Platelet Disorders

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Novel Thrombopoietic Agents

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Thrombocytopenia is a primary manifestation of immune thrombocytopenic purpura (ITP) and may occur as a result of hepatitis C, malignancy, and treatment with chemotherapy. There is a need for additional means to treat thrombocytopenia in these settings. Recombinant thrombopoietin-like agents became available after the cloning of thrombopoietin in 1994. In clinical trials, these agents showed some efficacy in chemotherapy-induced thrombocytopenia, but their use was ultimately discontinued due to the development of neutralizing antibodies that cross-reacted with endogenous thrombopoietin and caused thrombocytopenia in healthy blood donors and other recipients. Subsequently, "second-generation" thrombopoietic agents without homology to thrombopoietin were developed. In the past 5 years, these second-generation thrombopoeitic growth factors have undergone substantial clinical development and have demonstrated safety, tolerability and efficacy in subjects with ITP and hepatitis C–related thrombocytopenia. These completed studies, many of which are available only in abstract form, and other ongoing studies suggest that thrombopoietic agents will enhance the hematologist's ability to manage these and other causes of thrombocytopenia.

Introduction

Thrombopoietin, the primary circulating hematopoietic growth factor that regulates megakaryocytopoiesis and platelet production, was cloned and characterized in 1994.[1-6] It is the ligand for the thrombopoietin receptor (TPO-R), c-mpl, which is expressed on the surface of megakaryocytes, platelets and primitive stem cells. Based on *in vitro* and *in vivo* data, the kinetics of megakaryocyte differentiation and platelet production are such that stimulation of the TPO-R in a normal bone marrow would require 5 to 14 days to manifest itself as an increase in the platelet count, whereas it would require 2 to 3 weeks in an ablated marrow.[7]

Identification of human thrombopoietin was followed soon after by the development of recombinant molecules with thrombopoietin-like structure and activity.[8,9] Clinical trials with these recombinant products were initiated in patients undergoing chemotherapy and in healthy donors of platelets for transfusion. Further investigation was stopped due to the development of neutralizing antibodies that cross-reacted with endogenous thrombopoietin and caused protracted resistant thrombocytopenia. Thrombocytopenia was most evident in healthy platelet donors who had received 2 or 3 treatments, but was also seen in several patients after chemotherapy for cancer in whom the expected postchemotherapy platelet recovery never occurred.[10, 11]

The results seen, however, convinced investigators that TPO-R agonists could substantially increase platelet numbers. Therefore, development of less immunogenic peptide and small molecule TPO-R agonists was pursued (**Table 1**). These agents have shown substantial clinical promise in patients with immune thrombocytopenia purpura (ITP) and hepatitis C–related thrombocytopenia.[12-16] Ongoing clinical investigation with these new agents has revealed that they have the potential to be a safe and efficacious means to treat thrombocytopenia in these and other conditions.

Thrombopoietic Agents in Chemotherapy-Induced Thrombocytopenia

In the second half of the 1990s, the use of the now-discontinued recombinant thrombopoietins in the management of thrombocytopenia in patients undergoing both myeloablative and nonmyeloablative chemotherapy was investigated extensively (**Table 2**).

Table 1. Published studies of the second-generation thrombopoietic agents.

Agent, structure; route and frequency of administration	Ref.	Trial	No. of patients	Baseline platelet counts	Dose	Treatment period	Time to response	Platelet response	Toxicity	Thrombotic events?
AMG531 Recombinant TPO-R protein with carrier Fc domain; Once weekly subcutaneous injection	53	Phase 1	48 healthy controls	NA	0.3 to 10 mg/kg IV or 0.1 to 2.0 mg/kg SC or placebo	Single dose	Peak response 12-16 d	NA	Well tolerated, no serious adverse events. Most common toxicity mild headache or sore throat.	No
	12	Phase 1	24	Median platelet count 11 x 10^9/mL All had platelets <30 x 10^9/L	Cohorts ranging from 0.2 to 10 mg/kg/wk	6 wks	Median time to targeted response 5-10 d	Platelet count of ≥ 50 x 10^9/L achieved in 7/12 patients at doses ≥3 mg	Well tolerated, most frequent adverse effect was self-limiting mild headache. 4 had worsening of thrombocytopenia after treatment.	No
	12	Phase 2	21	Median platelet count 16 x 10^9/mL All had platelets <30 x 10^9/L	Randomly assigned to 1, 2 or 6 µg/kg/wk	6 wks	Median time to response 5-10 d	10/16 achieved target platelet count (50-450 x 10^9/L) with 1 or 3 mg/kg/wk		No
	13	Phase 1-2	16	Median platelet count 14.5; all had platelets <30 x 10^9/L	Cohorts ranging from 30 to 500 mg/wk	3 wks	Median of 10 d (range 5-13 d)	Platelet counts in 12/15 patients increased to ≥ 20 and in 8/15 to ≥ 100 x 10^9/L	Generally well tolerated, most common effect mild-mod headache. 1 patient had worsening of thrombocytopenia after treatment and 1 had transient increase in serum lactate dehydrogenase	No
Eltrombopag Small-molecule, nonpeptide TPO-R agonist; Once daily, oral capsule (on empty stomach)	58	Phase 1	73 healthy male controls	NA	Cohorts of 5-75 mg/d with placebo	10 d	Consistent increase started after 8 days of daily dosing; 16 days to peak increase	NA	No treatment-related adverse events reported.	No; no effect of treatment on platelet aggregation and activation
	54	Phase 3	104 enrolled; study ongoing	—	—	up to 96 wks	—	Mean platelet count 100 wk 1-24 x 10^9/L and 131 x 10^9/L wk 25-48; 6/12 pt able to discontinue concurrent steroids	Headache most common adverse event. 4 pts had serious treatment-related adverse events, 3 of whom were withdrawn.	Transverse sinus thrombosis in 1 pt
	14	Phase 2	117	Platelets ≤30 x 10^9/L	Randomly assigned to placebo, 30 mg, 50 mg or 75 mg	6 wks	Preliminary results encouraging, no major toxicity	—	—	No
	16	Phase 2	74 HEPC-ITP	Platelets 20-70 x 10^9/L	Randomly assigned to placebo, 30 mg, 50 mg or 75 mg	4 wks	Preliminary results encouraging, no major toxicity	—	—	No
AKR-501 Small-molecule TPO-R agonist; Oral, once daily	59	Phase 1	63 healthy volunteers	NA	Cohorts of 3-100 mg or placebo	14 d	Dose-dependent increases seen	NA	No serious drug-related adverse experiences reported at any dose.	No

Abbreviations: HEP C-ITP, hepatitis C virus–associated thrombocytopenia

Table 2. Trials involving exogenous TPO administration in chemotherapy-induced thrombocytopenia.

Trial	Form of exogenous thrombopoietin	No. pts.	Clinical setting	Was administration of exogenous TPO deemed useful overall?	Were platelet transfusions avoided?	Were platelet nadirs less low?	Were delays in chemotherapy administration or reduction in dose avoided?
Studies in nonmyeloablative chemotherapy regimens for solid tumors							
Basser et al[18]	PEG-rHuMGDF	41	Advanced cancer	Yes	NR	No	No
Fanucci et al[19]	PEG-rHuMGDF	53	Lung cancer	Yes	NR	Yes	NR
Crawford et al[20]	PEG-rHuMGDF	40	Lung cancer	Yes	Yes	Yes	NR
Basser et al[21]	PEG-rHuMGDF	68	Advanced cancer	Yes	No	Yes	NR
Vadhan-Raj et al[22]	rhTPO	29	Gynecological tumors	Yes	Yes	Yes	Yes
Vadhan-Raj et al[32]	rhTPO	66	Sarcoma	Yes	Yes	Yes	NR
Angiolillo et al[24]	rhTPO	12	Pediatric solid tumor	Yes	Yes	Yes	NR
Moskowitz et al[62]	PEG-rHuMGDF	41	Relapsed and refractory aggressive non-Hodgkin lymphoma receiving ICE prior to ASCT	Yes	Yes	Yes	Yes
Studies in chemotherapy regimens for leukemia							
Cripe et al[25]	rhTPO	28	AML induction	No	NR	NR	NR
Archimbaud et al[26]	PEG-rHuMGDF	108	AML induction, consolidation	No	No	No	NR
Schiffer et al[28]	PEG-rHuMGDF	57	AML induction, consolidation	No	No	No	NR
Geissler et al[27]	PEG-rHuMGDF	88	AML consolidation	No	No	No	NR
Studies in ablative chemotherapy prior to hematopoietic stem cell transplantation (HSCT)							
Nash et al[63]	rhTPO	38	Patients with delayed platelet recovery after HSCT	Kinetics of platelet recovery not dose related and therefore efficacy inconclusive.	NR	NR	NR
Wolff et al[64]	rhTPO	33	after HSCT	Kinetics of platelet recovery not dose related and therefore efficacy inconclusive.	NR	NR	NR
Schuster et al[31]	PEG-rHuMGDF	75*/64†	Autologous HSCT in patients with breast cancer	Inconclusive	Yes in phase 1/2 No in phase 3	NR	NR

*Phase 1/2 study.
†Phase 3 study.
Abbreviations: PEG-rHuMGDF, pegylated recombinant human megakaryocyte growth and development factor; rhTPO, recombinant human thrombopoietin; AML, acute myeloid leukemia; ICE, ifosphomide, carboplatin, etoposide; ASCT, autologous stem cell transplantation; NR, not reported

Although clinical development of the early thrombopoietic agents has ceased, no studies using the second-generation TPO-R agonists in CIT have been published. Therefore, the earlier studies will be briefly reviewed here to provide insight into the potential utility of thrombopoietic agents in this setting. In addition, since the earlier agents were removed from clinical trial, overviews of their use were not widely disseminated. Initially, there was much excitement regarding the use of the early recombinant agents as an adjunct to chemotherapy, given the adverse effects of chemotherapy-induced thrombocytopenia (CIT), such as treatment delays, dose reductions, and the side effects of platelet transfusions, most notably alloimmunization.[17] It was hoped that these agents would demonstrate similar effects on CIT as granulocyte colony-stimulating factor (G-CSF) had in ameliorating chemotherapy-induced neutropenia.

What if the patient were undergoing nonmyeloablative chemotherapy?

Generally favorable results were observed using recombinant thrombopoietin during nonmyeloablative chemotherapy for solid-tumor malignancies. Thrombopoietic therapy was found to be useful in each of 8 studies listed in the non-myeloablative section of **Table 2**.[18-24] In 7 of the 8 studies, an increase in the postchemotherapy platelet nadir and a reduced time to platelet recovery was observed. The requirement for platelet transfusions was reduced in 5 of 7 studies. However, many patients on the placebo arm did not require transfusion, and there was little significant bleeding at the platelet counts in these studies, so the need for exogenously administered thrombopoietin therapy remained unproven. Other clinically relevant endpoints, such as enhanced tumor response to chemotherapy and frequency of dose reductions or delayed initiation of chemotherapy, were generally not extensively studied, although this was specifically examined by Moskowitz et al.[60]

What if the patient were undergoing myeloablative chemotherapy?

These studies fall into two clinical settings: induction/consolidation of AML[25-28] and stem cell transplantation.[29-31,62] The 4 studies of recombinant TPO in patients with acute myelogenous leukemia (AML) undergoing induction and/ or consolidation chemotherapy showed no clinical benefit either in terms of ameliorating the platelet nadir or significantly reducing the need for platelet transfusion. One explanation for this difference may be that only very immature megakaryocyte progenitor cells survive the myelotoxic therapy used in leukemia treatment, and these progenitors require weeks to mature. Current studies are exploring administration of these agents prior to chemotherapy, as well as afterwards, in order to circumvent this problem.[32]

Three randomized trials of recombinant TPO given for autologous stem cell transplantation showed no overall benefit.[31,62-64] However, the data do support a role for thrombopoietic agents in combination with G-CSF to improve mobilization of hematopoietic progenitor cells to the peripheral blood and thereby enhance yields during peripheral stem cell harvesting.[33,34] Clinical consequences of the addition of thrombopoietic agents in this setting remain unknown.

Overall, the studies of thrombopoietic agents in the management of CIT are difficult to interpret due to intertrial variability in study design, study population, dosing strategy and clinical endpoints. The data suggest that thrombopoietic agents in the myeloablative setting may not be very useful; however, use in nonmyeloablative treatment regimens for solid-tumor malignancies shows promise. Given the potential for increasing platelet counts and thereby permitting chemotherapy dose augmentation, this usage of the newer generation of TPO-R agonists is being carefully investigated. The overall efficacy thus far does not appear too different from what one might conclude regarding the use of G-CSF in the chemotherapy-induced neutropenia setting.

Thrombopoietic Agents in Hepatitis C–Related Thrombocytopenia

Seventeen percent of patients with chronic liver disease from hepatitis C infection have thrombocytopenia,[35] and the prevalence of thrombocytopenia correlates with the severity of liver disease.[36] Although the thrombocytopenia is generally moderate, it increases the bleeding risk in this coagulopathic population during diagnostic studies (e.g., liver biopsies) and excludes patients from interferon-α (IFN-α)– based antiviral treatment or else requires dose modification. This is of particular concern given that adherence to the therapeutic regimen directly impacts viral containment.[37]

The etiology of thrombocytopenia in hepatitis C– related liver disease is multifactorial and includes hypersplenism, antiplatelet antibody-mediated platelet destruction,[38,39] and direct cytotoxicity by the virus on megakaryocytes.[40] Furthermore, although the thrombocytopenia may not usually be as severe as can be seen in ITP, bleeding occurs more frequently at higher counts, possibly related to comorbid factors such as cryoglobulins, disseminated intravascular coagulation, and impaired production of coagulation factors.[41] Patients with more severe disease have decreased TPO production,[42,43] and preclinical work showed that a small-molecule thrombopoietin mimetic, NIP-004, prevented IFN-α2b–induced thrombocytopenia in mice.[44] Taken together, these findings point to a potential role for TPO-R agonists in the management of patients with hepatitis C–related thrombocytopenia.

A recent randomized, placebo-controlled phase 2 clinical trial examined the efficacy of eltrombopag, an oral nonpeptide platelet growth factor, in increasing platelet counts and facilitating the maintenance of antiviral therapy in hepatitis C–infected patients with platelet counts below $70,000/\mu L$.[16] Although the results are preliminary, they suggest that thrombopoietic agents may play an important role in the management of this expanding patient population by preventing dose interruptions or reductions as a result of thrombocytopenia. Further clinical investigations with eltrombopag and other TPO-R agonists in this area have been initiated.

Thrombopoietic Agents in ITP

Historically, ITP has been classified as an autoimmune disease in which circulating antiplatelet antibodies accelerate peripheral destruction of platelets. Therefore, therapies directed at inhibiting platelet destruction have been the mainstay, including corticosteroids, intravenous immunoglobulin, intravenous anti-D, rituximab, and splenectomy. Recently, evidence has emerged demonstrating that ITP is also a state of platelet underproduction[45-47] in which thrombopoietin generation is not increased in proportion to the severity of thrombocytopenia. Marrow megakaryocytes are thought to be damaged, and their ability to produce platelets in response to thrombopoietin impaired,[48] thus dissociating the typically increased number of megakaryocytes in the marrow from an increase in platelet production.

Past attempts to increase platelet production by stimulation of the TPO-R involved a single patient with cyclic autoimmune thrombocytopenia maintained on pegylated megakaryocyte growth and development factor (PEG-MGDF) for 8 years,[49] 4 patients treated with a single dose of the same agent,[50] and work in HIV–related thrombocytopenia. These anecdotes, combined with the aforementioned difficulties in demonstrating unequivocal efficacy of thrombopoietic agents in CIT, were enough to initiate study of ITP with the novel thrombopoietic agents as the primary initial clinical target (**Table 1**). Phase 3 trials have primarily been conducted with two agents, AMG531 and eltrombopag, although trials have also been initiated with AKR501.

AMG531

AMG531 was the first of the second-generation thrombopoietic agents in clinical trials. It is a recombinant protein

composed of a carrier FcR domain and 4 peptide-containing domains that bind the thrombopoietin receptor, thereby stimulating megakaryocytopoiesis. AMG531 is given weekly by subcutaneous injection, and trials have included home injection by patients.

Preclinical studies have shown the ability of AMG531 to increase platelet counts in wild-type mice[51] and to reduce the duration and severity of thrombocytopenia in murine models of chemoradiation therapy–induced thrombocytopenia.[52] Phase 1 data from healthy human volunteers demonstrated that a single dose of intravenous or subcutaneous AMG531 was safe and tolerable, caused a dose-dependent increase in the platelet count, and did not induce anti-TPO antibodies.[53]

Two phase 1/2 multicenter clinical trials of AMG531 in ITP have been published.[12,13] Treated patients had chronic, relatively severe ITP and were typically the investigators' most difficult-to-treat patients, who could not otherwise be easily managed. Platelet counts at entry were required to be less than 30,000/μL unless the patient was on a stable dose of steroids. A response was defined as achieving a platelet count of 50,000/μL and doubling of the initial count. Both studies showed that weekly subcutaneous injections of at least 1 μg/kg were efficacious, with the median time to a platelet increase ranging from 5 to 10 days. At the highest dose, one recipient who had undergone splenectomy and had had ITP for more than 10 years had a platelet count higher than 10^6/μL.[12] Weekly dosing increased the response rate but also revealed fluctuating platelet counts on stable dosing in certain patients. No consistent effects on red blood cell or white blood cell counts were seen. These studies included primarily, but not solely, patients who had failed to respond to splenectomy. In both studies, AMG531 was well tolerated, with the most frequently reported adverse effect being self-limited headache that did not require drug discontinuation. Importantly, no anti-TPO or anti-AMG531 antibodies were detected.

A long-term, ongoing extension study has been reported in abstract form. It suggests that AMG531 will safely support an adequate platelet count (50-200,000/μL) in most patients, in some for more than 2 years, with weekly injections of doses up to 10 μg/kg/dose.[54] However, at least 2 patients on this long-term study developed reversible marrow reticulin fibrosis, raising questions regarding the frequency of this complication with long-term use. In preclinical and clinical studies using PEG-MGDF, an increase in bone marrow reticulin, which was reversible on discontinuation of the drug, has also been reported.[55-57] This is presumably a result of enhanced megakaryocyte secretion of growth factors inducing fibrosis, such as transforming growth factor-beta. The significance of this reversible, treatment-induced effect in the setting of ITP is unknown. Collectively, these clinical studies of AMG531 suggest that it appears to be a safe, therapeutic alternative to conventional immunomodulatory agents in most patients with ITP.

Recently completed AMG531 studies

Two multicenter, placebo-controlled, randomized studies enrolling 62 and 63 patients, respectively, investigated the efficacy of weekly dosing of AMG531 in adults with chronic ITP for a 6-month period. One study enrolled only patients who had undergone splenectomy, while the other enrolled only patients who had not had a splenectomy. The final results are not yet available, but the preliminary data appear promising.

Eltrombopag

Eltrombopag is an orally active, small-molecule TPO-R agonist. It is taken daily and requires an empty stomach for 2 hours both before and after ingestion. Initial studies showed that daily doses of 30 mg, 50 mg, and 75 mg were active in increasing the platelet count in healthy volunteers.[58] Platelet increases have been seen in responders at 1 week when the first on-treatment count has been obtained; earlier counts in patients with ITP have not been tested. The efficacy, safety, and tolerability of this agent were tested in a randomized controlled trial including the same 3 doses of eltrombopag compared with placebo in 117 adults with chronic ITP.[14] The 117 patients enrolled worldwide in this study had chronic ITP with had platelet counts 30,000/μL or less, and had failed at least one previous course of therapy for ITP. In addition, almost 50% had undergone splenectomy. A response was defined as a platelet count of 50,000/μL or more after 6 weeks of therapy. At this time, the preliminary results are promising but are available only in abstract form.

A second large randomized, placebo-controlled trial of eltrombopag has been completed that is similar in entry criteria and study endpoints to that of the first study.[15] Preliminary analysis suggests that it confirms the results of the first study, i.e., there is efficacy of 50 mg daily compared with placebo over 6 weeks of use in patients with chronic ITP. In addition, bleeding signs and symptoms were reduced compared with responders, and no important toxicity was observed.

Ongoing and future eltrombopag studies

Eltrombopag is being evaluated in several other ongoing studies: a randomized trial in which eltrombopag is being compared to placebo for 6 months; a long-term extension study in which patients who have completed previous trials are eligible to continue long-term use of the agent; and a study in which 6-week courses of study drug separated by 4-week washout periods allows comparison of the efficacy of the agent to itself with repeated use.

AKR-501

Results of a trial of another active oral thrombopoietic agent, AKR-501, showed that even single doses substantially raised the platelet count in healthy volunteers.[59] The drug was well tolerated, and studies in ITP are ongoing.

SB-559448

SB-559448 is an oral, nonpeptide, small-molecule TPO-R agonist. It has been investigated in healthy volunteers in a phase 1, single-blind, randomized fashion to cause a dose-dependent increase in platelet count with demonstrated safety.[60] Studies in ITP are planned.

Other agents with *in vitro* activity have also been developed by several companies. Which ones get developed and which, if any, will have specific clinical advantages is unknown at this time.

General Issues

There are several considerations involving the use of thrombopopietic agents in ITP and in other disease states. First, AMG531 is the only agent under investigation that has thus far been reported with long-term use. Therefore, whether the finding in a small number of treated patients of reticulin fibrosis is specific to this agent or would be observed with all thrombopoietic growth factors remains to be clarified. In this regard, AMG531 competes with TPO for the TPO-R and signals, as does TPO itself, via the JAK-STAT, P38 MAPK, and AKT pathways. The oral, small-molecule, thrombopoietic agents (eltrombopag and AKR501) do not compete with TPO for the TPO-R, and eltrombopag appears to signal via the JAK-STAT and P38 MAPK but not the AKT pathway. These differences suggest that the effects of these two compounds may not be identical, for better or worse, to those of TPO itself and AMG531. Second, the kinetics of platelet increase in responders appear to differ from those of IVIG or IV anti-D. A single dose of 1 g/kg of IVIG or 75 µg/kg IV anti-D might increase the platelet count overnight, with a peak effect seen at 4 to 6 days, but with a return to baseline by 2 to 3 weeks. In contrast, the platelet increase with a single dose of a thrombopoietic agent (or with daily doses beginning on day 1) would demonstrate the first clear platelet response by days 5 to 7, peak between then and day 14, and return to baseline at day 21. Finally, a major concern with these agents is that they will not only dramatically increase the platelet counts but also induce platelet activation, either because the newly produced platelets may be large and more functional or because of direct stimulation of the platelet via the TPO-R. Thus far, there has been no clinical evidence of increased thrombosis in treated patients, but further studies including analyses of platelet function in patients with ITP are required.

Conclusions

The newly developed thrombopoietic agents appear to dramatically increase the platelet count in most patients with ITP and with hepatitis C–induced thrombocytopenia. These platelet increases can be sustained by repeated (daily or weekly) drug administration, with dose adjustment depending upon the platelet count. Beneficial effects of these newer agents in CIT have yet to be demonstrated. It is anticipated that these agents will be clinically effective in patients who are not receiving myeloablative chemotherapy, but whether this will translate into a survival advantage or allow avoidance of platelet transfusions is uncertain and will require study. Safety and tolerability have thus far been demonstrated for the two most widely used agents, AMG531 and eltrombopag. Issues of concern include (1) whether thrombosis will be seen in responders in clinical practice, and (2) whether there will be a significant incidence of reticulin fibrosis with prolonged usage, whether this will be an effect seen with all thrombopoeitic agents, and whether it will be reversible in all cases. Regarding hypercoagulability, preliminary studies suggest that AMG531 and eltrombopag do not lead to a high incidence of clinical thrombotic events, but more experience is needed. Additional settings in which TPO-R agonists may be of use in the future include myelodysplastic syndromes, inherited thrombocytopenias, neonatal thrombocytopenia, and for enhancing the yield of platelets from healthy donors, if historic safety concerns are alleviated.[61]

The bottom line for the thrombopoietic agents, based on the clinical trials reported thus far, is that they appear to be safe, tolerable, and very effective. In the future, if further trials confirm the ones performed thus far, these agents may become widely used and have a major impact on the clinical care of patients with ITP, hepatitis C, and other thrombocytopenias.

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