2.5-100]	0 [0-97.5]
Partial reduction of SB at end of part 1, % [95% CI]	8.3 [0.2-38.5]	0 [0.0-70.8]	0 [0.0-45.9]	0 [0.0-97.5]	0 [0.0-97.5]	100 [2.5-100]

[1]According to predefined study criteria. [2]As evaluated by phase-contrast microscopy in a counting chamber. [3]Spontaneous bleeding during the week preceding evaluation according to the World Health Organization bleeding scale. §P<0.001 with respect to baseline. #P=0.001 with respect to baseline. *MYH9*-RD: *MYH9*-related disease; *ANKRD26*-RT: *ANKRD26*-related thrombocytopenia; XLT/WAS: X-linked thrombocytopenia/Wiskott-Aldrich syndrome; mBSS: monoallelic Bernard-Soulier syndrome; *ITGB3*-RT: *ITGB3*-related thrombocytopenia; n: number; 95% CI: 95% confidence interval; SD: standard deviation; SB: spontaneous bleeding..

was 64.5x10^9/L (95% CI: 43.7-85.3) overall, and 69.5x10^9/L in the 21 responders. Table 2 and Figure 1 report the average increase in platelet count in the responders according to the different forms of inherited thrombocytopenia.

Ten of the 12 patients with spontaneous bleeding at baseline (83.3%) obtained complete remission of bleeding. In particular, all responders (major or minor response) achieved disappearance of bleeding symptoms if present at baseline. Of the two non-responders, the patient with *ANKRD26*-RT did not obtain any improvement of bleeding manifestations, whereas the patient with *ITGB3*-RT experienced a reduction of spontaneous bleeding (WHO grade from 2 to 1) following a slight increase in platelet count (from 62 to 78 x10^9/L).

Eltrombopag dose

Ten patients (43.5%) achieved a major response with eltrombopag 50 mg/day and stopped therapy (Table 3). These patients were all the individuals with *MYH9*-RD or mBSS who obtained a major response and one subject with *ANKRD26*-RT. Thus, 13 patients (56.5%) switched to the dosage of 75 mg/day. Treatment with the higher dose resulted in the achievement of a better response according to the study criteria in four of these subjects (Table 3).

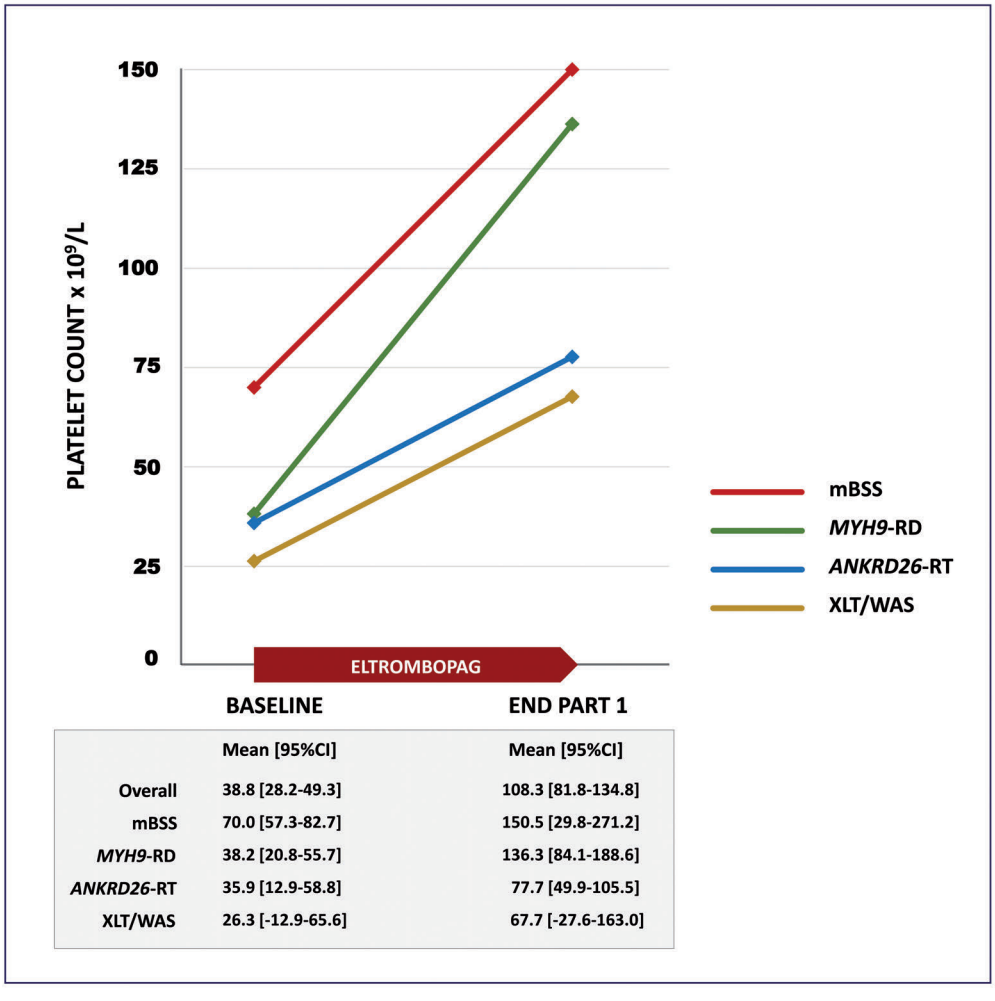
Exploratory endpoints

In vitro platelet aggregation in response to collagen, ADP, and ristocetin, was studied at the end of treatment in the 11 patients who achieved platelet counts >100x10^9/L.

Table 3. Doses of eltrombopag administered during part 1 of the study.

	Overall	*MYH9*-RD	*ANKRD26*-RT	WAS/XLT	mBSS	*ITGB3*-RT
Evaluable patients, n.	23	9	8	3	2	1
Major response with 50 mg/day, n. (%)	10 (43.5)	7 (77.8)	1 (12.5)	0	2 (100)	0
Switch to 75 mg/day, n. (%)	13 (56.5)	2 (22.2)	7 (87.5)	3 (100)	0	1
Improvement of response with 75 mg/day[1], n.	4	0	2§	2#	0	0

[1]Achievement of a better response according to the study criteria with respect to treatment with 50 mg/day. §One patient achieved a major response, one achieved a minor response. #Both patients achieved minor responses. *MYH9*-RD: *MYH9*-related disease; *ANKRD26*-RT: *ANKRD26*-related thrombocytopenia; XLT/WAS: X-linked thrombocytopenia/Wiskott-Aldrich syndrome; mBSS: monoallelic Bernard-Soulier syndrome; *ITGB3*-RT: *ITGB3*-related thrombocytopenia; n: number.



	Mean [95%CI]	Mean [95%CI]
Overall	38.8 [28.2-49.3]	108.3 [81.8-134.8]
mBSS	70.0 [57.3-82.7]	150.5 [29.8-271.2]
MYH9-RD	38.2 [20.8-55.7]	136.3 [84.1-188.6]
ANKRD26-RT	35.9 [12.9-58.8]	77.7 [49.9-105.5]
XLT/WAS	26.3 [-12.9-65.6]	67.7 [-27.6-163.0]

Figure 1. Mean increase in platelet count in the responders in part 1 of the study. Patients are categorized according to the diagnosis of the specific form of inherited thrombocytopenia. mBSS: monoallelic Bernard-Soulier syndrome; *MYH9*-RD: *MYH9*-related disease; . *ANKRD26*-RT: *ANKRD26*-related thrombocytopenia. XLT/WAS: X-linked thrombocytopenia/Wiskott-Aldrich syndrome. Mean values of platelet count at baseline and at the end of part 1 of the treatment along with their 95% confidence intervals (95%CI) are reported in the gray box.

Platelet aggregation was normal in all the cases, except for two patients with *MYH9*-RD and one with mBSS who had slightly reduced responses to the lowest ADP dose (*Online Supplementary Table S7*). In 12 patients, platelet activation in response to ADP and TRAP was also assessed through flow cytometry as the induction of surface expression of P-selectin and of the activated form of GPIIb-IIIa.[24] In these subjects, platelet activation at baseline was not significantly different from that of healthy controls. Overall, platelet responsiveness did not change significantly after eltrombopag treatment with respect to baseline in the investigated patients (*Online Supplementary Figure S2*).

The mean serum thrombopoietin level at baseline was 177.8 pg/mL [standard deviation (SD) 125]. Thrombopoietin levels were unchanged at the end of treatment both considering patients overall and stratifying them according to the different disorders or response to treatment (*Online Supplementary Table S8*).

Safety

We recorded seven adverse events in five patients (21%) (Table 4): all the adverse events were grade 1 (mild) according to Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Four patients reported mild and transient headache and/or diffuse bone pain during the first 2-3 days of treatment. One patient with *ANKRD26*-RT presented with increased plasma creatinine at the assessment after 3 weeks of treatment with 50 mg/day. Although this adverse event was grade 1 (creatinine 1.6 times above baseline), treatment was discontinued according to the study protocol. Further investigations showed that kidney dysfunction was due to urinary retention because of pre-existing benign prostatic hypertrophy and suggested that a causal relationship between eltrombopag administration and the adverse event was unlikely (see Table 4 for details). Results of an ophthalmic assessment at the end of therapy were unchanged in all cases, including the three patients with *MYH9*-RD who had cataracts at baseline.

Table 4. Adverse events recorded during part 1 of the study. All the adverse events were grade 1 according to the Common Terminology Criteria for Adverse Events, version 4.0.

Adverse event	N. of AE	N. of patients (%)	Description
Any adverse event	7	5 (21%)	
Headache	4	4 (17%)	Mild headache that resolved completely after 2 or 3 days. Some patients took low doses of acetaminophen with benefit.
Bone pain	2	2 (8%)	Mild diffuse bone pain that resolved completely after 3 or 4 days. Some patients took low doses of acetaminophen with benefit.
Increased plasma creatinine	1	1 (4%)	Increase 1.6-fold above baseline at the evaluation after 21 days of treatment. Treatment was stopped according to study protocol. Further investigations disclosed that the increased creatinine level was due to urinary retention because of concomitant benign prostatic hypertrophy. Creatinine level continued to increase progressively for 2 months after eltrombopag discontinuation and then completely resolved after specific urologic treatment. Worsening of prostatic hypertrophy has never been reported as an adverse reaction of eltrombopag treatment.[1] Based on the clinical course of the disorder and data from literature, the investigators suggest that a causal relationship between eltrombopag administration and the AE was unlikely.

[1]Eltrombopag (Revolade®) Product information available at *https://www.ema.europa.eu/en/medicines/human/* . N: number; AE: adverse event.

Table 5. Response in part 2 of the study in the four patients enrolled.

Patient ID (patient/family)[1]	1/1	12/10	17/13	22/16
Gender/age, years	F/49	F/45	F/45	M/24
Diagnosis	*MYH9*-RD	*MYH9*-RD	*ITGB3*-RT	WAS
Treatment duration, weeks	16	16	16	8
WHO bleeding score - baseline	3	3	2	2
Bleeding symptoms - baseline	Easy bruising Petechiae Gum bleeding Epistaxis	Easy bruising Gum bleeding Menorrhagia	Easy bruising Menorrhagia	Easy bruising Epistaxis Hematochezia
Platelet count - baseline, x10^9/L	14	38	62	9
WHO bleeding score - end of part 2[2]	1	1	1	0
Bleeding symptoms - end of part 2	Easy bruising	Easy bruising	Easy bruising	None
Platelet count - end of part 2,[3] x10^9/L	75	76	70	19
Eltrombopag dose - end of part 2, mg/day	50	25	25	50
Response in part 2[4]	Minor	Minor	Minor	Major

[1]See *Online Supplementary Table S4*. [2]Spontaneous bleeding during the 2 weeks preceding the last on-treatment visit according to the World Health Organization bleeding scale. [3]As evaluated at the last on-treatment visit by phase-contrast microscopy in a counting chamber. [4]According to predefined study criteria. ID: identity; *MYH9*-RD: *MYH9*-related disease; *ITGB3*-RT: *ITGB3*-related thrombocytopenia; WAS: Wiskott-Aldrich syndrome; WHO: World Health Organization.

Part 2 of the study

Six patients met the criteria for enrollment in part 2 of the study. Two of them did not consent to long-term treatment for logistic reasons, as they were not available to undergo the repeated visits planned by the study protocol. Thus, four patients entered part 2 (2 with *MYH9*-RD, 1 with WAS, 1 with *ITGB3*-RT). At baseline all of them had spontaneous mucosal hemorrhages WHO grade 2 or 3 (epistaxis, gum bleeding, menorrhagia, and/or hematochezia) (Table 5).

Primary endpoint

The outcome of part 2 of the study is summarized in Table 5 and Figure 2. Three patients completed the 16 weeks of therapy. All of them obtained a stable remission of mucosal bleeding throughout the treatment period. During eltrombopag administration, they experienced only very mild and occasional easy bruising (WHO grade 1), resulting in a minor response according to the study criteria. Concerning the patient with WAS, treatment was discontinued after 8 weeks because of exacerbation of cutaneous eczema (see below). During treatment, this patient obtained a complete remission of bleeding (WHO grade 0).

Eltrombopag dose and health-related quality of life

Two patients achieved a response with eltrombopag 25 mg/day, whereas two patients required 50 mg/day (Table 5, Figure 2).

The reduction of bleeding symptoms was associated with an overall increase in the scores obtained with the FACT-TH18 and FACIT-F questionnaires (*Online Supplementary Table S9*). The increase was evident in the two *MYH9*-RD patients presenting the highest degree of bleeding tendency at baseline (WHO grade 3), whereas the two other patients obtained mild or no improvements.

Exploratory endpoints

The thrombopoietin levels of the four patients did not change significantly during part 2 of the study. Platelet response to ADP and TRAP was assessed by flow cytometry in the two *MYH9*-RD patients and the WAS patient and did not show any significant changes with long-term eltrombopag (*data not shown*).

Safety

The patient with WAS reported exacerbation of a pre-existing cutaneous eczema, which is a typical manifestation of the genetic disease. For this reason, eltrombopag

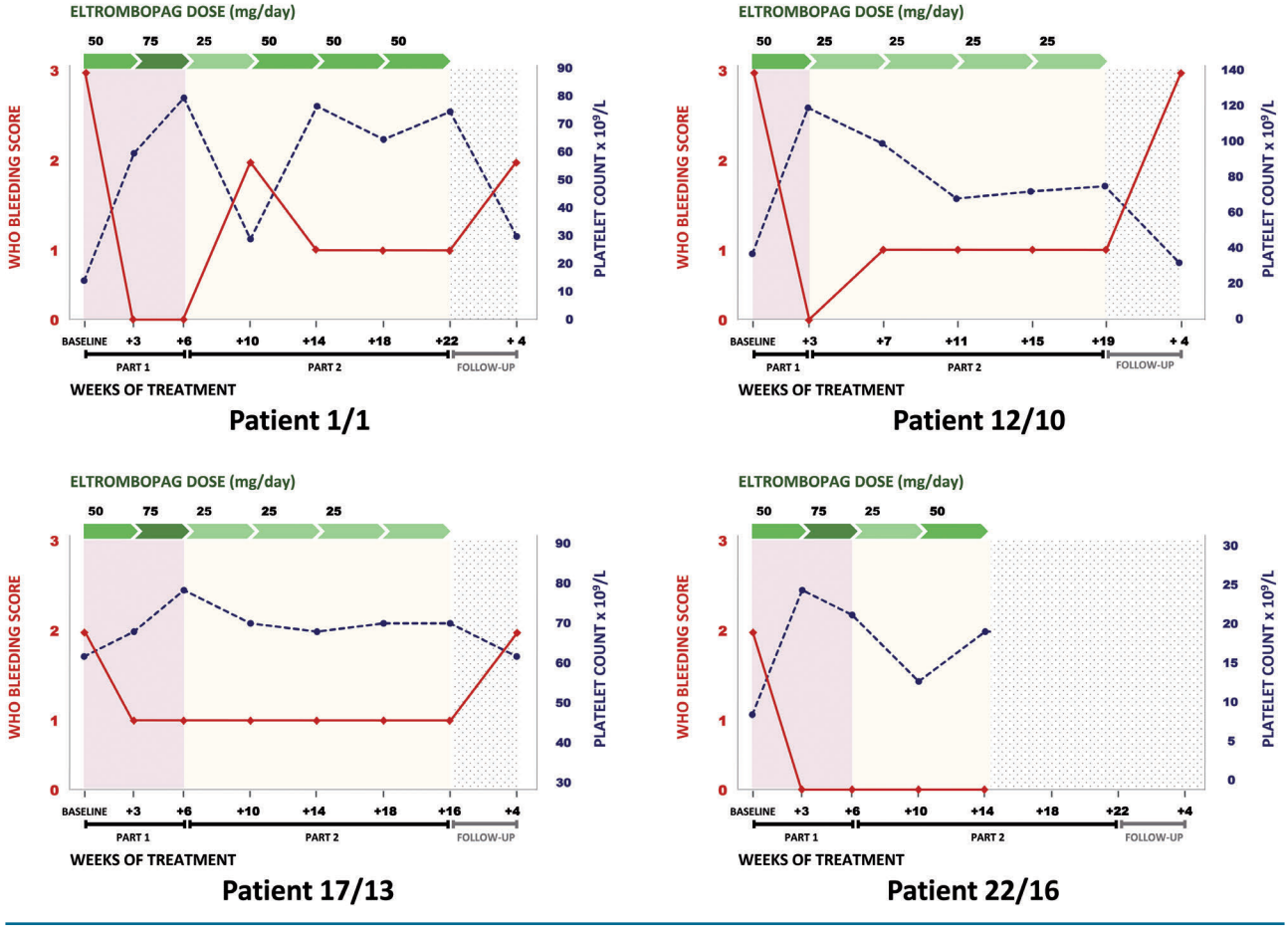


Figure 2. Effects of treatments in part 1 and part 2 of the study in the four individuals who received long-term eltrombopag therapy. The figure summarizes the effects of eltrombopag administration on bleeding symptoms according to the World Health Organization (WHO) bleeding scale and on platelet count. Patients 1/1 and 12/10 have *MYH9*-related disease, patient 17/13 has *ITGB3*-RT, and patient 22/16 has Wiskott-Aldrich syndrome (see Table 5).

was discontinued after 8 weeks of part 2 of the study. The adverse event was grade 2 according to CTCAE version 3.0. The patient described a similar exacerbation of the eczema before enrollment into this study, which occurred without any apparent causes. However, the eczema improved some weeks after eltrombopag discontinuation, supporting a causal relationship with the treatment. No additional adverse events were observed during part 2 therapy. In particular, no occurrence or worsening of cataracts was observed, even in the two patients with *MYH9*-RD who had cataracts at baseline.

Post-treatment assessments

Twenty patients were re-evaluated 30 days after the end of part 1 or 2 (3 patients refused the visit). Post-treatment assessments did not identify any adverse events. The mean platelet count was 47.0x10^9/L (SD 26), similar to that at baseline in the same patients (40.9x10^9/L, SD 23). The bleeding tendency returned to that recorded at baseline in all the cases.

Discussion

TPO-RA represent an appealing hypothesized treatment for the majority of patients with thrombocytopenias of genetic origin. In fact, in most forms of inherited thrombocytopenia, the megakaryocyte response to thrombopoietin is totally or partially preserved:[25,26] TPO-RA can therefore potentially increase platelet production in many of these disorders. Moreover, in most patients with inherited thrombocytopenia platelet function is normal or only partially impaired, so that increasing platelet count is expected to improve hemostasis.[25,27] Patients with an inherited thrombocytopenia may benefit from short-term courses of TPO-RA as well as prolonged treatment. Short-term courses may be given in preparation for elective surgery or other invasive procedures whenever the platelet count is below the safe threshold for the specific procedure. In this context, TPO-RA can replace perioperative platelet transfusions, thus preventing alloimmunization and the other risks of blood derivatives, and provide an option to increase platelet count even in patients refractory to platelet transfusions.[10-12,14,15] On the other hand, patients with clinically significant spontaneous bleeding may benefit from long-term TPO-RA administration to achieve an enduring remission of bleeding symptoms and reduce the risk of major hemorrhages. Despite these premises, clinical evidence on the efficacy and safety of TPO-RA in inherited thrombocytopenias is very scarce.[28]

The only previous prospective study investigated the short-term use of eltrombopag in patients with *MYH9*-RD and showed that most patients responded to treatment without major side effects.[9] The present trial investigates the response to short-term eltrombopag in a wider range of inherited thrombocytopenias, and provides information on the effects of prolonged treatment in those patients with clinically significant spontaneous hemorrhages.

We gave a short-term course of eltrombopag to patients affected with five different disorders: the large majority of them (91.3%) responded to the drug and the mean platelet count at the end of therapy was increased by 64.5x10^9/L compared to baseline (P<0.001). However, we observed some differences in the degree of platelet response between the patients with the different forms of inherited thrombocytopenia. Eltrombopag was highly effective in *MYH9*-RD, thus confirming and extending the results of the previous trial.[9] All the *MYH9*-RD patients responded, most of them (78%) reached a platelet count >100x10^9/L, and the mean increase in platelet count compared to baseline was 98.1x10^9/L. The two individuals with mBSS also achieved major responses with an increase in platelet count close to that of *MYH9*-RD subjects (80.5x10^9/L). Although seven of the eight evaluable patients with *ANKRD26*-RT responded to eltrombopag, the extent of platelet response was globally lower than that in *MYH9*-RD and mBSS patients: in fact, most *ANKRD26*-RT subjects obtained minor responses and the mean increase in platelet count in responders was 41.8x10^9/L. In the three XLT/WAS patients, results appeared similar to those of *ANKRD26*-RT: these patients reached a minor response with an average rise in platelet count of 41.4x10^9/L. In spite of these differences, we believe that in all the above disorders the response to eltrombopag is highly relevant with regards to the use of the drug in preparation for surgery in clinical practice. In fact, current guidelines define a platelet count of 50x10^9/L as the threshold level recommended for major surgery, with the exception of neurosurgery and posterior eye surgery that require a platelet count of 100x10^9/L.[4,5] In this view, while the response observed in *MYH9*-RD and mBSS appears a very good result, even the extent of the increase in platelet count obtained in *ANKRD26*-RT and XLT/WAS appears sufficient to avoid the use of platelet transfusions to prepare most patients for most surgical procedures.

Finally, we treated only one patient with *ITGB3*-RT, who failed to achieve a platelet response according to the study criteria.

All the patients who responded in part 1 of the study, even those achieving a minor response, had complete remission of bleeding symptoms whenever these were present at baseline. Even one of the two patients classified as non-responders according to the study criteria, experienced the remission of mucosal bleeding following a slight increase in platelet count. Remission of bleeding is consistent with the results of platelet function studies during eltrombopag treatment: since platelet responses to different agonists were normal or only slightly impaired, increasing platelet count was effective in improving hemostasis. Consistent with previous findings in XLT/WAS patients,[18] flow cytometry showed that platelet responsiveness to ADP and TRAP does not change significantly with eltrombopag administration.

Concerning the dosage of eltrombopag, ten of the 11 patients who achieved a major response obtained this result with a dose of 50 mg/day, while one patient required a dose of 75 mg/day. Overall, ten of the 13 patients who were switched from 50 to 75 mg/day obtained a further increase of platelet count with the higher dose: four subjects reached a better response according to the study criteria, while six patients achieved only slightly higher platelet counts. All the patients with *ANKRD26*-RT or XLT/WAS, but one, required the switch to the higher dose, which resulted in a higher platelet count in most cases. These data suggest that 75 mg/day is the most reasonable starting dose for preoperative eltrombopag in patients with either of these two disorders.

Effects of long-term eltrombopag administration were investigated in four patients who had frequent episodes of spontaneous mucosal bleeding. All of them achieved a sta-

ble remission of mucosal hemorrhages and the remission persisted throughout the treatment period. In two patients, the reduction of spontaneous bleeding was associated with a very slight increase of platelet count (around $10x10^9$/L). The same observation was previously made in a patient with WAS who was given long-term treatment with eltrombopag because of severe bleeding symptoms.[17] Two patients achieved remission of bleeding with the dosage of 25 mg/day, suggesting that, in some patients with inherited thrombocytopenia, clinical benefit can be maintained with prolonged administration of relatively low doses of eltrombopag. Interestingly, the two patients with the greatest bleeding tendency at baseline experienced not only stable improvements of HR-QoL related to bleeding, but also an increase of the score measuring the subjective perception of fatigue.

The observation that some patients obtained a significant reduction of bleeding tendency following a very slight increase in platelet count suggests that eltrombopag may improve some discrete platelet functions in addition to raising platelet concentration. As mentioned, overall we did not observe any significant change in platelet GPIIb-IIIa activation or P-selectin expression in response to ADP and TRAP after eltrombopag treatment compared to that at baseline, in 12 investigated patients. However, we cannot exclude that the drug could have improved some other mechanisms of platelet function[29] in some patients, and further investigations are required to explore this hypothesis.

Short-term treatment with eltrombopag was globally well tolerated, with 17% of patients reporting mild and transient headache and/or bone pain at the beginning of treatment. In one *ANKRD26*-RT patient, we observed a slight increase in plasma creatinine; clinical investigation of this subject suggested that a causal relationship between eltrombopag and this adverse event was unlikely. Regarding long-term treatment, the patient affected with WAS experienced worsening of a pre-existing cutaneous eczema that required eltrombopag discontinuation after 14 weeks. This adverse event had never been described in the previous retrospective reports on WAS patients who received eltrombopag.[17,18] No other adverse events were recorded with long-term therapy.

Eltrombopag has been associated with the occurrence of cataracts in patients with immune thrombocytopenia,[30] and *MYH9*-RD is a syndromic disorder predisposing to cataracts.[31] Thus, it is noteworthy that none of our *MYH9*-RD subjects showed development or progression of cataracts, not even the two patients who received long-term therapy and already had cataracts at baseline.

A previous study raised the suspicion that the TPO-RA romiplostim favors progression to myeloid leukemia in patients with myelodysplastic syndromes.[32] Subsequent trials of eltrombopag monotherapy in myelodysplastic syndromes did not reveal any safety issues in this regard:[33-35] however, a trend to an increased risk of disease progression was reported in a study in which eltrombopag was tested in association with azacitidine in intermediate- or high-risk myelodysplastic syndromes.[36] These observations raise concerns about the safety of TPO-RA in *ANKRD26*-RT, a condition that increases the risk of myeloid malignancies.[37] In the present study, short-term use of eltrombopag did not result in any changes of blood cell parameters or morphology (with the exception of platelet count) in *ANKRD26*-RT patients. However, further clinical data on this topic are needed, and caution should be used when treating individuals with *ANKRD26*-RT or other inherited thrombocytopenias predisposing to hematological malignancies[38] with TPO-RA, especially with long-term administration.

In conclusion, this study shows that eltrombopag was effective in increasing platelet count in four different forms of inherited thrombocytopenia, which, taken together, affect more than 55% of patients with genetic thrombocytopenias.[28] In most patients, short-term administration of eltrombopag increased platelet count above the threshold for major surgery recommended by current guidelines,[4,5] indicating that the drug can efficiently replace perioperative platelet transfusions in preparation for surgery or other invasive procedures. Although only four patients received long-term treatment, the results indicate that prolonged eltrombopag therapy can induce persistent remission of spontaneous bleeding. Both short- and long-term treatments were globally well tolerated. Although a greater amount of clinical data on the use of TPO-RA in inherited thrombocytopenias is certainly required, our results suggest that eltrombopag will probably have a central role in the treatment of thrombocytopenias of genetic origin.

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