From chronic immune thrombocytopenia to severe aplastic anemia: recent insights into the evolution of eltrombopag

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***Abstract*:** Thrombopoietin (TPO) is the most potent cytokine stimulating thrombopoiesis. Therapy with exogenous TPO is limited by the formation of antibodies cross-reacting with endogenous TPO. Mimetics of TPO are compounds with no antigenic similarity to TPO. Eltrombopag is an orally-active nonpeptide small molecule that binds to the transmembrane portion of the TPO receptor MPL. Initial trials of eltrombopag have centered on immune thrombocytopenia (ITP), which is due to both increased destruction and decreased production of platelets. Eltrombopag at 25–75 mg/day has been shown to be highly effective in raising the platelet count in ITP with suboptimal response to immunosuppression and splenectomy. These successful results led to the exploration of eltrombopag in other thrombocytopenic disorders. In hepatitis C viral infection, eltrombopag raises the platelet count sufficiently enough to allow treatment with ribavirin and pegylated interferon. Because MPL is expressed on hematopoietic cells, eltrombopag use in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) might enhance leukemic proliferation. Clinical trials of eltrombopag in MDS and AML, however, have shown amelioration of thrombocytopenia without promoting disease progression. In severe aplastic anemia (SAA) not responding to immunosuppression with anti-thymocyte globulin (ATG) and cyclosporine, eltrombopag as a single agent at 150–300 mg/day results in an overall response rate of 40–70%. At high doses, adverse effects including pigmentation, gastrointestinal upset and hepatic derangement have become evident. Current studies have examined the first-line use of eltrombopag in combination with ATG in SAA. In a recent study, eltrombopag used at 150 mg/day with horse ATG resulted in an overall response rate of 90% in newly diagnosed SAA patients, with a complete response rate of about 50%. Clonal karyotypic aberrations are, however, found in 10–20% of SAA patients treated with eltrombopag. The safety and efficacy of eltrombopag in SAA require further evaluation, particularly when it is used with less intensive immunosuppression.

Keywords: acute myeloid leukemia, eltrombopag, hematopoietic stem cell transplantation, hepatitis C virus, immune thrombocytopenia, myelodysplastic syndrome, severe aplastic anemia

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Introduction

Thrombopoietin (TPO) is physiologically the most potent molecule regulating megakaryopoiesis and thrombopoiesis. Several different strategies were used to purify TPO and subsequently clone the gene [Bartley *et al.* 1994; De Sauvage *et al.* 1994; Kuter *et al.* 1994; Lok *et al.* 1994; Kato *et al.* 1995; Kuter, 2013; Kaushansky, 2015]. Synthesized mainly by the liver under steady-state conditions, TPO is produced initially as a 353 amino acid precursor protein with a molecular weight of 36 kDa. It then undergoes further amino acid removal and glycosylation, resulting in a glycopeptide that is 95 kDa in size [Bartley *et al.* 1994; De Sauvage *et al.* 1994; Foster *et al.* 1994; Li *et al.* 2001; Kuter, 2013]. TPO is produced from the liver at a constant rate. In the circulation, it is rapidly adsorbed and internalized by platelets [Kuter, 2013; Kaushansky, 2015], so that the platelet count forms a feedback

loop controlling TPO concentrations [Nichol, 1998; Schrezenmeier *et al.* 1998; Kuter, 2013; Kaushansky, 2015]. In addition to this simple autoregulation, hepatic production of TPO is also influenced by cytokines, the most important being interleukin (IL)-6, which is produced in a variety of inflammatory conditions. IL-6 stimulates hepatic TPO production, explaining the thrombocytosis found in inflammatory conditions [Kaushansky, 2015]. Marrow stromal cells are another source of TPO. Many molecules influence marrow stromal TPO production, including platelet derived growth factor and fibroblast growth factor-2 that are stimulatory, and platelet factor 4, thrombospondin and transforming growth factor-β that are inhibitory [Kaushansky, 2015].

Physiological functions of TPO

TPO binds *via* its amino-terminal to its receptor MPL, so named because it was originally identified as the proto-oncogene homolog of the murine myeloproliferative leukemia virus oncogene [Kaushansky, 2015]. MPL is a type I cytokine receptor and lacks intrinsic kinase activity. However, ligation to TPO leads to MPL tyrosine phosphorylation and activation of its associated proteins JAK2 and TYK2 [Bacon *et al.* 1995; Drachman *et al.* 1995], which in turn activates STAT3 and STAT5 [Drachman and Kaushansky, 1997]. The PI3K/AKT [Sattler *et al.* 1997; Miyakawa *et al.* 2001] and RAF-1/MAP kinase pathways [Nagata and Todokoro, 1995; Yamada *et al.* 1995] are also stimulated. These stimulatory pathways are negatively regulated by several mechanisms. TPO stimulation of MPL also leads to activation of LYN, LNK SOCS that serve as negative regulators [Kaushansky, 2015]. Furthermore, the TPO–MPL complex is rapidly internalized and targeted to both lysosomal and proteosomal degradation.

MPL is expressed on hematopoietic stem cells (HSCs), megakaryocyte colony-forming units (CFU-MKs), myeloid and erythroid precursors, early and late megakaryocytes and mature platelets [Kaushansky, 2015]. TPO is important in HSC maintenance, and individuals with MPL mutation develop aplastic anemia. For CFU-MKs, TPO increases their development and enhances their proliferative rate in collaboration with other cytokines including IL-3, IL-11 and stem cell factor [Kuter, 2013]. TPO also stimulates megakaryocyte maturation, increasing megakaryocyte size, ploidy and expression of glycoprotein (GP)Ib and GPIIb/IIIa. In platelets, TPO primes their interaction with agonists and promotes their aggregation, and may therefore play a role in thrombosis.

Therapeutic effects of TPO

Early therapeutic trials of TPO involved two drugs, recombinant human TPO (rh-TPO) and pegylated megakaryocyte growth and development factor (PEG-rHuMGDF) [Hitchcock and Kaushansky, 2014]. In cancer patients receiving chemotherapy, treatment with intravenous rh-TPO led to an increase in platelet count, which started after 5 days and peaked at 10–14 days, thereby accelerating platelet recovery and reducing the need of platelet transfusion [Vadhan-Raj, 2010]. Subcutaneous PEG-rHuMGDF resulted in similar effects. On the other hand, for patients undergoing hematopoietic stem cell transplantation (HSCT) and induction chemotherapy for acute leukemia, rh-TPO and PEG-rHuMGDF did not result in improvement of platelet engraftment or recovery. These results suggested that the intensity of chemotherapy and hence the number of residual HSCs might affect the response to TPO.

However, in a study where PEG-rHuMGDF was administered to normal platelet donors to try to improve platelet collection [Kuter, 2014], about 2.5% of patients developed thrombocytopenia, due to the formation of antibodies to the recombinant drug that cross-reacted with endogenous TPO [Li *et al.* 2001; Basser *et al.* 2002; Kuter and Begley, 2002]. Consequently, rh-TPO and PEG-rHuMGDF have not been further tested clinically.

TPO mimetics

To overcome these early setbacks, TPO mimetics or agonists that do not resemble TPO have been developed. These include TPO nonpeptide mimetics, TPO peptide mimetics and TPO agonist antibodies [Kuter, 2013]. TPO nonpeptide mimetics are small chemical molecules developed for high-affinity binding to MPL, activating it by mechanisms different from TPO. Eltrombopag is currently the only approved TPO nonpeptide mimetic. TPO peptide mimetics comprise a 14-amino acid peptide (Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala) with high affinity for MPL but have no sequence homology with TPO, which is dimerized and

linked to human Fab and Fc constructs or PEG moieties. Romiplostim is the currently approved TPO peptide mimetic. TPO agonist antibodies are antibodies raised against MPL. They are genetically engineered to bind with high affinity to and stimulate MPL. There is no current example of a TPO agonist antibody in clinical trials.

Eltrombopag

Eltrombopag binds to the transmembrane and juxtamembrane domain of MPL, which results in activation of downstream JAK/STAT and MAPK pathways [Kuter, 2013]. As its binding site is different from that of TPO, eltrombopag and TPO exhibit mutual additive effects. Eltrombopag is rapidly absorbed after oral administration; the maximum plasma concentrations achieved after 2–6 h [Gibiansky *et al.* 2011]. It should not be taken within 4 h of consumption of food rich in polyvalent cations, which chelate eltrombopag and decrease its absorption. It is mainly metabolized in the liver by the cytochrome P450 system, with a plasma elimination half-life of 21–32 h. Dose adjustment therefore is needed for patients with severe liver function derangement [Hayes *et al.* 2011]. Clearance of eltrombopag is 33–52% lower in Asian people, probably due to cytochrome P450 allele polymorphisms [Gibiansky *et al.* 2011]. Hence, the starting dose of eltrombopag for Asian patients should be approximately half that recommended for other populations.

Immune thrombocytopenia

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by a platelet count of $<100 \times 10^9$/l. It may be a primary disorder, or secondary to systemic autoimmune diseases, viral infections, drugs and other conditions [Kaushansky, 1995; Von Dem Borne *et al.* 2002; McMillan *et al.* 2004]. Clinically, ITP can be divided into three groups: newly diagnosed (diagnosis to 3 months); persistent (3–12 months from diagnosis); and chronic (lasting for >12 months) [Neunert *et al.* 2011].

Pathogenetically, ITP is caused by autoantibodies directed against platelet glycoproteins, leading to platelet destruction. In addition to platelet destruction, autoantibodies might impair megakaryocyte production and maturation [Mcmillan *et al.* 2004]. Furthermore, TPO levels in ITP patients are lower than those found in patients with comparable degrees of thrombocytopenia resulting from chemotherapy or bone marrow failure. This may be related to the binding of TPO to platelets, resulting in its increased clearance together with platelets in the macrophage system [Von Dem Borne *et al.* 2002]. Hence, the pathogenesis of ITP involves increased destruction and decreased production of platelets.

The realization that under-production of platelets contributes to the thrombocytopenia in ITP forms the basis for the therapeutic use of TPO mimetics [Kosugi *et al.* 1996; Porcelijn *et al.* 1998]. In addition, TPO mimetics may have immunomodulatory functions [Schifferli *et al.* 2016], inducing immune tolerance and modulating the functions of T-regulatory cells.

Eltrombopag in ITP

The efficacy of eltrombopag in adults with chronic ITP has been demonstrated by many randomized and nonrandomized, multicenter studies (Table 1). In a phase II dose-finding study, 118 adult patients with chronic ITP for at least 6 months and a platelet count of $<30 \times 10^9$/l who had not responded to at least one prior therapy, including splenectomy, or had relapsed within 3 months of previous therapy, were randomized in a 1:1:1:1 ratio to receive 30, 50, 75 mg of eltrombopag or placebo daily for 6 weeks [Bussel *et al.* 2007]. Treatment with eltrombopag at a dose of 30 mg, 50 mg or 75 mg daily resulted in a platelet count of $\geqslant 50 \times 10^9$/l on day 43 in 28%, 70% and 81% of cases, as compared with 11% in the placebo treatment group, indicating a dose-dependent response. In a subsequent double-blind phase III trial using similar inclusion criteria, 110 adult ITP patients were randomized in a 2:1 ratio to receive either eltrombopag ($n = 73$) or placebo ($n = 37$) to evaluate the efficacy, safety, and tolerability of eltrombopag at 50 mg/day, and to explore the efficacy of a dose increase to 75 mg/day [Bussel *et al.* 2009]. The results showed that patients on eltrombopag achieved a platelet response ($\geqslant 50 \times 10^9$/l) more frequently than patients on placebo (59% *versus* 16%, $p < 0.0001$). Of the 34 patients in the efficacy analysis, who increased their dose of eltrombopag to 75 mg/day on or after day 22 due to lack of response at 50 mg/day, 10 patients (29%) responded and achieved a platelet response. Patients receiving eltrombopag had a significantly lower risk of developing bleeding symptoms at each time point during treatment [odds ratio (OR): 0.49; 95% confidence interval (CI): 0.26–0.89; $p = 0.021$].

Table 1. Selected studies of eltrombopag in immune thrombocytopenia and aplastic anemia.

Studies	Key observations	References
Immune thrombocytopenia (ITP)		
118 patients randomized 1:1:1 to receive daily doses of eltrombopag at 30 mg, 50 mg and 75 mg	Platelet count of $\geq 50 \times 10^9$/l on day 43 achieved in 28%, 70% and 81% of cases on eltrombopag, as compared with 11% in the placebo treatment group	Bussel *et al.* [2007]
110 patients randomized 2:1 to receive eltrombopag at 50 mg/day ($n = 73$) or placebo ($n = 37$)	Eltrombopag-treated patients achieved a platelet response ($\geq 50 \times 10^9$/l) in 59% of cases, as compared with placebo (in 16%) ($p < 0.001$)	Bussel *et al.* [2009]
197 patients were randomized 2:1 to receive eltrombopag ($n = 135$) or placebo ($n = 62$)	Eltrombopag-treated compared with placebo-treated patients were significantly more likely to achieve a platelet count between 40 and 400×10^9/l (OR: 8.20; 95% CI: 3.59–18.73; $p < 0.001$) and to experience a longer duration of response (9.5 *versus* 2.2 weeks)	Cheng *et al.* [2011]
302 patients received continued eltrombopag treatment	At a median exposure of 2.4 years, 86% of patients achieved platelets $\geq 50 \times 10^9$/l	Bussel *et al.* [2016]
Phase II (PETIT) study, 67 patients aged 1–17 years randomized 2:1 to receive eltrombopag ($n = 45$) or placebo ($n = 22$) for 7 weeks	From weeks 1–6, 62% eltrombopag-treated patients, compared with 32% placebo-treated patients achieved platelets $\geq 50 \times 10^9$/l (OR: 4·31; 95% CI: 1·39–13·34; $p = 0·011$)	Bussel *et al.* [2015]
Phase III (PETIT2) study, 92 patients aged 1–17 years randomized 2:1 to receive eltrombopag ($n = 63$) or placebo ($n = 29$) for 13 weeks	40% of eltrombopag-treated patients compared with 3% placebo-treated patients achieved platelets $\geq 50 \times 10^9$/l for 6 of the last 8 weeks of the double-blind period (OR: 18·0; 95% CI: 2·3–140·9; $p = 0·0004$)	Bussel *et al.* [2015]
Chronic hepatitis C viral (HCV) infection		
74 patients with HCV-related cirrhosis and platelet counts between 20–70 $\times 10^9$/l received 4 weeks of eltrombopag	Anti-viral therapy could be initiated in 71–91% of patients, with 36–65% of patients able to complete 12 weeks of therapy	McHutchison *et al.* [2007]
ELEVATE study, for patients with chronic HCV infection requiring interventional procedures	Platelet transfusions were significantly less frequent in eltrombopag-treated patients compared with placebo-treated patients (72% *versus* 19%, $p < 0.001$)	Afdhal *et al.* [2012]
Phase III ENABLE-1 and ENABLE-2 trials, HCV-infected patients with platelet counts $<75 \times 10^9$/l were treated with eltrombopag for initiation and maintenance of antiviral therapy with ribavirin and pegylated interferon-α 2A or 2B	Significantly more eltrombopag-treated patients were able to receive treatment and at a higher dose, and maintain platelets $>50 \times 10^9$/l during antiviral treatment	Afdhal *et al.* [2014]
Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS)		
98 AML or MDS patients with platelet counts $<30 \times 10^9$/l randomized 2:1 to receive eltrombopag ($n = 64$) or placebo ($n = 34$), starting dose at 50 mg/day and increased to 300 mg/day	A trend towards efficacy in the eltrombopag *versus* placebo group in reduction of hemorrhage (≥grade 3) (16% *versus* 26%), platelet transfusion independence (38% *versus* 21%), red blood cell transfusion independence (20% *versus* 6%) and improved median overall survival (27.0 *versus* 15.7 weeks)	Platzbecker *et al.* [2015]
Severe aplastic anemia (SAA)		
25 patients with therapy-refractory SAA treated with eltrombopag at a starting dose of 50 mg/day and increased to a maximum of 150 mg/day	11 patients (44%) met primary response criteria with improvement in at least one lineage at 12 weeks	Olnes *et al.* [2012]
10 patients with therapy-refractory SAA treated with eltrombopag at a starting dose of 100 mg/day and increased to a maximum of 300 mg/day	7 patients (70%) achieved response in at least on lineage	Gill *et al.* [2016]

AML, acute myeloid leukemia; CI, confidence interval; HCV, hepatitis C virus; ITP, immune thrombocytopenia; MDS, myelodysplastic syndrome; OR, odds ratio; SAA, severe aplastic anemia.

In another double-blind placebo-controlled phase III study evaluating the efficacy and safety of a 6-month treatment of eltrombopag in adult ITP patients, 197 cases were randomized in a 2:1 ratio to receive either eltrombopag ($n = 135$) or placebo ($n = 62$) [Cheng *et al.* 2011]. Study patients were started with eltrombopag at 50 mg/day or placebo. At the end of 3 weeks of treatment, dose escalation to 75 mg/day was allowed if the platelet counts were less than 50×10^9/l. If the platelet counts were more than 200×10^9/l, eltrombopag was reduced to 25 mg/day. Tapering or discontinuation of eltrombopag was allowed if platelet counts were more than 100×10^9/l on two consecutive visits after 6 weeks of therapy. In the primary efficacy analysis, eltrombopag-treated compared with placebo-treated patients were significantly more likely to achieve a platelet count between 40 and 400×10^9/l (OR: 8.20; 95% CI: 3.59–18.73; $p < 0.001$); to experience a longer duration of continuous response (mean: 9.5 *versus* 2.2 weeks), and to have a reduced need for concomitant therapy or rescue medication and a lower risk of bleeding. The incidence of rebound thrombocytopenia after treatment discontinuation was similar in both groups (7%). No increased incidence of serious bleeding episodes was observed in the post-treatment period in the eltrombopag and placebo groups (4% and 10%).

A subsequent open-label extension study (the EXTEND trial) was designed to evaluate the safety and efficacy of prolonged eltrombopag treatment. Adult patients with chronic ITP previously enrolled into eltrombopag trials and had not experienced any eltrombopag-related serious adverse events (AEs) or drug intolerance were enrolled [Bussel *et al.* 2016]. The study started in June 2006 and ended in July 2015, and involved 302 patients. The overall median duration of exposure was 2.4 years (range: 2 days to 8.8 years), and mean average daily dose was 50.2 mg/day (range, 1–75 mg). Median platelet counts increased to $\geqslant 50 \times 10^9$/l by the second week. Overall, 86% (259/302) of patients achieved platelets $\geqslant 50 \times 10^9$/l in the absence of rescue therapy. Non-splenectomized and less-pretreated patients responded better than splenectomized and heavily pretreated patients. Incidence of bleeding symptoms [World Health Organization (WHO) grade 1–4] decreased from 57% (171/302) at the beginning of the study to 16% (13/80) at 1 year. WHO grade 3 and 4 bleeding events were infrequent. Of 101 patients receiving some form of ITP treatment at baseline, 34 stopped at least one ITP medication, and 39 had a sustained reduction or permanently stopped at least one ITP medication taken at baseline.

The efficacy of eltrombopag compared with placebo had also been evaluated in children with ITP in two randomized, double-blind, multicenter clinical studies (PETIT and PETIT-2) [Bussel *et al.* 2015; Grainger *et al.* 2015]. In a phase II study (PETIT), patients aged 1–17 years with ITP lasting for 6 months or longer and platelets $<30 \times 10^9$/l, who had received at least one previous treatment, were recruited [Bussel *et al.* 2015]. After an open-label, dose-finding phase, 67 patients were randomized in a 2:1 ratio to receive eltrombopag ($n = 45$) or placebo ($n = 22$) for 7 weeks. From weeks 1–6, 62% patients who received eltrombopag, compared with 32% who received placebo, achieved the primary endpoint of platelet count of $\geqslant 50 \times 10^9$/l at least once without rescue (OR: 4·31; 95% CI: 1·39–13·34; $p = 0{\cdot}011$). In the phase III study (PETIT2), patients were randomly assigned (2:1) to receive eltrombopag ($n = 63$) or placebo ($n = 29$) for 13 weeks. A total of 25 (40%) patients receiving eltrombopag compared with 1 (3%) patient receiving placebo achieved the primary outcome of platelet counts of $\geqslant 50 \times 10^9$/l for 6 of the last 8 weeks of the double-blind period (OR: 18·0; 95% CI: 2·3–140·9; $p = 0.0004$). In both studies, a clinical benefit was demonstrated by a reduction in the need for rescue therapy with eltrombopag *versus* placebo. In addition, a reduction of clinically significant bleeding was also seen in patients randomized to receive eltrombopag in the PETIT study [Bussel *et al.* 2015]. Both PETIT and PETIT2 studies allowed patients who completed the randomized phase to receive open-label treatment for up to 24 weeks. During the extension phase of eltrombopag treatment, platelet counts were maintained above 50×10^9/l in the majority of patients and about half of patients on concomitant drugs were able to discontinue or reduce these medications.

Adverse effects of eltrombopag in ITP

Eltrombopag is generally well tolerated. In the final analysis of the EXTEND study, AEs were reported in about 92% of patients while on therapy, but 67% of patients reported AEs of grades 1–2 only [Bussel *et al.* 2016]. The most commonly reported AEs were headache (28%), nasopharyngitis (25%), upper respiratory tract infection (23%), fatigue (17%) and diarrhea

(16%). Hepatobiliary laboratory abnormalities (HBLAs: increased serum alanine transaminase, aspartate transaminase and bilirubin) were reported in about 10% of patients receiving eltrombopag compared with about 3% in the placebo groups in various randomized clinical trials [Bussel *et al.* 2007, 2009; Cheng *et al.* 2011]. In the EXTEND study, 7% of patients reported HBLAs [Bussel *et al.* 2016]. These abnormalities were usually mild, and resolved either during continuation or on withdrawal of therapy. Furthermore, some patients who had experienced HBLAs during eltrombopag treatment might not have recurrence upon re-treatment. There is currently no evidence of severe irreversible liver damage associated with eltrombopag treatment at the recommended dosage for ITP.

In a pooled analysis of the eltrombopag clinical trials, there was no evidence of a correlation between platelet count increases and the occurrence of thromboembolic events [Bussel *et al.* 2010]. In the EXTEND study, 19 patients (6%) reported thromboembolic events including deep vein thrombosis, cerebral infarction and myocardial infarction [Bussel *et al.* 2016]. The incidence of thromboembolic events reported in the EXTEND study was similar to that reported in ITP patients [Sarpatwari *et al.* 2010]. In fact, in a study evaluating thrombophilia risk markers in previously treated patients with chronic ITP receiving eltrombopag, 81% of 167 patients had abnormal levels of at least one known or suspected thrombotic risk marker or coagulation cascade activation marker [Wong *et al.* 2015]. These findings supported the theory that chronic ITP was a prothrombotic disease. However, in a retrospective multicenter study, for 986 ITP patients during a 3888 patient-year follow up, 43 (arterial, $n = 28$; venous, $n = 15$) thrombotic events occurred, resulting in a cumulative incidence of 3.2% for arterial (95% CI, 2.0–5.0) and 1.4% for venous (95% CI, 0.8–2.5) thrombosis at 5 years. Splenectomy (in 136 patients, 13.7%) increased thrombotic risk (HR = 3.5, 95% CI, 1.6–7.6) compared with non-splenectomized patients. Age greater than 60 years, more than two risk factors for thrombosis at diagnosis and steroid use were independent thrombotic risks. These risks did not appear significantly higher than predefined thresholds, suggesting that venous and arterial thromboembolism were not frequent complications in ITP, except in splenectomized patients and those over 60 years old [Ruggeri *et al.* 2014]. Therefore, in patients with underlying risk factors for thrombosis, eltrombopag should be started at a lower dose with close monitoring to achieve a platelet count adequate for hemostasis [Cheng, 2012].

Cataracts were observed in rodents treated with eltrombopag, which were time- and dose-dependent [Cheng, 2012]. In the RAISE study, the incidences of newly diagnosed or progressive cataracts were similar between patients randomized to eltrombopag or placebo [Cheng *et al.* 2011]. In the EXTEND study, 9% patients experienced cataracts resulting in drug withdrawal in 4 patients [Bussel *et al.* 2016]. As most of these patients had been treated with corticosteroids before, the association of eltrombopag at the recommended doses for ITP with cataract could not be definitely established.

Bone marrow fibrosis might occur after TPO mimetic treatment [Douglas *et al.* 2002]. In a longitudinal prospective study evaluating the effects of eltrombopag treatment on bone marrow in patients with chronic ITP, a mild increase of reticulin fibrosis was observed in 10% of patients [Brynes *et al.* 2015a]. No patient had an on-treatment marrow biopsy of ⩾grade 2 reticulin fibrosis (European Consensus Scale of Marrow Fibrosis) at 2 years; or clinical signs and symptoms indicative of marrow dysfunction. These data were similar to those reported for the EXTEND study, and suggested that eltrombopag treatment was generally not associated with clinically relevant increases in bone marrow reticulin or collagen [Brynes *et al.* 2015b; Bussel *et al.* 2016].

Practice recommendations of eltrombopag in ITP

Current evidence suggests that eltrombopag is a valuable option for the management of chronic ITP in both adults and children. Accordingly, eltrombopag has been approved for the treatment of thrombocytopenia in patients with chronic ITP who have an insufficient response to immunosuppressive therapy or splenectomy. Eltrombopag should be initiated at 50 mg/day for most adult and pediatric patients 6 years and older, and at 25 mg/day for pediatric patients aged 1–5 years. Dose reductions are needed for patients with hepatic derangement, and patients of East Asian ancestry (starting dose: 25 mg/day). Complete blood counts should be monitored weekly until a stable dose is reached. The effect of a particular dose should be achieved within 2 weeks. Dosage may be adjusted up to 75 mg/day

to maintain platelet counts of $\geq 50 \times 10^9$/l. If platelet count increases to $\geq 400 \times 10^9$/l, eltrombopag should be withheld and re-initiated at a lower dose after the platelet count falls to below 150×10^9/l.

Liver function should be monitored regularly. Dosages of concomitant ITP medications is modified, as clinically indicated, to avoid excessive increases in platelet counts during eltrombopag treatment. Most patients require long-term maintenance therapy, although successful drug discontinuation might be possible in some cases [Gonzalez-Lopez *et al.* 2015]. Platelet counts and bleeding symptoms must be closely monitored following discontinuation of eltrombopag [Cheng, 2012].

Eltrombopag in chronic liver disease

Thrombocytopenia is present in up to 70% of patients with liver cirrhosis and in 6% of noncirrhotic patients with chronic liver diseases [Giannini, 2006]. The mechanisms of thrombocytopenia are multifactorial. Liver cirrhosis causes portal hypertension and splenomegaly, resulting in increased platelet sequestration. In alcoholic liver disease, alcohol-derived reactive aldehydes induce myelosuppression, whereas in patients with chronic viral hepatitis, viral-related immune complexes increase platelet destruction in the reticuloendothelial system [Ballard, 1989; Peck-Radosavljevic, 2000]. It has also been shown that the titer of platelet-associated immunoglobulin G is increased in more than 80% of patients with chronic hepatitis C virus (HCV) infection [Nagamine *et al.* 1996]. Furthermore, increased auto-antibodies against GP IIb/IIIa are found in chronic liver disease [Kajihara *et al.* 2003]. Finally, interferon used for treating cirrhosis inhibits hematopoietic progenitors and causes myelosuppression [Peck-Radosavljevic *et al.* 2002; Schmid *et al.* 2005; Brouwer *et al.* 2015].

Eltrombopag for thrombocytopenia-complicating chronic liver disease

In a phase II study, the efficacy of eltrombopag to increase platelet counts in order to initiate ribavirin and pegylated interferon treatment was tested in 74 patients with HCV-related cirrhosis and platelet counts between 20–70 $\times 10^9$/l [Mchutchison *et al.* 2007] (Table 1). With 4 weeks of eltrombopag therapy, anti-viral therapy could be initiated in 71–91% of patients, with 36–65% of patients able to complete 12 weeks of therapy. There were three pivotal randomized controlled trials subsequently conducted. In the ELEVATE study conducted in patients with chronic liver diseases requiring interventional procedures, platelet transfusions were significantly less frequent in patients randomized to receive eltrombopag than placebo (72% *versus* 19%, $p < 0.001$) [Afdhal *et al.* 2012]. However, portal vein thrombosis developed in six patients receiving eltrombopag (five of who had a platelet count of $>200 \times 10^9$/l) as compared with one patient receiving placebo. In two other phase III trials ENABLE-1 and ENABLE-2, the efficacy of eltrombopag as compared with placebo was tested in HCV-infected patients (with platelet counts $<75 \times 10^9$/l) for increasing platelet counts to allow initiation and maintenance of antiviral therapy with ribavirin and pegylated interferon-α 2A or 2B [Afdhal *et al.* 2014]. Significantly more patients receiving eltrombopag were able to receive treatment and at a higher dose. Furthermore, significantly more eltrombopag-treated patients maintained platelet counts of $>50 \times 10^9$/l during antiviral treatment. AEs were similar, but eltrombopag compared with placebo led to more hepatic decompensation (10% *versus* 5%) and thromboembolic events (3% *versus* 1%).

Eltrombopag is currently approved for use in chronic HCV infection to allow initiation and maintenance of pegylated interferon-based antiviral therapy. Clinical use should be restricted to this specific indication, owing to concerns of hepatic decompensation and portal vein thrombosis (eltrombopag therapy should be stopped once platelet counts are $\geq 200 \times 10^9$/l).

Eltrombopag in thrombocytopenia in other hematologic diseases

Thrombocytopenia is a prevalent and an important cause of morbidity and mortality in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [Kantarjian *et al.* 2007]. In MDS, thrombocytopenia occurred as a consequence of ineffective platelet production secondary to disordered differentiation and proliferation of megakaryocytes, increased megakaryocyte programmed cell death, abnormal TPO signaling and increased platelet destruction [Li *et al.* 2016]. Thrombocytopenia in MDS is not only associated with bleeding, but is also recognized as an independent predictor of progression to AML and inferior overall survival [Kantarjian *et al.* 2008]. In

AML, ineffective hematopoiesis and generation of a hematopoiesis-suppressing microenvironment by the malignant cell clone in the bone marrow result in thrombocytopenia [Estey *et al.* 2006].

The high efficacy of TPO mimetics in ITP led to interests in their use in thrombocytopenia-complicating hematopoietic neoplasms. MPL is expressed on many malignant hematopoietic cell types including blast cells and bone marrow mononuclear cells from MDS patients [Vigon *et al.* 1993; Kalina *et al.* 2000; Luo *et al.* 2000; Schroder *et al.* 2000], so that there are theoretical risks that TPO mimetics might exacerbate leukemia or accelerate progression of MDS to leukemia. Interestingly, eltrombopag had been shown to modestly inhibit cellular proliferation in samples of AML and MDS patients at clinically achievable concentrations [Will *et al.* 2009]. Additional studies with various AML and MDS cell lines showed anti-leukemia effect of eltrombopag through an MPL independent, non-apoptotic mechanism, by decreasing intracellular iron levels leading to decreased cell proliferation [Erickson-Miller *et al.* 2010; Roth *et al.* 2012]. Furthermore, eltrombopag decreases the levels of reactive oxygen species, leading to a disruption of AML intracellular metabolism and hence cell death [Kalota *et al.* 2015].

Eltrombopag therapy in MDS and AML

In a pilot phase I dose-finding safety study of eltrombopag in azacitidine-treated MDS patients with platelet counts of $<75 \times 10^9$/l, 9 of 12 patients maintained stable platelet counts despite azacitidine therapy, with no increase in blast count, disease progression or marrow fibrosis [Svensson *et al.* 2014].

The safety and tolerability of eltrombopag for the treatment of thrombocytopenia in patients with advanced MDS, secondary AML, or *de novo* AML were evaluated in a randomized, placebo-controlled, double-blind, phase I/II trial [Platzbecker *et al.* 2015] (Table 1). A total of 98 patients with MDS or AML, bone marrow blast counts of 10–50%, platelet counts $<30 \times 10^9$/l or platelet transfusion dependence, and relapsed or refractory disease or ineligibility to receive standard treatment, were randomly assigned in a 2:1 ratio to receive eltrombopag ($n = 64$) or placebo ($n = 34$). The starting dose was 50 mg/day, increased every 2 weeks to a maximum of 300 mg/day (or 150 mg/day for patients of East Asian ancestry). Drug-related AEs of grade 3 or higher were reported in six (9%) patients in the eltrombopag group and four (12%) patients in the placebo group, indicating an acceptable safety profile. There was no difference in the proportion of peripheral blasts in the two groups. The results showed a trend towards efficacy in the eltrombopag *versus* placebo group in reduction of hemorrhage ($\geqslant$ grade 3) (16% *versus* 26%), platelet transfusion independence (38% *versus* 21%), red blood cell transfusion independence (20% *versus* 6%) and improved median overall survival (27.0 *versus* 15.7 weeks).

Various randomized clinical trials are currently ongoing to further evaluate the role of eltrombopag as monotherapy in patients with high-risk MDS or AML (ASPIRE; ClinicalTrials.gov identifier: NCT01440374), or in combination with azacitidine in patients with intermediate or high-risk MDS (SUPPORT; ClinicalTrials.gov identifier: NCT02158936) or decitabine in patients with AML (DELTA; ClinicalTrials.gov identifier: NCT02446145).

Current evidence suggests that the use of eltrombopag to up to 300 mg/day (150 mg/day in East Asians) appears to be well tolerated in MDS and AML, with some potential benefits including reduction of bleeding symptoms, less platelet transfusion and better survival. Further data are required before the role of eltrombopag can be defined either as monotherapy or in combination with other agents in these patients. Currently, eltrombopag may be considered in MDS and AML patients who have severe thrombocytopenia and significant bleeding symptoms, who are otherwise not suitable for other treatment such as chemotherapy or hypomethylating agents.

Eltrombopag in thrombocytopenia in other conditions

About 3–4% of patients with solid tumors develop grade-4 thrombocytopenia after chemotherapy [Ten Berg *et al.* 2011; Hassan and Waller, 2015]. Small-scale studies have suggested that prophylactic use of eltrombopag might reduce the duration and severity of chemotherapy-induced thrombocytopenia. In a randomized phase I study, the administration of eltrombopag in patients with advanced solid tumors receiving gemcitabine-based

chemotherapy was well tolerated, and resulted in improvement in thrombocytopenia and reduction in delays in chemotherapy schedule or decrease in chemotherapy dosage [Winer *et al.* 2015]. Eltrombopag had also been shown to improve platelet counts in patients with advanced solid tumors receiving carboplatin/paclitaxel based chemotherapy, and in patients with soft tissue sarcomas receiving doxorubicin and ifosfamide [Kellum *et al.* 2010; Chawla *et al.* 2013]. The therapeutic application of eltrombopag in solid tumors is limited due to concerns of thromboembolism and the theoretical risk of cytokine-associated tumor proliferation. Intriguingly, there is *in vitro* evidence to show anti-tumor effect of eltrombopag in hepatocellular carcinoma [Kurokawa *et al.* 2015]. Furthermore, it has been shown that breast, lung and ovarian tumor samples and cell lines lack MPL mRNA or protein expression [Erickson-Miller *et al.* 2012]. Despite these findings, there are currently no established guidelines for the use of eltrombopag in solid tumor patients developing chemotherapy-induced thrombocytopenia.

HSCT is associated with prolonged thrombocytopenia requiring frequent platelet transfusions and increased risks of bleeding. In a phase I trial of 19 patients undergoing HSCT with total body irradiation as part of the conditioning regimen, eltrombopag was evaluated at 75 mg, 150 mg, 225 mg and 300 mg daily for 27 days [Liesveld *et al.* 2013]. Overall, 15 patients were able to complete the treatment protocol, confirming tolerability at these dosages. In another small retrospective study of eltrombopag therapy in 12 patients undergoing allogeneic HSCT, eltrombopag was started at 12.5 mg/day and increased by 12.5 mg/day every week until the endpoint of platelet counts $>50 \times 10^9$/l [Tanaka *et al.* 2016]. Overall, three of five patients with prolonged isolated thrombocytopenia (defined as platelet transfusion dependence for more than 90 days post-HSCT) and five of seven patients with secondary failure of platelet recovery (defined as platelet counts of $<20 \times 10^9$/l lasting at least 7 days or the need for platelet transfusion within 7 days after primary platelet engraftment) were able to achieve the endpoint. The number of megakaryocytes in the bone marrow before eltrombopag therapy was associated with better response to treatment. Therefore, eltrombopag appears to be relatively well tolerated and may be efficacious for thrombocytopenia after HSCT, although formal studies are required to validate this indication.

Eltrombopag in refractory aplastic anemia

Aplastic anemia (AA) is a rare disorder defined as pancytopenia with a hypocellular bone marrow, without evidence of abnormal cellular infiltration or marrow fibrosis [Killick *et al.* 2016]. Severe AA (SAA) is defined as a marrow cellularity of $<25\%$ (or 25–50% with $<30\%$ residual hematopoietic cells) with at least two of the following parameters: absolute neutrophil count $<0.5 \times 10^9$/l; platelet count $<20 \times 10^9$/l; and reticulocyte count $<20 \times 10^9$/l [Killick *et al.* 2016]. AA is thought to be due to immune attack against hematopoietic stem and progenitor cells mediated by cytotoxic T-lymphocytes. In younger patients with SAA, allogeneic HSCT from an HLA-matched sibling is curative in up to 90% of cases [Storb *et al.* 1994; Deeg *et al.* 1998; Desmond *et al.* 2015; Killick *et al.* 2016]. Otherwise, horse anti-thymocyte globulin (ATG) and cyclosporine are the current standard treatment. In 30–40% of cases, relapse following first-line treatment with horse ATG and cyclosporine [Marsh *et al.* 2013; Desmond *et al.* 2015] occurs. In relapsed or refractory AA, only about 30–35% of patients respond to a second course of an alternative source of ATG (rabbit ATG) and cyclosporine. Thus, a significant proportion of patients with SAA remains refractory, resulting in transfusion dependence and other complications of pancytopenia.

MPL is expressed on hematopoietic stem and progenitor cells (HSPCs) [Zeigler *et al.* 1994]. Therefore, HSPCs proliferate in response to TPO and other cytokines [Ku *et al.* 1996; Sitnicka *et al.* 1996]. These observations led to the application of TPO agonist in SAA. Eltrombopag possesses favorable characteristics. As a small molecule it enters the bone marrow niche more effectively that endogenous TPO [Roth *et al.* 2012; Sugita *et al.* 2013]. It is parenteral, making prolonged administration feasible.

In a phase II study conducted by the National Institute of Health (NIH, USA), 25 patients with SAA who had failed one or more courses of ATG and cyclosporine were treated with eltrombopag, at a starting dose of 50 mg/day, stepping up by 25 mg every 2 weeks until a maximum dose of 150 mg/day [Olnes *et al.* 2012] (Table 1). Primary endpoints were hematologic responses and toxic effects at 12 weeks. A platelet response was defined as an increase of 20×10^9/l over baseline, or independence from platelet transfusions for a minimum of 8 weeks. An erythroid response was

defined as a hemoglobin increase of 1.5 g/dl for patients with a baseline of <9 g/dl, or reduction of pack cell transfusion by ⩾4 units in 8 weeks as compared with the previous 8 weeks. A neutrophil response was defined as an increase in absolute neutrophil count by 0.5×10^9/l, or two times increase in baseline neutrophil count if it was $<0.5 \times 10^9$/l. Patients with a response at 12 weeks continued to receive eltrombopag for an additional 4 weeks. If the response was stable, patients continued to receive eltrombopag provided that the response was maintained.

All but one patients received the maximum dose of 150 mg/day. A total of 11 patients (44%) met the primary response criteria in at least one lineage at 12 weeks. Overall, 9 patients had a platelet response (concomitant neutrophil response, $n = 2$; concomitant hemoglobin response, $n = 2$), and two patients had a neutrophil response. A total of seven patients went on to receive eltrombopag (150 mg/day) for a median of 16 (8–32) months. Bone marrow examination in patients with a response showed normalization of trilineage hematopoiesis, without increase in fibrosis. These findings showed that eltrombopag was capable of inducing multilineage hematologic response in patients with SAA.

These results were later updated with an additional 18 patients [Desmond *et al.* 2014]. In this updated cohort of 43 patients, the overall response rate was 40% (17/43) at 3–4 months, including bilineage and trilineage responses. An important observation was that for patients who had a unilineage or bilineage response at 12 weeks, continuation of eltrombopag treatment might ultimately lead to trilineage response. Of 17 patients who had a response, 5 patients with near-normalization of blood counts discontinued eltrombopag after a median of 28.5 (9–37) months of treatment. With a median follow up of 13 (1–15) months, these patients had maintained their responses without further eltrombopag treatment.

In another retrospective analysis of 10 (eight Chinese and two Portuguese) patients with AA/SAA who had failed multiple courses of therapy, eltrombopag treatment led to an apparently higher overall response rate of 70%, with 30% of patients achieving trilineage response [Gill *et al.* 2016] (Table 1). Notably, these patients had maximum eltrombopag doses of 50–300 mg/day. Owing to the lower eltrombopag clearance in Asian people [Gibiansky *et al.* 2011], the maximum eltrombopag exposure in these Chinese patients was equivalent to White patient doses of 67–450 mg/day. In three cases of trilineage response, two occurred in Chinese patients receiving eltrombopag at maximum doses of 150 mg/day and 300 mg/day (equivalent White patient doses: 200–225 mg/day, 400–450 mg/day); and one occurred in a Portuguese patient receiving a maximum dose of 200 mg/day. Another Portuguese patient receiving a maximum dose of 150 mg/day achieved bilineage response. On the other hand, two Chinese patients receiving maximum doses of 50–75 mg/day only achieved platelet response. Therefore, the hematologic response appeared to be dose-dependent. Furthermore, eltrombopag doses of >150 mg/day and up to a White patient equivalent of 450 mg/day were feasible without unacceptable toxicities.

The findings of these studies have clearly shown that eltrombopag has single-agent activity in stimulating trilineage hematopoiesis in AA/SAA. In the NIH studies, dose escalation stopped at 150 mg/day. The biologic rationale for this dose capping is unclear. In the Chinese study, much higher doses of eltrombopag were used, which apparently led to a higher overall response rate. Therefore, more studies are required to determine if patients not responding to lower doses of eltrombopag may respond to higher doses. Furthermore, these results have paved the way for employing eltrombopag as a frontline treatment.

Eltrombopag in frontline treatment of SAA

In a phase II study, 88 patients with newly diagnosed SAA undergoing horse ATG and cyclosporine induction therapy received in addition eltrombopag at 150 mg/day [Townsley *et al.* 2015]. Patients were divided into three cohorts according to eltrombopag dosing: cohort 1 ($n = 30$), third week to 6 months; cohort 2 ($n = 31$), third week to 3 months; cohort 3 ($n = 27$), day 1–6 months. The primary endpoint was complete response at 6 months. In total, treatment was prematurely stopped (before 6 months) in 8 patients (cutaneous sensitivity, $n = 2$; evolution to MDS, $n = 2$; non-response, $n = 2$). The overall response/complete response of the three cohorts at 6 months were respectively 80%/33% (cohort 1); 87%/36% (cohort 2); and 92%/54% (cohort 3). These results were considered to be superior to those of historical controls receiving horse ATG and cyclosporine. For responders, the median

time to transfusion independence was 32 days for platelets and 42 days for red blood cells. Serial marrow biopsies showed improved cellularity in 80% of cases without increased fibrosis. Bone marrow CD34+ cell count also significantly increased (median increase from baseline at 3 months, cohort 1: 17-fold; cohort 2: 4-fold) ($p < 0.001$). Overall, 12 patients underwent HSCT due to relapse ($n = 3$), refractoriness ($n = 6$) or MDS ($n = 3$).

These findings suggested that eltrombopag used with standard immunosuppression might salvage and expand residual HSC, and accelerate the rate and quality of hematopoietic recovery. Conceptually, the efficacy of eltrombopag is dependent on the number of residual HSCs, implying that it should be used early in the treatment of AA/SAA.

Adverse effects of eltrombopag used in high doses in AA/SAA

The AEs of eltrombopag are significantly accentuated in high doses. Transaminitis was most common, and was reversible after short treatment interruption without dose reduction [Desmond *et al.* 2014; Gill *et al.* 2016]. Gastrointestinal side effects including dyspepsia could usually be controlled symptomatically without treatment disruption [Gill *et al.* 2016]. At doses >150 mg/day, a slate-grey pigmentation occurred almost universally [Gill *et al.* 2016]. However, it was fully reversible on dose reduction.

Clonal evolution associated with eltrombopag use in AA/SAA

AA has been conventionally viewed as arising from deficiency of HSC consequent on an autoimmune attack or previous exposures to toxic drugs or agents. However, recent molecular analysis showed that genetic mutations typically associated with myeloid neoplasms were found in up to one-third of patients with AA/SAA [Yoshizato *et al.* 2015]. Clonal selection of cells with gene mutations is regarded to be the predominant pathogenetic mechanism.

Against this genetic backdrop, eltrombopag treatment in AA/SAA has also been associated with clonal cytogenetic evolution. In the NIH refractory SAA study of refractory patients, 8 of 43 patients developed new cytogenetic abnormalities, including partial deletion or loss of chromosome 7 in 5 patients [Desmond *et al.* 2014]. In the Chinese AA/SAA study, 1 of 10 patients developed AML with monosomy 7 [Gill *et al.* 2016]. In the NIH eltrombopag frontline study [Townsley *et al.* 2015], after a median follow up 15 (1–42) months, cytogenetic abnormalities were observed in 7 of 88 patients, which included monosomy or partial deletion of chromosome 7 in 5 cases (with concomitant t(3;3)(q21;q26) in 1 case), transient deletion 13q in 1 case, and trisomies 6 and 15 in 1 case.

Several mechanisms might account for clonal evolution. Patients with AA have a 15% chance of developing cytogenetic aberrations in 10 years [Desmond *et al.* 2015]. A damaged marrow microenvironment might be conducive to the acquisition of genetic alterations. Minor preexisting clones undetectable at diagnosis might be stimulated by eltrombopag. Alternatively, excessive stimulation of HSC might destabilize the genome and lead to cytogenetic aberrations. Some of the cases might have been hypoplastic MDS indistinguishable from AA at diagnosis. In this respect, eltrombopag treatment for thrombocytopenia-complicating MDS has so far not been associated with disease progression. Therefore, the phenomenon of clonal evolution in AA consequent on eltrombopag treatment remains to be further studied.

Conclusions and future perspectives

In the decade since eltrombopag has been used clinically, it has evolved from a medication primarily for increasing thrombopoiesis in ITP to an agent with multiple roles in stimulating hematopoiesis. A better definition of its safety when used in patents with myeloid neoplasms will further expand its role in these disorders. Eltrombopag is poised to play an increasingly important part in the treatment of AA. The efficacy of eltrombopag in combination with less immunosuppressive treatment such as cyclosporine for SAA, and on its own for less severe AA, warrants further exploration.

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