haematologica
Journal of The Ferrata Storti Foundation

Eltrombopag for the treatment of inherited thrombocytopenias: a phase 2 clinical trial

by Carlo Zaninetti, Paolo Gresele, Antonella Bertomoro, Catherine Klersy, Erica De Candia, Dino Veneri, Serena Barozzi, Tiziana Fierro, Maria Adele Alberelli, Valeria Musella, Patrizia Noris, Fabrizio Fabris, Carlo L Balduini, and Alessandro Pecci

Haematologica 2019 [Epub ahead of print]

Citation: Carlo Zaninetti, Paolo Gresele, Antonella Bertomoro, Catherine Klersy, Erica De Candia, Dino Veneri, Serena Barozzi, Tiziana Fierro, Maria Adele Alberelli, Valeria Musella, Patrizia Noris, Fabrizio Fabris, Carlo L Balduini, and Alessandro Pecci. Eltrombopag for the treatment of inherited thrombocytopenias: a phase 2 clinical trial.
Haematologica. 2019; 104:xxx
doi:10.3324/haematol.2019.223966

Eltrombopag for the treatment of inherited thrombocytopenias: a phase 2 clinical trial

Carlo Zaninetti,[1,2] Paolo Gresele,[3] Antonella Bertomoro,[4] Catherine Klersy,[5] Erica De Candia,[6,7] Dino Veneri,[8] Serena Barozzi,[1] Tiziana Fierro,[3] Maria Adele Alberelli,[6] Valeria Musella,[5] Patrizia Noris,[1] Fabrizio Fabris,[4] Carlo L. Balduini,[1,9] and Alessandro Pecci[1]

[1] Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation and University of Pavia, Pavia, Italy.
[2] PhD course in Experimental Medicine, University of Pavia, Pavia, Italy.
[3] Department of Medicine, University of Perugia, Perugia, Italy.
[4] Department of Medicine, University of Padova, Padova, Italy.
[5] Service of Clinical Epidemiology & Biometry, IRCCS Policlinico San Matteo Foundation and University of Pavia, Pavia, Italy.
[6] Institute of Internal Medicine and Geriatrics, IRCCS Policlinico Universitario A. Gemelli Foundation, Roma, Italy.
[7] Università Cattolica del Sacro Cuore, Roma, Italy.
[8] Department of Medicine, Section of Hematology, University of Verona, Verona, Italy.
[9] Ferrata-Storti Foundation, Pavia, Italy.

Correspondence:
Alessandro Pecci, Department of Internal Medicine, IRCCS Policlinico San Matteo Foundation and University of Pavia, Piazzale Golgi, 27100 Pavia, Italy. Tel.: +39.0382.501358. Fax: +39.0382.526223. e-mail: alessandro.pecci@unipv.it

Shortened title as running head: Eltrombopag in inherited thrombocytopenias

Trial registration:
ClinicalTrials.gov identifier: NCT02422394

Main text word count: 3659
Abstract word count: 249
Figure count: 2
Table count: 5
Supplemental File: 1

ABSTRACT

Patients with inherited thrombocytopenias often require platelet transfusions to raise platelet count before surgery or other invasive procedures; moreover, subjects presenting 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 2 clinical trial to investigate the efficacy of the oral thrombopoietin-mimetic eltrombopag in different forms of inherited thrombocytopenia. We enrolled 24 patients affected with *MYH9*-related disease, *ANKRD26*-related thrombocytopenia, X-linked thrombocytopenia/Wiskott-Aldrich syndrome, monoallelic Bernard-Soulier syndrome, or *ITGB3*-related thrombocytopenia. Average pre-treatment platelet count was 40.4 $x10^9$/L. Patients received a 3- to 6-week course of eltrombopag in a dose-escalation manner. Of 23 patients evaluable for response, 11 (47.8%) achieved a major response (platelet count >100 $x10^9$/L), 10 (43.5%) a minor response (platelet count at least twice than baseline), whereas 2 patients (8.7%) did not respond. Average increase of platelet count compared to baseline was 64.5 $x10^9$/L ($p<0.001$). Four patients who presented clinically significant spontaneous bleeding were admitted to a long-term eltrombopag administration (16 additional weeks): all of them obtained remission of mucosal hemorrhages that persisted throughout the treatment period. Treatment was globally well tolerated: 5 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.

INTRODUCTION

Inherited thrombocytopenias (ITs) are a heterogeneous group of disorders characterized by a reduced number of blood platelets that can result in a bleeding tendency of variable severity. Although ITs 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 ITs in the Italian population is estimated to be 2.7 per 100,000.[1]

Most patients with ITs present 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 latter is a particularly critical event in these patients with lifelong thrombocytopenia. Moreover, the availability of platelet units is affected by the scarceness of blood donors. Conversely, some IT patients present 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 (TPO)-receptor agonists (TPO-RAs) are targeted agents that can stimulate megakaryopoiesis and platelet production through the activation of the TPO receptor, MPL. These drugs are currently approved for the treatment of a few forms of acquired thrombocytopenia.[8] Although the hypothesis that TPO-RAs can increase platelet count also in patients with ITs is appealing, the evidence on this topic is very scarce, mainly due to the difficulties in carrying out clinical trials in these orphan diseases. As a matter of fact, only one prospective study has been conducted so far: moreover, it enrolled patients affected with only one of the many forms of ITs. In this trial, a short course of the oral TPO-RA eltrombopag was given to 12 patients with the thrombocytopenia deriving from mutations in *MYH9* (*MYH9*-related disease, *MYH9*-RD): 11 patients showed an increase of platelet count.[9] Based on these results, short-term eltrombopag was used anecdotally to prepare *MYH9*-RD patients for major surgery.[10-12] The remaining available clinical information on the effects of TPO-RAs in ITs 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 TPO-RAs in genetic thrombocytopenias. We investigated the efficacy of eltrombopag in increasing platelet count in patients

affected by different forms of IT. Patients received short-term eltrombopag to test if this treatment can raise platelet count up to safe levels for major surgery. Moreover, in those patients presenting with clinically significant spontaneous bleeding, we also tested if a prolonged administration of eltrombopag can induce a persistent remission of the spontaneous hemorrhages.

METHODS

Patients

Patients were enrolled at 5 Italian centres (Supplemental Table S1). The study protocol was approved by the institutional review boards of all centres. Patients or their legal guardians signed written informed consent for the study, which was conducted according to the Declaration of Helsinki.
Inclusion and exclusion criteria for this trial are detailed in Supplemental Methods.

Study design

This was a phase 2, open-label, dose-escalation trial. The study consisted of two Parts.

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

Part 2. The main aim of Part 2 was to test the efficacy of long-term eltrombopag in achieving an enduring remission of bleeding symptoms in patients presenting with clinically significant spontaneous hemorrhages. Criteria for entering the 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. Then, they were re-evaluated every 4 weeks, and eltrombopag dose was adjusted based on bleeding tendency and platelet count according to the schedule reported in Supplemental Figure S1.

Endpoints and outcome measures

The primary endpoint of Part 1 was the achievement of a platelet count >100 x10^9/L, i.e. the 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. Major response was defined as a complete remission of hemorrhages. Minor response was defined as a reduction of bleeding according to the WHO bleeding scale without reaching the criteria for major response.

Secondary endpoints included safety and tolerability of the treatments; dosages of eltrombopag required for achieving the primary endpoints; 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 TPO 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 Supplemental Methods.

Statistical analysis

Statistical analysis was performed as reported in Supplemental Methods.

RESULTS

Study population

Twenty-four patients were enrolled between April 2015 and May 2017. They included 9 patients affected with *MYH9*-RD; 9 with *ANKRD26*-related thrombocytopenia (*ANKRD26*-RT);[19] 3 with thrombocytopenia caused by *WAS* mutations (2 with X-linked thrombocytopenia, XLT, and 1 with Wiskott-Aldrich syndrome, WAS);[20,21] 2 with monoallelic Bernard-Soulier syndrome (mBSS) caused by the Ala156Val mutation of GPIbα;[22] 1 with thrombocytopenia deriving from an *ITGB3* mutation (*ITGB3*-RT).[23] Patients' mean platelet count was 40.4 x10^9/L. Tables 1 and S4 describe the features of the study population at baseline.

Part 1

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

Primary endpoint. Response to Part 1 is summarized in Table 2 and detailed in Tables S5 and S6. Twenty-one of the 23 evaluable patients (91.3%, 95%CI 72.0-98.9) obtained a response according to the study criteria: 11 patients (47.8%) achieved a major response and 10 (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 was 104.9 $x10^9$/L ($p<0.001$ compared to baseline). The mean increase in platelet count with respect to baseline was 64.5 $x10^9$/L (95%CI 43.7-85.3) overall, and 69.5 $x10^9$/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 IT.
Ten of the 12 patients presenting 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 mild 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 4 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 >100 $x10^9$/L. Platelet aggregation was normal in all the cases, except for two patients with *MYH9*-RD and one with mBSS who presented slightly reduced responses to the lowest ADP dose (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 compared to healthy controls. Overall, platelet responsiveness did

not significantly change after eltrombopag treatment with respect to baseline in the investigated patients (Figure S2).
Mean serum TPO level at baseline was 177.8 pg/mL (SD 125). TPO levels were unchanged at the end of treatment both considering patients overall and stratifying them according to the different disorders or response to treatment (Table S8).

Safety. We recorded 7 AEs in 5 patients (21%) (Table 4): all the AEs were grade 1 (mild) according to CTCAEv4.0. 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 increased plasma creatinine at the assessment after 3 weeks of treatment with 50 mg/day. Although the AE was grade 1 (creatinine 1.6x 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 AE is unlikely (see Table 4 for details). Results of ophthalmic assessment at the end of therapy were unchanged in all cases, including the 3 *MYH9*-RD patients presenting cataracts at baseline.

Part 2

Six patients had the criteria for enrollment to the 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, 4 patients entered the Part 2 (2 with *MYH9*-RD, 1 with WAS, 1 with *ITGB3*-RT). All of them presented at baseline with spontaneous mucosal hemorrhages WHO grade 2 or 3 (epistaxis, gum bleeding, menorrhagia, and/or hematochezia) (Table 5).

Primary endpoint. Outcome of Part 2 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, he obtained a complete remission of bleeding (WHO grade 0).

Eltrombopag dose and HR-QoL. Two patients achieved the 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 (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. TPO levels of the 4 patients did not significantly change during Part 2. 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 referred exacerbation of a pre-existing cutaneous eczema, which is a typical manifestation of the genetic disease. For this reason, eltrombopag was discontinued after 8 weeks of Part 2. The AE was grade 2 according to CTCAEv3.0. The patient referred similar exacerbations of the eczema before enrollment to 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 AEs were observed during Part 2 therapy. In particular, no occurrence or worsening of cataracts was observed, including the two patients with *MYH9*-RD presenting cataracts at baseline.

Post-treatment assessments

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

DISCUSSION

TPO-RAs represent an appealing treatment hypothesis for the majority of patients with thrombocytopenias of genetic origin. In fact, in most forms of IT, the megakaryocyte response to TPO is totally or partially preserved:[25,26] therefore, TPO-RAs can potentially increase platelet production in many of these disorders. Moreover, in most IT patients, platelet function is normal or only partially impaired, so that increasing platelet count is expected to improve hemostasis.[25,27] Patients with ITs may benefit from short-term courses of TPO-RAs as well as a prolonged treatment. Short-term courses may be given in preparation for elective surgery or other invasive procedures whenever platelet count is below the safe threshold for the specific procedure. In this context, TPO-RAs 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 presenting with

clinically significant spontaneous bleeding may benefit from a long-term TPO-RAs 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-RAs in ITs is very scarce.[28]

The only previous prospective study tested short-term eltrombopag in *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 ITs, and provides information on the effects of a prolonged treatment in those patients presenting with clinically significant spontaneous hemorrhages.

We gave short-term eltrombopag to patients affected with 5 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 64.5 $x10^9$/L compared to baseline ($p<0.001$). However, we observed some differences in the degree of platelet response between the different forms of IT. 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 >100 $x10^9$/L, and the mean increase in platelet count compared to baseline was 98.1 $x10^9$/L. The two individuals with mBSS also achieved major responses with an increase in platelet count close to that of *MYH9*-RD subjects (80.5 $x10^9$/L). Although 7 of the 8 evaluable patients with *ANKRD26*-RT responded to eltrombopag, the extent of platelet response was globally lower than in *MYH9*-RD and mBSS: in fact, most *ANKRD26*-RT subjects obtained minor responses and the mean increase in platelet count in responders was 41.8 $x10^9$/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 41.4 $x10^9$/L. In spite of these differences, we believe that in all the above disorders the response to eltrombopag is highly significant for the use of the drug in preparation for surgery in clinical practice. In fact, current guidelines define a platelet count of 50 $x10^9$/L as the threshold level recommended for major surgery, with the exception of neurosurgery and posterior eye surgery that require 100 $x10^9$/L platelets.[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 to Part 1, even achieving a minor response, showed a 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 mild increase in platelet count. Remission of bleeding is consistent with the results of platelet function

studies during eltrombopag treatment: since platelet response to different agonists was 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 significantly change with eltrombopag administration.

Concerning the dosage of eltrombopag, 10 of the 11 patients achieving a major response obtained this result with 50 mg/day, while one patient required the dose of 75 mg/day. Overall, 10 of the 13 patients who were switched from 50 to 75 mg/day obtained a further increase of platelet count with the higher dose: 4 subjects reached a better response according to the study criteria, while 6 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 that 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 these two disorders.

Effects of long-term eltrombopag administration were investigated in 4 patients presenting with frequent episodes of spontaneous mucosal bleeding. All of them achieved a stable remission of mucosal hemorrhages that persisted throughout the treatment period. In two patients, the reduction of spontaneous bleeding was associated with a very mild increase of platelet count (around 10×10^9/L). The same observation was previously made in a patient affected with WAS and treated with long-term eltrombopag because of severe bleeding symptoms.[17] Two patients achieved remission of bleeding with the dosage of 25 mg/day, suggesting that, in some IT patients, clinical benefit can be maintained with prolonged administration of relatively low doses of eltrombopag. Interestingly, the two patients with the highest degree of bleeding tendency at baseline experienced not only a stable improvement 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 mild increase in platelet count may suggest the hypothesis that eltrombopag improves some discrete platelet functions in addition to raise 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 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 test this hypothesis.

Short-term 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 mild increase in plasma creatinine; clinical investigation of this subject suggested that a causal relationship

between eltrombopag and this AE is unlikely. Regarding the long-term treatment, the patient affected with WAS experienced worsening of a pre-existing cutaneous eczema that required eltrombopag discontinuation after 14 weeks. This AE has never been described with the previous retrospective reports on WAS patients who received eltrombopag.[17,18] No other AEs 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 presented cataracts at baseline.

A previous study raised the suspicion that the TPO-RA romiplostim favors the progression to myeloid leukemia in patients with myelodysplastic syndromes (MDS).[32] Subsequent trials of eltrombopag monotherapy in MDS did not observe safety issues in this regard:[33-35] however, a trend for an increased risk of disease progression was reported in a study testing eltrombopag in association with azacitidine in intermediate- or high-risk MDS.[36] These observations raise a concern on safety of TPO-RAs in *ANKRD26*-RT, a condition that increases the risk of myeloid malignancies.[37] In the present study, short-term eltrombopag did not result in any changes of blood cells 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 *ANKRD26*-RT or other ITs predisposing to hematological malignancies[38] with TPO-RAs, especially with long-term administration.

In conclusion, this study shows that eltrombopag is effective in increasing platelet count in 4 different forms of ITs, which, taken together, affect more than 55% of patients with genetic thrombocytopenias.[28] In most patients, short-term 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 4 patients received long-term treatment, the results indicate that a prolonged eltrombopag therapy can induce a persistent remission of spontaneous bleeding. Both short- and long-term treatments were globally well tolerated. Although it is certainly required a greater amount of clinical data on the use of TPO-RAs in ITs, our results suggest that eltrombopag will probably have a central role in the treatment of thrombocytopenias of genetic origin.

ACKNOWLEDGEMENTS

The authors would like to thank all the patients and their families. We thank the Clinical Trial Quality Team of the IRCCS Policlinico San Matteo Foundation for the continuing assistance and cooperation, Dr. Ruggero Panebianco (Novartis) for critically revising the study proposal, and Prof. Antonio Ruggiero (Department of Pediatrics, IRCCS Policlinico A. Gemelli Foundation) for his helpful collaboration.
This study was supported by grants from the IRCCS Policlinico San Matteo Foundation (to A.P.) and the Telethon Foundation (GGP17106 to A.P. and GGP10155 to P.G.).
Novartis made eltrombopag available for the study and partially supported the clinical and laboratory analyses required by the trial.

AUTHOR CONTRIBUTIONS

CZ and AP designed research, collected clinical and laboratory data, analyzed and interpreted data, and wrote the manuscript. CLB designed research, analyzed and interpreted data, and wrote the manuscript. PG, AB, EDC, DV, SB, TF, MAA, PN, and FF collected clinical and laboratory data, analyzed and interpreted data. CK analyzed and interpreted data and performed statistical analysis. VM analyzed and interpreted data and coordinated data management. All authors had access to primary clinical trial data. All the authors critically revised the manuscript and accepted the final version.

CONFLICT OF INTERESTS DISCLOSURE

The authors declare no competing financial interests.

REFERENCES

1. Balduini CL, Pecci A, Noris P. Diagnosis and management of inherited thrombocytopenias. Semin Thromb Hemost. 2013;39(2):161-171.

2. Orsini S, Noris P, Bury L, et al. Bleeding risk of surgery and its prevention in patients with inherited platelet disorders. Haematologica. 2017;102(7):1192-1203.

3. Dupuis A, Gachet C. Inherited platelet disorders: Management of the bleeding risk. Transfus Clin Biol. 2018;25(3):228-235.

4. Estcourt LJ, Birchall J, Allard S, et al. Guidelines for the use of platelet transfusions. Br J Haematol. 2017;176(3):365-394.

5. Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: a clinical practice guideline from the AABB. Ann Intern Med. 2015;162(3):205-213.

6. Bolton-Maggs PH, Chalmers EA, Collins PW, et al. A review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO. Br J Haematol. 2006;135(5):603-633.

7. Pecci A. Diagnosis and treatment of inherited thrombocytopenias. Clin Genet. 2016;89(2):141-153.

8. Rodeghiero F, Carli G. Beyond immune thrombocytopenia: the evolving role of thrombopoietin receptor agonists. Ann Hematol. 2017;96(9):1421-1434.

9. Pecci A, Gresele P, Klersy C, et al. Eltrombopag for the treatment of the inherited thrombocytopenia deriving from MYH9 mutations. Blood. 2010;116(26):5832-5837.

10. Pecci A, Barozzi S, d'Amico S, Balduini CL. Short-term eltrombopag for surgical preparation of a patient with inherited thrombocytopenia deriving from MYH9 mutation. Thromb Haemost. 2012;107(6):1188-1189.

11. Favier R, Feriel J, Favier M, Denoyelle F, Martignetti JA. First successful use of eltrombopag before surgery in a child with MYH9-related thrombocytopenia. Pediatrics. 2013;132(3):e793-795.

12. Favier R, De Carne C, Elefant E, Lapusneanu R, Gkalea V, Rigouzzo A. Eltrombopag to treat thrombocytopenia during last month of pregnancy in a woman with MYH9-related disease: a case report. A A Pract. 2018;10(1):10-12.

13. Gröpper S, Althaus K, Najm J, et al. A patient with Fechtner syndrome successfully treated with romiplostim. Thromb Haemost. 2012;107(3):590-591.

14. Fiore M, Saut N, Alessi MC, Viallard JF. Successful use of eltrombopag for surgical preparation in a patient with ANKRD26-related thrombocytopenia. Platelets. 2016;27(8):828-829.

15. Westbury SK, Downes K, Burney C, et al. Phenotype description and response to thrombopoietin receptor agonist in DIAPH1-related disorder. Blood Adv. 2018;2(18):2341-2346.

16. Yamanouchi J, Hato T, Kunishima S, Niiya T, Nakamura H, Yasukawa M. A novel MYH9 mutation in a patient with MYH9 disorders and platelet size-specific effect of romiplostim on macrothrombocytopenia. Ann Hematol. 2015;94(9);1599-1600.

17. Gabelli M, Marzollo A, Notarangelo LD, Basso G, Putti MC. Eltrombopag use in a patient with Wiskott-Aldrich syndrome. Pediatr Blood Cancer. 2017;64(12).

18.Gerrits AJ, Leven EA, Frelinger AL 3rd, et al. Effects of eltrombopag on platelet count and platelet activation in Wiskott-Aldrich syndrome/X-linked thrombocytopenia. Blood. 2015;126(11):1367-1378.

19. Noris P, Perrotta S, Seri M, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood. 2011;117(24):6673-6680.

20. Albert MH, Bittner TC, Nonoyama S, et al. X-linked thrombocytopenia (XLT) due to WAS mutations: clinical characteristics, long-term outcome, and treatment options. Blood. 2010;115(16):3231-3238.

21. Massaad MJ, Ramesh N, Geha RS. Wiskott-Aldrich syndrome: a comprehensive review. Ann N Y Acad Sci. 2013;1285:26-43.

22. Noris P, Perrotta S, Bottega R, et al. Clinical and laboratory features of 103 patients from 42 Italian families with inherited thrombocytopenia derived from the monoallelic Ala156Val mutation of GPIbα (Bolzano mutation). Haematologica. 2012;97(1):82-88.

23. Gresele P, Falcinelli E, Giannini S, et al. Dominant inheritance of a novel integrin beta3 mutation associated with a hereditary macrothrombocytopenia and platelet dysfunction in two Italian families. Haematologica. 2009;94(5):663-669.

24. Michelson AD, Barnard MR, Krueger LA, Frelinger AL 3rd, Furman MI. Evaluation of platelet function by flow cytometry. Methods. 2000;21(3):259-270.

25. Pecci A. Pathogenesis and management of inherited thrombocytopenias; rationale for the use of thrombopoietin-receptor agonists. Int J Hematol. 2013;98(1):34-47.

26. Pecci A, Balduini CL. Lessons in platelet production from inherited thrombocytopenias. Br J Haematol. 2014;165(2):179-192.

27. Balduini CL, Savoia A, Seri M. Inherited thrombocytopenias frequently diagnosed in adults. J Thromb Haemost. 2013;11(6):1006-1019.

28. Rodeghiero F, Pecci A, Balduini CL. Thrombopoietin receptor agonists in hereditary thrombocytopenias. J Thromb Haemost. 2018;16(9):1700-1710.

29. Daskalakis M, Colucci G, Keller P, et al. Decreased generation of procoagulant platelets detected by flow cytometric analysis in patients with bleeding diathesis. Cytometry B Clin Cytom. 2014;86(6):397-409.

30. Wong RSM, Saleh MN, Khelif A, et al. Safety and efficacy of long-term treatment of chronic/persistent ITP with eltrombopag: final results of the EXTEND study. Blood. 2017;130(23):2527-2536.

31. Pecci A, Klersy C, Gresele P, et al. MYH9-related disease: a novel prognostic model to predict the clinical evolution of the disease based on genotype-phenotype correlations. Hum Mutat. 2014;35(2):236-247.

32. Giagounidis A, Mufti GJ, Fenaux P, et al. Results of a randomized, double-blind study of romiplostim versus placebo in patients with low/intermediate-1-risk myelodysplastic syndrome and thrombocytopenia. Cancer. 2014;120(12):1838-1846.

33. Platzbecker U, Wong RS, Verma A, et al. Safety and tolerability of eltrombopag versus placebo for treatment of thrombocytopenia in patients with advanced myelodysplastic syndromes or acute myeloid leukaemia: a multicentre, randomised, placebo-controlled, double-blind, phase 1/2 trial. Lancet Haematol. 2015;2(10):e417-426.

34. Oliva EN, Alati C, Santini V, et al. Eltrombopag versus placebo for low-risk myelodysplastic syndromes with thrombocytopenia (EQoL-MDS): phase 1 results of a single-blind, randomised, controlled, phase 2 superiority trial. Lancet Haematol. 2017;4(3):e127-e136.

35. Mittelman M, Platzbecker U, Afanasyev B, et al. Eltrombopag for advanced myelodysplastic syndromes or acute myeloid leukaemia and severe thrombocytopenia (ASPIRE): a randomised, placebo-controlled, phase 2 trial. Lancet Haematol. 2018;(1):e34-e43.

36. Dickinson M, Cherif H, Fenaux P, et al. Azacitidine with or without eltrombopag for first-line treatment of intermediate- or high-risk MDS with thrombocytopenia. Blood. 2018;132(25):2629-2638.

37. Noris P, Favier R, Alessi MC, et al. ANKRD26-related thrombocytopenia and myeloid malignancies. Blood. 2013;122(11):1987-1989.

38. Noris P, Pecci A. Hereditary thrombocytopenias: a growing list of disorders. Hematology Am Soc Hematol Educ Program. 2017; 2017(1):385-399.

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

	Overall	*MYH9*-RD	*ANKRD26*-RT	XLT/WAS	mBSS	*ITGB3*-RT
Patients, no.	24	9	9	3	2	1
M/F, no. 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] no. of patients	13	3	7	1	1	1
WHO grade = 1, no.	*7*	*0*	*7*	*0*	*0*	*0*
WHO grade = 2, no.	*4*	*1*	*0*	*1*	*1*	*1*
WHO grade = 3, no	*2*	*2*	*0*	*0*	*0*	*0*
Previous splenectomy, [3] no. of patients	2	2	0	0	0	0

Notes: [1] = as evaluated by phase-contrast microscopy in a counting chamber. [2] = spontaneous bleeding presented during the week preceding baseline evaluation according to World Health Organization (WHO) bleeding scale. [3] = previous splenectomy because of a mistaken diagnosis of immune thrombocytopenia.

Table 2. Response to Part 1 (primary endpoint), overall and according to the different forms of IT.

	Overall	*MYH9*-RD	*ANKRD26*-RT	WAS/XLT	mBSS	*ITGB3*-RT
Evaluable patients, no.	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]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 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 (SB)[3]						
Patients with SB at baseline, no.	12	3	6	1	1	1
Complete remission of SB end 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 end 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]

Notes: [1] = according to predefined study criteria. [2] = as evaluated by phase-contrast microscopy in a counting chamber. [3] = spontaneous bleeding presented during the week preceding evaluation according to World Health Organization (WHO) bleeding scale.
[§] $p<0.001$ with respect to baseline. [#] $p=0.001$ with respect to baseline.

Table 3. Doses of eltrombopag administered during the Part 1.

	Overall	*MYH9*-RD	*ANKRD26*-RT	WAS/XLT	mBSS	*ITGB3*-RT
Evaluable patients, no.	23	9	8	3	2	1
Major response with 50 mg/day, no. (%)	10 (43.5)	7 (77.8)	1 (12.5)	0	2 (100)	0
Switch to 75 mg/day, no. (%)	13 (56.5)	2 (22.2)	7 (87.5)	3 (100)	0	1
Improvement of response with 75 mg/day[1], no.	4	0	2[§]	2[#]	0	0

Notes: [1] = achievement of a better response according to the study criteria with respect to treatment with 50 mg/day. [§] =1 patient achieved major response, 1 achieved minor response. [#] = both patients achieved minor response.

Table 4. Adverse events (AEs) recorded during the Part 1. All the AEs were grade 1 according to CTCAEv4.0.

Adverse event	No. of AEs	No. 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 progressively increase for two 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 is unlikely.

Notes: [1] Eltrombopag (Revolade®) Product information available at https://www.ema.europa.eu/en/medicines/human/

Table 5. Response to Part 2 in the 4 enrolled patients.

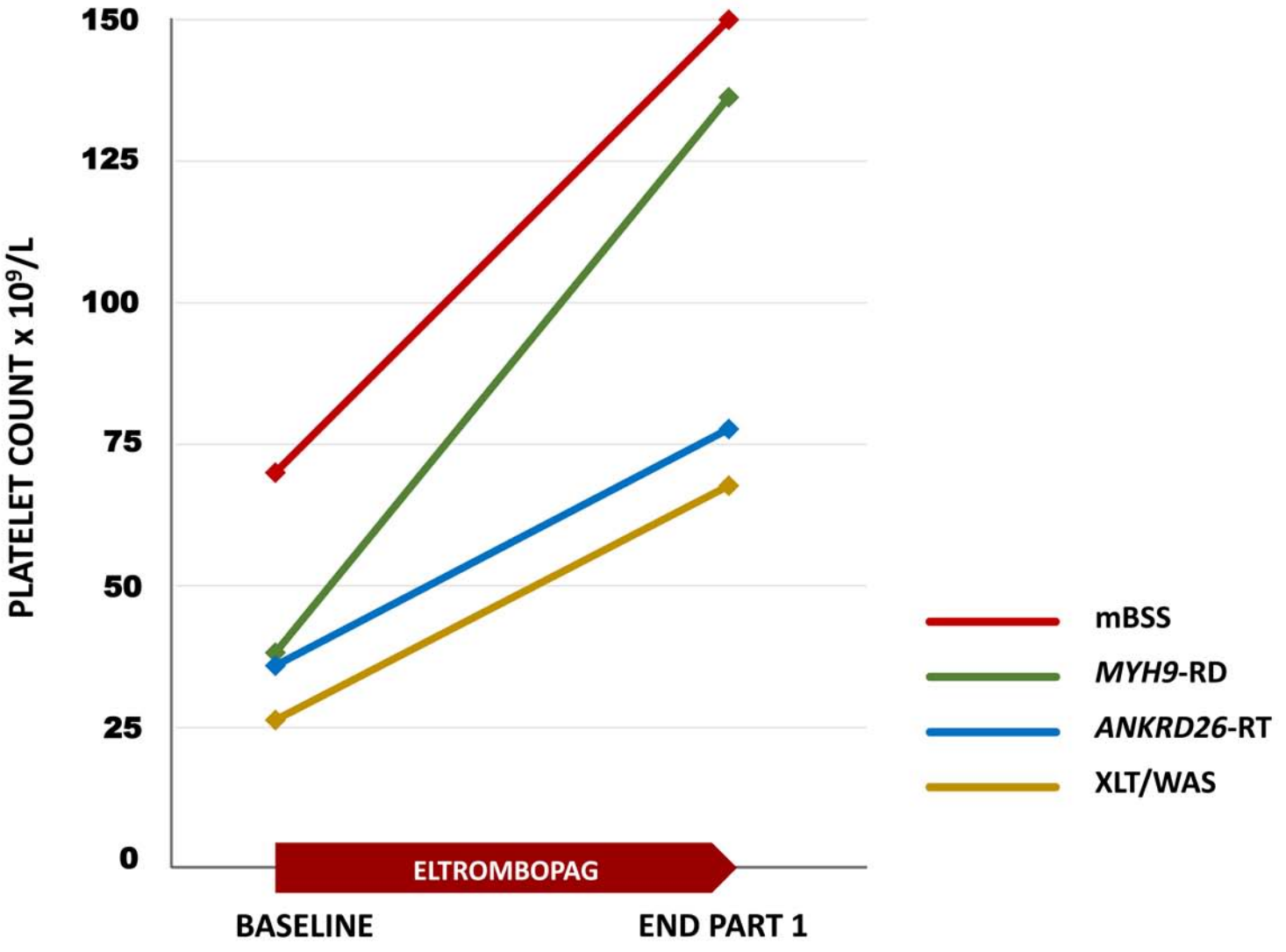
Patient ID (Patient/Family)[1]	1/1	12/10	17/13	22/16
Gender/age	F/49	F/45	F/45	M/24
Diagnosis	*MYH9*-RD	*MYH9*-RD	*ITGB3*-RT	WAS
Treatment duration, weeks	16	16	16	8
WHO bleeding score - baseline	3	3	2	2
Bleeding symptoms - baseline	Easy bruising Petechiae Gum bleeding Epistaxis	Easy bruising Gum bleeding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

Notes: [1] = please see Table S4. [2] = spontaneous bleeding presented during the 2 weeks preceding the last on-treatment visit according to WHO 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.

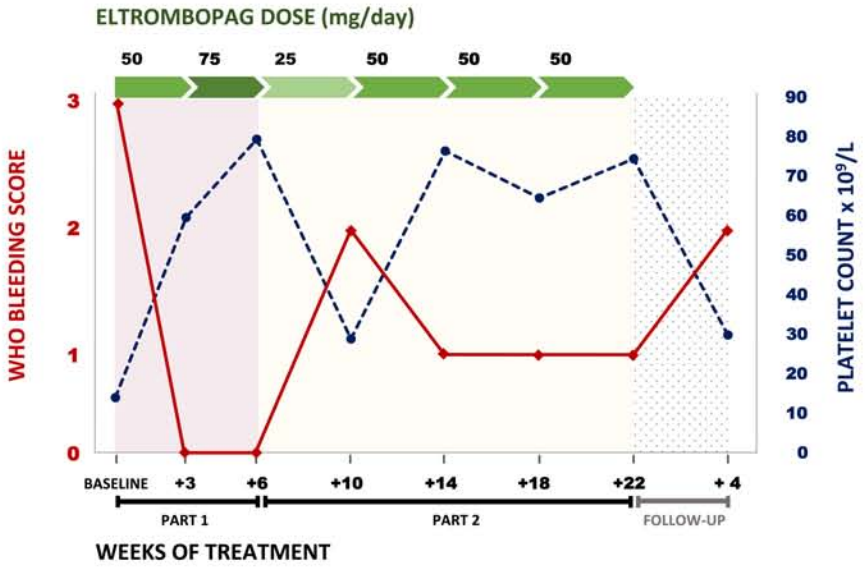
FIGURE LEGENDS

Figure 1. Mean increase in platelet count in the responders to the Part 1 treatment. Patients are categorized according to the diagnosis of the specific form of IT. *MYH9*-RD: *MYH9*-related disease. mBSS: monoallelic Bernard-Soulier syndrome. *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 treatment along with their 95% confidence intervals (95%CI) are reported in the grey box.

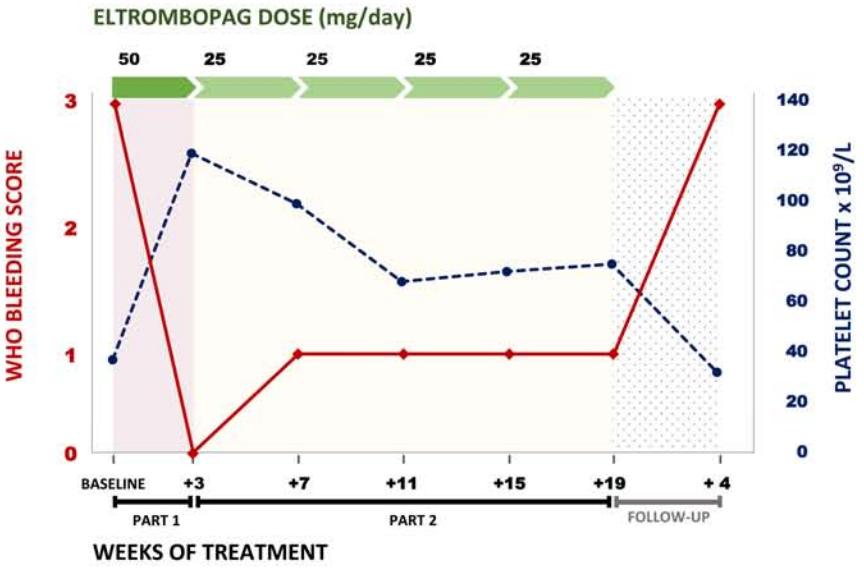
Figure 2. Effects of Part 1 and Part 2 treatments in the 4 individuals who received long-term eltrombopag. Figure summarizes the effects of eltrombopag administration on bleeding symptoms according to the World Health Organization (WHO) bleeding scale and on platelet count. Patients 1/1 and 12/10 are affected with *MYH9*-related disease, patient 17/13 with *ITGB3*-RT, and patient 22/16 with Wiskott-Aldrich syndrome (see Table 5).



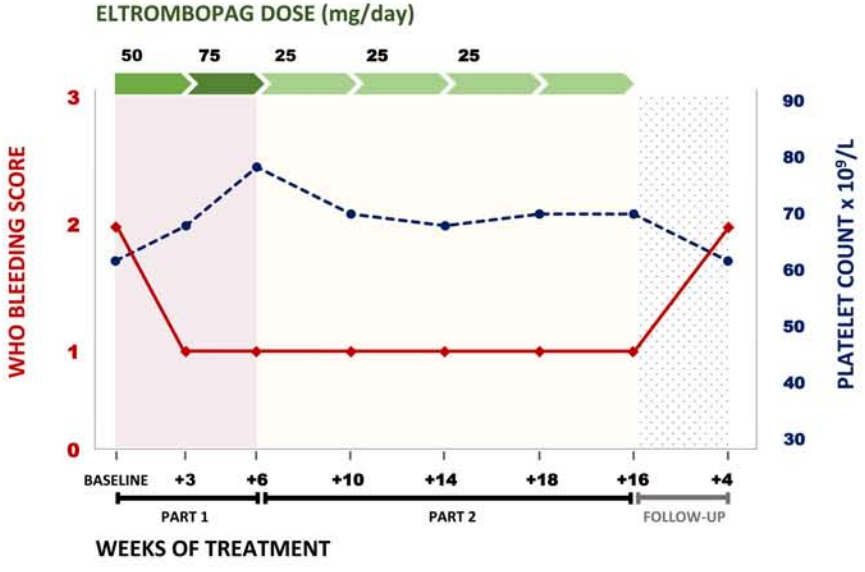
	Mean [95%CI]	Mean [95%CI]
Overall	38.8 [28.2-49.3]	108.3 [81.8-134.8]
mBSS	70.0 [57.3-82.7]	150.5 [29.8-271.2]
MYH9-RD	38.2 [20.8-55.7]	136.3 [84.1-188.6]
ANKRD26-RT	35.9 [12.9-58.8]	77.7 [49.9-105.5]
XLT/WAS	26.3 [-12.9-65.6]	67.7 [-27.6-163.0]



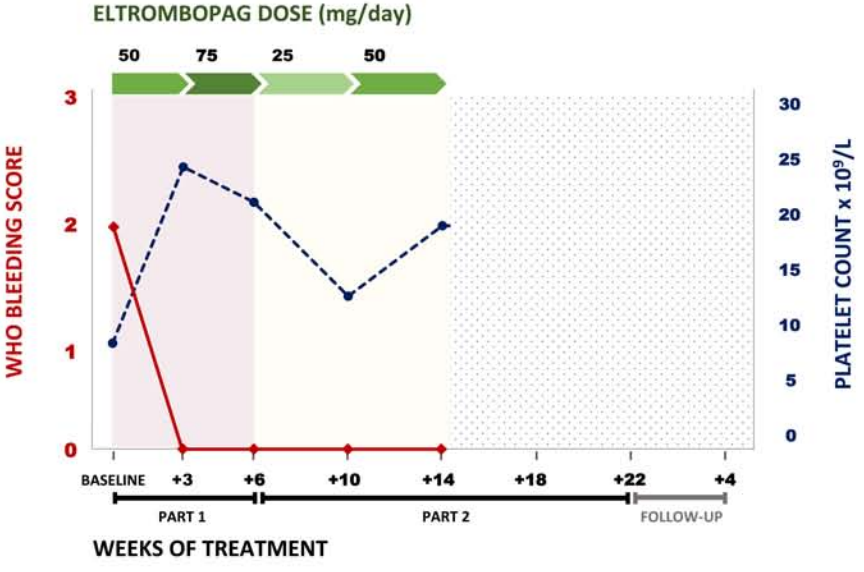
Patient 1/1



Patient 12/10



Patient 17/13



Patient 22/16

Supplemental data of the paper entitled "Eltrombopag for the treatment of inherited thrombocytopenias: a phase 2 clinical trial".

SUPPLEMENTAL METHODS

Inclusion and exclusion criteria

Patients were eligible for the study if they fulfilled all of the following criteria: diagnosis of one of the forms of IT listed in Table S2 confirmed by molecular analysis; age 16-70 years; platelet count <80 $x10^9$/L. Exclusion criteria are listed in Table S2.

Investigation of patients

Table S3 details the studies performed to investigate patients at baseline and at each subsequent visit (Part 1: after 3 weeks of treatment and, in some patients, 6 weeks of treatment, and post-treatment assessment at 30 days unless patients entered Part 2. Part 2: after 4, 8, 12, and 16 weeks of treatment and post-treatment assessment at 30 days). Platelet count was measured by both automated cell counters and phase-contrast microscopy in a counting chamber. Since electronic cell counters underestimate platelet count in patients with ITs characterized by marked platelet macrocytosis,[1,2] only platelet count measured by microscopy was used for the purposes of this study. Spontaneous bleeding was measured according to the WHO bleeding scale:[3] grade 0, no bleeding; grade 1, cutaneous bleeding only; grade 2, mild blood loss; grade 3, gross blood loss; grade 4, debilitating blood loss.
Adverse events (AEs) were coded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAEv4.0). Patients' health-related quality of life (HR-QoL) was assessed in patients eligible to Part 2 at baseline and at each subsequent visit as detailed below.

Measurement of serum thrombopoietin levels

Measurement of serum thrombopoietin (TPO) levels was centralized at the IRCCS Policlinico San Matteo, Pavia. Serum TPO levels were measured using the Quantikine ELISA Human Thrombopoietin Immunoassay Kit (R&D system, Minneapolis, USA) according to the manufacturer's instructions.[4]

In vitro platelet aggregation

In vitro platelet aggregation in response to collagen, adenosine diphosphate (ADP) and ristocetin was assessed in patients who obtained platelet count >100 x10^9/L, with the densitometric method of Born, as described.[5]

Flow cytometry investigation of platelet activation

Platelet activation in response to different agonists was investigated by flow cytometry as reported.[6] Briefly, aliquots of whole blood were incubated with moAbs and either ADP 1 µM, ADP 5 µM, TRAP 25 µM, or vehicle HEPES buffer alone, for 10 minutes at 37°C, and then fixed with paraformaldehyde. The following moAbs were used: PAC1, which specifically binds to the activated conformation of GPIIb-IIIa (Becton Dickinson, San Josè, CA, USA); CLB-Thromb/6 against P-selectin (CD62P) (Immunotech, Marseille, France); P2 against GPIIb-IIIa (CD41) (Immunotech). Platelets were gated by GPIIb-IIIa (CD41) expression. Platelet activation was expressed as the ratio between mean fluorescence intensity (MFI) measured after stimulation with each agonist and MFI measured after incubation with the buffer alone. Patients' samples were processed in parallel with those of 25 healthy controls. Data represent the mean ± SD of two independent analyses.

Assessment of patients' health-related quality of life

Patients' health-related quality of life (HR-QoL) was assessed in patients eligible to Part 2 at baseline and at each subsequent visit. HR-QoL was measured through the administration of three validated questionnaires with complementary significance. [3,7-10] The 18-item Functional Assessment of Cancer Therapy-Thrombocytopenia (FACT-Th18) was used to assess the effect of bleeding on HR-QoL.[7,9] The fatigue subscale of the Functional Assessment of Chronic Illness Therapy (FACIT-F) questionnaire was used to focus on the perception of fatigue.[8,9] The acute recall version of the short form-36, version 1 (SF-36v1) measured general HR-QoL.[10]

Statistical analysis

Stata 15.1 (StataCorp, College Station, TX) was used for all analyses. The rate of response and its 95% exact binomial confidence interval (95%CI) was computed. We compared baseline to end of Part 1 platelet counts with the Student t test for paired data (after graphically assessing normality of the distribution) and computed the mean change and its 95%CI (normal based).

REFERENCES FOR SUPPLEMENTAL METHODS

1. Seri M, Pecci A, Di Bari F, et al. MYH9-related disease: May-Hegglin anomaly, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome are not distinct entities but represent a variable expression of a single illness. Medicine (Baltimore). 2003;82(3):203-215.

2. Noris P, Klersy C, Gresele P, et al. Platelet size for distinguishing between inherited thrombocytopenias and immune thrombocytopenia: a multicentric, real life study. Br J Haematol. 2013;162(1):112-119.

3. Cheng G, Saleh MN, Marcher C, et al. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. Lancet. 2011;377(9763):393-402.

4. Pecci A, Ragab I, Bozzi V, et al. Thrombopoietin mutation in congenital amegakaryocytic thrombocytopenia treatable with romiplostim. EMBO Mol Med. 2018;10(1);63-75.

5. Pecci A, Gresele P, Klersy C, et al. Eltrombopag for the treatment of the inherited thrombocytopenia deriving from MYH9 mutations. Blood. 2010;116(26):5832-5837.

6. Melazzini F, Palombo F, Balduini A, et al. Clinical and pathogenic features of ETV6-related thrombocytopenia with predisposition to aucte lymphoblastic leukemia. Haematologica. 2016;101(11):1333-1342.

7. Cella D, Beaumont JL, Webster KA, Lai JS, Elting L. Measuring the concerns of cancer patients with low platelet counts: the Functional Assessment of Cancer Therapy-thrombocytopenia (FACT-Th) questionnaire. Support Care Cancer 2006;14:1220-1231.

8. Signorovitch J, Brainsky A, Grotzinger KM. Validation of the FACIT-fatigue subscale, selected items from FACT-thrombocytopenia, and the SF-36v2 in patients with chronic immune thrombocytopenia. Qual Life Res. 2011;20(10):1737-1744.

9. Webster K, Cella D, Yost K. The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: properties, applications, and interpretation. Health Qual Life Outcomes. 2003;1:79.

10. Apolone G, Mosconi P. The Italian SF-36 Health Survey: translation, validation and norming. J Clin Epidemiol. 1998;51(11):1025-1036.

SUPPLEMENTAL TABLES

Table S1. Centres that participated in this study.

- IRCCS Policlinico San Matteo Foundation, Pavia, Italy (sponsor and coordinating centre).
- Azienda Ospedaliera di Perugia, Perugia, Italy.
- Azienda Ospedaliera di Padova, Padova, Italy.
- IRCCS Policlinico Agostino Gemelli Foundation, Roma, Italy.
- Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy.

Table S2. Inclusion and exclusion criteria for the present study.

INCLUSION CRITERIA

Patients were considered for enrollment if they fulfilled all of the following 4 criteria.

1 - Diagnosis of one of the following forms of inherited thrombocytopenia confirmed by molecular analysis:
- *MYH9*-related disease (OMIM 155100, 605249, 153640, 153650)
- Bernard-Soulier Syndrome deriving from monoallelic mutations (OMIM 153670)
- Wiskott-Aldrich syndrome (OMIM 301000).
- X-linked thrombocytopenia (OMIM 313900).
- X-linked thrombocytopenia with thalassemia (OMIM 314050).
- Dyserythropoietic anemia with thrombocytopenia (OMIM 300367).
- *ITGA2B*/*ITGB3*-related thrombocytopenia (OMIM 187800).
- *ANKRD26*-related thrombocytopenia (OMIM 188000).
- *TUBB1*-related thrombocytopenia (OMIM 613112)
- *ACTN1*-related thrombocytopenia (OMIM 615193)
- *GFI1B*-related thrombocytopenia (OMIM 187900)
- *CYCS*-related thrombocytopenia (OMIM 612004)
- *SLFN14*-related thrombocytopenia (OMIM not available)

2 - Age ≥ 16 years and ≤ 70 years
3 - Average platelet count at baseline and during the previous year less than 80 $x10^9$/L
4 - Written informed consent

EXCLUSION CRITERIA

Patients were excluded from enrollment if they presented one or more of the following criteria.

- Hypersensitivity to eltrombopag or one of the excipients.
- History of thromboembolic events.
- Treatment with anti-platelet drugs or other drugs affecting platelet function and/or with anticoagulants.
- Concurrent diseases or conditions that significantly increase the risk of thromboembolic events.
- Moderate to severe liver failure (Child-Pugh score ≥ 5).
- Altered renal function as defined by creatinine ≥ 2 mg/dL
- Previous or concurrent clonal disorders of the myeloid series (acute myeloid leukemias and myelodysplastic syndromes).
- Females who are pregnant or nursing (a negative pregnancy test was required before enrolment of fertile women).
- Formal refusal of any recommendations for a safe contraception.
- Alcohol or drug addiction.
- Any other disease or condition that by the advice of the responsible physician would make the treatment dangerous for the patient or would make the patient ineligible for this study, including physical, psychiatric, social and behavioral problems.

Table S3. Studies for investigation of patients at baseline and at each subsequent on-treatment and post-treatment assessments (unless otherwise specified in notes).

- Medical history
- Physical examination
- Evaluation of bleeding tendency according to WHO bleeding scale during the previous 1 or 2 weeks [1]
- Complete blood counts and differential by automated cell counter
- Measurement of platelet count by phase-contrast microscopy in a counting chamber
- Peripheral blood smear examination
- Measurement of plasma aspartate transaminase (AST), alanine transaminase (ALT), total and fractionated bilirubin, and creatinine
- Urine analysis
- Ophthalmic assessment to monitor for cataracts or other ocular changes[2]
- Measurement of serum thrombopoietin level
- Assessment of health-related quality of life with the FACT-Th18, FACIT-F, and SF-36v1 questionnaires[3]
- Investigation of *in vitro* platelet aggregation in response to collagen (5 and 20 µg/mL), ADP (2 or 5 and 20 µM), and ristocetin (1.5 mg/mL)[4]

Notes: [1] = previous 1 week at baseline and during Part 1, previous 2 weeks during Part 2.
[2] =performed only at baseline, at the end of Parts 1 and 2, and at the assessment 30 days after the end of Parts 1 and 2.
[3] = performed only at baseline, during the Part 2, and at the assessment 30 days after the end of Part 2.
[4] = performed at the end of the Parts 1 and 2 whenever platelet count was over 100 $x10^9$/L.

Table S4. Features of the study population at baseline.

Patient no./ Family no.	Gender / age	Diagnosis	Causative mutation	Automated platelet count,[1] x10^9/L	Microscopic platelet count,[2] x10^9/L	WHO bleeding score[3]	Bleeding symptoms[4]	Other disease features[5]
1/1	F/49	*MYH9*-RD	*MYH9* c.279C>G	4	14	3	EB, Pe, GB, Ep	Nephropathy, sensorineural deafness, cataracts
2/2	M/57	*ANKRD26*-RT	*ANKRD26* c.-125 T>G	22	17	1	EB	-
3/3	F/55	*MYH9*-RD	*MYH9* c.3493C>T	58	69	2	EB, GB	Sensorineural deafness
4/4	M/63	*ANKRD26*-RT	*ANKRD26* c.-118C>A	37	33	1	EB	-
5/5	M/43	*ANKRD26*-RT	*ANKRD26* c.-125T>G	13	12	1	EB, Pe	-
6/6	M/46	mBSS	*GPIBA* c.515C>T	65	69	2	GB, Ep, Hm	-
7/7	F/24	*ANKRD26*-RT	*ANKRD26* c.-128G>A	42	37	1	EB	-
8/8	M/54	mBSS	*GPIBA* c.515C>T	67	71	0	-	-
9/9	M/40	*ANKRD26*-RT	*ANKRD26* c.-116C>T	67	63	1	EB	-
10/2	M/54	*ANKRD26*-RT	*ANKRD26* c.-125T>G	33	33	1	EB	-
11/2	M/19	*ANKRD26*-RT	*ANKRD26* c.-125T>G	55	53	1	EB	-
12/10	F/45	*MYH9*-RD	*MYH9* c.4270G>C	23	38	3	EB, GB, Me	Nephropathy, sensorineural deafness, cataracts
13/11	M/37	XLT/WAS	*WAS* c.257G>A	11	30	0	-	-
14/11	M/27	XLT/WAS	*WAS* c.257G>A	52	40	0	-	-
15/12	M/19	*MYH9*-RD	*MYH9* c.2104C>T	16	12	0	-	Nephropathy, sensorineural deafness
16/12	M/46	*MYH9*-RD	*MYH9* c.2104C>T	67	70	0	-	Nephropathy, sensorineural deafness

17/13	F/45	*ITGB3*-RT	*ITGB3* c.2134+1G>C	55	62	2	EB, Me	-
18/7	F/39	*ANKRD26*-RT	*ANKRD26* c.-128G>A	78	75	0	-	-
19/14	F/47	*MYH9*-RD	*MYH9* c.5797C>T	61	57	0	-	-
20/14	F/34	*MYH9*-RD	*MYH9* c.5797C>T	30	27	0	-	-
21/15	F/25	*MYH9*-RD	*MYH9* c.3485G>C	23	18	0	-	
22/16	M/24	XLT/WAS	WAS c.777+3inst	10	9	2	EB, Ep, Hm	Cutaneous eczema, immunodeficiency
23/17	F/66	*MYH9*-RD	*MYH9* c.4270G>A	37	39	0	-	Cataracts
24/18	M/29	*ANKRD26*-RT	*ANKRD26* c.-126T>G	14	14	0	-	-

Notes: [1] = as evaluated by standard automated cell counters. [2] = as evaluated by phase-contrast microscopy in a counting chamber. Only platelet count measured with this method was used for the purposes of this study. [3] = spontaneous bleeding presented during the week preceding baseline evaluation according to World Health Organization (WHO) bleeding scale. [4] = EB, easy bruising. Pe, petechiae. GB, gum bleeding. Ep, epistaxis. Me, menorrhagia. Hm, hematochezia. [5] = other disease features in patients with syndromic forms of ITs.

Table S5. Response to Part 1 of the study.

Patient no./ Family no.	Gender / age	Diagnosis	Maximal eltrombopag dose[1]	Platelet count - baseline,[2] x10^9/L	Platelet count - end treatment,[2] x10^9/L	WHO bleeding grade - baseline[3]	WHO bleeding grade - end treatment[3]	Response[4]
1/1	F/49	*MYH9*-RD	75 mg	14	80	3	0	Minor
2/2	M/57	*ANKRD26*-RT	75 mg	17	49	1	0	Minor
3/3	F/55	*MYH9*-RD	50 mg	69	300	2	0	Major
4/4	M/63	*ANKRD26*-RT	75 mg	33	82	1	0	Minor
5/5	M/43	*ANKRD26*-RT	75 mg	12	35	1	0	Minor
6/6	M/46	mBSS	50 mg	69	141	2	0	Major
7/7	F/24	*ANKRD26*-RT	75 mg	37	91	1	0	Minor
8/8	M/54	mBSS	50 mg	71	160	0	0	Major
9/9	M/40	*ANKRD26*-RT	75 mg	63	109	1	0	Major
10/2	M/54	*ANKRD26*-RT	50 mg	33	35	1	0	Not evaluable
11/2	M/19	*ANKRD26*-RT	75 mg	53	60	1	1	No response
12/10	F/45	*MYH9*-RD	50 mg	38	120	3	0	Major
13/11	M/37	WAS/XLT	75 mg	30	96	0	0	Minor
14/11	M/27	WAS/XLT	75 mg	40	83	0	0	Minor
15/12	M/19	*MYH9*-RD	75 mg	12	77	0	0	Minor
16/12	M/46	*MYH9*-RD	50 mg	70	122	0	0	Major
17/13	F/45	*ITGA2B/ITGB3*-RT	75 mg	62	78	2	1	No response
18/7	F/39	*ANKRD26*-RT	50 mg	75	115	0	0	Major
19/14	F/47	*MYH9*-RD	50 mg	57	110	0	0	Major
20/14	F/34	*MYH9*-RD	50 mg	27	178	0	0	Major

21/15	F/25	*MYH9*-RD	50 mg	18	114	0	0	Major
22/16	M/24	WAS/XLT	75 mg	9	24	2	0	Minor
23/17	F/66	*MYH9*-RD	50 mg	39	126	0	0	Major
24/18	M/29	*ANKRD26*-RT	75 mg	14	63	0	0	Minor

Notes: [1] = 50mg, 50 mg/day for 3 weeks. 75mg, 50 mg/day for 3 weeks followed by 75 mg/day for 3 additional weeks. [2] = as evaluated by phase-contrast microscopy in a counting chamber. [3] = spontaneous bleeding presented during the week preceding evaluation according to World Health Organization (WHO) bleeding scale. [4] = according to predefined study criteria.

Table S6. Results of platelet count measurements during the Part 1 of the study.

			Platelet count $x10^9/L^1$			
Patient no./ Family no.	**Gender / age**	**Diagnosis**	**Baseline**	**After 3 weeks treatment with eltrombopag 50 mg/day**	**After 3 additional weeks treatment with eltrombopag 75 mg/day**	**30 days after treatment discontinuation**
1/1	F/49	*MYH9*-RD	14	60	80	nd[2]
2/2	M/57	*ANKRD26*-RT	17	40	49	28
3/3	F/55	*MYH9*-RD	69	300	-	80
4/4	M/63	*ANKRD26*-RT	33	57	82	30
5/5	M/43	*ANKRD26*-RT	12	23	35	15
6/6	M/46	mBSS	69	141	-	90
7/7	F/24	*ANKRD26*-RT	37	68	91	46
8/8	M/54	mBSS	71	160	-	64
9/9	M/40	*ANKRD26*-RT	63	82	109	nd[3]
10/2	M/54	*ANKRD26*-RT	33	35	-	-
11/2	M/19	*ANKRD26*-RT	53	58	60	56
12/10	F/45	*MYH9*-RD	38	120	-	nd[2]
13/11	M/37	WAS/XLT	30	54	96	30
14/11	M/27	WAS/XLT	40	56	83	37
15/12	M/19	*MYH9*-RD	12	64	77	22
16/12	M/46	*MYH9*-RD	70	122	-	110
17/13	F/45	*ITGA2B/ITGB3*-RT	62	68	78	nd[2]

18/7	F/39	*ANKRD26*-RT	75	115	-	69
19/14	F/47	*MYH9*-RD	57	110	-	52
20/14	F/34	*MYH9*-RD	27	178	-	24
21/15	F/25	*MYH9*-RD	18	114	-	22
22/16	M/24	WAS/XLT	9	21	24	nd[2]
23/17	F/66	*MYH9*-RD	39	126	-	nd[3]
24/18	M/29	*ANKRD26*-RT	14	47	63	16

Notes: [1] = as evaluated by phase-contrast microscopy in a counting chamber. [2] = not determined (nd) as the patient was admitted to the Part 2 of the study (see Figure 2). [3] = not determined (nd) as the patient refused the follow-up visit.

Table S7. *In vitro* platelet aggregation at the end of Part 1 in the 11 patients who achieved a platelet count above 100 x10^9, maximal extent (percentage). Patients are reported according to the laboratories that performed the analysis, as the normal ranges of the assay are slightly different according to the laboratories of the different participating centres.

	Patient ID[1]	**Patient diagnosis**	**Platelet count,[2] x10^9/L**	**Collagen, 4 µg/mL**	**Collagen, 20 µg/mL**	**ADP, 2 or 5 µM[3]**	**ADP, 20 µM**	**Ristocetin, 1.5 mg/mL**
Pavia laboratory	3/3	*MYH9*-RD	300	87%	nd	67%	nd	85%
	6/6	mBSS	141	96%	nd	21%	86%	96%
	8/8	mBSS	160	85%	nd	58%	84%	100%
	9/9	*ANKRD26*-RT	120	74%	nd	70%	nd	84%
	12/10	*MYH9*-RD	109	88%	nd	85%	nd	98%
Perugia laboratory	16/12	*MYH9*-RD	122	nd	182%	nd	104%	nd
Padova laboratory	18/7	*ANKRD26*-RT	115	94%	nd	98%	nd	98%
	19/14	*MYH9*-RD	110	95%	nd	89%	nd	96%
	20/14	*MYH9*-RD	178	89%	nd	20%	85%	99%
	21/15	*MYH9*-RD	114	92%	nd	15%	79%	104%
Roma laboratory	23/17	*MYH9*-RD	126	74%	75%	75%	73%	69%

Normal values (range):
Pavia laboratory: collagen 66-88%, ADP 43-76%, ristocetin 67-90%.
Perugia laboratory: collagen 57.8-80.2%, ADP 43.2-73.2%, ristocetin 70-90%
Padova laboratory: collagen 44-86%, ADP 57-101%, ristocetin 76-90%
Roma laboratory: collagen 70-130%, ADP 58-90 %, ristocetin >60 %
Notes: [1] = please see Table S4. [2] = platelet count at the end of Part 1. [3] = the lowest dose of adenosine diphosphate (ADP) was 5 µM in the Pavia and Roma laboratories, and the 2 µM in the Padova laboratory.
Abbreviation: nd = not determined.

Table S8. TPO levels at baseline, at the end of Part 1, and at the post-treatment assessment 30 days after the end of Part 1.

	Overall	***MYH9*-RD**	**ANKRD26-RT**	**WAS/XLT**	**mBSS**	***ITGB3*-RT**
Baseline (n=23 patients) mean (SD), pg/mL	177.8 (125)	139.3 (104)	274.3 (129)	141.8 (33)	54.1 (13)	69 (-)
End treatment (n=23 patients) mean (SD), pg/mL	182.1 (209)	109.6 (117)	315.8 (287)	128.5 (33)	72.0 (20)	73 (-)
Post-treatment (n=17 patients) mean (SD), pg/mL	173.9 (178)	104.7 (73)	310.5 (259)	151.0 (65)	63.0 (15)	nd

Notes: [1] = 6 patients did not undergo assessment 30 days after the end of Part 1 (2 refused the post-treatment visit and 4 entered the Part 2).
Normal values, as determined in a cohort of 100 consecutive healthy individuals, are 72.7 pg/mL (mean, SD 47.1).

Table S9. Results of the assessment of health-related quality of life (HR-QoL) in the 4 patients enrolled to the Part 2.

	Mean (SD)	**Patient 1 (*MYH9*-RD)**	**Patient 2 (*MYH9*-RD)**	**Patient 3 (WAS)**	**Patient 4 (*ITGB3*-RT)**
FACT-TH18 (Trial Outcome Index)					
Baseline	83.7 (23.9)	55.7	72	102	105
Week 4	102.2 (6.6)	98.4	96	103.4	111
Week 8	100 (8.6)	96.3	92	99.8	112
Week 12	101.4 (8.4)	98.3	95	nd	111
Week 16	102.4 (8.4)	96.3	99	nd	112
Post-treatment	81.7 (27.6)	53.2	63	102	108.5
% at week 16 *vs.* baseline[1]	113%	173%	137%	98%	107%
FACIT-F					
Baseline	36.0 (12.2)	25	26	48	45
Week 4	43.5 (5.3)	39	39	49	47
Week 8	44.7 (5.1)	38	44	50	47
Week 12	44.3 (4.0)	40	45	nd	48
Week 16	44.7 (5.9)	38	49	nd	47
Post-treatment	39.0 (10.5)	25	37	48	46
% at week 16 *vs.* baseline[1]	124%	152%	188%	104%	104%
SF-36v.1					

	PCS	MCS	PCS	MCS	PCS	MCS	PCS	MCS	PCS	MCS
Baseline	44.9 (9)	50.7 (5)	47.3	43.3	30.9	51.1	50.7	55.9	50.8	52.7
Week 4	50.8 (3)	48.7 (6)	46.8	48.6	50.4	40.0	51.3	53.8	54.7	52.6
Week 8	49.8 (5)	52.7 (3)	42.7	49.7	50.2	51.7	51.5	57.0	54.7	52.6
Week 12	49.6 (7)	52.7 (3)	42.9	49.7	49.2	54.4	nd	nd	56.6	53.9
Week 16	50.3 (7)	54.1 (5)	43.5	49.7	50.5	59.6	nd	nd	57.0	53.0
Post-treatment	46.0 (8)	45.6 (8)	47.3	43.3	37.5	38.9	nd	nd	53.3	54.7
% week 16 *vs.* baseline[1]	112%	107%	92%	115%	163%	117%	102%	102%	112%	101%

Notes:
FACT-Th18 = The 18-item Functional Assessment of Cancer Therapy-thrombocytopenia questionnaire, which measures the effect of bleeding on HR-QoL.
FACIT-F = The Fatigue subscale of the Functional Assessment of Chronic Illness Therapy questionnaire, which measures the perception of fatigue.
SF-36v1 = The acute recall version of the Short Form-36, version 1, which measures the general HR-QoL. Data are reported separately for the Physical Component Summary (PCS) and the Mental Component Summary (MCS).
[1] = percentage variation at week 16 (end of Part 2) with respect to the baseline value.

SUPPLEMENTAL FIGURES

FIGURE S1

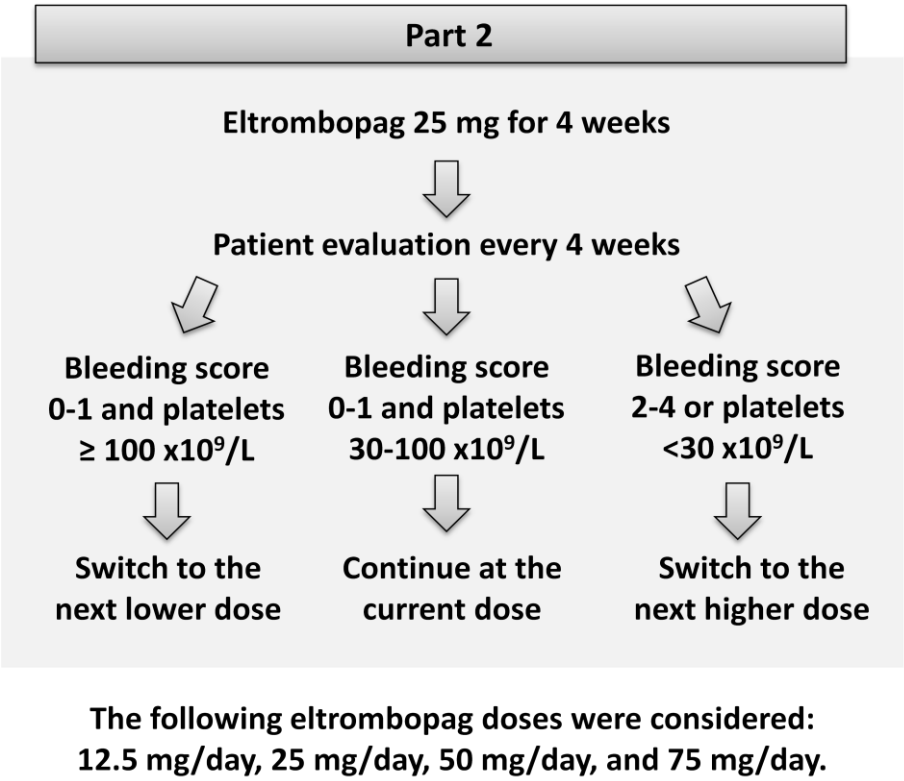


Figure S1. Schedule for dose adjustments of eltrombopag during the Part 2 of the study.

FIGURE S2

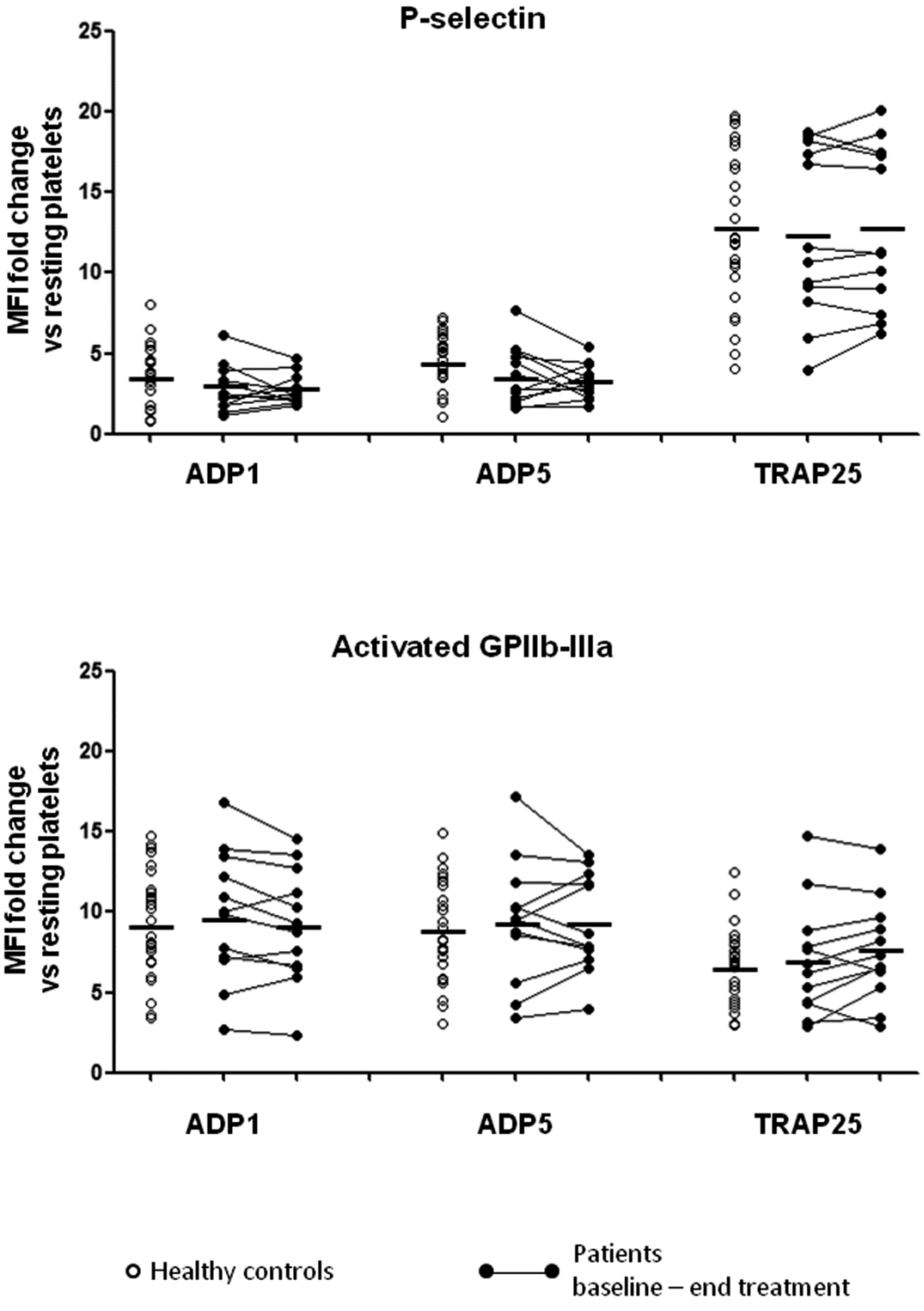


FIGURE S2. Platelet responsiveness to ADP and TRAP in 12 patients at baseline and at the end of Part 1. Flow cytometry study of platelet activation in response to ADP and TRAP was carried out in 12 patients at baseline and at the end of Part 1: investigated patients were 4 subjects with *MYH9*-RD, 5 with *ANKRD26*-RT, 2 with mBSS, and 1 with WAS. Results obtained in patients were compared with those of 25 healthy

controls who were processed in parallel. Platelet surface expression of P-selectin and of the activated form of GPIIb-IIIa (PAC1 antibody binding) was measured after incubation with ADP 1 µM, ADP 5 µM, TRAP 25 µM, or the vehicle HEPES buffer alone. Platelet activation is expressed as the ratio between the mean fluoresce intensity (MFI) measured after stimulation with each agonist and the MFI measured after incubation with the buffer alone (resting platelets). Filled circles with connecting lines represents the values obtained in individual patients at baseline and after treatment; open circles represent the values obtained in healthy controls. Thick lines indicate the mean values. Platelet responses to ADP and TRAP were not significantly different in patients at baseline compared to controls. Platelet responses to the agonists did not significantly change at the end of Part 1 treatment with respect to the baseline (Student t test for paired data).