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Title: Eltrombopag restores tri-lineage hematopoiesis in refractory severe aplastic anemia which can be sustained on discontinuation of drug

Running title: Eltrombopag in refractory severe aplastic anemia

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Key points:

1. Eltrombopag promotes hematopoiesis in patients with severe aplastic anemia by stimulating stem and progenitor cells.
2. Eltrombopag can be discontinued safely in robust responders with maintenance of hematopoiesis.

Abstract

About a quarter of patients with severe aplastic anemia (SAA) remain pancytopenic despite immunosuppressive therapy. We have previously demonstrated that eltrombopag has efficacy in this setting with 44% (11/25) of patients having clinically significant hematologic responses. We now report safety and efficacy data on a further 18 patients and long term follow up on the entire cohort of 43 patients. The overall response rate was 17/43 (40%) at 3-4 months, including tri and bi-lineage responses. The majority of patients who remained on eltrombopag in an extension study (14/17) continued to show improvement, and 7 eventually had significant increases in neutrophil, red cell and platelet lineages. Five patients with robust near-normalization of blood counts had drug discontinued at a median of 28.5 months after entry (range 9 to 37), and all maintained stable counts a median of 13 months (range 1-15) off eltrombopag. 8 patients, including 6 non-responders and 2 responders, developed new cytogenetic abnormalities on eltrombopag, including 5 with chromosome 7 loss or partial deletion. None evolved to acute myeloid leukemia to date. Eltrombopag is efficacious in a subset of patients with aplastic anemia refractory to IST, with frequent multilineage responses, and maintenance of normalized hematopoiesis off treatment. This study is registered at www.clinicaltrials.gov, identifier: NCT00922883.

Introduction

Aplastic anemia is a bone marrow failure syndrome characterized by marrow hypoplasia and hematopoietic stem cell deficiency. An immune-mediated pathophysiology has been inferred from response to immunosuppressive therapy (IST), demonstration of immune activation, and animal models[1-3]. Depletion of primitive hematopoietic stem and progenitor cells (HSPC) is profound, and may persist even in patients responding to IST [4]. While outcomes for patients have markedly improved with the advent of allogeneic stem cell transplantation and IST [5],about 30% of patients with severe acquired aplastic anemia (SAA) remain pancytopenic after horse antithymocyte globulin (ATG) and cyclosporine[6,7]. A fraction of patients who fail to improve with one course of IST will respond to a second round. In the remaining two-thirds non-transplantation options are limited and include growth factors, transfusional support and androgen therapy [7]. Management of these patients is challenging. Allogeneic stem cell transplantation is an option for patients who have suitable donors, but infectious complications, graft versus host disease, and graft failure often intervene, especially in older patients and in those without a well-matched family or unrelated donor[5].

Thrombopoietin (TPO) is known to be a critical regulator of hematopoiesis[8-10]. The TPO receptor c-mpl is expressed on hematopoietic stem (HSC) and progenitor cells[11] and knock-out mice that lack c-mpl are

deficient in HSCs[12,13]. Eltrombopag is an oral TPO receptor agonist and was originally developed to stimulate thrombopoiesis in patients with immune thrombocytopenias[14]. However, animal models [15] and insights from congenital marrow failure syndromes indicate involvement of TPO signaling in hematopoietic stem cell homeostasis as well as in thrombopoiesis[10,16,17].

We treated an initial cohort of 25 patients with SAA refractory to IST on a pilot dose escalation study, administering eltrombopag for 3-4 months. Hematologic response was observed in 44%[18]. Blood count improvement was often robust and durable. Study design allowed responding patients to continue on eltrombopag in an extension arm, and we noted continued count improvement in multiple lineages over time. Two non-responding patients evolved to monosomy 7 at the 3-4 month response assessment.

To confirm and expand these initial observations, we added a second cohort. Here we report on a larger cohort with longer follow-up with focus on duration and quality of responses, the impact of eltrombopag discontinuation in robust responders, and the incidence of clonal progression.

Methods

Study participants and oversight

Consecutive patients with refractory SAA who fulfilled eligibility criteria were enrolled between July 2009 and February 2013. This pilot, investigator-initiated, non-randomized trial was registered at www.clinicaltrials.gov as NCT00922883, was approved by the Institutional Review Board of the National Heart, Lung and Blood Institute, and was monitored by a data safety and monitoring board. Drug was provided by GlaxoSmithKline (Collegeville, PA), but the company provided no financial support, and did not play a role in data collection or analysis. All authors had full and independent access to data.

Patients eligible for inclusion were 12 years or older and previously diagnosed with severe aplastic anemia as defined by standard criteria [19], refractory to at least one course of ATG-based IST initiated at least six months previously, and with platelet counts <30,000/μL. Fanconi's anemia was excluded by DEB testing in patients under 40, and no patients with familial marrow failure or clinical characteristics of dykeratosis congenita were included. Primary refractory patients had never responded to IST, relapsed refractory patients had responded to at least one prior cycle of IST, but were refractory to the most recent course of IST. Bone marrow aspiration and biopsy were performed prior to enrollment to exclude myelodysplastic syndrome (MDS) by morphology and cytogenetic analysis. Paroxysmal nocturnal hemoglobinuria (PNH) was assessed by flow cytometry, measuring the frequency of red cells and neutrophils lacking glycosylphosphatidylinositol(GPI) -anchored proteins, and a PNH clone was considered present if red cells or neutrophils deficient in GPI-anchored proteins were >1%. The characteristics of the first 25 patients have been described[18], and entry criteria for the additional 18 patients enrolled in the expanded trial were identical, with the exception of decreasing the lower age limit to 12 years from the prior 18 years, based on the availability of more pharmacologic information in pediatric patients treated with eltrombopag in trials for immune thrombocytopenic purpura (ITP)[20]. Informed consent was obtained in accordance with the Declaration of Helsinki.

Study design

The initial treatment plan was unchanged from the original protocol[18]. Subjects commenced eltrombopag at a dose of 50 mg, which was increased by 25 mg every two weeks if the platelet count had not increased by 20,000/µl, to a maximum dose of 150 mg. Patients continued supportive care with platelet and red blood cell transfusions as required. Continuation of CSA was permitted.

Blood counts and chemistries were monitored weekly through the primary endpoint and response assessment was at 3-4 months. Liver enzyme elevation greater than 6 times normal required suspension of eltrombopag, with reinstitution at the next lowest dose level when transaminases had returned to < 5 times normal. Adverse events (AE) were monitored and graded according to the Common Terminology for Adverse Events version 3.0. Bone marrow aspirate and biopsy with cytogenetic analysis was done at study entry, and repeated at the 3-4 month response assessment, and then every six months in responding patients remaining on drug. Reticulin staining was graded according to standard guidelines[21].

Those patients who responded were offered the option of continuing on eltrombopag in an extension study. In August 2012, a modification to the extension study was approved which included a taper of eltrombopag over 16 weeks in those who achieved a robust response, defined as platelets >50,000/µl, hemoglobin >10g/dL and neutrophils >1,000/µl for greater than 8 weeks without transfusion support.

Study end points

The primary endpoint was hematologic response at 3-4 months and defined as uni- or multi-lineage recovery by one or more of the following criteria: 1) platelet response -- increase to 20 x 10^9/L above baseline, or stable platelet counts with transfusion independence for a minimum of 8 weeks in those who were transfusion-dependent on entry into the protocol; 2) erythroid response -- when pretreatment hemoglobin was less than 9 g/dL defined as an increase in hemoglobin by 1.5g/dL or, in transfused patients, a reduction in the units of packed red blood cell (PRBC) transfusions by an absolute number of at least 4 transfusions for eight consecutive weeks - compared with the pretreatment transfusion number in the previous 8 weeks; 3) neutrophil response -- when pretreatment absolute neutrophil count (ANC) of <0.5 x 109/L as at least a 100% increase in ANC, or an ANC increase > 0.5 x 10^9/L, and the toxicity profile as measured using the CTCAE criteria.

Secondary endpoints were change in blood counts as a continuous variable, incidence of bleeding, change in serum TPO, health related quality of life (as measured by the Medical Outcomes Study 36-Item Short Form General Health Survey, version 2; Quality-Metric) and clonal evolution to monosomy 7 or complex cytogenetics. Telomere lengths in peripheral blood cells were measured as previously described, and analyzed as a predictor of response or clonal progression[22]. DNA was extracted from bone marrow mononuclear cells for comparative genomic hybridization (CGH) using the single nucleotide polymorphism (SNP) based CytoScan high density microarrays from Affymetrix.

Statistical analysis

We used the two-stage "minimax" design [23] with a response probability of 10% or less as the null hypothesis and a response probability of 30% or more as the alternative hypothesis in the initial patient cohort. We added a second cohort of 18 patients to explore the rate of clonal evolution for this patient population and to better define the response rate, adding as an exploratory secondary endpoint clonal evolution to monosomy 7 or complex cytogenetics. Univariate and multivariate logistic regression models were used to evaluate the effects of risk factors on the probabilities of response and clonal evolution. The R and S-plus software packages were used compute the numerical results.

Results

Patient characteristics: Forty-four patients were enrolled on study, with 43 patients receiving eltrombopag. Initial results on the first 25 patients have been previously reported[18]. One patient's diagnosis was re-classified as hypoplastic MDS after study entry but prior to eltrombopag initiation. The characteristics of the entire cohort are shown in Table 1 . Six patients met criteria for very severe aplastic anemia at study entry, defined as SAA and neutrophil count <200/µl. Three patients were on stable doses of cyclosporine throughout the study. One patient received G-CSF for a short period, and this patient was not assessed for a neutrophil response.

Hematologic responses: We previously reported that 11 of the initial 25 (44%) patients had a hematological response to eltrombopag, at 3-4 months[18]. In the second cohort, we enrolled an additional 18 patients. Of these, 6/18 responded in at least one lineage at response assessment for an overall response rate of 17/43 (40%). Fig 1a summarizes the response characteristics of the 17 responders at the initial response assessment. One patient achieved a tri-lineage response and there were 5 bi-lineage responses. Nine of 15 patients who were receiving platelet transfusions became transfusion independent. Of the 8 neutrophil responders, 4 initially had severe neutropenia (<500/µl). Median time to initial response was 12 weeks (range 8-14). Nine responders met standard response criteria, defined as no longer meeting criteria for SAA, after a median time on drug of 6 months (range 3-21) months. There was no significant difference in the number of bleeding episodes and infections between responders and non-responders.

Fourteen of 17 responders continued on eltrombopag in the extension arm, with a median time on drug of 12 months (range 6-37). The majority of patients who remained on eltrombopag continued to show hematologic improvement, and 7 eventually achieved tri-lineage responses. Longitudinal responses are shown in figure 2. Figure 1b summarizes best hematologic responses achieved during follow-up. One non-responder reached response criteria 4 weeks after discontinuing eltrombopag, without institution of any additional therapies remaining so for more than 4 months to date, with platelet counts 15-20x10^9. A second patient who was red cell transfusion dependent for a year prior to protocol entry did not meet response criteria at 16 weeks and eltrombopag was discontinued. Her reticulocyte count continued to increase and she has not required red cell transfusion for over two years, with hemoglobin

stable at 10 g/dL, as of this writing. Thus taking into account these two delayed responses, 19/43 (44%) of patients showed significant clinical improvement after protocol entry.

Eltrombopag discontinuation: As reported previously[18], one patient who discontinued drug at 10 weeks because of a cataract misdiagnosis had a trilineage response and continues to be transfusion-independent, nearly three and a half years following protocol entry. We modified the protocol in August 2012 to include tapering of eltrombopag and discontinuation in patients with platelets >50 x 10^9/L, Hb >10g/dL and neutrophils >1 x 10^9/L for more than 8 weeks, without transfusions. Five patients fulfilled these criteria, and eltrombopag was tapered and then discontinued after a median of 28.5 months (range 9 to 37). All five have maintained stable counts with a median follow up off drug of 13 months (range 1-15) (supplementary Fig. 2a, b, and c). Their bone marrows have remained normocellular (Fig 3).

Three patients lost their responses while on the extension arm. All were on the full dose of 150 mg at the time of relapse. Two had achieved only erythroid responses, defined by a reduction in red cell transfusion requirements, and after 6 months on drug returned to their previous transfusion requirements. One patient had a haplo-cord transplant and the other continued supportive care with transfusions. The third relapse occurred when a neutrophil response was lost after 6 months and the patient succumbed to an infectious episode.

Response predictors: We confirmed that absolute reticulocyte count was the only pre-treatment predictor for response (responders vs. non-responders, 41.8 vs. 24.2; p=0.023). Age, neutrophil count, age-adjusted telomere length, presence or absence of more than 1% GPI-deficient neutrophils, number of prior cycles of immunosuppression, time since the last cycle of immunosuppression, duration of aplastic anemia diagnosis, and primary refractory disease versus relapsed refractory disease were not predictive of response.

Quality of life: Quality of life scores were measured using the Medical Outcomes Study 36-Item Short Form General Health Survey. At study entry, physical health (PCS) scores were significantly lower (p<0.001) than the U.S. general population mean specifically in the areas of physical function, role function (physical and emotional), social function, and general health (Supplemental Figure 2); all differences were clinically relevant. Mental health (MCS) scores did not differ (p=0.12). 27 patients had surveys completed at the 3-4 month primary response assessment time point, and at that time, there was no significant changes in pre- versus post- eltrombopag physical or mental scores.

Toxicity: Similar to our prior report on the initial cohort, we observed a favorable toxicity profile in this expanded cohort, and with longer follow-up period. There were no dose-limiting toxicities other than reversible transaminitis[18], similar to the much larger safety and efficacy studies in patients with ITP, despite a higher dose used in our study[24]. Two patients had reversible transaminitis related to drug; both required dose interruption and one is currently maintained on 75mg with a sustained response. Liver function abnormalities in the other patient returned to baseline after stopping drug for 4 days and he tolerated reinstitution at 150 mg.

Contrary to reports suggesting that TPO receptor agonist use can increase bone marrow reticulin[25], we found no significant increase in fibrosis in biopsy specimens, throughout our entire study cohort after a median follow-up of 13 months (range, 3-51) with biopsies performed every 6 months. There were no thrombotic events while receiving eltrombopag, but one robust responding patient experienced a lower extremity deep venous thrombosis 14 months after eltrombopag had been discontinued, with a platelet count of 60 x 10^9/L and normal hemoglobin and neutrophil levels.

Clonal evolution: Eight patients developed clonal cytogenetic abnormalities during eltrombopag administration (Table 2). Seven had a normal karyotype confirmed within 3 months of starting drug, assessed by conventional cytogenetics. One patient (#42) had insufficient metaphases on his sample prior to entering the study, but a normal karyotype 9 months prior to entering the study. Only 2/8 had dysplastic changes when new cytogenetic abnormalities appeared, but some samples were severely hypocellular, making assessment of morphology difficult. None had increased myeloblasts. Six of 8 clonal evolution events occurred in non-responding patients, and the new cytogenetic changes were detected on the marrow performed at response assessment. Two responding patients evolved. One responder (#23), whose counts had been gradually increasing while on the extension arm, was noted to have falling counts at 13 months, and the marrow showed mild dyserythropoeisis and 13q deletion. A second responder (#32) had stable blood counts and on routine evaluation marrow at 10 months had also developed del 13q, but no dysplastic features seen. Five of 8 evolvers developed chromosome 7 abnormalities. Many of these evolvers proceeded immediately to transplant, therefore sequential cytogenetics were available on only 4, showing no significant change in clone size 1 to 9 months later.

We retrospectively performed comparative genomic hybridization (CGH) using the single nucleotide polymorphism (SNP) based CytoScan high density microarrays on DNA extracted from pretreatment and evolution bone marrow mononuclear cells from these patients, and could not detect chromosome 7 abnormalities in the pre-treatment samples. The combined SNP/CGH array technology is reportedly more sensitive to mosaicism than standard cytogenetics and can detect levels as low as 5%, however, the pretreatment samples were profoundly hypocellular and therefore much of the DNA assayed may have originated from non-hematopoietic cells such as fibroblasts or adipocytes[26,27].

No predictive factors for evolution were identified (Table 3). In previous work, leukocyte telomere content at SAA diagnosis predicted risk of clonal evolution: patients with telomere length at diagnosis in the shortest age-adjusted quartile had the highest proportion of clonal evolution[22]. In the current study all eight patients that clonally evolved were in the shortest quartile of telomere content at study entry. Of the 33 non-evolvers, 29 were also on the shortest quartile, and telomere content in the lowest quartile did not predict clonal evolution in the current study (p=0.262 by Fisher's exact test), likely due to the limited numbers and overall very short telomeres possibly related to a longer history of disease and prior therapies. Patients with relapsed refractory disease showed a trend of being more likely to evolve than those with primary refractory disease (4/33 vs. 4/10; p=0.052).

One patient died from progressive cytopenias, and five have undergone cord blood/haplo or matched unrelated donor hematopoietic stem cell transplantation. All engrafted with no relapses.

Discussion

Patients with SAA refractory to IST have a poor prognosis and represent an unmet clinical need. Infectious complications are common and can be fatal [28]. Intensive transfusion support is necessary for the majority of patients and can be complicated by hemosiderosis, alloimmunization, and transfusion transmitted infections. New therapies are needed for this challenging clinical situation. We confirm that eltrombopag therapy resulted in clinically significant increases in blood counts and/or decreased transfusion requirements in 40% of patients with refractory SAA. Some patients achieved multi-lineage responses by 3 months of dose-escalating therapy, however, further improvements in blood counts were observed in patients who remained on drug and bi and tri-lineages responses were common and more likely with greater duration of drug exposure. As previously reported in the large ITP cohorts patients tolerated eltrombopag extremely well.

The responses seen in our clinical trial provide strong evidence for the stimulation of human HSCs by eltrombopag in vivo. In ITP, patients relapse when eltrombopag is stopped[24]. In contrast, continual exposure to eltrombopag may not be necessary to sustain adequate hematopoiesis in patients with SAA. The clinically relevant mechanisms in the two diseases may be different, with direct stimulation of HSPCs in SAA to restore physiologic HSC numbers versus supraphysiologic stimulation of megakaryocytes by eltrombopag in ITP. The eltrombopag dosages resulting in response in the ITP trials were lower than in this study. No patients responded to doses less than 100mg per day. It is possible that stimulation of HSPCs versus megakaryocytes requires higher eltrombopag concentrations and longer treatment time to push quiescent HSPCs into active hematopoiesis but differentiating which is more important is difficult because of the dose escalation design. In the current SAA study, five patients have remained transfusion-free with normal or near normal blood counts and marrow cellularity after discontinuation of eltrombopag, (4 followed off therapy for over 12 months). Three of these patients were particularly heavily pre-treated, failing 3 previous rounds of IST, suggesting that abrogation of immune attack may be necessary for a sustained response to eltrombopag in patients with SAA.

Stem cell depletion is a central pathological feature of SAA[4], and our results suggest that pharmacologic expansion of this compartment can be effective in addressing this pathophysiology. Baseline reticulocyte count was the only factor predicting response in this trial. The importance of higher reticulocyte numbers as predictors of response to IST have been noted previously , perhaps reflecting residual HSC numbers [29,30]. Why endogenous pathways do not stimulate normalization of the HSPC compartment, if immune attack has abated, particularly given the very high endogenous circulating levels of TPO in patients with SAA, is unclear[31,32]. It is likely that a critical mass of HSPCs is required for bone marrow recovery in patients, although HSPC numbers remain low even in those who normalize blood counts[33]. In an ongoing trial we have observed responses to eltrombopag in patients with moderate aplastic anemia (MAA) not previously treated with IST, who presumably have higher residual numbers of HSCs. These responses suggest that baseline HSPC numbers are a crucial determinant of response, and that some patients with MAA may not have an underlying or active immune etiology for their marrow failure, consistent with data suggesting lower response rates to immunosuppression in patients with moderate as compared to severe AA[34]. There are ongoing efforts to elucidate any possible

impact of eltrombopag on immune cell function however we did not find any changes in T cell subsets, including Tregs, comparing samples collected before and following 3-4 months of treatment from the first 25 patients.

The number of patients who clonally evolved on this study is concerning. To date, 8/43 (19%) patients have developed new cytogenetic abnormalities, 3-13 months after beginning eltrombopag. Only two had morphologic dysplasia, and none had increased myeloblasts. None progressed to AML, although 5 of 8 have undergone allogeneic stem cell transplantation. Patients with SAA are at risk of progression to clonal marrow dysfunction, with the largest experience reporting up to 15% of patients clonally progressing by 10 years from diagnosis [35]. Refractoriness to IST has been shown to be a risk factor for evolution, but it is difficult to discern true rates for evolution in this population, due to the problem of competing outcomes, because refractory patients are referred for transplant at different time points, and therapies for refractory patients are varied and applied in only small single arm trials[36]. The emergence of clones with chromosome 7 abnormalities confers a poor prognosis in SAA [19] and is most common in patients who have failed to respond to therapy[37]. We have treated 81 patients with experimental immunosuppression for refractory disease at our institution, of which clonal evolution was observed in 9 subjects (an additional two evolutions were observed amongst cases receiving immunosuppression followed by eltrombopag therapy). Among these 9 subjects, only 2 developed frank leukemia, with the remaining having monosomy 7 (4), trisomy 6 (2) and 1 subject with t (6;14). Median time to evolution from first IST was approximately 2 years and median time to evolution from second IST was approximately 1 year. Five patients in the current study developed chromosome 7 abnormalities with three monosomy 7, one del 7p and one der (1;7) (Table 2). All were non-responders and just one had evidence of dysplasia.

Any hematopoietic cytokine, particularly one that directly affects hematopoietic stem and progenitor cells via the c-mpl receptor, could impact on proliferation or self-renewal and theoretically on the emergence of abnormal clones. G-CSF therapy has been associated with monosomy 7 in retrospective studies of patients with aplastic anemia[36]. Although large prospective multi-institutional studies did not confirm an impact of G-CSF on clonal progression, follow up may not have been sufficiently long to accurately assess risk[38,39]. In studies of patients with MDS, cytokine therapy has not been associated with an increased risk of progression to AML[40,41]. Eltrombopag increases megakaryocytic proliferation when added to mononuclear cells from patients with AML and MDS, but in the majority of samples inhibited blast cell proliferation[42]. Romiplostim, a subcutaneous TPO receptor agonist, has shown promise in improving thrombocytopenia in low risk MDS patients enrolled in a single arm study[43]. A subsequent phase 3 study treating patients with low to intermediate risk-1 MDS with romiplostim was terminated early due to more cases of progression to AML in the treatment arm. However, longer follow up of this cohort, published as an abstract in 2012, found no difference in rates of progression between the placebo and romiplostim-treated groups[44]. Eltrombopag is also being investigated for the treatment of cytopenias in MDS and AML and one patient with primary refractory AML and monosomy 7 has been reported to have reached a clinical, morphologic and cytogenetic response to eltrombopag, sustained for 6 months[45]. Preliminary results from a randomized controlled trial of eltrombopag versus placebo in 98 patients with advanced MDS/AML have not shown any increased rate of disease progression[46].

Two responders evolved both acquiring deletion of 13q. Patients with both de novo MDS and MDS evolved from SAA with deletions of 13q are considered to have an excellent prognosis, generally without progression to frank MDS/AML[37,47]. The first patient underwent transplant, and we are currently observing the second. His counts fell initially but have remained stable for 2 months and he remains transfusion independent. Although transient cytogenetically-abnormal clones have been reported in aplastic anemia[48], this phenomenon is infrequent[37]. As the majority of patients who evolved in this study underwent hematopoietic stem cell transplantation we cannot rule out transience of these clones or assess the rate of progression to frank MDS/AML.

Two hypotheses may be offered to explain clonal evolution. Firstly, eltrombopag therapy may have stimulated the expansion of dormant clones. Perhaps these clones were initially below the limit of detection for metaphase cytogenetics, or were quiescent and only became apparent when eltrombopag stimulation initiated cycling, providing metaphases for analysis. To investigate this possibility, we screened pre-treatment marrow samples using combined CGH-SNP arrays. This technique was used to detect cryptic clonal genomic aberrations in aplastic anemia, undetectable by traditional metaphase cytogenetics[49]. We did not find evidence for pre-existing clones in our cohort, although this technique will not detect clones making up less than 5% of the DNA sample. Alternatively, chronic pharmacologic stimulation may have driven HSPC proliferation, and in the presence of shortened telomeres or other perturbations due to stem cell deficits or chronic immune attack, subsequent destabilization of the genome is accelerated and abnormal clones emerge. Evolution may have been inevitable in this group but was hastened by the use of eltrombopag. We note that prior to institution of drug discontinuation in robust responders, 6 patients remained on 150mg eltrombopag for a median of 20 months (range 7-30), without clonal evolution, so if eltrombopag does hasten clonal evolution, only a subset of refractory patients appear to be susceptible.

Eltrombopag is efficacious in a substantial subset of patients with aplastic anemia refractory to IST. Multilineage responses are frequently seen, particularly with extended therapy. We now report sustained and durable hematopoiesis in patients who discontinued eltrombopag. Dosing schedules incorporating drug taper when robust responses are achieved may be optimal for safety. Clonal evolution rates are of concern, although a direct association with eltrombopag is unclear. Identification of patients with pre-existing clonal hematopoiesis using newer more sensitive techniques or delineation of other factors predisposing patients to clonal progression may prove helpful to guide physicians in referring patients for early allogeneic transplantation versus a trial of eltrombopag, and should be the focus of future clinical trials. Given these concerns, eltrombopag should be used in patients with refractory AA with careful attention to potential risks and benefits. Ongoing clinical research trials will help answer some of these questions. We are currently conducting clinical trials with this very promising drug in moderate aplastic anemia and unilineage bone marrow failure syndromes, low to intermediate risk MDS and in upfront therapy in combination with IST. Our results open new strategies for treatment of acquired and potentially congenital bone marrow failure states and suggest that eltrombopag is a potent stimulator of in vivo HSPC function.

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Authorship

Contribution: Ronan Desmond was the principal investigator for this study and participated in amending the protocol, execution of the study, data collection, analysis, and interpretation, and drafting of the manuscript. C.D., N.Y, M.O. and P.S. participated in the primary conception of the study; K.B., M.B., B.D. participated in collecting data. C.D., N.Y., M.O., B.D. participated in analyzing the data. B.D. performed the CGH. K.B cared for the patients. D.T., M.O., P.S. and A.P. participated in interim discussions, data interpretation, and critically revised the manuscript. K.C. read the pathology specimens. C.W. provided statistical analysis.

Conflict of interests

No authors have conflict of interest to declare.

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Tables

Baseline characteristic	No.
Age, years	
Median	44
Range	17-77
Sex, n (%)	
Male	24 (56)
Female	19 (44)
Ethnic group, n (%)	
White	20 (47)
Black	13 (30)
Hispanic	9 (21)
Asian	1 (2)
Time since last IST, months	
Median	9
Range	6-117
Prior number of courses of IST	
Median	2
Range	1-4
Response to prior IST, n (%)	

Primary refractory	33 (77)
Relapsed refractory	10 (23)
Transfusion dependency, n (%)	
Red cells	40 (93)
Platelets	42 (98)
PNH clones, n (%)	
Yes	26 (60)
No	17 (40)
Laboratory parameters	
-Platelets ($x10^{-3}/mm^3$)	
Median	8
Range	2-22
-Hemoglobin (g/dl)	
Median	7.9
Range	6-13.3
-Neutrophils ($x10^{-3}/mm^3$)	
Median	0.57
Range	0.07-2.8

Table 1. Patient characteristics at baseline.

Patient #	Age		Baseline	Clone	Time on eltrombopag (months)	Dysplasia	Outcome	Time post transplant (months)
7	60	NR	46XY[20]	**-7[20]**	3	N	Died of progressive cytopenias	N/A
8	18	NR	46XX[6]	+8[9]/46XX[11]	3	N	Transplanted successfully	31
19	20	NR	46XY[20]	**-7[5]t(1;16) [3]/46XY[12]**	3	N	Transplanted successfully	18
26	67	R	46XY[20]	del(13)[19]/46XY[1]	13	Mild dyserythropoeisis	Transplanted	8
31	41	NR	46XY	+21(3)/46XY(17) **-7[2]/46XY[19]**	3 6	Mild dyserythropoeisis	Awaiting transplant	N/A
32	66	R	46XY[20]	46XYdel13q[2]/46XY[18]	9	N	Under observation	N/A
36	23	NR	46XY[20]	**-7[5],XY[15]**	3	N	Transplanted successfully	3
42	17	NR	No metaphases	**+1,der(1;7) [4]/46XY[16]**	3	N	Transplanted successfully	4

Table 2. Characteristics of patients who evolved while on eltrombopag.

Factor	Univariate Logistic Regression Analysis: Evolution			Multivariate Logistic Regression Analysis: Evolution		
	Coefficient	SD	P-value	Coefficient	SD	P-value
Age	-0.0225	0.0211	0.2873	-0.0122	0.0279	0.6603
Male Gender	2.0031	1.1212	0.0740	2.3316	1.7582	0.1848
ANC	-0.1307	0.7649	0.8643	0.7459	0.9201	0.4176
ARC	-0.0188	0.0193	0.3301	-0.0229	0.0256	0.3697
Relapsed Refractory	1.5755	0.8373	0.0599	2.2455	1.9191	0.2420
Prior IST Treatments						
One IST Course	--------	---------	---------	---------	---------	--------
Two IST Courses	15.0620	906.943	0.9868	13.0273	866.145	0.9880
Three IST Courses	15.5852	906.943	0.9863	14.3640	866.149	0.9868
Four IST Courses	15.8729	906.944	0.9860	14.7658	866.155	0.9864
Years since Last IST	-0.0078	0.0241	0.7463	-0.3367	0.4787	0.4818
Years since Diagnosis	0.0866	0.1059	0.4133	-0.0337	0.3942	0.9318
PNH Clone	-0.8109	0.8851	0.3595	-2.3858	1.9912	0.2308

Table 3. Univariate and multivariate logistic regression analysis for clonal evolution

Figure Legends

Fig 1a/1b

Responses to eltrombopag by lineage.

These Venn diagrams show the numbers of patients with uni- and multilineage responses at response assessment (Fig. 1a) and best response at follow-up (Fig 1b). All biologic best responses were included in Fig 1b, including a >1.5g increase in Hb even if began at >9 gr/dl. The lineage affected is indicated by the shade of the circle.

Fig 2

Box plots of hematological responses over time by lineage, showing erythroid response (2a), neutrophil response (2b) and platelet response (2c).

Fig 3

Bone marrow cellularity in patients 1 and 2. The first panel shows trephine core biopsies at baseline. The second panel shows the cellularity just prior to discontinuing eltrombopag in these two robust responders. The samples taken at 6 months off eltrombopag demonstrate that the marrows remain cellular in both patients. The images were taken on an Olympus BX41 microscope with an Olympus DP72 camera, using a 4x UPlanFL N Olympus objective, magnification is 40X.

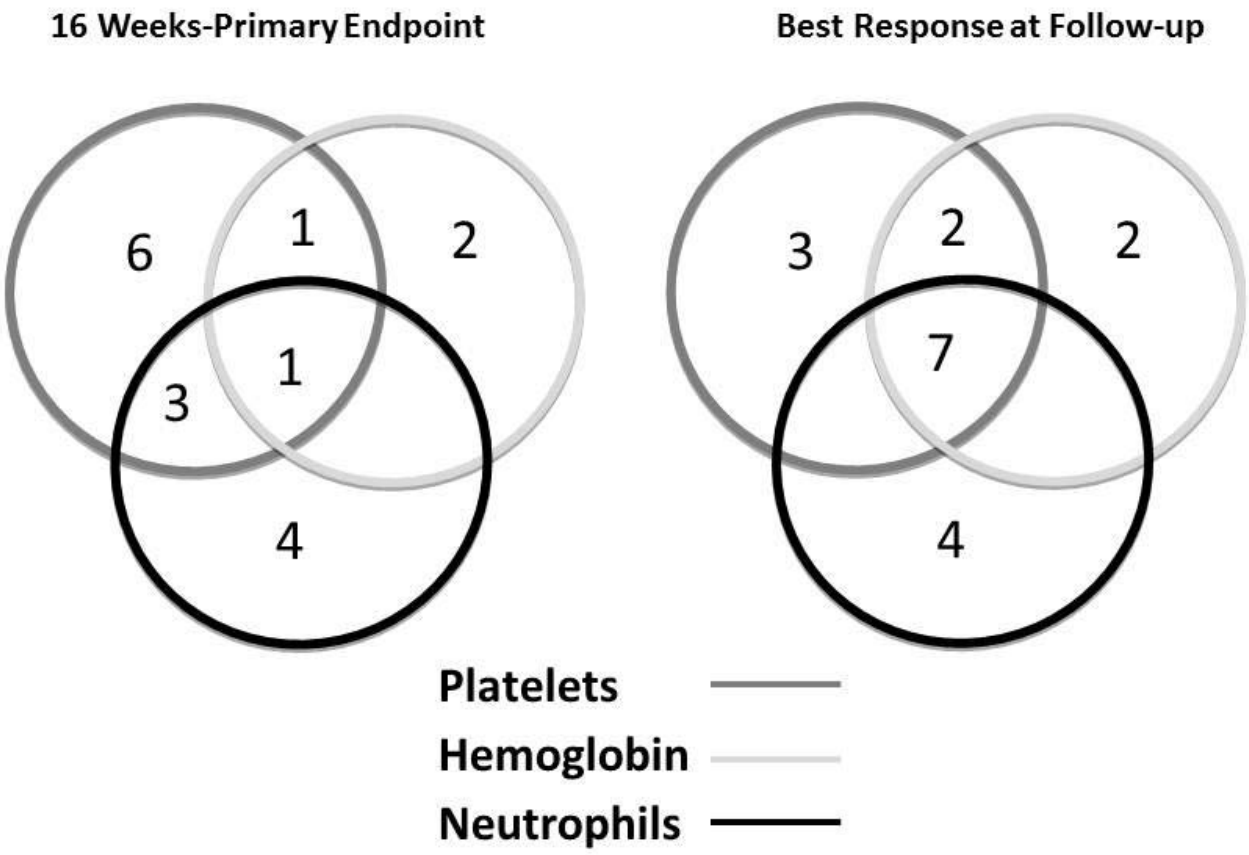

Fig 1

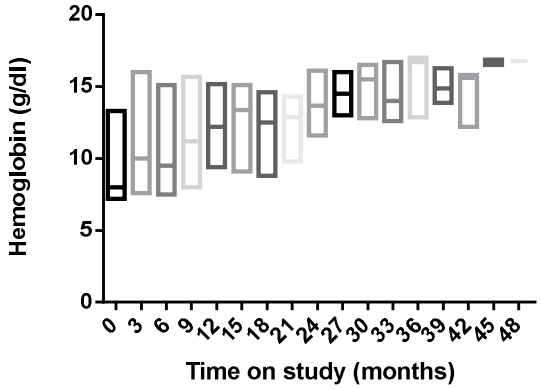

Figure 2a.

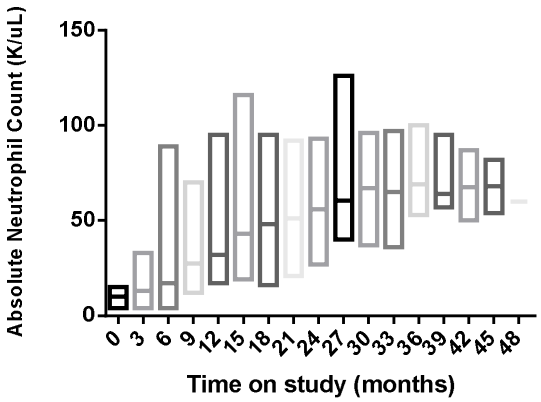

Figure 2b.

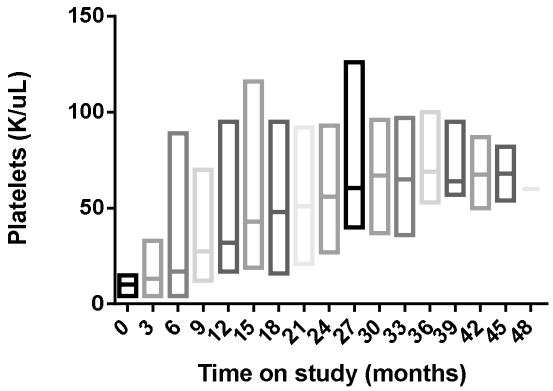

Figure 2c.

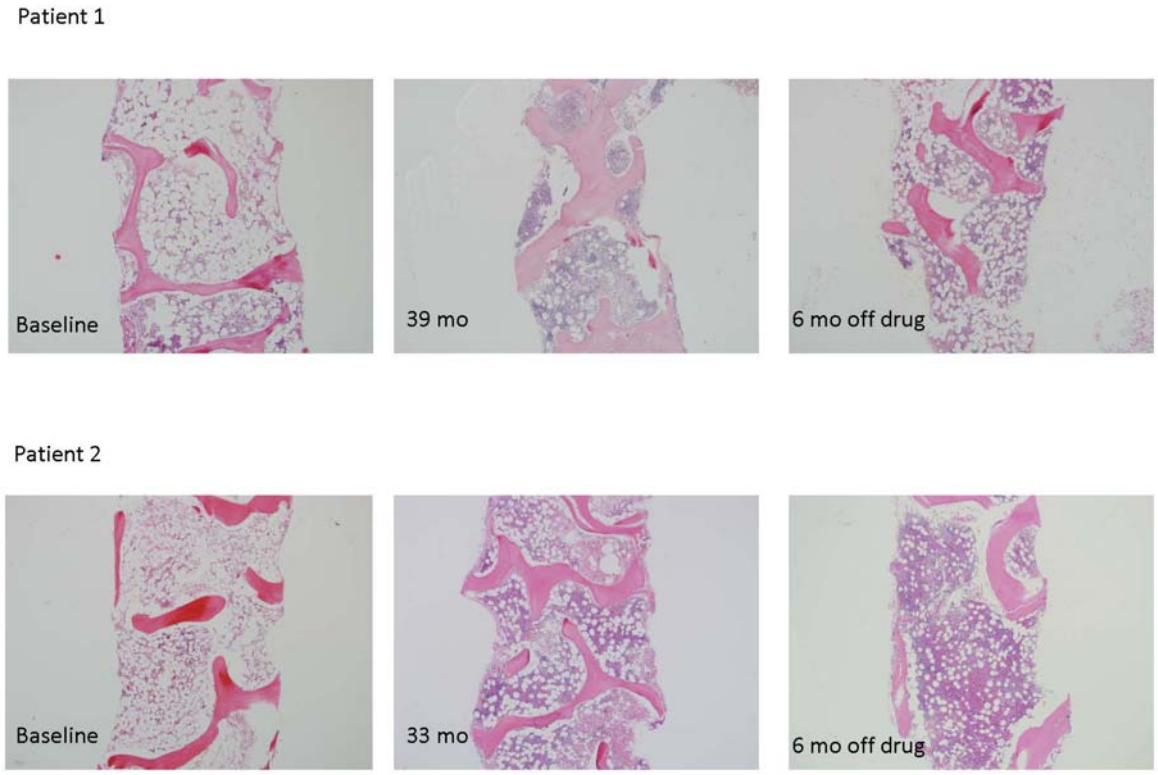

Fig 3