Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Townsley DM, Scheinberg P, Winkler T, et al. Eltrombopag added to standard immunosuppression for aplastic anemia. N Engl J Med 2017;376:1540-50. DOI: 10.1056/NEJMoa1613878

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Study Eligibility: Inclusion and Exclusion

All subjects age 2 and older with SAA who have not received prior ATG-based immunosuppressive therapy and lack a suitable matched sibling marrow donor, or are not allogeneic transplantation candidates due to patient choice, advanced age, or infeasibility of transplantation will be considered for enrollment. Due to the nature of SAA, counts may fluctuate depending on transfusions. Because of this, the lowest clinical laboratory result (ANC, platelet, and/or absolute reticulocyte count) obtained within 30-days prior to treatment initiation will be used for eligibility determination.

Inclusion Criteria

- Severe aplastic anemia characterized by bone marrow cellularity <30% (excluding lymphocytes) and at least two of the following:
  - Absolute neutrophil count< 500/ μL
  - Platelet count< 20,000/ μL
  - Absolute reticulocyte count<60,000/ μL
- Age ≥ 2 years old
- Weight> 12 kg

Exclusion Criteria

- Known diagnosis of Fanconi anemia
- Evidence of a clonal disorder on cytogenetics performed within 12 weeks of study entry. Patients with super severe neutropenia (ANC < 200 /μL) will not be excluded initially if cytogenetics are not available or pending. If evidence of a clonal disorder consistent with myelodysplasia is later identified, the patient will go off study – see section 9.6.
- Prior immunosuppressive therapy with any ATG, alemtuzumab, or high dose cyclophosphamide
- SGOT or SGPT >5 times the upper limit of normal
- Subjects with known liver cirrhosis in severity that would preclude tolerability of cyclosporine and eltrombopag as evidenced by albumin < 35g/L
- Hypersensitivity to eltrombopag or its components
- Infection not adequately responding to appropriate therapy
- Moribund status or concurrent hepatic, renal, cardiac, neurologic, pulmonary, infectious, or metabolic disease of such severity that it would preclude the patient's ability to tolerate protocol therapy, or that death within 7-10 days is likely
- Potential subjects with cancer who are on active chemotherapeutic treatment or who take drugs with hematological effects will not be eligible
- Current pregnancy, or unwillingness to take oral contraceptives or use a barrier method of birth control or practice abstinence to refrain from pregnancy if of childbearing potential during the course of this study

- Inability to understand the investigational nature of the study or to give informed consent or does not have a legally authorized representative or surrogate that can provide informed consent

Horse Anti-thymocyte Globulin (h-ATG) Administration

A single treatment course of h-ATG was administered at a dose of 40 mg/kg/day for 4 consecutive days. Dose was calculated based on actual body weight. H-ATG was infused intravenously for approximately 4 hours and be extended up to 24 hours to improve tolerance of infusional side effects. Serum sickness prophylaxis with oral prednisone at 1 mg/kg/d began prior to the first dose of h-ATG, and was continued for 10 days total and then tapered over the subsequent 7-14 days. Subjects who developed serum sickness sometimes required a longer tapering schedule and were dosed individually as clinically indicated.

Cyclosporine Dosing and Adjustments

Cyclosporine was administered at a dose of 6 mg per kilogram per day (12 mg per kilogram per day for children under 12 years of age), as divided doses every 12 hours from day 1 and continued for at least 6 months as tolerated in all cohorts. The dose was adjusted to maintain trough blood levels of 200 to 400 ng per milliliter as tolerated. All subjects in cohort 1 and the first 14 subjects in cohort 2 had cyclosporine stopped at 6 months. The protocol was amended starting with subject # 46 on cohort 2, so that cyclosporine was continued at a

fixed daily dose, 2mg/kg/day, for an additional 18 months in order to prevent relapse.

Eltrombopag Dosing and Adjustments

In the first of three consecutive cohorts, eltrombopag was initiated two weeks after beginning ATG and cyclosporine, due to concern for overlapping hepatotoxicity of the three agents, and eltrombopag was continued for 6 months. As most responses appeared within 3 months and in order to limit eltrombopag exposure and possible toxicity, in the second cohort eltrombopag was administered for 3 months only. As hepatotoxicity was infrequent, and also due to an apparently lower CR in the second cohort, in the third cohort eltrombopag was initiated with ATG on day 1 and continued for 6 months.

The daily starting dose of eltrombopag was determined according to age and ethnicity. Subjects between 12 and 17 years of age received the adult dose of 150 mg and to adjust for the higher expected exposure, if East Asian or South East Asian ancestry, the dose was reduced by 50% to 75mg. Those between 6 and 11 years 75 mg/day, and children between 2 and 5 years of age 2.5 mg/kg/day. The starting dose for East Asian and South East Asian subjects between 12 and 17 years of age was 75 mg once daily. For East Asian and South East Asian subjects between 6 and 11 years of age, the starting dose was 37.5 mg once daily, and for children between 2 and 5, the starting dose was 1.25

mg/kg. Dietary adjustments to permit its absorption in the absence of calcium were necessary.

The daily dose of eltrombopag was decreased to prevent thrombocytosis according to the following rules:

Platelet Count	**Dose Adjustment or Response**
>200,000/ µL (untransfused) at any time on study	Decrease dosage by 25mg every 2 weeks to lowest dosage that maintains platelet count ≥ 50,000/ µL. In children under 12, the dose will be decreased by 12.5 mg.
>400,000/ µL (untransfused) at any time on study	Discontinue eltrombopag for one week, if platelets fall to <200,000/ µL; restart at dosage decreased by 25 mg/day (or 12.5 mg in children under 12).

Cohort 3 Dose Delays of Eltrombopag During Days 1 through 14

Dose delays for transient transaminase elevations were performed when eltrombopag was administered with hATG, in cohort 3, to prevent exacerbation of liver toxicity. Transient hepatotoxicity is an expected and common side effect of h-ATG that occurs during the first 14 days following the start of h-ATG administration. In the event of an increase in the ALT level to > 6 times the ULN

during Days 1 – Days 14, eltrombopag was held until ALT was < 5 times the ULN and then resumed at the same dose.

Infection Prophylaxis

Pneumocystis jiroveci prophylaxis: Aerosolized pentamidine was used as prophylaxis against *Pneumocystis jiroveci,* 300 mg approximately every 4 weeks by inhalation beginning the first month of therapy and to continue for 6 months total for subjects 5 years of age and older for all cohorts. Atovaquone was substituted at the discretion of the PI. Bactrim (TMP/SULF) was avoided because trimethoprim is a moderate to strong inhibitor of CYPs that may theoretically result in enhanced activity of eltrombopag. Children under 5 years of age received dapsone or another prophylactic regimen at the discretion of the PI.

Antiviral prophylaxis: Valacyclovir, 500 mg once daily, was administered for at least 1 month in all subjects regardless of HSV serology status. Pediatric subjects less than 40 kg, received acyclovir (or equivalent) at 20mg/kg PO q12h to a maximum dose of 800mg q12h. Prophylaxis was extended at the discretion of the PI as clinically indicated.

Antibacterial and antifungal prophylaxis was not included systematically with the immunosuppressive regimen, but administered at the discretion of the PI or treating physician on a case-by-case basis.

Permitted Supportive Care

- Transfusion support (blood and platelets) as clinically indicated.
- Hematopoietic growth factors (e.g., G-CSF, GM-CSF, or erythropoietin) if deemed necessary by the investigator or treating physician. Romiplostim (N-Plate) or IL-11 (Neumega) was not administered.
- Estrogens, combination OCP's or leuprolide as indicated for uterine bleeding

Bone Marrow Imaging and Processing

Images of the H&E stained core biopsy sections were taken at 40x magnification on an Olympus BX41 microscope using an Olympus DP72 camera.

Bone marrow samples from patients and healthy donors were collected after obtaining written informed consent on treatment protocols approved by the National Institutes of Health (NIH) Institutional Review Board in accordance with the Declaration of Helsinki. Mononuclear cells (MNC) were separated using Ficoll-Hypaque density gradient centrifugation (MP Biomedicals LLC, Solon, OH). In select experiments, MNC were enriched for CD34+ cells by immunomagnetic bead affinity elution as per manufacturer's instructions (Miltenyi Biotec Inc., San Diego, CA).

CD34+ Cell Enumeration

<u>Cohort 1</u>

Patient and control MNC samples were stained at 4^{o}C for 30 minutes with anti-human CD45-APC and CD34-PE antibodies (BD Biosciences, San Jose, CA) and dead cells were excluded using a violet fluorescent reactive dye (ViVID, Invitrogen, Grand Island, NY). CD34+ cells were enumerated after initial gating on live CD45+ cells using a FACS Canto II Flow Cytometer Analyzer (BD Biosciences, San Jose, CA).

Cohort 2 - 3

Multiparameter flow cytometric analysis of bone marrow aspirates was performed using a fluorescence- activated cell sorter (FACS) Canto II Analyzer (BD Biosciences) equipped with 3 lasers and 8 fluorescent detectors, as previously described (Ganapathi K et al Blood 2015). Bone marrow aspirate cells were stained with CD34 antibodies (PerCP-Cy5.5, PE-Cy7, APC-H7, BD Biosciences) in appropriate dilutions for 15 minutes. After red blood cell lysing with BD FACS lysing solution, cells were washed with phosphate-buffered saline containing 1% albumin. Cells were fixed in a 1% paraformaldehyde solution, and 1×10^5 events were acquired using FACS Diva software (BD Biosciences). The list mode files were analyzed with FCS Express (DeNovo Software). CD34 positive cells were quantified as a percentage of all nucleated cells in the bone marrow aspirate samples.

Assay of Hematopoietic Progenitors Methods

Human CD34+ cells were stained with Rhodamine 123 (Invitrogen) at a concentration of 0.1ug/mL at 37^{o}C for 30 minutes. Rhodamine 123 uptake was interrupted by addition of ice-cold medium and effluxed by incubation at 37^{o}C for 30 minutes in dye-free buffer. Cells were subsequently stained with anti-human CD34-PE, CD38-APC, CD45RA-APC-H7, CD90-PECy7, and CD49f-PECy5 (BD Biosciences) for 30 minutes at 4^{o}C. Cells were analyzed using a FACS LSRII Flow Cytometer Analyzer (BD Biosciences) and percentages were determined for cells with an MPP (CD34+CD38-CD45RA-CD90-CD49f-) and HSC (CD34+CD38-CD45RA-CD90+CD49f+) phenotype.

Self-reported Health Outcomes

Methods

Adult English-speaking or Spanish speaking patients were eligible to complete surveys. Self-reported health outcomes [PROMIS Global Physical (GPH) and Mental (GMH) Health)][1], Health related quality of life [Functional Assessment of Cancer Therapy-Neutropenia[2] (FACT-N)], PROMIS sleep disturbance (Sleep) and applied cognitive abilities (Cog)[1] were secondary endpoints. All available data was analyzed using linear mixed modeling.

Results

Adult English or Spanish speaking patients (n=69) completed surveys. 23 subjects were not eligible to complete surveys; 19 patients were under 18 years

of age; and 4 patients due to non-English or non-Spanish speaking. Of the 69 patients who were eligible to complete the survey, all surveys were competed at baseline, and 97% and 93% were completed at 3 and 6 months, respectively. At study entry, SAA patients reported GPH and Cog scores significantly lower ($p<0.001$, $p=0.009$ respectively) than the US general population mean; the GPH difference was clinically relevant. Sleep disturbance and GMH scores were not significantly different ($p>0.05$). At the 3-month time point all scores were similar to pre-treatment scores or improved (FACT-N; $p=0.011$); 6-month scores were stable except for the GPH and FACT-N scores which improved ($p=0.004$, $p=0.0005$ respectively). Although improving, the GPH score remained significantly lower ($p=0.014$) than the US general population. Patients with an overall response at 6 months had significantly higher GPH ($p=0.017$) and FACT-N ($p=0.001$) compared to those with no response; although the significant improvement with physical health should be interpreted with caution due to post-hoc analyses. There was no effect of cohort for any outcome. Response bias may affect these results due to failure to complete surveys following treatment, however the effect is likely limited since 97% and 93% of surveys were completed at 3 and 6 months.

Self-reported Health Outcomes at Baseline (n=69)

Self-reported Outcome	Pre-eltrombopag[1]	6-Month[2]
FACT-N	124.2 (±20.2)	141.8 (±23.3)
Global PH[3]	43.9 (±8.1)	47.6 (±7.4)
Global MH[3]	49.2(±7.4)	48.8 (±7.9)
Sleep Disturbance[3]	51.3 (±9.3)	50.3 (±9.7)
Cognitive Abilities[3]	47.5 (±7.8)	49.8 (±7.0)

PH=PROMIS Global Physical; MH = PROMIS Global Mental Health), FACT-N = Functional Assessment of Cancer Therapy-Neutropenia [1]n=66; [2]n=64; [3]General Population Norm = Mean 50 (±10);

Pharmacokinetic Analysis

Methods

Subjects enrolled in Cohort 1, had PK assessments at the landmark 3-month study visit. Subject must have received once daily eltrombopag for at least 7 days prior to this visit (i.e., be at PK steady-state with no recent dose interruptions). If the subject was not currently receiving eltrombopag at the time of this visit (because of a dose interruption) or eltrombopag was reinitiated after a dose interruption within the 7 days prior to this visit, PK assessments were not performed. The eltrombopag dosing history for the 2 weeks prior to the PK visit will be recorded (any dose interruptions, actual dose administered).

Blood samples (2 mL) for PK analysis was collected in K2EDTA-containing tubes. One sample was collected at each of the following times: within 30 min prior to eltrombopag dosing (pre-dose sample), and at 2, 4, 6, and 8 h after eltrombopag dosing. Plasma concentrations of eltrombopag were measured using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) with a lower limit of quantification of 100 ng/mL. Pharmacokinetic parameters (area under the concentration-time curve – AUC, and peak plasma concentration – Cmax) were calculated using non-compartmental methods with WinNonLin Phoenix software.

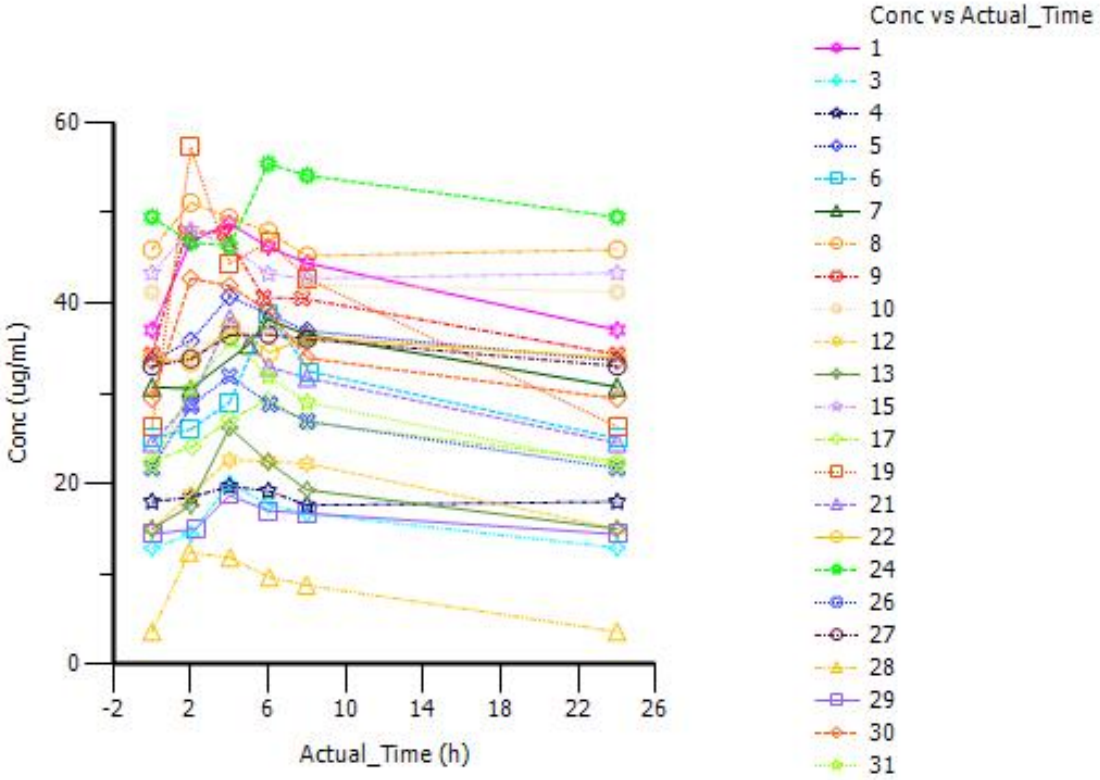
Results

Twenty-three subjects on Cohort 1 had PK assessments performed and 7 subjects were ineligible for PK assessment due to dose-adjustments prior to their visit. 21 subjects were on 150mg daily dose in the seven days prior, one subject was on 125 mg daily dose prior, and one subject was on 100 mg. The mean area under the curve (AUC) and maximum concentration (Cmax) according to eltrombopag dose is shown in panel A. In panel B, the concentration of eltrombopag in the plasma (x-axis) for each subject is graphed over time (y-axis). The first collection was obtained 30 minutes prior to eltrombopag (pre-dose sample), and at 2, 4, 6, and 8 hours after eltrombopag dosing. A 24-hour concentration value was imputed based on the pre-dose sample (steady-state assumption). Panel C shows the mean concentration for all 23 subjects. Panel D shows the trend towards higher exposure in older subjects and female subjects. Adolescent subjects consistent with adult subjects.

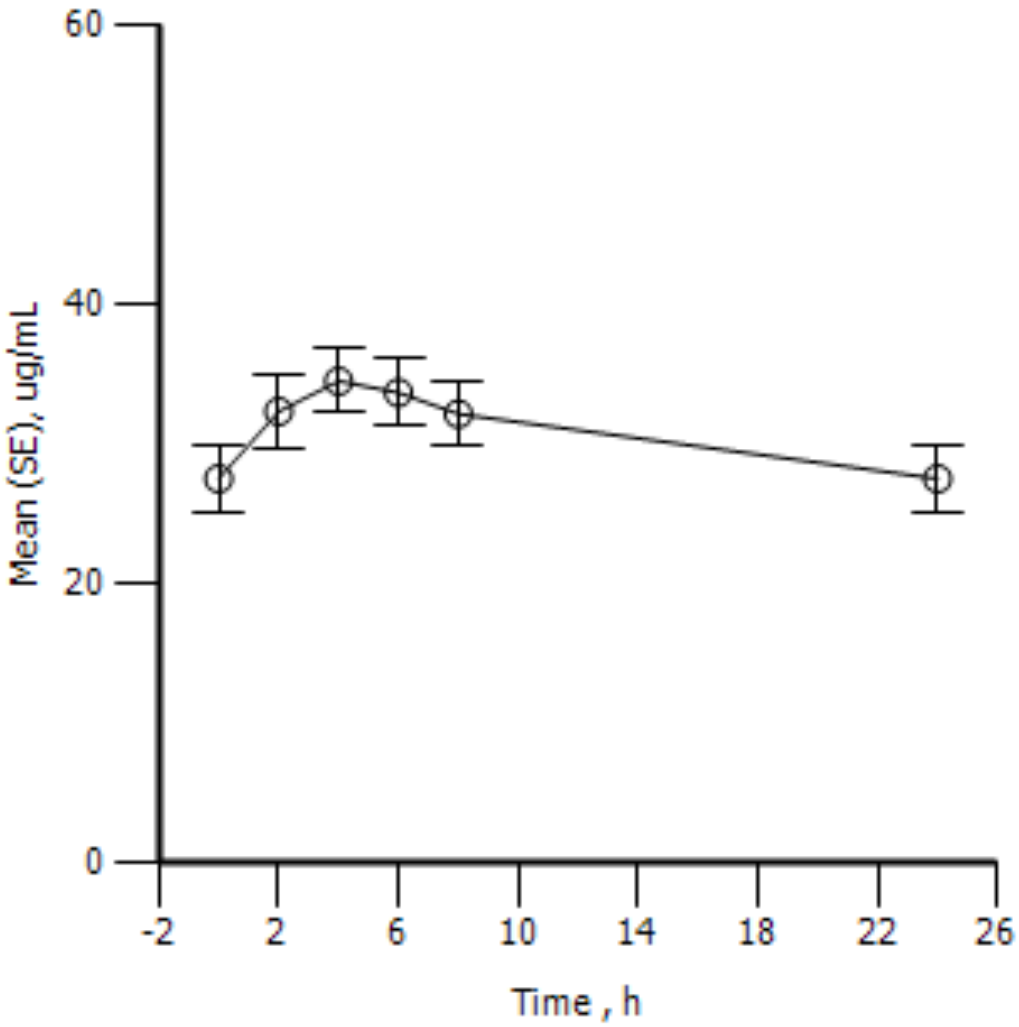
A

	Dose at month 3						
	100 mg		125 mg		150 mg		
Parameter	N	Geometric Mean	N	Geometric Mean	N	Geometric Mean	CV%
AUC(0-tau) ug.h/mL	1	841	1	703	21	668	50.6
Cmax ug/mL	1	37.4	1	38.8	21	33.8	43.2

B.


Conc vs Actual_Time
1
3
4
5
6
7
8
9
10
12
13
15
17
19
21
22
24
26
27
28
29
30
31
Conc (ug/mL)
60
40
20
0
-2
2
6
10
14
18
22
26
Actual_Time (h)

C.



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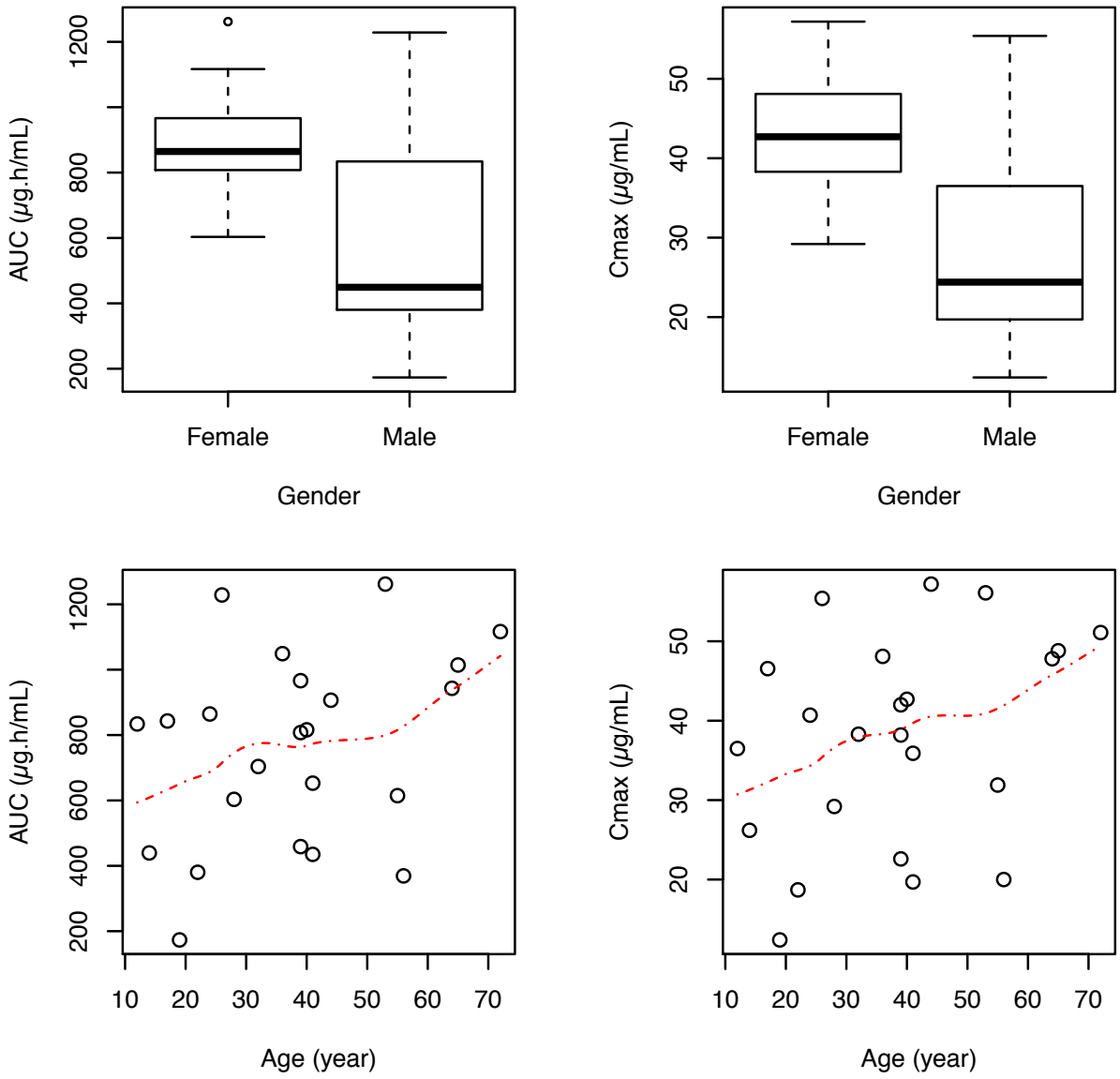


Statistical Analysis

Summary statistics, including proportions, means, standard deviations, and confidence intervals, were used to describe the primary and secondary end points. Nominal p-values for testing the primary end point of complete response rate at 6 months were computed using the t-tests for proportions with normal

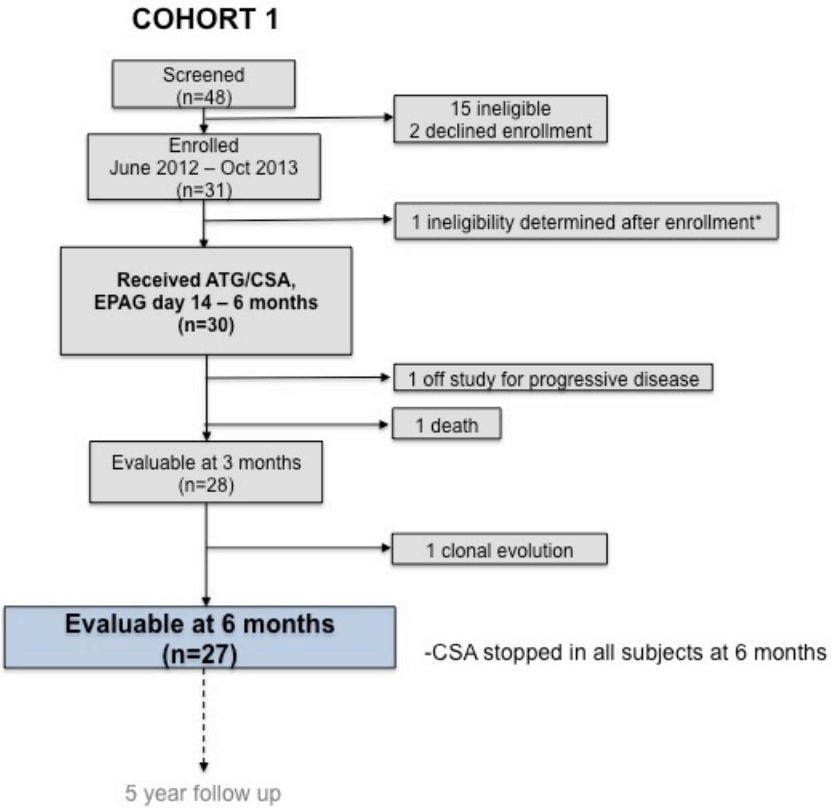
approximations. Statistical inferences with regard to mean changes in the secondary end points were described with 95% confidence intervals and two-sided t-tests for the null hypothesis that the mean changes are zero. Univariable and multivariable logistic regression models were used to evaluate the predictive effects of covariates on the complete and overall response rates at 6 months. The Cox Proportional Hazard Model was used to evaluate the effects of covariates on the distribution functions of time to event variables, including times to death, relapse and clonal evolution, with time to stem-cell transplantation treated as censoring time in some instances. Because the counts of neutrophil, reticulocyte and platelet have skewed distributions, logarithmic transformations of the form log(x+1) were applied to these variables when computing the p-values of their corresponding test statistics and their effects in regression analysis. Self-reported health outcomes were analyzed using linear mixed modeling.

Description of Historic Cohort

Secondary and exploratory endpoints were compared to a historical cohort of patients who received hATG/CSA while on randomized control arms of our two most recent clinical trials. The first protocol compared hATG/CsA ± sirolimus[3] and was conducted from 2003-2005, and the second trial compared hATG/CsA to rabbit ATG/CsA[4] and was conducted from 2005 to 2010. The standard horse ATG/CsA control arm of the first (N=42) and second trial (N=60) were used as the historical comparator (N=102) for this study. The overall hematologic

response at 6 months was 66% and complete response 17% in this historical cohort of 102 patients. Baseline characteristics of the historic cohort as compared to the current study are shown in Table S1. Since 1989 horse ATG plus cyclosporine has been administered as control in sequential protocols at the NHLBI. The hATG dose, protocol administration, management of infusion related toxicities, prophylactic therapies, and hATG supplier have been identical in the context of these. protocols. In addition, cyclosporine target levels, monitoring, and dose adjustments have been unchanged with this regimen in a single center at the NIH. The criteria for defining study entry, hematologic response and the timing of this determination has also been unchanged in severe aplastic anemia prospective protocols at the NIH. As a consequence, the hematologic response rate of h-ATG/CsA has been consistent since 1989 at our institution.

Figure S1. Enrollment Flow Diagram
Panel A thru C depicts flow through the study per cohort. The Clinical Center of NIH is a quaternary referral center, making application of a CONSORT analysis problematic due to screening at multiple levels. Of patients seen in our clinic with a putative diagnosis of severe aplastic anemia and determined to be eligible for this protocol, all but three were enrolled into the study. *One patient (subject #11) who enrolled on the protocol was misdiagnosed at baseline and discovered to have an unusual presentation of acute myeloid leukemia; she did not receive the study drug. A.



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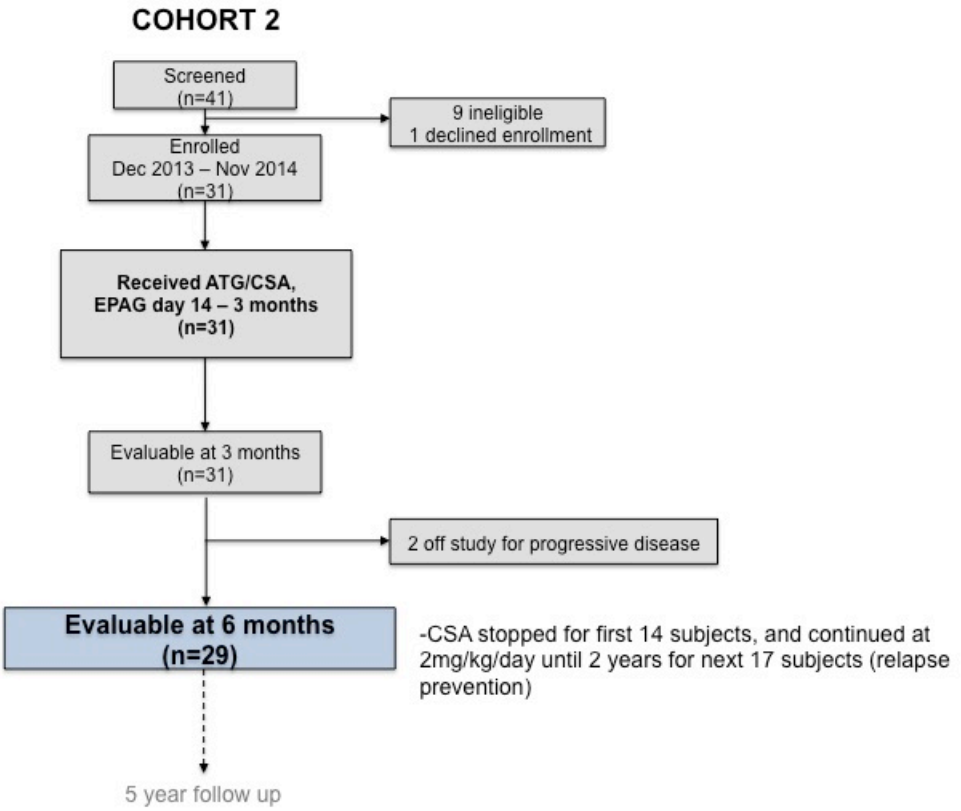

COHORT 2
Screened
(n=41)
9 ineligible
1 declined enrollment
Enrolled
Dec 2013 – Nov 2014
(n=31)
Received ATG/CSA,
EPAG day 14 – 3 months
(n=31)
Evaluable at 3 months
(n=31)
2 off study for progressive disease
Evaluable at 6 months
(n=29)
-CSA stopped for first 14 subjects, and continued at 2mg/kg/day until 2 years for next 17 subjects (relapse prevention)
5 year follow up

C.

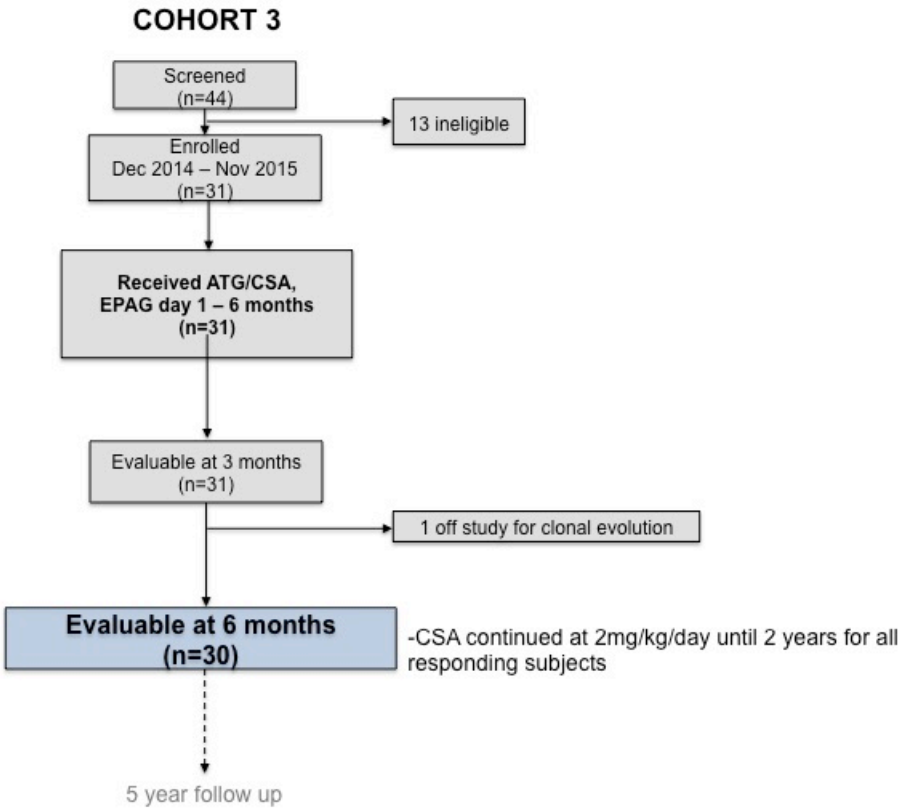

COHORT 3
Screened
(n=44)
13 ineligible
Enrolled
Dec 2014 – Nov 2015
(n=31)
Received ATG/CSA,
EPAG day 1 – 6 months
(n=31)
Evaluable at 3 months
(n=31)
1 off study for clonal evolution
Evaluable at 6 months
(n=30)
-CSA continued at 2mg/kg/day until 2 years for all responding subjects
5 year follow up

Figure S2. Blood counts of Patients Treated with Eltrombopag and IST Compared to Historic Cohort Treated without Eltrombopag

Peripheral-blood counts at various time points during the study are shown for patients who met criteria for a hematologic response at 6 months. Blood counts of patients who achieved a partial response (PR), complete response (CR), and overall response (OR) at 6 months are graphed left to right. The blue lines denote the trajectory of blood counts for patients treated on the current study, and in red are those treated in our historic cohort. Circles represent the mean blood count at baseline, 3 months, and 6 months on the study. The error bars above and below represent the standard error of the mean (summary statistics are provided in Table S1 in the Supplements).

*p<0.05, **p<0.01, ***p<0.001, two-sample T test for means.

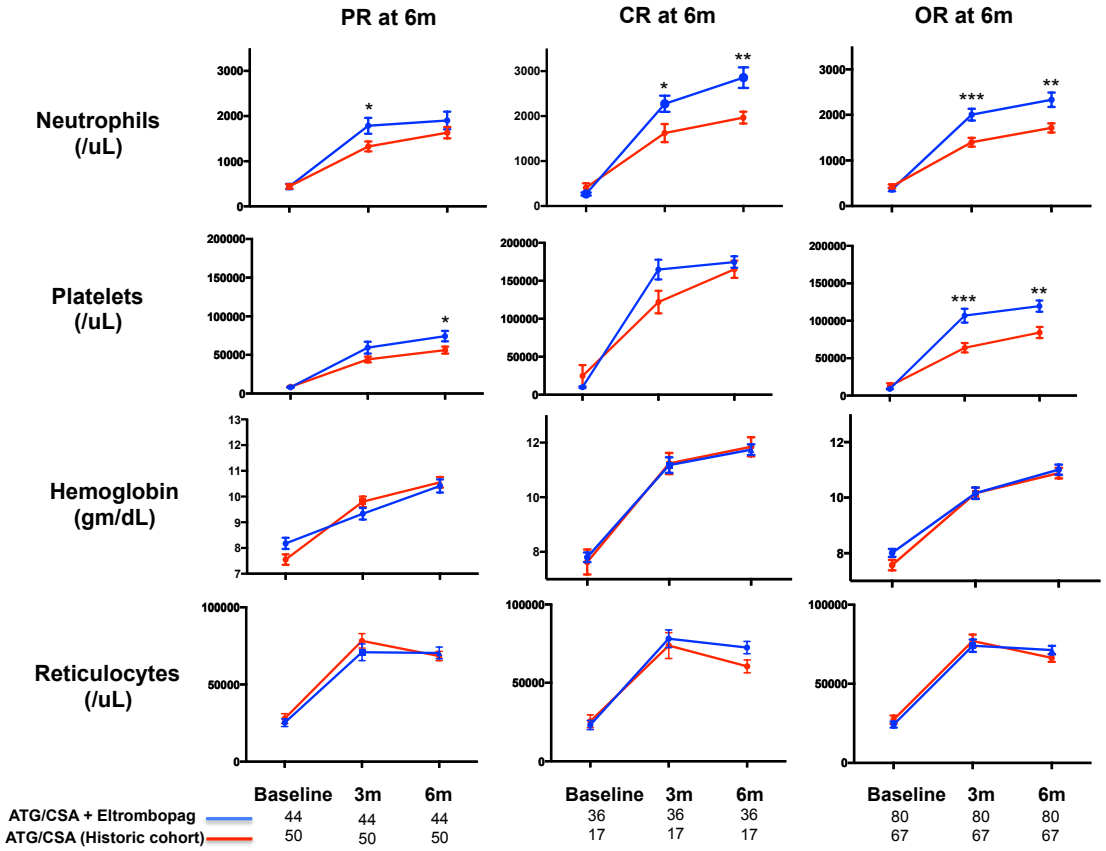
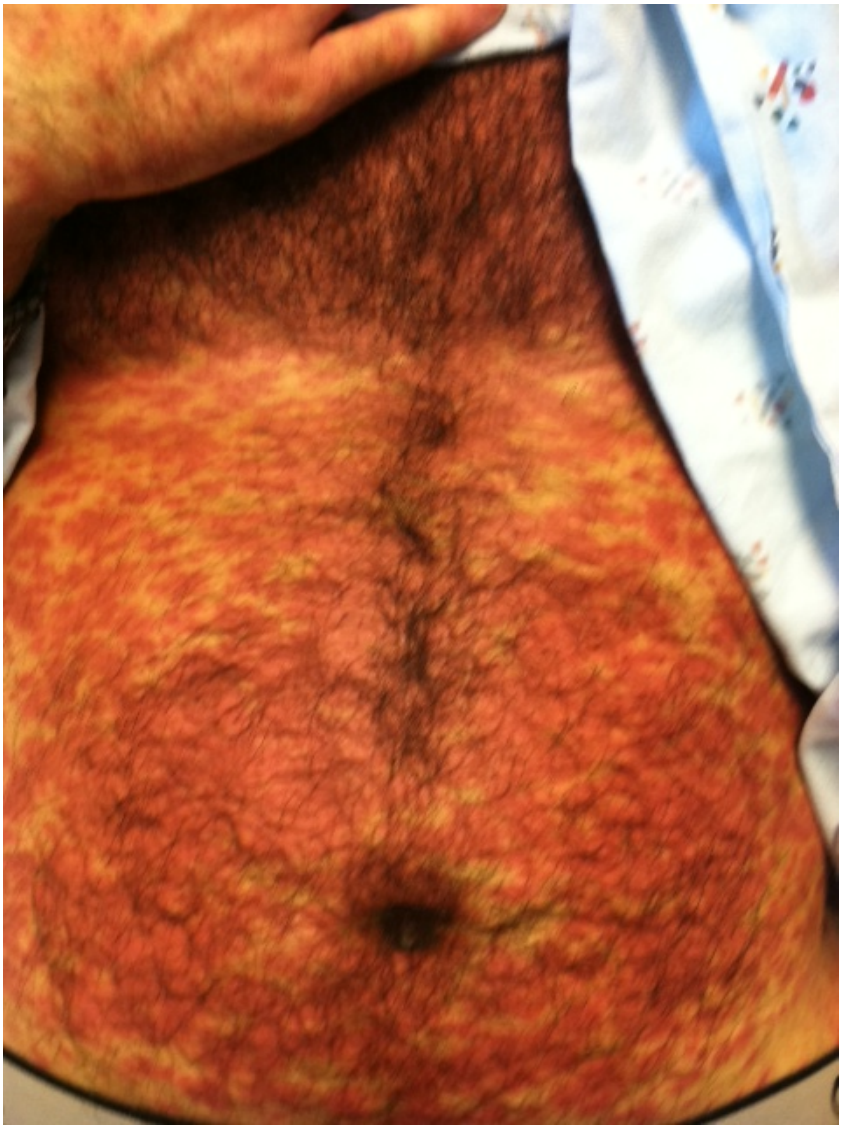


Figure S3. Pictures of Cutaneous Reaction to Eltrombopag
Severe cutaneous reactions attributed to eltrombopag occurred in two patients on the study at day 40 for patient # 54 and day 27 for patient # 86. Photography of the rash is available for patient # 54. Panel A shows a macular papular rash affecting the trunk, and panel B shows the rash affecting bilateral lower extremities and feet.

A.



B.

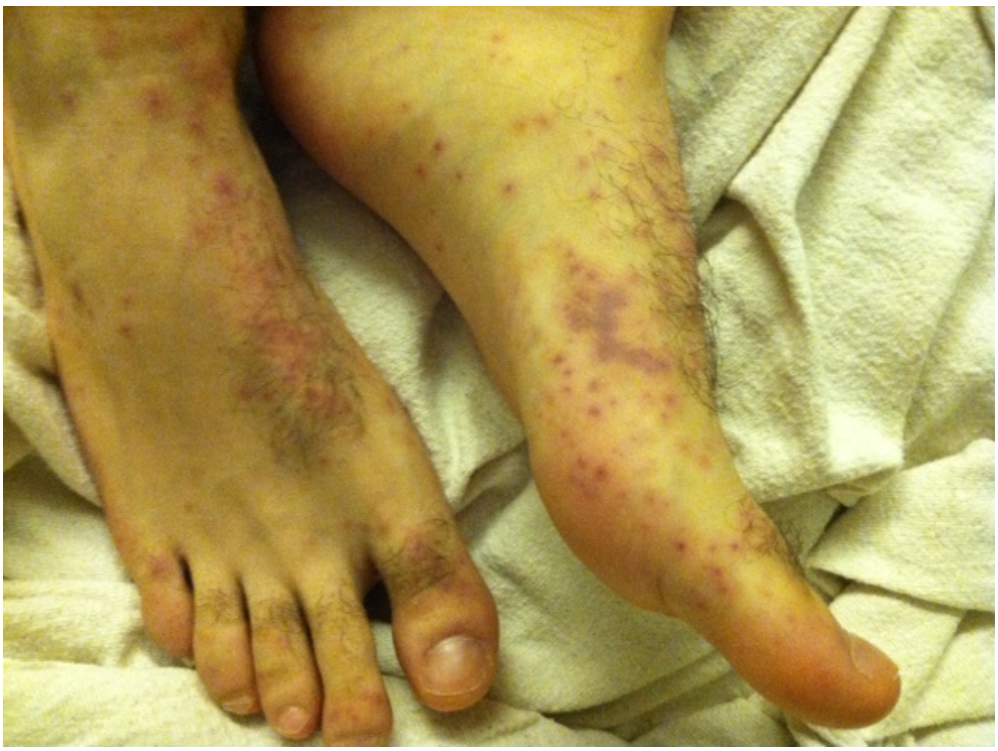
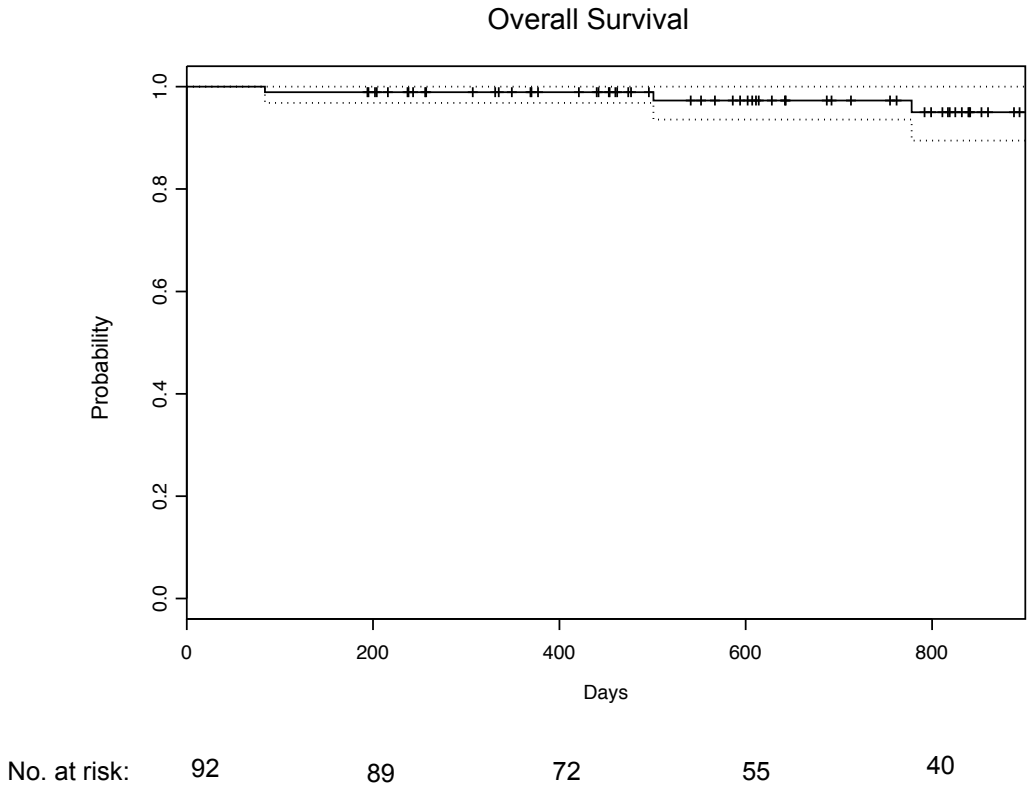


Figure S4. Survival, Relapse and Clonal Evolution
Kaplan Meier curves are shown for overall survival, panel A, and for survival with time of stem-cell transplantation censored, panel B. Panel C, time to relapse according to cyclosporine treatment beyond 6 months. Panel D depicts the cumulative incidence of clonal evolution (blue) as compares to incidence of hematopoietic stem cell transplantation (BMT in figure legend) and death by competing risk analysis using Fine and Grey methodology for all patients on the study (panel D, left) and for patients in the historic cohort (panel D, right). At 2 years, the incidence of clonal evolution is 7.7% for patients treated with eltrombopag and 7.9% for those treated without eltrombopag, in the historic cohort. Standard error is provided in parenthesis. Numerical computation was done using the “cmprsk” package in R.

A



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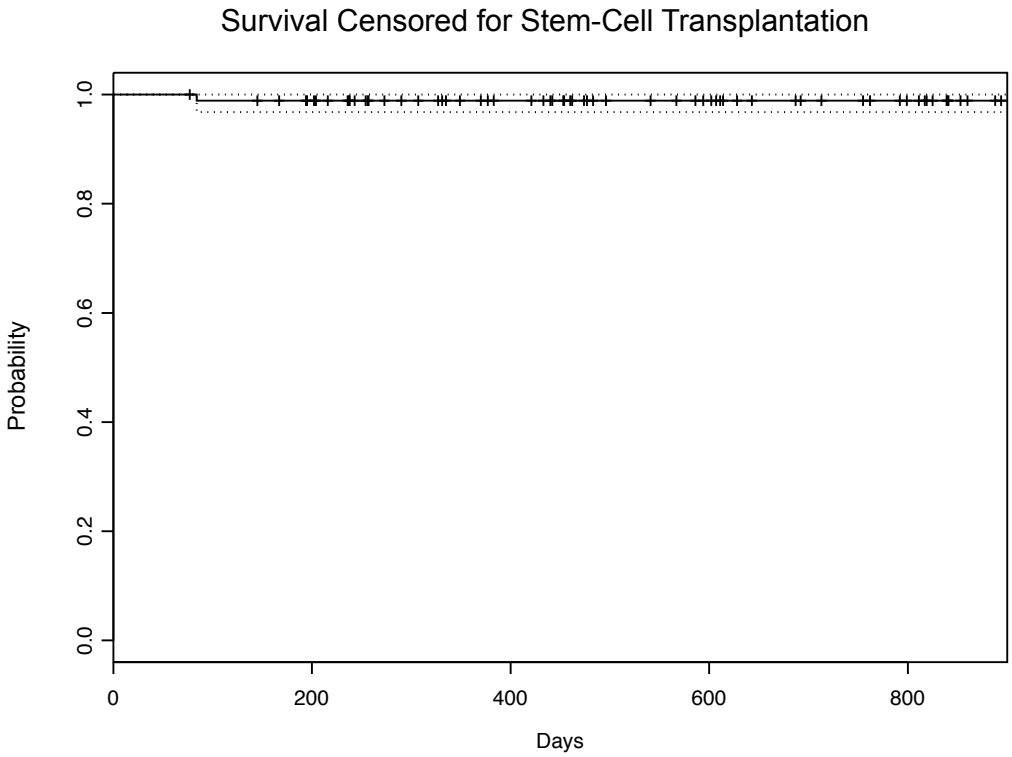
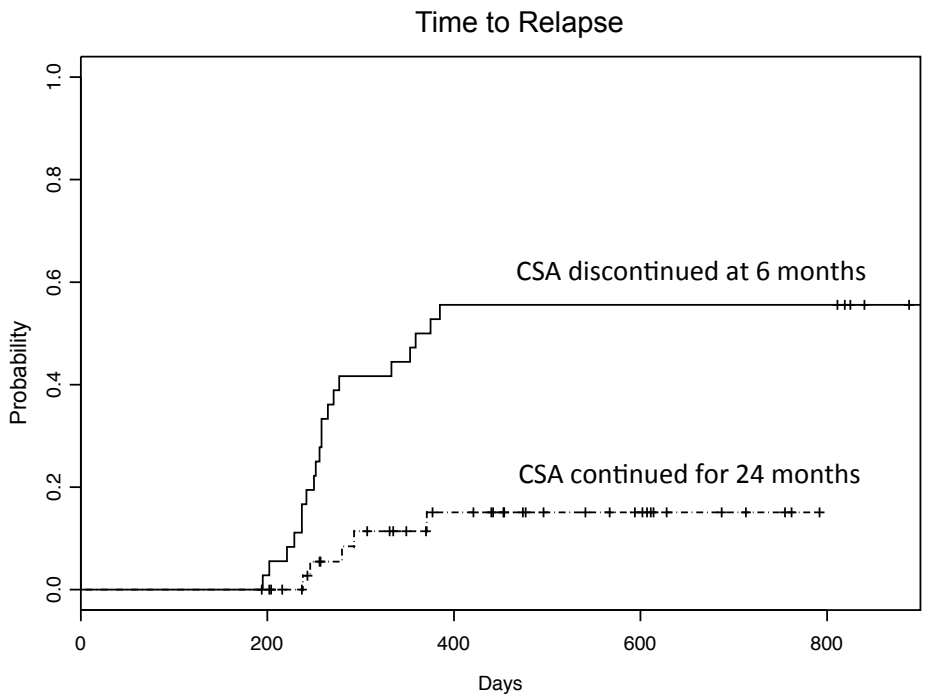


No. at risk:	92	84	64	46	33

C.



No. at risk:					
CSA discontinued	92	35	17	16	16
CSA continued	92	43	24	11	1

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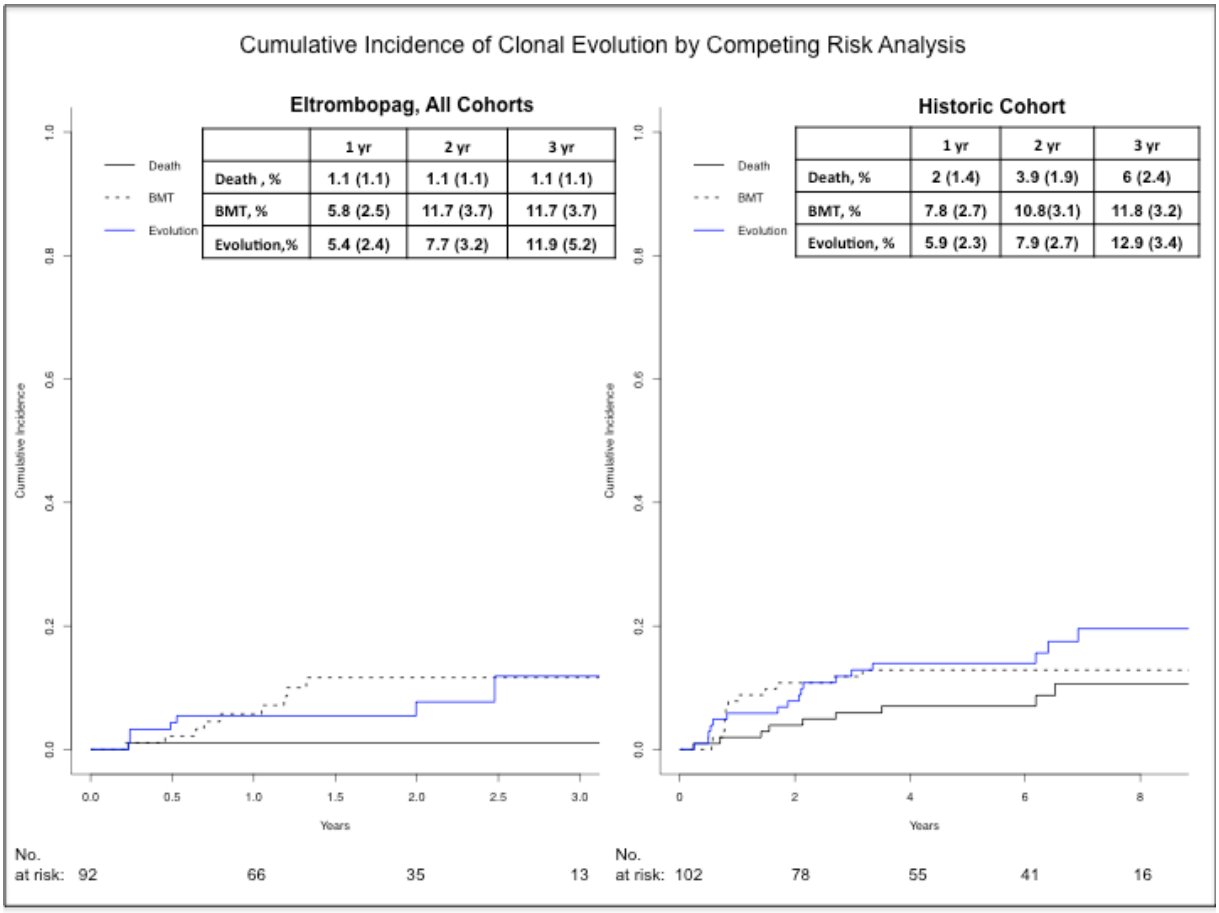
D.

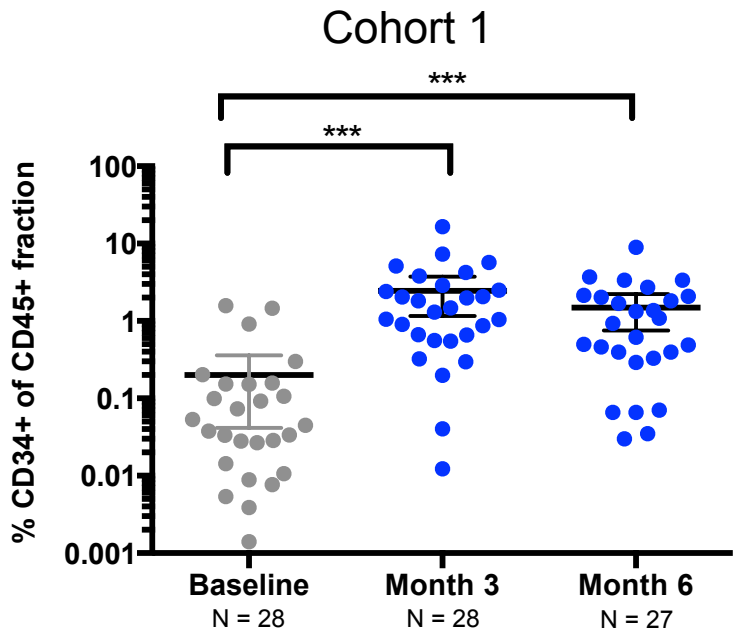


	1 yr	2 yr	3 yr
Death , %	1.1 (1.1)	1.1 (1.1)	1.1 (1.1)
BMT, %	5.8 (2.5)	11.7 (3.7)	11.7 (3.7)
Evolution,%	5.4 (2.4)	7.7 (3.2)	11.9 (5.2)

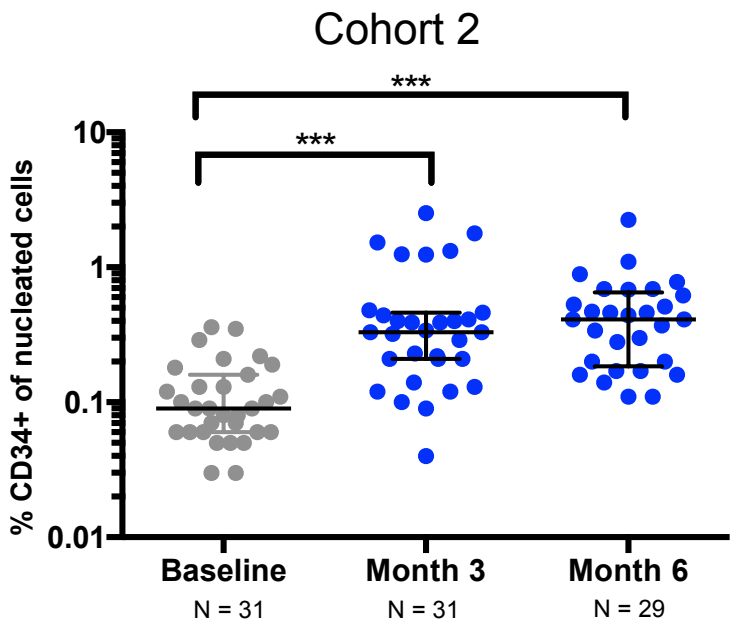
	1 yr	2 yr	3 yr
Death, %	2 (1.4)	3.9 (1.9)	6 (2.4)
BMT, %	7.8 (2.7)	10.8(3.1)	11.8 (3.2)
Evolution, %	5.9 (2.3)	7.9 (2.7)	12.9 (3.4)

Figure S5. CD34 Enumeration and Assay for Hematopoietic Progenitors
(A) Serial enumeration of CD34+ cells from bone marrow aspirate in patients enrolled to Cohort 1, CD34+ cells are shown as a percentage of the CD45+ fraction of cells. (B) For patients on cohort 2 and (C) patients on cohort 3, the percentage of CD34+ cells were enumerated from all nucleated cells. The median with interquartile range is depicted for baseline, 3 and 6 months. CD34+ percentage of the CD45+ fraction at baseline was 0.04%, 1.39% at 3 months, and 0.93% for Cohort 1. For Cohort 2, the CD34+ percentage of nucleated cells at baseline was 0.09%, 0.33% at 3 months, and 0.41% at 6 months. For Cohort 3, the median CD34+ percentage of nucleated cells at baseline was 0.13%, 0.45% at 3 months, and 0.37% at 6 months. (D) Representative serial flow cytometry analyses of phenotypically defined MPPs ($CD34^{+}CD38^{-}CD45RA^{-}CD90^{-}CD49f^{-}$) in the bone marrow of patient #29 and a healthy individual (normal). Nucleated bone marrow cells were initially enriched for CD34+ cells by MACS separation and analyzed by flow cytometry for markers expressed on HSCs. Numbers in boxes represent percentages of cells within the indicated fractions. Due to the paucity of CD34+ cells at baseline, most cells recovered had a CD34- phenotype.
***p<0.001, Wilcoxon matched pairs

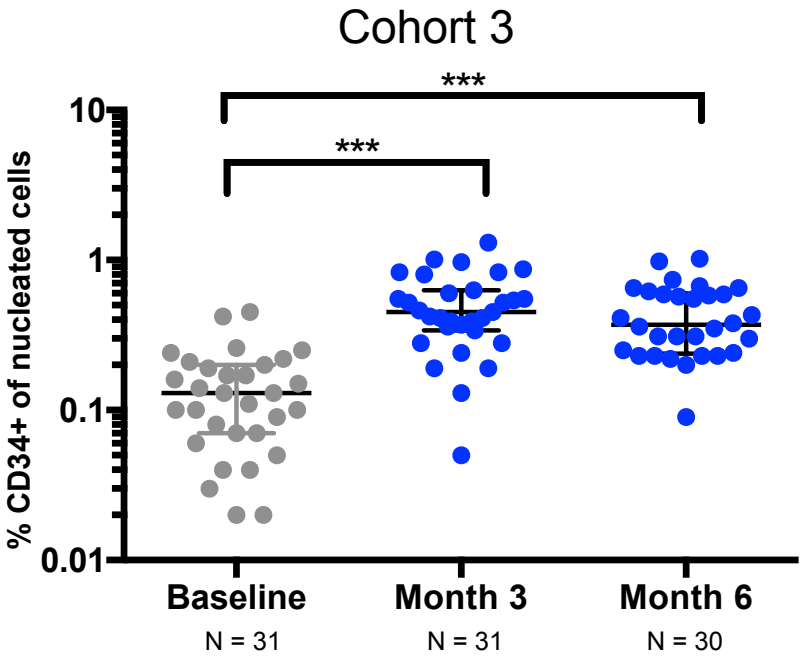
A.



B.


Cohort 2
***
***
10
1
0.1
0.01
% CD34+ of nucleated cells
Baseline
N = 31
Month 3
N = 31
Month 6
N = 29

C.


Cohort 3
***
***
10
1
0.1
0.01
% CD34+ of nucleated cells
Baseline
N = 31
Month 3
N = 31
Month 6
N = 30

D.

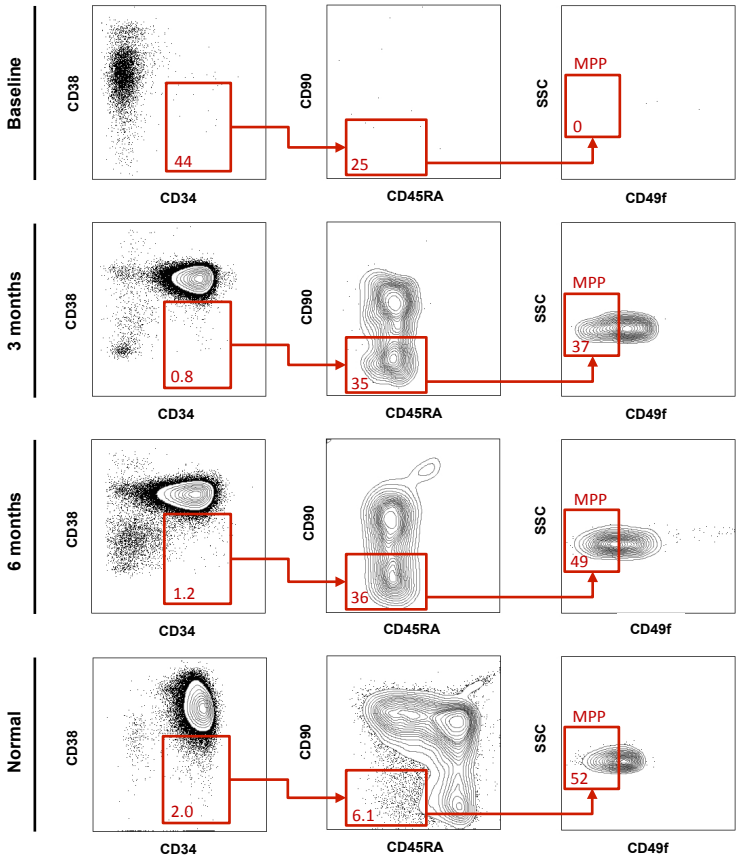

Baseline
CD38
44
CD34
CD90
25
CD45RA
SSC
MPP
0
CD49f
3 months
CD38
0.8
CD34
CD90
35
CD45RA
SSC
MPP
37
CD49f
6 months
CD38
1.2
CD34
CD90
36
CD45RA
SSC
MPP
49
CD49f
Normal
CD38
2.0
CD34
CD90
6.1
CD45RA
SSC
MPP
52
CD49f

Table S1. Comparison of Baseline Characteristics with Historic Cohort

Characteristic	All Patients IST+ Eltrombopag	Historic Cohort	P-value*
	N= 92	N=102	
Age (years)			
Median	32	30	0.511
Min-Max	3 - 82	5 - 79	
Age category (years) - n (%)			
<18	19 (21)	23 (23)	0.750
18-64	61 (66)	67 (66)	0.928
≥ 65	12 (13)	12 (11)	0.789
Gender - n (%)			
Male	50 (54)	57 (56)	0.831
Female	42 (46)	45 (44)	
Laboratory values			
GPI-neutrophils			
Range	<1 – 99%	<1 – 93%	
<1% - n	53	59	0.468
≥1% - n	31	43	
Neutrophil count - per uL			
Median	310	305	0.311**
Range	0 – 1,810	0 – 1,760	
Reticulocyte count - per uL			
Median	19,950	15,450	0.097**
Range	1,600 – 60,400	1,400 – 106,000	
Platelet count - per uL			
Median	9,000	9,000	0.477**
Range	0 – 37,000	1,000 – 254,000	

*P-values were obtained from t-tests for means or proportions.

**Transformations log(neutrophil count + 1), log(reticulocyte count +1) and log(platelet count +1) were used because of the skewness of the distributions of neutrophil, reticulocyte and platelet counts.

Table S2. Univariate and Multivariate Analysis

	Univariate logistic model			Multivariate logistic model*		
Baseline Risk	Coefficient (β)	SE	*P*-value	Coefficient (β)	SE	*P*-value
Age	-0.01296	0.01533	0.39811	-0.07370	0.02962	0.01285
Female^	0.18619	0.62701	0.76647	-0.40994	0.85343	0.63123
Log (ALC + 1)	0.20356	0.26191	0.43716	0.00733	0.36599	0.98404
Log (ANC + 1)	0.20687	0.15056	0.16944	0.46314	0.32764	0.15736
Log (ARC + 1)	0.49090	0.33435	0.14210	0.49596	0.52392	0.34364
Telomere length	0.64604	0.33779	0.05575			
Telomeres-NL^	1.37055	0.64156	0.03268	3.05654	1.15554	0.00817
TPO	-0.00009	0.00045	0.84774	0.00002	0.00066	0.97766
PNH^	-0.14885	0.68918	0.82899	-0.72076	0.86867	0.40654

Univariate and multivariate logistic regression analysis of continuous and categorical risk factors on the response rate at 6 months. Log transformed variables are used where indicated. Telomere length and age are related covariates; a higher percentage of younger patients had shorter telomeres compared to older patients.

*Effect of cohort was analyzed in the multivariate analysis and not included in the table because the variable was not significant.

^Denotes categorical variable where specified.

ARC, absolute reticulocyte count; ALC, absolute lymphocyte count; ANC, absolute neutrophil count; PNH, paroxysmal nocturnal hemoglobinuria; TPO, thrombopoietin; NL, normal

Table S3. Severe Adverse Events Not Attributed to Eltrombopag

Serious Adverse Event	Any Grade	Grade ≥ 3
	number (%)	
Any event	66 (72)	59 (64)
Infection	32 (35)	29 (32)
Febrile Neutropenia –infectious source not identified	9 (10)	9 (10)
Bacteremia	5 (5)	4 (4)
Fungal Infection	1 (1)	1 (1)
Catheter related infection	2 (2)	2 (2)
Pneumonia	2 (2)	2 (2)
Urinary infection	2 (2)	2 (2)
Upper respiratory infection	3 (3)	3 (3)
Cellulitis	2 (2)	2 (2)
Dental Infection	2 (2)	2 (2)
Gastrointestinal	4 (4)	4 (4)
Serum sickness	6 (7)	6 (7)
Hematologic	5 (5)	5 (5)
Immune thrombocytopenia	1 (1)	1 (1)
Thrombosis, splenic artery^	1 (1)	1 (1)
Bleeding	3 (3)	3 (3)

Neurologic	5 (5)	5 (5)
Headache	3 (3)	3 (3)
Lower extremity weakness, pain	1 (1)	1 (1)
Transient global amnesia	1 (1)	-
Encephalopathy – thymoma related*	1 (1)	1 (1)
Cardiovascular	3 (3)	3 (3)
Reversible cardiopulmonary failure	1 (1)	1 (1)
Syncope	1 (1)	1 (1)
Hypertension	1 (1)	1 (1)
Miscellaneous		
Musculoskeletal pain	1 (1)	1 (1)
Squamous cell skin cancer	1 (1)	1 (1)
Basal cell skin cancer	1 (1)	-
Abdominal pain	2 (2)	2 (2)
Hyperkalemia	2 (2)	2 (2)
Hypoglycemia	1 (1)	-
Rhabdomyolysis	1 (1)	1 (1)
Renal calculi	1 (1)	1 (1)
Suicidal ideation**	1 (1)	1 (1)
Peripheral edema	1 (1)	1 (1)
Pleural effusion	1 (1)	1 (1)

Data for the safety population, n=92; the data cutoff date was May 25, 2016. All serious adverse events unrelated to eltrombopag that occurred at any time while on study are shown (see Table 3 for events related to eltrombopag). Events were

graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4).
*Patient #23 died on study from a nonhematologic cause, autoimmune encephalopathy associated with thymoma. He had neurologic deficits prior to enrollment and the cause of death was confirmed by autopsy.
**Patient #10 had suicidal ideation and did not take study medications as prescribed for 3 months while on study.
^Patient #35 had multiple risk factors for thrombosis. He had preexisting atrial fibrillation and developed hemolytic anemia related to paroxysmal nocturnal hemoglobinuria when he suffered a splenic artery thrombosis. He was receiving eltrombopag for relapsed disease and the drug was discontinued at the time of the event. He was initiated on oral anticoagulation and was hospitalized less than 48 hours.

Table S4. Clonal Evolution in Patients During the Study

Age (yr)	Heme Response	Time to event (months)	Cytogenetics	BM dysplasia	Outcome
Cytogenetic abnormality of unclear significance					
68	CR	3	46, XX, del(13)(q12q22)[cp3]/46,XX[17]	No	Cytogenetics normalized
39	CR	30	48, XX +6 +15 [2]/ 46,XX[18]	No	CR stable
Chromosome 7 abnormality					
64	PR	3	45,XX,t(3;3)(q21;q26),-7[3]/ 46, XX[17]	Yes	AML, death post-HSCT*
72	PR	30	45, XY, -7[20]	Yes	PR stable
48	CR	6	46,XX,del (7)(p13p15)[3]/46,XX[19]	No	HSCT
61	PR	6	45, XX,-7[7]/46,XX[16]	Yes	Awaiting HSCT
16	NR	3	45, XY,-7[6]/46,XY[14]	No	HSCT*

*Two subjects with high-grade cytogenetic evolutions were found to have a cryptic inherited bone marrow failure disorder following enrollment consistent with a telomere disease (gene mutation in *RTEL1*).
CR, complete response; PR, partial response; Heme, hematologic; BM, bone marrow; AML, acute myeloid leukemia; HSCT, hematopoietic stem cell transplantation

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3. Scheinberg P, Wu CO, Nunez O, et al. Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with or without sirolimus: a prospective randomized study. Haematologica 2009;94:348-54.

4. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med 2011;365:430-8.