Review article: pharmacological approaches for the treatment of thrombocytopenia in patients with chronic liver disease and hepatitis C infection

N. H. AFDHAL* & J. G. MCHUTCHISON†

SUMMARY

Background
Patients with chronic liver disease and hepatitis C virus (HCV) frequently experience thrombocytopenia that complicates the management of their disease. Traditional therapy for thrombocytopenia consists of platelet transfusion, which can be associated with significant safety and economic issues. Consequently, efforts have been directed toward developing novel approaches for the treatment of thrombocytopenia.

Aim
To summarize the available data on the limitations of traditional therapies and the effects of novel therapies currently in clinical development for the treatment of thrombocytopenia.

Results
Recent research has begun to reveal the complex mechanisms that regulate thrombopoiesis. Cytokines and growth factors, such as interleukin-11 and thrombopoietin (TPO), play a key role in the production of platelets. A number of recent clinical studies have provided evidence that pharmacologic agents that target megakaryocyte precursors and stimulate thrombopoiesis can effectively reverse thrombocytopenia. Here, we review the regulation of thrombopoiesis, the role of TPO, and a number of novel compounds that stimulate platelet production by acting through the TPO receptor. Agents that stimulate TPO include the orally available nonpeptidic agonists eltrombopag and AKR-501, peptidic agonists AMG-531 and Peg-TPOmp, and small engineered antibodies.

Conclusion
Results from clinical trials with these agents in healthy subjects confirm that activation of thrombopoiesis via the TPO pathway is an effective method of stimulating platelet production. This approach may provide safer, more effective treatment for thrombocytopenia in patients with chronic liver disease. Several of these agents are currently being tested in large scale trials.

Aliment Pharmaco Ther 26 (Suppl 1), 29–39
INTRODUCTION

Thrombocytopenia (platelet counts below $150 \times 10^3/\mu L$) because of chronic liver disease and hepatitis C virus (HCV) affects between 15% and 70% of patients with cirrhosis, depending on the advanced stage of the liver disease and criteria used to define the condition. Its prevalence also appears to increase with the severity of liver disease. Thrombocytopenia has been reported in 64% of cirrhotic patients and 6% of non-cirrhotic patients with chronic liver disease, while advanced thrombocytopenia ($<10000/\mu L$) that requires platelet transfusions is observed in <1% of patients.

While thrombocytopenia itself is rarely life-threatening, it complicates the medical management of patients with advanced liver disease, cancer, immune thrombocytopenia purpura (ITP) and other diseases. Thrombocytopenia may increase the risk of bleeding associated with invasive diagnostic or therapeutic medical procedures including surgery and other medically necessary procedures. Patients with low platelet counts ($<75000$ to $100000/\mu L$) may not be able to receive or maintain pegylated interferon (PEG-IFN) and ribavirin treatment for HCV, thus reducing the overall effectiveness of treatment.

In chronic liver disease patients, several factors are thought to contribute to thrombocytopenia. In cirrhotic patients with portal hypertension, increased splenic sequestration of platelets is considered the most significant factor but other mechanisms may also be involved. HCV infection and IFN treatment used to treat HCV can both cause bone marrow suppression. Alcoholic liver disease can also lead to impaired bone marrow function through a direct effect of alcohol on the marrow. In advanced liver disease, the loss of functional liver tissue that can occur from cirrhosis may result in reduced levels of thrombopoietin (TPO), the major growth factor that regulates platelet production. Finally, in patients with hepatocellular carcinoma, chemotherapy can induce bone marrow suppression that contributes to thrombocytopenia and can have direct toxic effects on the liver leading to decreased TPO production.

Historically, platelet transfusions are the most effective means of managing thrombocytopenia. However, the associated costs and risks of platelet transfusions can be substantial. Up to 50% of patients become refractory after multiple rounds of platelet transfusions because of human leucocyte antigen (HLA) alloimmunization. And a significant proportion of patients who undergo platelet transfusion develop side effects including febrile non-haemolytic reactions and transfusion-associated infections. Given the limitations and risks associated with platelet transfusions, more effective and novel therapies that increase peripheral functional platelet concentrations are needed. Recent research has increased our understanding of the biology of thrombopoiesis and the regulation of platelet production leading to the development of novel therapies that may avoid the limitations of traditional approaches. In this review, the biological basis for exploiting the TPO pathway is discussed, along with novel therapies that may provide effective treatments for thrombocytopenia in patients with chronic liver disease.

THROMBOPOIESIS

Megakaryocyte development is a complex process that involves the interaction of haematopoietic stem cells, bone marrow stromal cells and the surrounding micro-environment and soluble cytokines. It can be divided into three major processes: commitment to the megakaryocyte lineage, proliferation, and differentiation. Soluble cytokines play a significant role in increasing the numbers of committed progenitor cells, decreasing cell cycling time, increasing the number of cycles per progenitor cell and promoting differentiation. In vitro, a number of factors have been shown to play a role in megakaryopoiesis including TPO, stem cell factor (SCF; Kit ligand), stromal cell-derived factor-1 (SDF-1), interleukin-1 (IL-1), IL-3, IL-6, IL-11, granulocyte-macrophage colony-stimulating factor (GM-CSF), leukaemia inhibitory factor and erythropoietin (EPO; Figure 1). Only knock-out mice deficient in SCF or TPO (but not IL-3, IL-6, the IL-11 receptor or leukaemia inhibitory factor) have defects in megakaryocyte and platelet production in vivo.

While many cytokines play a role in megakaryopoiesis, TPO, SCF, IL-3 and IL-11 are thought to be the most significant. IL-3 appears to act primarily as a proliferation factor, and SCF and IL-11 synergize with TPO to increase the number and maturation of megakaryocyte precursors. TPO and IL-11 have been shown to stimulate megakaryocyte maturation. TPO, however, appears to be the most important cytokine for the generation of mature megakaryocytes and platelets. TPO$^{+/−}$ and Mpl$^{−/−}$ mice have 90% fewer platelets and megakaryocyte progenitors compared with...
normal mice. Conversely, daily infusion of TPO into normal animals induced a fourfold increase in platelet count, increased the number of megakaryocytes in the marrow and expanded the number of megakaryocyte progenitor cells 20-fold. TPO is a potent megakaryocyte colony-stimulating and maturation factor, inducing colony formation from as many as two-thirds of all megakaryocyte progenitors. In suspension cultures containing murine or human bone marrow cells, treatment with TPO increases the size and number of megakaryocytes, and stimulates the expression of platelet-specific markers such as CD41 and CD61. TPO is also the most potent known stimulator of endomitosis and polyploidy in megakaryocytes. While TPO has profound effects on the proliferation and maturation of megakaryocytes, it has little effect on the release of platelets from the mature megakaryocyte. However, at relatively high concentrations, it does appear to sensitize platelets to aggregation through a variety of stimuli.

### BIOLOGY OF TPO

The presence of a TPO-like molecule had long been postulated but eluded identification until 1994, when TPO was purified and cloned by several laboratories. As discussed above, TPO has been shown to be the predominant growth factor responsible for the production of megakaryocytes, playing a role in each of the steps from megakaryocyte-precursor stem cell to maturation of the differentiated megakaryocyte (Figure 1). Indeed, both TPO- and c-Mpl-deficient mice have substantially reduced levels of megakaryocytes and platelets. The specific cell-surface receptor for TPO is c-Mpl, which was first identified as the oncogene v-Mpl responsible for murine lymphoproliferative leukaemia.

Thrombopoietin is synthesized as a 353-amino acid pro-protein, with a 21-amino acid pro-domain that is cleaved prior to secretion. The mature full-length TPO is a 332-amino acid glycosylated protein with two domains. The receptor-binding domain (RBD) resides in the N-terminal half of the protein, while the C-terminal domain is highly glycosylated and appears to be responsible for stability and secretion of the protein. The RBD is highly homologous to EPO at the primary sequence level and in its tertiary structure. TPO is synthesized predominantly by hepatocytes in the liver, and its expression has also been detected in kidney cells and stromal cells in the bone marrow and spleen. Circulating levels of TPO are regulated by receptor-mediated internalization and degradation by platelets and megakaryocytes. Hepatocytes constitutively produce and secrete TPO, and circulating levels increase when platelet numbers or megakaryocyte mass is low leading to decreased rates of internalization and degradation. High levels of TPO activate megakaryocyte production, increasing the number of Mpl-bearing megakaryocytes and platelets, thereby normalizing TPO levels through feedback regulation.

---

**Figure 1. Role of thrombopoietin (TPO) in thrombopoiesis.** The progressive development of megakaryocytes from bone marrow stem cells is driven by cytokines that act at various stages in the process. Unlike the other cytokines that regulate this process, TPO has a central role at each of these stages. Adapted with permission from Kaushansky (NEJM 1998; 339: 746–54).
When liver function is impaired (e.g. due to cirrhosis), TPO secretion decreases, which partly results in a reduction in platelet counts. The direct relationship between circulating TPO levels and platelet counts has been confirmed in murine gene knock-out studies, in which deletion or inactivation of one TPO allele resulted in half the normal level of TPO and a 50% reduction in the number of circulating platelets.31

**TPO SIGNALLING**

The TPO receptor, Mpl, is a member of the haematopoietic cytokine receptor superfamily and is expressed on the surface of platelets, megakaryocytes and megakaryocyte precursor cells. Mpl contains two 200-amino acid cytokine receptor homology modules (CRMs). TPO initiates signal transduction through binding to the distal CRM (CRM1), which leads to receptor dimerization.32, 33 CRM1 appears to inhibit receptor dimerization until TPO binds (Figure 2a), which relieves this inhibition, allowing receptor dimerization, JAK2 transphosphorylation and phosphorylation of two critical tyrosine residues.34 Mpl also has two conserved intracellular motifs, box 1 and box 2, which are essential for signalling through JAK2, and for a separate pathway that leads to c-myc transcription.35 JAK2 and Mpl phosphorylation results in the recruitment of a variety of SH2 domain-containing adaptor molecules, leading to activation of several signalling cascades that ultimately result in cellular

![Figure 2. Thrombopoiesis activation and mechanism of action of platelet-centric thrombopoietic agents. (a) Megakaryocyte precursors express receptors for IL-11 and thrombopoietin (Mpl) to which their cognate ligands bind, thereby activating signal transduction cascades that result in proliferation and maturation of megakaryocytes. (b) Novel thrombopoietic agents act through binding and activation of various receptors on the megakaryocyte precursor to stimulate proliferation, maturation and platelet production.](image)

© 2007 The Authors, Aliment Pharmacol Ther 26 (Suppl 1), 29–39
Journal compilation © 2007 Blackwell Publishing Ltd
proliferation and survival. Several studies have demonstrated that JAK2 phosphorylation of STAT3 is responsible for Mpl-dependent expansion of hematopoietic progenitors and regulation of hematopoietic stem cells. Phosphorylated STATs dimerize then translocate to the nucleus, leading to cell proliferation and survival. In addition to activating STATs, phosphorylated Mpl activates Ras and subsequently the Raf/MEK/ERK and PI3K pathways that stimulate cell proliferation and inhibit apoptosis. Interestingly, it has been proposed that transient activation of ERK promotes proliferation, but prolonged activation, as seen with TPO, stimulates differentiation of responsive cells. This is supported by the observation that MEK inhibition blocks the development of higher ploidy classes of primary megakaryocytes. Thus, TPO binding to Mpl leads to the activation of a variety of distinct cellular pathways, all of which converge to regulate cellular proliferation, maturation and survival.

PHARMACOLOGICAL APPROACHES TO THE TREATMENT OF THROMBOCYTOPENIA

There have been two recent pharmacological approaches to treating thrombocytopenia: (i) cytokines and growth factors that enhance the bone marrow and (ii) treatments that activate the TPO receptor and promote megakaryocyte or platelet production.

Targeting general thrombopoiesis – cytokines and growth factors

TPO.

Based on the successful development of EPO for anaemia, and granulocyte colony-stimulating factor (G-CSF) and GM-CSF for neutropenia, a substantial effort has been made to develop TPO for the treatment of thrombocytopenia. Two forms of recombinant TPO (rhTPO) have been evaluated in clinical trials. When administered intravenously to normal healthy volunteers and cancer patients, rhTPO produced a dose-dependent increase in platelet counts beginning 5 days after administration and peaking 10–14 days later. A recombinant truncated form of TPO, known as megakaryocyte-derived growth factor (rHuMDGF), consists of the RBD-containing amino-terminal 163 amino acids. rHuMDGF, produced in Escherichia coli, was pegylated to improve pharmacokinetic parameters. Treatment with PEG-rHuMDGF also produced a dose-dependent rise in platelet counts with the same kinetics as rhTPO. Clinical development of PEG-rHuMDGF was halted because several human subjects who received PEG-rHuMDGF developed thrombocytopenia and pancytopenia because of the formation of neutralizing antibodies to TPO. Although there was no evidence of a similar immune response to rhTPO, its development was also terminated. As PEG-rHuMDGF was administered subcutaneously while rhTPO was given intravenously, it is not known whether differences in physical properties or route of administration were responsible for the immune response generated.

Before the development of rhTPO and PEG-rHuMDGF was terminated, a variety of clinical studies were performed in patients that provide insight into the utility of this class of compounds. In patients undergoing stem cell transplantation, induction chemotherapy or remission consolidation for acute leukaemia, rhTPO and PEG-rHuMDGF had little effect over a variety of doses and schedules, possibly due to a lack of Mpl-bearing megakaryocyte precursors. However, in patients receiving non-myeloablative chemotherapy, treatment with either compound increased the nadir platelet count, shortened the duration of thrombocytopenia and reduced the number of platelet transfusions. Similarly, in aphaeresis donors and patients with ITP and cyclic immune thrombocytopenia, PEG-rHu-MDGF treatment resulted in increased platelet counts. Clinical development of these compounds did provide important clinical proof-of-principle for the use of TPO agonists in the treatment of various types of thrombocytopenia.

IL-1, IL-3, IL-6 and GM-CSF.

As discussed earlier, IL-1, IL-3, IL-6 and GM-CSF have been shown to play a role in the generation of megakaryocytes in animals and have demonstrated thrombopoietic activity in clinical studies. However, each either exhibits unacceptable toxicity profiles or does not produce significant increases in platelet counts, and further therapeutic use of these cytokines in the treatment of thrombocytopenia has been discontinued.

Promegapoietin.

Promegapoietin, a TPO/IL-3 chimaeric molecule, was genetically engineered based on the observed synergy of IL-3 and TPO on megakaryocyte proliferation and maturation. When administered in a non-human
primate model of severe radiation-induced myelosuppression, platelet regeneration was restored, virtually eliminating the need for whole blood transfusions. However, in a phase I clinical study, antibody formation resulted in severe thrombocytopenia, terminating further development of promegapoietin.

**IL-11.**

*In vitro*, IL-11 has been shown to work synergistically with other cytokines to promote multiple stages of megakaryocyte development. Megakaryocytes and megakaryocyte precursors express IL-11 receptors. When IL-11 receptors are stimulated by ligand binding (Figure 2a), they undergo phosphorylation and activate the STAT3 transcription factor. IL-11 promotes megakaryocyte maturation, stimulates platelet production in normal animals, and can enhance haematopoietic recovery following myelosuppression. In mice, IL-11 increased megakaryocyte ploidy in the bone marrow and megakaryocyte colony-forming units (CFUs) in the spleen. Interestingly, the IL-11 receptor is not present on platelets.

Clinically, recombinant human IL-11 (rhIL-11; oprelvekin) has been successful in some patients. In a phase I study in advanced breast cancer patients treated with myelosuppressive chemotherapy, treatment with rhIL-11 produced dose-dependent increases in bone marrow progenitor cells, megakaryocytes and mean platelet counts. In a randomized, placebo-controlled trial in patients with solid tumours who were severely thrombocytopenic because of myelosuppressive chemotherapy and had previously received platelet transfusions, treatment with oprelvekin provided positive results to support approval for this indication. Thirty per cent of patients who received 50 µg/kg rhIL-11 for 14–21 days after initiation of chemotherapy did not need transfusions compared with 4% in placebo-treated controls ($P < 0.05$). One case study has demonstrated that oprelvekin can correct HCV-associated thrombocytopenia, which would increase the number of patients who are able to complete anti-viral therapy.

**Pharmacological therapies – TPO agonists**

Based on the successful clinical proof-of-concept of treating thrombocytopenia with rhTPO and PEG-rHu-MDGF, a variety of novel approaches to stimulating the TPO receptor (Mpl) have been evaluated. Of these, the non-peptidic small-molecule agonists, eltrombopag and AKR-501, and the peptide agonists, AMG-531 and Peg-TPOmp, have been evaluated in clinical trials. Antibodies to Mpl that have agonist activity have also been developed, and while they have not been evaluated clinically, show interesting potential.

**Eltrombopag.**

Eltrombopag is an orally bioavailable, low molecular weight non-peptidic growth factor that is a selective Mpl agonist. Eltrombopag was discovered through modification of lead compounds identified in a high-throughput reporter gene assay based on the activation of STATs in Mpl-expressing cells. It interacts with the transmembrane domain of Mpl, rather than the ligand-binding domain of the receptor, leading to the activation of the JAK/STAT and MEK/ERK signalling pathways (Figure 2b). Preclinically, it has been shown to induce the proliferation and differentiation of cells in the megakaryocytic lineage with the same kinetics and potency as TPO. *In vivo*, eltrombopag increases platelet counts in chimpanzees, but unlike TPO, does not potentiate ADP-mediated platelet aggregation or induce P-selectin expression on platelets. In contrast to peptidic Mpl agonists, eltrombopag is active against human and chimpanzee Mpl, but not rat, mouse, ferret or cynomolgus monkey Mpl. Eltrombopag likely either facilitates the dimerization of Mpl or directly activates the signalling mechanism.

In a phase I study in healthy human volunteers, oral administration of eltrombopag (for 10 days) was safe and well tolerated, and resulted in a dose-dependent increase in circulating platelet counts. Platelet counts began to rise after 8 days of dosing, and the peak platelet count was observed at 16 days; counts returned to baseline by day 22. Similar to the preclinical studies, there was no effect of eltrombopag on platelet aggregation or activation.

The ability of eltrombopag to facilitate initiation and maintenance of IFN-based anti-viral therapy in patients with thrombocytopenia associated with chronic HCV infection is currently being evaluated (Figure 3). Eligibility criteria in this randomized, double-blind, placebo-controlled phase II study included evidence of liver damage based on liver biopsy indicating chronic hepatitis and cirrhosis, or radiographic evidence of cirrhosis, or non-bleeding gastro-oesophageal
varices and compensated liver disease (Child-Pugh A).\textsuperscript{68} Patients had pre-existing thrombocytopenia (platelet count of 20 000 to 70 000/μL) and no history of thrombosis, HIV infection or active hepatitis B infection. Patients were treated 30, 50 or 75 mg eltrombopag or placebo daily for 4 weeks (part 1). After 4 weeks, patients were evaluated before initiation of the anti-viral treatment phase of the trial. Criteria for initiation of anti-viral therapy with PEG-IFN and ribavirin included platelet counts of >70 000/μL for Pegasys\textsuperscript{®} (Roche Pharmaceuticals, Nutley, New Jersey, USA) (peginterferon alpha-2b) or >100 000/μL for PEG-Intron\textsuperscript{®} (Schering Corporation, Kenilworth, New Jersey, USA) (peginterferon alpha-2b). Patients who met these criteria received anti-viral therapy plus eltrombopag for an additional 12 weeks (part 2). Interim results from part 1 of this study are presented in Table 1. Eltrombopag treatment resulted in a dose-dependent increase in platelet counts on week 4. The number of patients able to initiate anti-viral therapy ranged from 71% to 91% with eltrombopag vs. 22% in the control arm. Sixty-five per cent of patients in the 75-mg dose group completed anti-viral therapy compared with 6% of placebo-treated patients. Eltrombopag treatment was generally well tolerated. The most frequent adverse events were nausea, headache and diarrhoea, none of which required treatment discontinuation. Final analysis of these data is ongoing and will be fully reported in the near future.

Eltrombopag has also been demonstrated to increase platelet counts in patients with chronic ITP.\textsuperscript{69} One hundred and eighteen patients with chronic ITP and platelet counts <30 000/μL who were refractory or relapsed with at least one ITP treatment were randomized to receive 30, 50 or 75 mg eltrombopag or to receive placebo for up to 6 weeks. More patients in the eltrombopag treatment arms reached the primary end point (platelet count ≥50 000) than placebo

Table 1. Efficacy and toxicity data on day 28

<table>
<thead>
<tr>
<th>Eltrombopag</th>
<th>Placebo (n = 5)</th>
<th>30 mg (n = 8)</th>
<th>50 mg (n = 9)</th>
<th>75 mg (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders* on day 28, n (%)</td>
<td>0 (0)</td>
<td>4† (67)</td>
<td>7 (78)</td>
<td>9‡ (90)</td>
</tr>
<tr>
<td>Median platelet count (\times 10^3/\mu\text{L}) (n)</td>
<td>38</td>
<td>119</td>
<td>174</td>
<td>246</td>
</tr>
<tr>
<td>Subjects initiating anti-viral therapy, n (%)</td>
<td>0 (0)</td>
<td>6 (75)</td>
<td>7 (78)</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Toxicity, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any event</td>
<td>1 (20)</td>
<td>4 (50)</td>
<td>5 (56)</td>
<td>6 (55)</td>
</tr>
<tr>
<td>Headache</td>
<td>0 (0)</td>
<td>3 (38)</td>
<td>2 (22)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0 (0)</td>
<td>1 (13)</td>
<td>2 (22)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td>1 (11)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>0 (0)</td>
<td>1 (13)</td>
<td>1 (11)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Responders defined as platelet count ≥100 000/μL after 4 weeks of therapy.
† Two patients were excluded from the primary analysis because of protocol violations on baseline platelet counts.
‡ One patient was excluded from the primary analysis because of protocol violations on baseline platelet counts.
patients (28%, 70% and 81% in the 30-, 50- and 75-mg eltrombopag groups vs. 11% in the placebo group). Eltrombopag was well tolerated in this patient population.

**AKR-501.**

Another orally active TPO agonist identified through modification of lead compounds in a similar cell-based screen is AKR-501 (formerly YM477). AKR-501 is a small molecule mimetic of TPO. Like eltrombopag, this compound stimulates the growth of TPO-dependent cell lines, increases the growth of megakaryocytes from human CD34+ cells, and is active only against the human and chimpanzee receptor. In mice engrafted with human CD34+ cells, AKR-501 stimulated the production of human platelets.70 AKR-501 appears to be active based on clinical studies in healthy volunteers. Daily oral administration of AKR-501 at 3, 10 or 30 mg for 14 days increased the platelet count by 1.3-, 2.25- and 2.8-fold, respectively. AKR-501 was well absorbed and exhibited a dose-linear pharmacokinetic profile, and none of the treated subjects experienced serious adverse events.71 Further development of this drug candidate is underway.

**AMG-531.**

AMG-531 is a rationally designed TPO peptide agonist consisting of two identical peptide sequences covalently linked to two disulphide-bonded human IgG1 heavy chain and kappa light chain constant regions. To reduce the likelihood of an anti-TPO immune response and identify a compound that would bind and activate Mpl, the peptide sequence was identified by screening peptide libraries with no sequence homology to human TPO. AMG-531 was found to bind to Mpl, induce the phosphorylation of JAK2 and STAT5, promote the growth of TPO-dependent cell lines and development of megakaryocyte CFUs, and increase megakaryocyte ploidy and maturation in vitro.72 Pharmacokinetic studies in rhesus monkeys demonstrated that AMG-531 has a long circulating half-life, and that a single dose results in a dose-dependent rise in platelet counts starting on day 5 and peaking at days 7–9.73 AMG-531 has not been tested in patients with chronic liver disease or HCV infection but treatment with AMG-531 has been shown to increase platelet counts in other patient populations. Phase I studies in both normal healthy subjects and patients with ITP revealed that single subcutaneous injections of AMG-531 gave rise to a dose-dependent increase in platelet count, starting at day 5 and peaking at days 12–15.74 A subsequent phase II study in ITP patients demonstrated that AMG-531, administered once weekly for 6 weeks, resulted in a dose-dependent increase in platelet counts in 12 of 16 patients.74 Finally, interim data from 36 patients with ITP in an open-label phase III study (with treatment up to 96 weeks) indicated that mean platelet counts were stable at >100 000/µL, and patients were able to maintain a stable dose of AMG-531. More than half were able to reduce or discontinue concurrent corticosteroid treatment.75 Safety data from these studies indicate that AMG-531 is well tolerated, with no serious adverse events.74, 75

**PEG-TPOmp.**

PEG-TPOmp is a pegylated TPO peptide agonist that is active in cell-based assays at picomolar concentrations. In small animals, this agent increased platelet counts in a dose-dependent manner.76 Administration of single intravenous doses to healthy male subjects also produced a dose-dependent increase in platelet counts that peaked at days 10–12. The compound was well tolerated, and platelet function was normal.76

**Agonist antibodies.**

An alternate approach to treatment of thrombocytopenia is the use of agonist antibodies, which are thought to dimerize and activate TPO via their bivalent binding properties. Although engineered antibodies are not orally available, they typically have long circulating half-lives that can be expected to provide long-lasting therapeutic utility following a single dose. Monoclonal antibodies that bind Mpl have been genetically engineered to create small, bivalent TPO agonist ‘minibodies’.77 These were shown in vitro to activate a TPO-expressing cell line and were able to induce phosphorylation of JAK2, STAT5 and Mpl as potently as rhTPO. In vivo administration of such minibodies to cynomolgus monkeys increased platelet counts. A second engineered anti-Mpl monoclonal antibody, generated by incorporating the constant heavy region (CH) of IgG4 and the hinge region (CH2–CH3) of IgG3, stimulated a TPO-dependent cell line and increased development of megakaryocyte CFUs in vitro.76 When
injected into human Mpl-expressing mice, this antibody increased platelet counts for over a month.\textsuperscript{78}

CONCLUSION

In conclusion, recent research has provided substantial evidence that activating megakaryocyte progenitor cells with cytokines or through activation of the TPO receptor stimulates platelet production and can reverse thrombocytopenia in patients with cancer, ITP and HCV-associated liver disease. Preclinical and clinical evidence demonstrate that peptidic compounds including AMG-531 and PEG-TPOmp, non-peptidic compounds including eltrombopag and AKR-501 and engineered antibody agonists of Mpl lead to the proliferation of megakaryocyte progenitors and a rise in platelet counts, however, many of these agents have not been tested in patients with chronic liver disease or HCV infection. Clinical development of earlier agents (e.g. rhTPO and PEG-rHu-MDGF) has been discontinued because of an increased risk of immune reactions. However, this has not been observed with other compounds (e.g. eltrombopag) that appear to be safe and well tolerated.

Rationally based approaches to drug discovery utilizing an increased understanding of the role of TPO in thrombopoiesis have led to the development of numerous agents that produce increased platelet counts. These novel agents offer promise for the treatment of thrombocytopenia in patients with chronic liver disease, obviating traditional approaches such as platelet transfusions and their associated risks.

ACKNOWLEDGEMENT

Declaration of personal interests: NHA has received grant support and acted as a paid consultant to GlaxoSmithKline. JGM has received research grant support from or has been a consultant to Akros Pharma Inc., Amgen Inc., Anadys Pharmaceuticals Inc., Aus Bio PTL, Bayer Pharmaceuticals, Bio-Medicines Inc., Bristol-Myers Squibb Company, Centocor Inc., Coley Pharmaceuticals, First Circle Medical, Fujisawa, Gilead Sciences, GlaxoSmithKline, Human Genome Sciences, Idenix, IDUN, InterMune Pharmaceuticals, Inc., Isis Pharmaceuticals, National Genetics Institute, Novartis Pharmaceuticals Inc., Nucleonics, Ortho Diagnostic Systems, Otsuka Pharmaceuticals, Peregrine, Pfizer Pharmaceuticals Inc., PPD, Prometheus Laboratories, Ribozyme Pharmaceuticals, Rigel, Roche Pharmaceuticals, Schering-Plough Corporation, SciClone, SIRNA Therapeutics, Triangle Pharmaceuticals, United Therapeutics, Vertex Pharmaceuticals and XTL. This article appeared in a supplement whose development was supported by GlaxoSmithKline.

REFERENCES

15 Farese AM, Hunt P, Boone T, MacVittie TJ. Reombinant human megakaryocyte growth and development factor stimu-


Kuter DJ, Rosenberg RD. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. Blood 1995; 85: 2720–30.


Kuter DJ, Rosenberg RD. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. Blood 1995; 85: 2720–30.


