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研究成果の刊行物・別冊

Chapter 7

Thrombopoietin receptor agonists

Yoshiaki Tomiyama

Thrombopoietin receptor agonists are now available for the treatment of 'refractory' primary immune thrombocytopenia. This chapter has focused on the mechanism, efficacy and safety of thrombopoietin receptor agonists.

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Aa c-Mpl: the receptor for thrombopoietin (TPO) and its dimerization is prerequisite for signal transduction.

Neutralizing antibodies: antibodies induced by recombinant TPOs that cross-react and inhibit the activity of endogenous TPO.

Thrombopoietin & recombinant thrombopoietins

Thrombopoietin (TPO) was cloned by five independent groups in 1994. TPO is a single 95-kDa glycoprotein consisting of 332 amino acids and was the ligand for c-Mpl that had already been cloned as an orphan receptor in 1991 [1]. The N-terminus of TPO has a receptor-binding domain showing considerable homology to erythropoietin, while the C-terminus of TPO is highly glycosylated and contributes to protein stability. The TPO receptor, c-Mpl, contains two cytokine receptor homology modules (CRM1 and CRM2). TPO exclusively binds to CRM1 (the distal CRM) of c-Mpl in the ratio 1:2 and then activates several transduction pathways, such as JAK2 and STAT5, to increase the production of mature megakaryocyte and platelets [2]. Although TPO could stimulate proliferation of stem cells and early progenitor cells of all lineages, its major effect is to stimulate proliferation of early and late stages of megakaryocyte maturation. Recombinant human TPO (rhTPO) and pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) were developed for clinical studies in thrombocytopenic disorders. PEG-rHuMGDF is a truncated form of TPO that contains the first 163 amino acids of endogenous TPO. However, PEG-rHuMGDF paradoxically induced persistent thrombocytopenia in 13 out of 325 healthy volunteers. The thrombocytopenia was caused by the antibody to PEG-rHuMGDF that cross-reacted with endogenous TPO and neutralized its biological activity [3]. Although rhTPO did not show such adverse effects, the development of rhTPO as well as PEG-rHuMGDF was stopped in 1998.

Structure of TPO receptor agonists

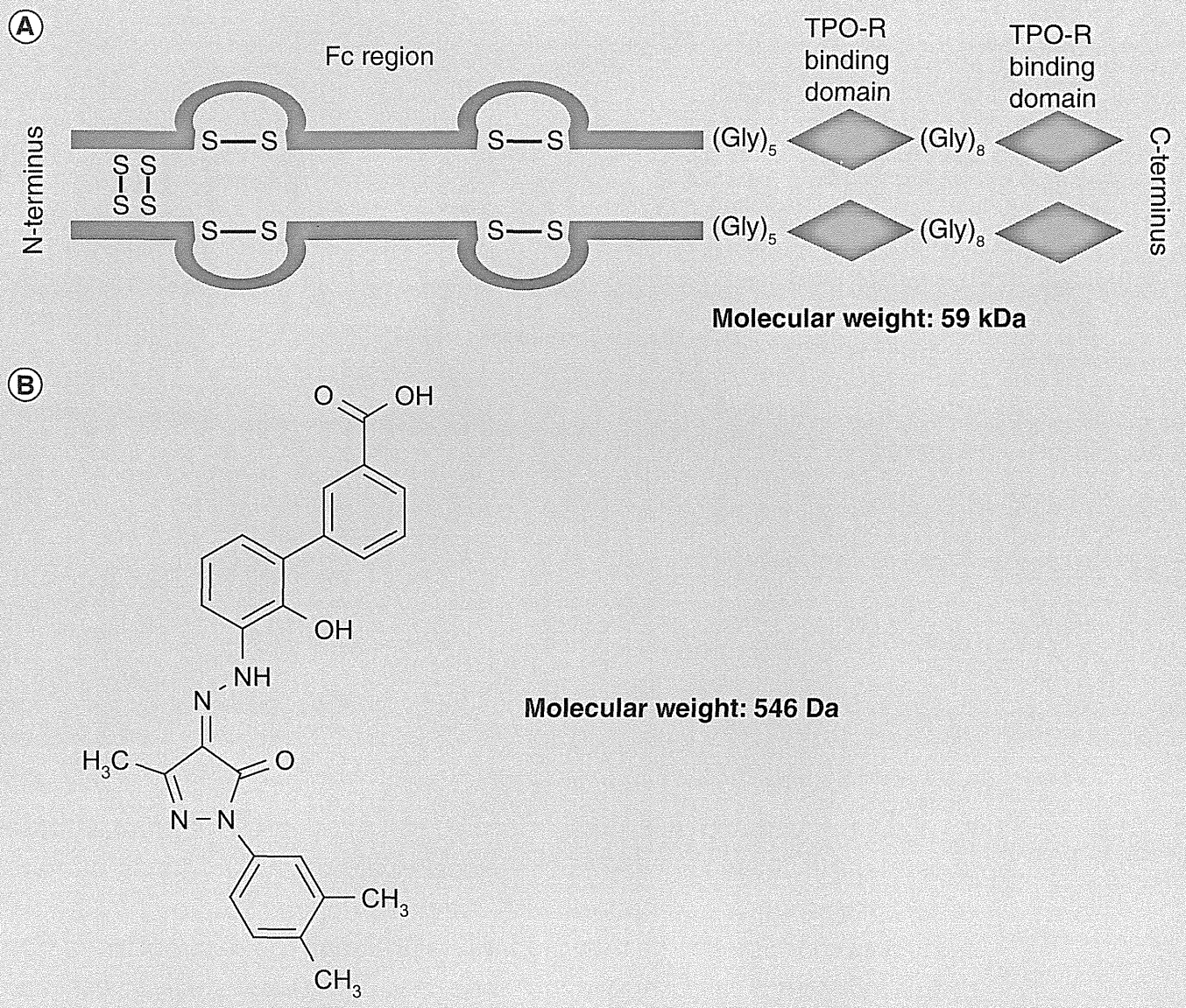
To prevent and overcome the development of neutralizing antibodies induced by recombinant TPOs, several drugs that stimulate c-Mpl without sequence homology with endogenous TPO have been developed. Among them two TPO receptor agonists are now available: romiplostim and eltrombopag **(Figure 7.1)**. These novel drugs are obtained by screening of peptide or small nonpeptide libraries for the ability to stimulate the growth or reporter genes such as the STAT protein in TPO-dependent cell lines.

Romiplostim

Romiplostim (N-plate®, AMG531; Amgen, CA, USA) is subcutaneously administered once a week for clinical use. Romiplostim is a peptide-based drug with a molecular weight of 59 kDa and

Stem cells and early progenitor cells of all lineages also express c-Mpl and could react with TPO.

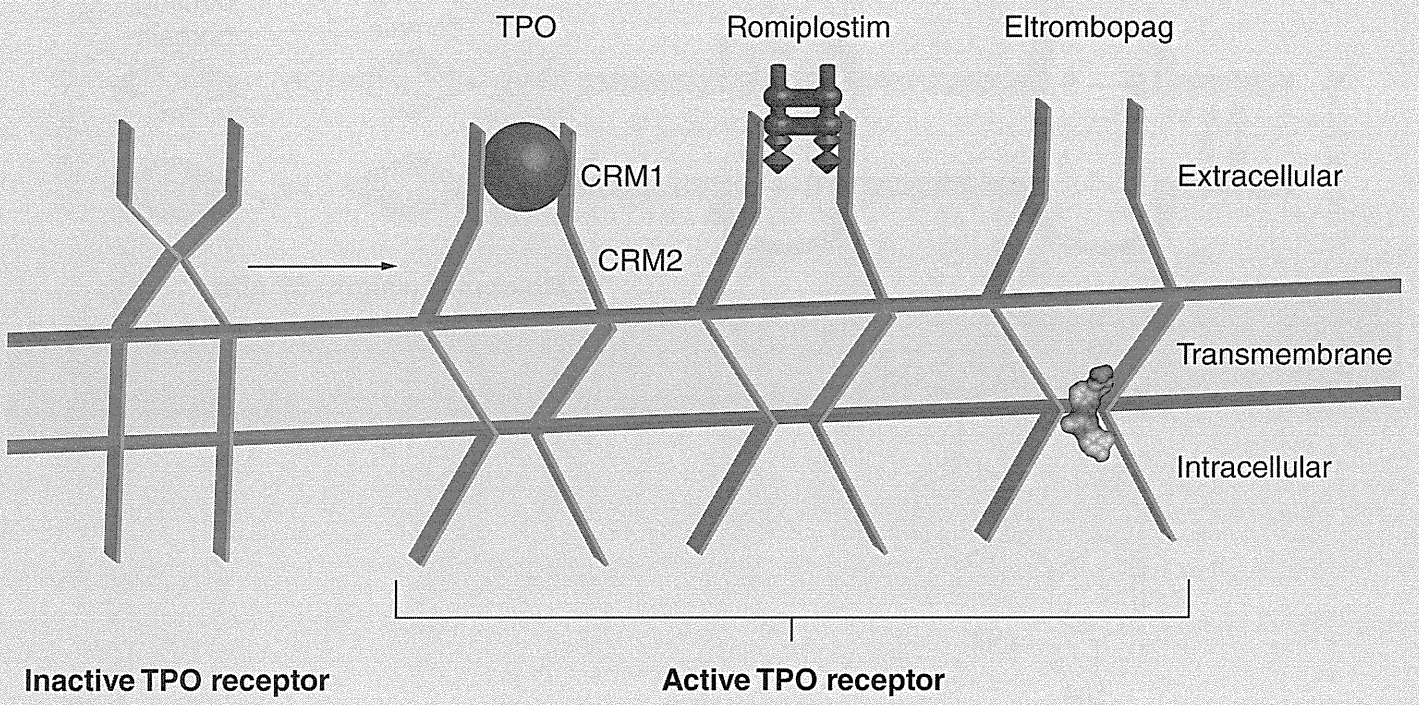
Figure 7.1. Structure of romiplostim and eltrombopag.



(A) Structure of romiplostim. Romiplostim is a 'peptibody' and TPO-R binding domain consists of 14 amino acids (Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala). **(B)** Structure of eltrombopag. Eltrombopag is a nonpeptide compound.
TPO-R: Thrombopoietin receptor.

designated as a 'peptidody' that is a fusion protein of IgG_1 Fc fragments and TPO mimetic peptides. Active sites of romiplostim are two identical TPO mimetic peptides (Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala) linked via an eight-glycine bridge as a linker [4,5]. IgG_1 Fc fragments covalently bound with the peptide-containing domain are dimerized via disulfide bonds. The Fc fragments extend the half-life of romiplostim in the circulation, since peptides tend to have poor stability and pharmacokinetic properties. Romiplostim bound to TPO receptor and the binding was inhibited by rhTPO, suggesting that romiplostim bind to CRM1 of cMpl **(Figure 7.2)**.

Figure 7.2. Binding sites for thrombopoietin, romiplostim and eltrombopag on the thrombopoietin receptor, c-Mpl.



CRM: Cytokine receptor homology module; TPO: Thrombopoietin.

Romiplostim was active in mice, rats, rabbits, monkeys, and induced dose-dependent increase in platelets in all species, although nonhuman primates were less responsive compared with other tested species. The first human study was conducted in healthy volunteers based on the data obtained from rhesus monkeys. A single intravenous dose of 10 µg/kg was initially investigated in human volunteers, which was anticipated to be a no-effect dose. However, this dose was found to increase platelet count almost sixfold. This distinct effect between humans and rhesus monkeys was probably due to much higher affinity of romiplostim for human c-Mpl than the monkey c-Mpl. A clinically effective dose that increases platelet count twofold was identified as 1 µg/kg. Intravenous administration of 1 µg/kg gave a peak of 12,900 pg/ml, while subcutaneous administration gave undetectable levels of romiplostim concentrations probably due to slow adsorption (less than 18 pg/ml). Intravenous and subcutaneous routes produced almost identical effects in healthy volunteers and peaked at days 14–15, and in its current formulation, romplostim is administered as subcutaneous injection every week [2,5].

Eltrombopag

Eltrombopag (Promacta® in the USA or Revolade® in the EU, SB497115; GSK) is an orally administered, low-molecular-weight (546 Da), nonpeptide

TPO receptor agonist. In human studies in healthy male volunteers, single oral dose of eltrombopag did not increase platelet count. However, daily oral administration for 10 days increased platelet counts dose dependently. For subjects at the 75-mg dose, the increase in platelet count started on day 6 and peaked at days 14–16.

Eltrombopag has unique characteristics regarding *in vivo* activity between species. Eltrombopag showed activity in human and chimpanzee TPO receptors, but not cynomolgus monkey, mouse or rat TPO receptors. This species difference is due mostly to a single amino acid difference in the transmembrane domain of c-Mpl. Human and chimpanzee TPO receptors have a histidine at residue 499, whereas other species have a leucine 499. Exchanging of leucine 499 for histidine 499 made the cynomolgus monkey TPO receptor responsive to eltrombopag. These data suggest that the binding site of eltrombopag is not CRM1 but the transmembrane domain of human TPO receptor. Thus, the binding sites of eltrombopag and rhTPO seem quite different **(Figure 7.2)** [2].

Plasma TPO levels in primary immune thrombocytopenia

TPO is synthesized primarily in the liver and is secreted into circulation. Hepatic transcription and translation of the TPO gene appears constant, and TPO is removed from circulation by binding of the TPO receptor on platelets and bone marrow megakaryocytes. Thus, plasma TPO levels usually increase in response to the decreased in platelet/megakaryocyte mass. Actually, in patients with aplastic anemia or chemotherapy-induced thrombocytopenia in which platelet/megakaryocyte mass was markedly decreased plasma TPO levels were markedly elevated, and platelet transfusion into thrombocytopenic patients resulted in a decrease in plasma TPO levels [6,7]. By contrast, in patients with immune thrombocytopenia (ITP), plasma TPO levels were within normal range or only slightly elevated [7]. The reason why TPO levels were normal in ITP is not fully understood. It is possible that TPO may be rapidly removed by ITP platelets due to the shortened platelet lifespan. In addition, total platelet/megakaryocyte mass may not be decreased in ITP. Recent studies also showed that platelet production may be impaired by antiplatelet autoantibodies [8]. Under these pathological conditions, ITP is a good candidate for applying TPO receptor agonists because of mostly normal TPO levels in ITP.

Therapeutic efficacy

For use in adult patients with chronic ITP, romiplostim received US FDA approval in August 2008, and in late 2009 in the EU. Eltrombopag received

accelerated approval in November 2008 in the USA, and in March 2010 in the EU. In the USA, each drug is only available for patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy and who have an increased risk of bleeding. In the EU, indication of TPO receptor agonists is much more limited and each drug is only available for splenectomized patients who are refractory to other treatments or may be available for nonsplectomized patients where surgery is contraindicated.

The therapeutic goal of ITP is not to normalize the platelet count, but to elevate platelet count to a safe range (mostly above $50 \times 10^3/\mu l$) to minimize the risk of bleeding with minimal side effects of drugs such as corticosteroids and TPO receptor agonists [9]. In this context, patients with ITP were eligible if platelet counts were less than $30 \times 10^3/\mu l$ and they had failed at least one prior treatment in most clinical studies employing either of TPO receptor agonist. Platelet count $>50 \times 10^3/\mu l$ by TPO agonists was considered as platelet response in most studies. These clinical studies have indicated that each drug is very effective in the treatment of chronic ITP.

Romiplostim

In early clinical studies for chronic ITP, two Phase I/II clinical trials were performed. In the US trial, patients received romiplostim dosed by bodyweight at 0.2–10 µg/kg. Seven out of 12 patients treated at 3, 6 or 10 µg/kg achieved a platelet count $>50 \times 10^3/\mu l$ after two doses, and the peak platelet count was dose dependent. Continuation of the trial for 6 weeks at 1 or 3 µg/kg resulted in platelet count within $50–450 \times 10^3/\mu l$ in 10/16 patients, and an additional two patients at 3 µg/kg were above $450 \times 10^3/\mu l$. Four patients showed a transient rebound thrombocytopenia when treatment was stopped. In the European trial, romiplostim was administered as a fixed dose between 30 and 500 µg. From these two trials, 1 µg/kg of romiplostim was chosen as a starting dose in the following studies [5].

Long-term (24-week) administration of romiplostim in 63 splenectomized and 62 nonsplenectomized patients with ITP was assessed in two parallel trials both of which are in a double-blind, randomized, controlled trial conducted in multiple centers internationally [10]. All patients were randomized 2:1 (romiplostim:placebo) to receive a starting dose of 1 µg/kg. Doses of drug were adjusted to maintain platelet count of $50–200 \times 10^3/\mu l$. The primary end points were to get a durable platelet response (platelet count $\geq 50 \times 10^3/\mu l$ during 6 or more of the last

The area under the plasma concentration–time curve is the measure of total plasma exposure of a drug over a given time period.

8 weeks of treatment) and treatment safety. A durable platelet response was achieved by 16 out of 42 (38%) splenectomized patients with romiplostim, while that was achieved by 25 out of 41 (61%) nonsplenetomized patients with romiplostim. The overall platelet response including the durable or transient platelet response was 79% (33/42) of splenectomized and 88% (36/41) of nonsplenectomized patients. In the placebo groups, none out of 21 splenectomized patients and three out of 21 nonsplenectomized patients responded. Mean doses of romiplostim for splenectomized patients were 4–5 µg/kg, while those for nonsplenectomized patients were approximately 3 µg/kg. In total, 12 out of 23 (52%) splenectomized or nonsplenectomized patients with romiplostim discontinued all of their concurrent ITP drugs.

A recent clinical study with nonsplenectomized ITP patients suggested that as compared with standard of care, romiplostim treatment may induce greater improvement in quality of life [11]. A long-term extension study has been performed [12]. The possible adverse effects of TPO receptor agonists will be discussed in the section 'Clinical safety'.

Eltrombopag

In contrast to subcutaneous administration of romiplostim, eltrombopag is orally administrated and is absorbed with a peak concentration occurring 2–6 h after oral administration. Eltrombopag pharmacokinetics are altered when the drug is administered with polyvalent cations, such as iron, calcium and magnesium. Eltrombopag should be taken at least 4 h before or after any products such as antacids, daily products or other calcium-containing food products, or mineral supplements containing polyvalent cations. *In vitro* studies indicate that eltrombopag is metabolized in the liver, and systemic exposure to eltrombopag is increased in patients with mild or moderate to severe hepatic impairment (41 and 80–93% increases in AUC_{∞}). Thus, administration of eltrombopag to patients with moderate-to-severe hepatic impairment should be undertaken with caution and should be closely monitored [13].

In a Phase II clinical study, optimal dose of eltrombopag was determined. TRA10073A compared 30-, 50- and 75-mg doses of eltrombopag and placebo to 118 patients with refractory ITP for 6 weeks [14]. The primary end point was the proportion of responders at day 43. Responders were defined as subjects whose platelet count reached $\geq 50 \times 10^3/\mu l$ at day 43 or who achieved a platelet count $>200 \times 10^3/\mu l$ prior to day 43. A dose-dependent effect was observed: 27.6, 70.4, and 80.8% in the 30-, 50- and 75-mg treatment groups, respectively. The results of 50-mg and 70-mg

doses were significantly different from placebo, and 50-mg dose was chosen as a starting dose in the following studies.

A 6-week administration [15] and 6-month administration (RAISE study) of eltrombopag [16] was assessed in Phase III clinical studies in a double-blind, randomized, controlled trial conducted in multiple centers internationally. The doses for these studies were 50 mg or matching placebo once daily, and patients with platelet count $<50 \times 10^3/\mu l$ on day 22 or after may have had their dose increased to 75 mg. In addition, in RAISE dose decreases to 25 mg once daily were required for patients with platelet count $>200 \times 10^3/\mu l$. For patients with platelet count $>400 \times 10^3/\mu l$, study treatment was interrupted and resumed at the next lowest dose when platelet count fell to $<150 \times 10^3/\mu l$. The primary end point was the odds of achieving platelet count $50–400 \times 10^3/\mu l$ during the 6-month treatment periods. 79% (106/135) patients in the eltrombopag group responded at least once, compared with 28% (17/62) patients in the placebo group. The odds of responding over the 6-month treatment period were greater (odds ratio: 8.2; 99% CI: 3.59–18.73; $p < 0.0001$) for eltrombopag group. 59% (37/63) in the eltrombopag group reduced or discontinued baseline treatments compared with 32% (10/31) patients in the placebo group. Thus, eltrombopag is effective for management of chronic ITP, and starting dose is 50 mg once daily for most patients, and could be increased to 75 mg once daily.

Patient ethnicity may affect the pharmacokinetics of eltrombopag. AUC exposure to eltrombopag was approximately twofold greater among Japanese healthy volunteers than among non-Asian (predominantly Caucasian) volunteers and 87% greater among ITP patients of East Asian descent compared to non-East Asian ITP patients. From these data, it has been recommended that for patients of East Asian ancestry (e.g., Japanese, Korean, Chinese or Taiwanese) the initial dose of eltrombopag should be reduced to 25 mg once daily. It is noteworthy that a recent Japanese clinical trial evaluated the efficacy and safety of eltrombopag at a starting dose of 12.5 mg and a maximum dose of 50 mg in the treatment of Japanese patients with previously treated chronic ITP [17]. During the first 3 weeks treated with 12.5 mg eltrombopag, 22% (5/23) of Japanese patients responded. Since the disease state is of a chronic nature and the 12.5-mg tablet is available only in Japan so far, it is possible that 25 mg every-other-day administration as a starting dose may be suitable for some chronic ITP patients of East Asian ancestry to prevent overshooting of platelet count.

Clinical safety

Romiplostim and eltrombopag were generally well tolerated during clinical

Venous thrombosis as well as arterial thrombosis could be induced by TPO receptor agonists.

studies [5,13]. In romiplostim studies, headache was the most commonly reported adverse event, followed by nasopharyngitis, contusion and fatigue. In eltrombopag studies, headache was also the most commonly reported, followed by nausea, increased alanine aminotransferase and increased aspartate aminotransferase, diarrhea and fatigue. These adverse events were all mild to moderate in severity. Eltrombopag may cause hepatotoxicity, and alanine aminotransferase, aspartate aminotransferase and bilirubin should be monitored during treatment.

In terms of the development of neutralizing antibodies against endogenous TPO, no patient developed such antibodies. Although two patients treated with romiplostim developed antibodies that neutralized romiplostim, but resolved after drug withdrawal, the antibodies did not cross-react with TPO or affect platelet count [5].

Since long-term treatment with TPO receptor agonists would be expected in chronic ITP, one should pay special attention to possible severe complications as listed in **Box 7.1**.

Thrombotic complications

It has been suggested that ITP itself may be a prothrombotic disease, and in retrospective analysis thrombotic events in ITP were identified by history in 5% of patients. Arterial thrombotic and venous thromboembolic events have been reported for patients with both romiplostim groups and eltrombopag groups, but also in placebo groups. Most of the patients with thromboembolic events were elderly and had pre-existing risks for thrombosis (e.g., factor V Leiden, antiphospholipid syndrome). Although it is still uncertain that TPO receptor agonists may apparently induce arterial thrombotic/venous thromboembolic events, special attention should be paid for patients having pre-existing risks for thrombosis when starting TPO receptor agonists.

Induction of reticulin formation in bone marrow

IL-11, GM-CSF, and TPO agents may increase bone marrow reticulin, possibly through local release of TGF-β from the increased number of bone marrow megakaryocytes. Prolonged administration of large doses of

Box 7.1. Possible adverse effects of thrombopoietin receptor agonists.

- Thrombotic complications
- Induction of reticulin formation in bone marrow
- Increase in blast cell count
- Rebound thrombocytopenia upon stopping treatment

romiplostim to mice produced bone marrow fibrosis that was reversible within 4 weeks by stopping romiplostim. The presence of or increase in bone marrow reticulin was reported in some patients with long-term treatment with romiplostim, and most of them had reticulin levels that were mild to moderate [18]. Because of this possible adverse effect, all patients treated with TPO receptor agonists continue to be monitored for clinical signs of any progressive bone marrow abnormalities.

Increase in blast cell count

TPO receptor agonists may stimulate the growth of hematopoietic malignancies, or increased progression of myelodysplastic syndrome. In a recent randomized, double-blind, placebo-controlled study for International Prognostic Scoring System low/int-1 myelodysplastic syndrome with thrombocytopenia, 750 μg/body romiplostim was administered. Although platelet count increased in romiplostim group, romiplostim group showed a tendency to increase risk to transform to acute myelogenous leukemia (AML). AML rates through 58 weeks were romiplostim 6.0%, placebo 2.4% (hazard ratio: 2.51; 95% CI: 0.55–11.47). Due to study-independent safety data-monitoring committee concerns regarding the potential for transient increases in blast cell counts and the risk for progression to or treatment for AML, the study drug was discontinued in February 2011 [19].

Chronic ITP in children

TPO receptor agonists are so far indicated for the treatment of only adult (over 18 years of age) chronic ITP patients. In a randomized, double-blind study have been performed for children with chronic ITP (from 12 months to 18 years of age [n = 17]) for 12 weeks. In this short-term study, romiplostim increased platelet count in 88% of children with ITP and was well tolerated and apparently safe [20]. The long-term safety, however, still remains to be determined.

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