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

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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).

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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

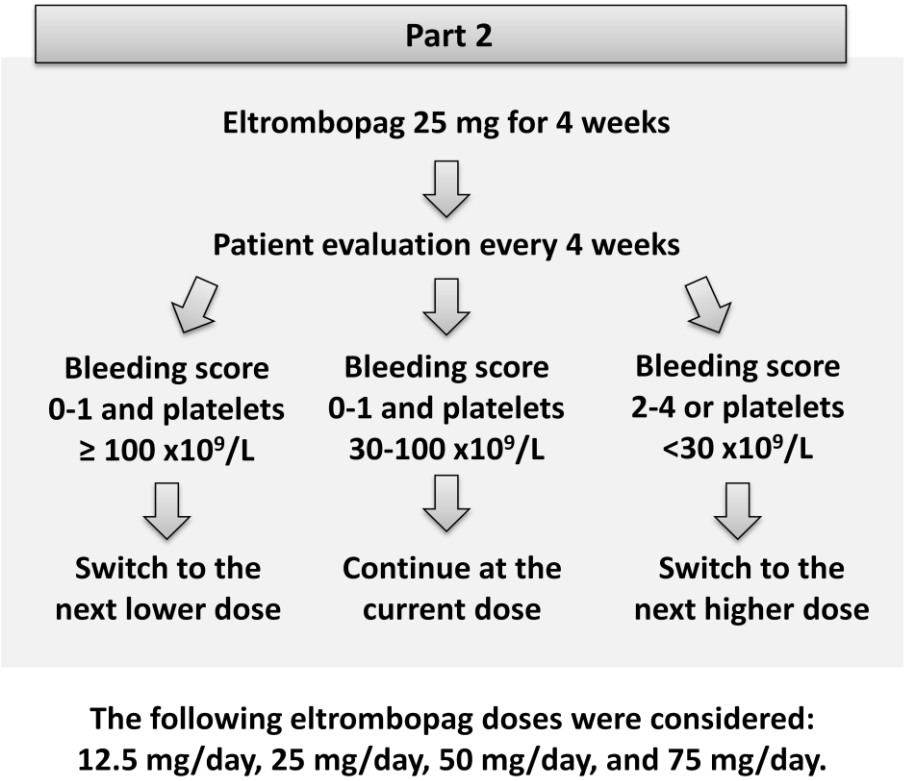

Figure S1. Schedule for dose adjustments of eltrombopag during the Part 2 of the study.

FIGURE S2

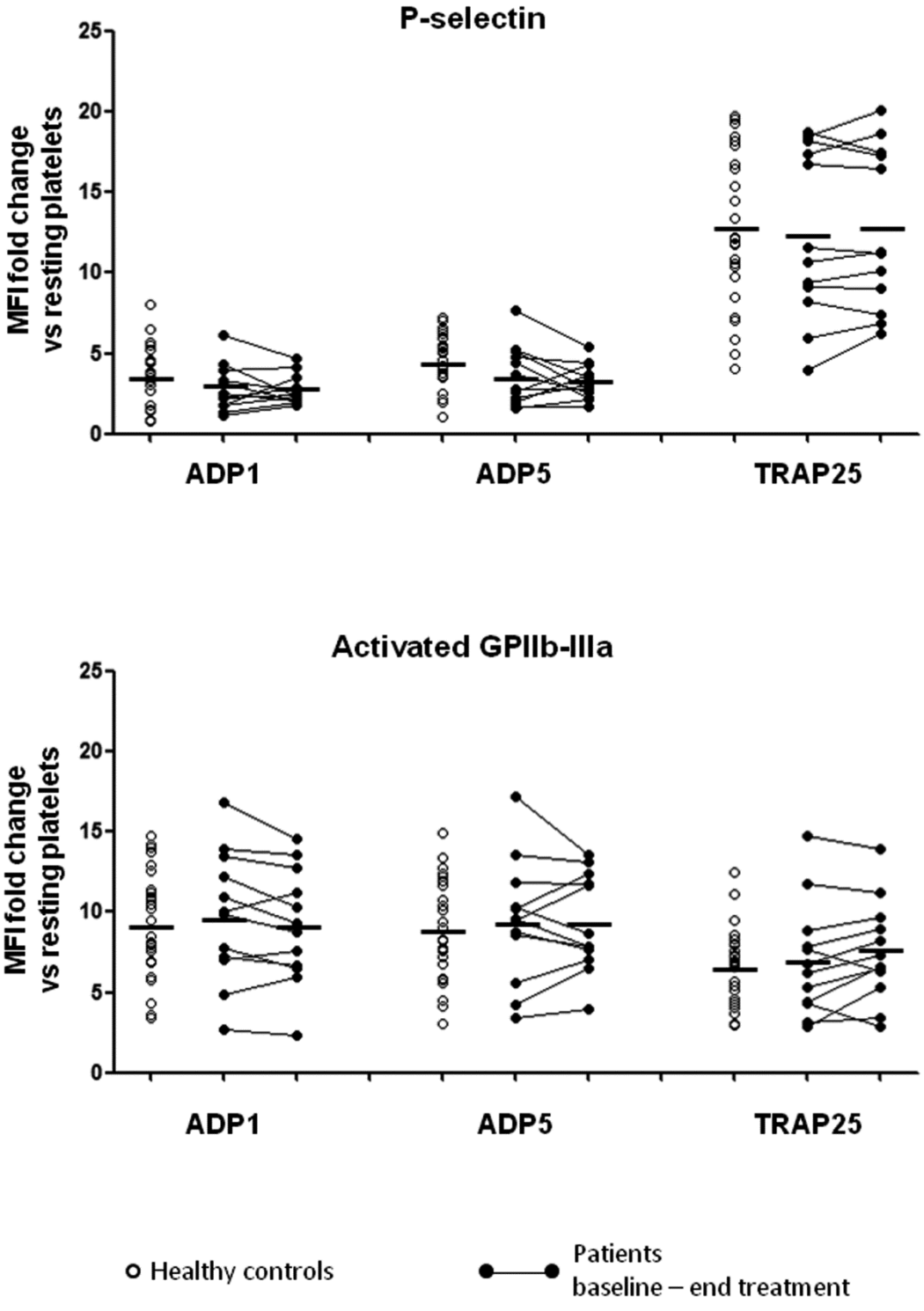

FIGURE S2. Platelet responsiveness to ADP and TRAP in 12 patients at baseline and at the end of Part 1. Flow cytometry study of platelet activation in response to ADP and TRAP was carried out in 12 patients at baseline and at the end of Part 1: investigated patients were 4 subjects with *MYH9*-RD, 5 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).