Drugs of the Future 2007, 32(9): 781-787

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.09.1135519

Romiplostim

Prop INN

Thrombopoietin Receptor Ligand
Antithrombocytopenic Agent
Treatment of Idiopathic Thrombocytopenic Purpura

AMG-531

L-Methionyl[human immunogloblin heavy constant γ 1-(227 *C*-terminal residues)-peptide (Fc fragment)] fusion protein with 41 amino acids peptide, (7-7':10,10')-bisdisulfide dimer

Recombinant protein consisting of a carrier Fc domain linked to multiple Mpl-binding domains

CAS: 267639-76-9
EN: 325552

Abstract

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune blood disorder in which there is platelet destruction mediated by autoantibodies. In many patients platelet production by the marrow is impaired, which may also be caused by autoantibodies. Current therapy for ITP is unsatisfactory and relies on generalized immunosuppression using a variety of drugs; other therapies include human blood products such as immunoglobulin or splenectomy. Recently, a novel, genetically engineered second-generation peptibody thrombopoietic growth factor, romiplostim (AMG-531), has shown efficacy in the treatment of adults with ITP. The molecule appears to demonstrate benefit in patients both pre- and post-splenectomy. Its mode of action is via the thrombopoietin (TPO) receptor and involves the same signaling pathways as native TPO. However, romiplostim has no homology with native TPO, which should reduce the possibility of anti-TPO antibodies being generated. Data from phase I and II studies in healthy volunteers and patients with ITP are encouraging. For patients with ITP, treatment with romiplostim should reduce the need for immunosuppressive therapy, thus reducing the significant morbidity and mortality associated with existing drugs. Furthermore, romiplostim may be useful in a wide range of thrombocytopenic states, such as chemotherapy-induced thrombocytopenia, myelodysplasia and hepatitis C-related thrombocytopenia. It may also be useful in stimulating yield from normal donors. Studies involving the use of romiplostim in these settings are under way or planned. Romiplostim is currently in late-stage development and is expected to be approved for use in ITP in the near future.

Background

Within the bone marrow, megakaryocyte proliferation and differentiation rely on several interleukins, colony-stimulating factors and hormones. IL-3 and stem cell factor (SCF) have been shown to play a role in platelet production. Until fairly recently, the hormone controlling the final stage of megakaryocyte maturation had not been identified, but as long ago as 1958 it was termed thrombopoietin. In 1994, the gene encoding the ligand for Mpl was cloned and named thrombopoietin (1-9).

Human TPO is a polypeptide of 353 amino acids which shares 21% sequence identity and 40% sequence homology with human erythropoietin (Epo). This portion of the molecule binds to the TPO receptor and has been shown to be the principal growth factor for the regulation of platelet production by the marrow (10-12).

Mpl (the TPO receptor) is a member of the type I hematopoietic growth factor family (10, 13). These molecules contain a transmembrane domain of around 20-25 amino acids with a 70-500-amino-acid intracellular portion. The latter contains sequences that bind intracellular kinases and other signal-transducing molecules. The TPO receptor is present on megakaryocyte precursors, megakaryocytes themselves and platelets.

After binding TPO, the TPO receptor is activated and transmits signals within the cell (Fig. 1). These signals influence hematopoietic stem cells, megakaryocytes and platelets. TPO activates both JAK2 (Janus kinase 2) and TYK2, but it appears that only JAK2 is required for signaling. After TPO complexes with SHP2, Gab/IRS and the phosphatidylinositol 3-kinase (PI3K) p85 regulatory subunit, PI3K and Akt (PKB) become activated (14, 15).

In addition to stimulating PI3K, TPO also activates a number of other pathways, including two MAPK (mitogen-activated protein kinase) pathways, *i.e.*, p42/p44 ERK1 (extracellular signal-regulated kinase) and ERK2 (16) and p38 MAPK (17), with phosphorylation of a number of molecules, including GRB2 (growth factor receptor-bound protein 2), SHC (Src homologous collagen protein) and

Drew Provan, Adrian C. Newland*. Department of Haematology, Bart's & The London, Queen Mary's School of Medicine and Dentistry, Whitechapel, London El 2ES, U.K. *Correspondence: e-mail: a.c.newland@qmul.ac.uk

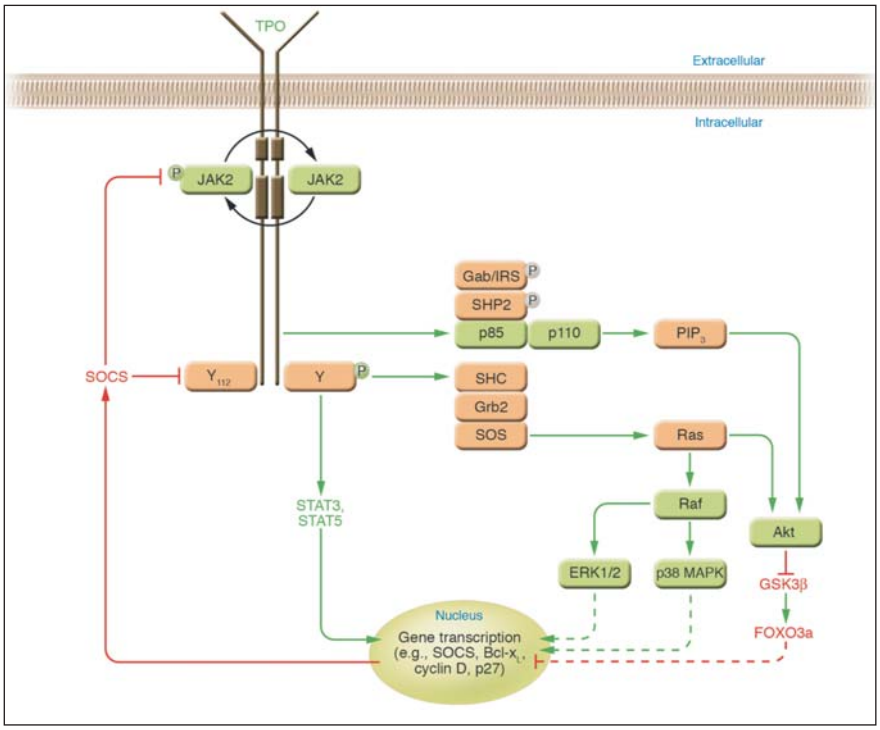

Fig. 1. Signaling pathways activated by thrombopoietin (TPO). The TPO receptor is shown in its activated form. From Ref. 13.

SOS (son of sevenless). The net result of this activation and phosphorylation is induction of the transcription factor HoxB4 and stem cell expansion by p38 MAPK (17). There is also translocation of transcription factors to the nucleus with release of phosphorylase A_2 and subsequent platelet activation (18).

TPO is synthesized constitutively by the liver, and studies to date show that TPO messenger RNA (mRNA) levels remain relatively constant irrespective of platelet count (19-21). However, plasma TPO concentrations vary with platelet count. For example, in aplastic anemia and other underproduction disorders, TPO levels are high; in idiopathic thrombocytopenic purpura (ITP), where increased numbers of platelets are often produced by the marrow, TPO levels are normal despite the blood thrombocytopenia. This may be explained by the platelets' ability to bind TPO. Where the platelet count is low, little TPO is platelet-bound, leaving large amounts of free TPO available to stimulate the marrow. Where there are increased numbers of platelets produced, such as in ITP, there is relatively little free TPO since most is bound to the platelet mass, and hence the TPO level is low in relation to the degree of thrombocytopenia (22, 23).

The principal role of platelets is to secure primary hemostasis. In healthy subjects, the normal platelet count is 150-400 x 10^9/l. When platelets fall significantly below normal, spontaneous bruising and bleeding may occur. Patients with platelet counts of < 50 x 10^9/l may bleed excessively when there is a major hemostatic challenge such as trauma or surgery. However, spontaneous bleeding is uncommon until the platelet count drops below 30 x 10^9/l; more commonly, few problems are seen until the count is < 20 x 10^9/l. Bleeding in ITP is usually mucocutaneous (nose and gum bleeds, menorrhagia) (24, 25). Intracerebral hemorrhage is usually the cause of fatal bleeding in patients with ITP and is estimated to occur in 1-3% of adults with chronic severe ITP. A number of disease states and drugs can induce thrombocytopenia. ITP, which is also known as immune thrombocytopenic purpura, is an autoimmune disorder in which platelets, opsonized with antiplatelet autoantibodies, are destroyed prematurely by the spleen and other sites. This leads to a reduced peripheral blood platelet count. Although bone marrow megakaryocytes are often increased, relative platelet production failure may play a role in some patients (26). The cause of ITP in adults is unknown and the clinical course is variable and unpredictable. The pathogenesis and treatment of ITP have been reviewed by several authors and will not be discussed in detail here (27-35).

Treatment of ITP may be required if the platelet count is sufficiently low and/or if the patient is deemed at high risk for bleeding. Medical therapies include corticosteroids, immunoglobulins (intravenous pooled human immunoglobulin or anti-Rh_0[D]), immunosuppressive drugs, attenuated hormones and vinca alkaloids, among others. Surgical treatment with splenectomy may be used for patients who fail to respond to medical therapies (36, 37).

Most drugs used for the treatment of ITP are not approved for this indication, and studies have shown that the morbidity and mortality associated with therapy may

be higher than the disease itself (38). There is therefore major interest in therapies which elevate the peripheral blood platelet count but do not induce immunosuppression or lead to problems typically associated with existing therapies.

The first-generation thrombopoietic drugs, such as recombinant human TPO and PEG-rHuMGDF, underwent clinical evaluation some years ago and were shown to elevate platelet counts in patients with thrombocytopenia. However, because of the immunogenicity associated with PEG-rHuMGDF and the development of neutralizing anti-TPO antibodies in subjects receiving this molecule leading to worsening thrombocytopenia, clinical development was stopped (39-42). Recently, several second-generation thrombopoietic growth factors have been developed, including nonpeptide molecules and peptide-based drugs such as romiplostim (AMG-531) (43). TPO mimetics and other molecules that interact with the TPO receptor, such as romiplostim, are attractive since they augment natural TPO and may raise the platelet count in patients unresponsive to other therapies. Because they play no obvious role in modulating the immune system, there should be no associated immunosuppressive side effects, although the long-term effects of chronic marrow stimulation are unknown.

Romiplostim is a novel Mpl ligand designed to overcome the development of cross-reacting (neutralizing) antibodies against native TPO seen with first-generation thrombopoietic growth factors. The molecule was chosen by screening peptide libraries for molecules that would bind to the TPO receptor but have no homology with native TPO. Romiplostim is a genetically engineered peptibody expressed in *Escherichia coli*, with a molecular weight of 60 kDa (44). The molecule is comprised of two domains: 1) the peptide domain that binds to the TPO receptor; and 2) the antibody (Fc) domain that prolongs the half-life of romiplostim.

The portion of romiplostim that binds to the TPO receptor contains multiple copies of the Mpl-binding peptide. The function of the Fc portion of the molecule is to extend the half-life of romiplostim. This region of the molecule contains disulfide-bonded human IgG_1 heavy chains and κ light chain constant regions, with two identical peptide sequences linked by polyglycine at residue 228 of the heavy chain (45). The Fc carrier domain binds to the FcRn salvage receptor, which results in endothelial re-circulation (46), thus prolonging the half-life of the molecule (Fig. 2).

Preclinical Pharmacology

Studies have been carried out in a variety of species, including mice, rats, rabbits, dogs and monkeys. Romiplostim increases platelet counts in most of the species investigated, although interspecies variation in the platelet response has been observed. *In vivo* work using rhesus monkeys showed that a single dose of romiplostim led to a dose-dependent increase in platelet counts. The platelet rise was seen at day 5 and peaked between days 7 and 9. No rebound thrombocytopenia was observed and neutralizing anti-TPO antibodies were not detected (47).

Romiplostim has been shown to stimulate murine megakaryopoiesis, and demonstrated a dose-dependent effect on megakaryocyte colony-forming units in murine marrow culture. The molecule binds to Mpl and activates signaling pathways, including tyrosine phosphorylation of Mpl, JAK2 and STAT5 (signal transducer and activator of transcription 5) (47), and induces the development of megakaryocytes from low-density bone marrow cells. Romiplostim is also able to induce endomitosis of megakaryocytes, resulting in nuclear ploidy of up to 128N.

In order to confirm that romiplostim was binding to the TPO receptor, and to determine whether it competes with native TPO in its binding to the receptor, Broudy and Lin incubated [^{125}I]-TPO with Ba/F3 cells expressing human Mpl (Baf3-hMpl) in the presence or absence of unlabeled TPO or romiplostim at varying concentrations. The data obtained confirmed that romiplostim was binding to the TPO receptor and was indeed competing with TPO for the receptor. They repeated the experiments using human platelets (which are known to bind native TPO) and again confirmed that romiplostim was competing with

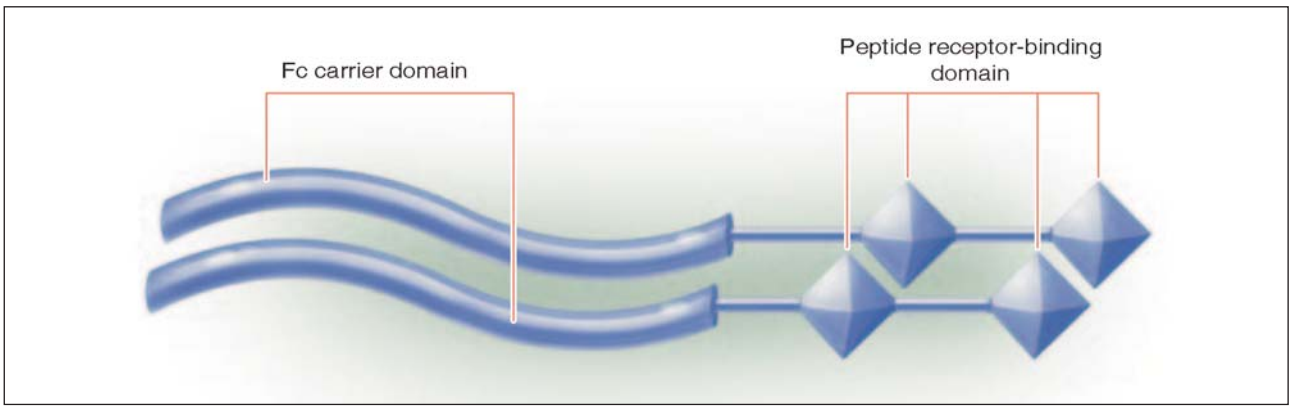

Fig. 2. Romiplostim (AMG-531) showing the four TPO receptor binding sites. From Ref. 45.

TPO for binding to the TPO receptor on the platelet membrane (47).

Pharmacokinetics and Metabolism

After intravenous (i.v.) administration of romiplostim at doses of 100-1000 μg/kg to wild-type (WT) and FcRn receptor knockout (KO) mice, nonlinear pharmacokinetics were seen. The clearance of romiplostim was higher in the KO mice (128 and 318 ml/kg/h after doses of 100 and 1000 μg/kg, respectively) than the WT mice (8.23 and 13.1 ml/kg/h, respectively). The half-life of romiplostim was 1.3-2.2 h in the KO mice but longer in the WT mice (6.5-12.5 h). The steady-state volume of distribution was 193-464 ml/kg in the WT mice but lower (104-158 ml/kg) in the knockout animals. The increase seen in the WT mice is postulated to be due to the presence of the FcRn receptor in these animals (48).

Phase I data obtained from 48 subjects (44) administered a single i.v. dose of romiplostim showed a biphasic disposition profile (Fig. 3). The dose range studied was 0.3-10.0 μg/kg and pharmacokinetics were nonlinear. Serum concentrations of romiplostim generally dropped below 18 pg/ml (the lowest quantitation limit of the assay) in normal volunteers in the subcutaneous (s.c.) cohorts. Absorption of romiplostim following s.c. administration was slow, with peak serum levels detected at 24-36 h after administration.

Safety

Romiplostim was well tolerated in all the studies described below. The most common adverse event was headache (mild to moderate). There were no clinically significant changes in laboratory parameters, including urinalysis, blood coagulation and platelet aggregation. No neutralizing antibodies against either romiplostim or native TPO were detected. Although the ITP patients' platelet counts returned to their pretreatment levels, a few subjects had rebound thrombocytopenia where the platelet count was lower than before treatment. This period of rebound thrombocytopenia lasted for a maximum of 2 weeks before returning to baseline level (43). Another AE noted in 2 subjects receiving relatively high doses of romiplostim (> 10 μg/kg) was an increase in bone marrow reticulin. A repeat bone marrow biopsy taken 14 weeks after discontinuation of romiplostim showed improvement, with a decrease in reticulin formation. The second subject has not had a repeat bone marrow biopsy to date. Reversibility of romiplostim-associated bone marrow reticulin has been documented previously in humans and animals exposed to romiplostim (43, 49). A similar phenomenon was seen in mice treated with high doses of PEG-rHuMGDF, which was similarly reversible once PEG-rHuMGDF was stopped (23).

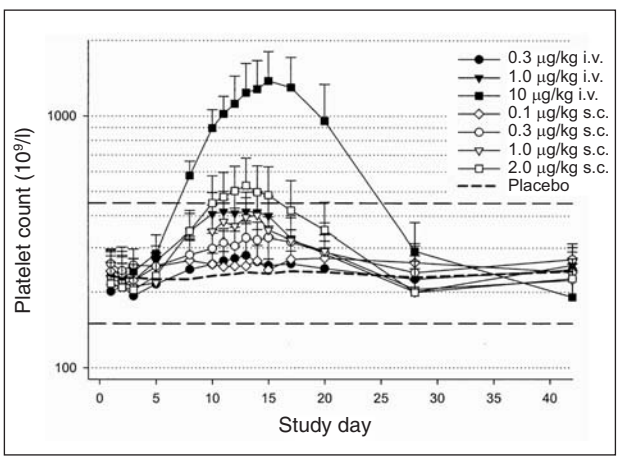

Fig. 3. Pharmacokinetic profile (mean) of romiplostim after single i.v. or s.c. bolus doses in healthy subjects. From Ref. 44.

Thrombocytosis, with platelet counts exceeding 400 x 10^9/l, has been documented in both healthy volunteer and ITP studies of romiplostim, but on discontinuing drug the platelet counts returned to normal. As might be expected, thrombocytosis was more common at higher doses of romiplostim in both groups. Because of this, patients on romiplostim require close monitoring (*e.g.*, weekly) during the early stages of therapy.

Rebound thrombocytopenia, *i.e.*, worsening of thrombocytopenia on stopping romiplostim, has been observed in studies to date. Such rebound thrombocytopenia may last for up to 2 weeks before platelets return to baseline levels (43). As far as we aware, this has not been of any major clinical consequence.

Clinical Studies

Platelet counts peak between days 8 and 15 after stimulation with exogenous TPO in healthy volunteers and individuals receiving chemotherapy (50-52). The temporal relationship between TPO administration and platelet increase is determined by the rate of platelet production.

A randomized, blinded, placebo-controlled study was performed to determine the safety, tolerability, pharmacokinetics and pharmacodynamics of a single i.v. or s.c. dose of romiplostim (44). Forty-eight healthy volunteers were given either romiplostim (n=32) or placebo (n=16). Romiplostim was shown to raise the platelet response in a dose-dependent manner following both i.v. and s.c. dosing. The peak platelet counts were achieved on days 12-16. The greatest platelet rise was seen in the 10 μg/kg i.v. dose group. There was no significant difference between the s.c. and i.v. groups (Fig. 4). The change in platelet count (mean ratio of peak over baseline counts) was 1.15 for the placebo group and 1.43, 2.09 and 5.80, respectively, for the 0.3, 1.0 and 10 μg/kg i.v. groups and 1.23, 1.34, 1.86 and 2.48, respectively, for the 0.1, 0.3, 1.0 and 2.0 μg/kg s.c. groups. Following administration of romiplostim, a rise in platelet count was seen after 3-5 days.

An open-label, dose-escalating phase I/II study was carried out to evaluate the safety and efficacy of romiplostim (53). The study was also used to determine dose

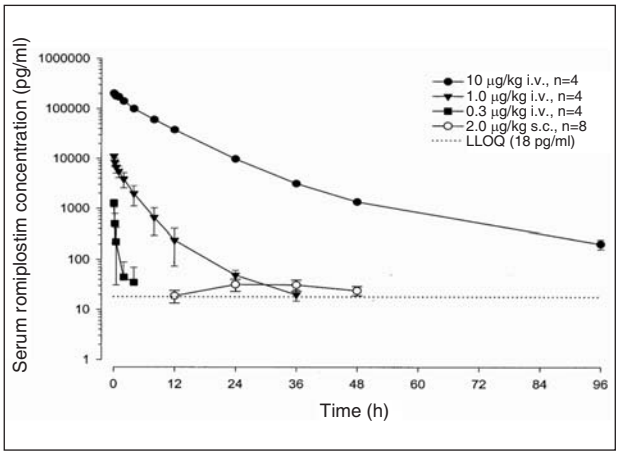

Fig. 4. Mean platelet counts in healthy subjects receiving a single dose of romiplostim (i.v. or s.c.) or placebo. Dashed lines represent the normal platelet count (150-400 x 10^9/l). From Ref. 44.

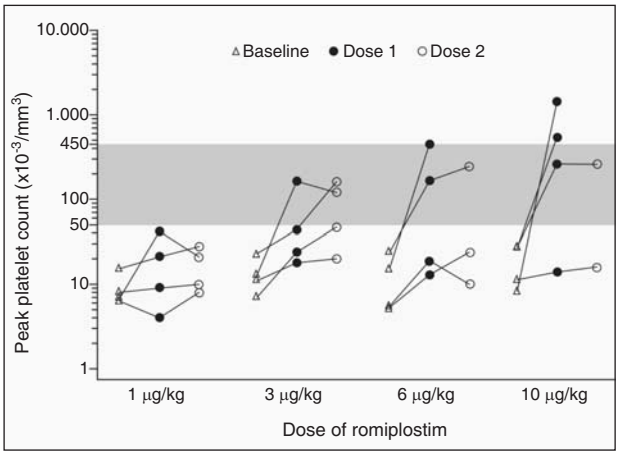

Fig. 5. Peak platelet counts in a phase I study. Baseline and peak platelet counts after doses 1 and 2 are shown. Each cohort involved 4 patients. Three patients received only the first dose. Shaded area represents the target platelet range. From Ref. 45.

in patients with ITP. Patients were assigned to one of four sequential dose cohorts: romiplostim 30, 100, 300 or 500 μg. There was a 2-4-week pretreatment period, a 3-week treatment period and an 8-week post-treatment observation period. Romiplostim was given on days 1 and 15 (or day 22 if the patient's platelet count was > 50 x 10^9/l on day 15). The primary objective was safety, as assessed by adverse event monitoring, laboratory studies and antibody assays. The patients enrolled were adults with ITP in whom 2 of 3 platelet counts during the prescreening period were < 30 x 10^9/l if not receiving corticosteroids, or < 50 x 10^9/l if taking concomitant steroids. Fourteen of 16 (87.5%) patients received both doses of romiplostim. Two patients were withdrawn after the first injection because their platelet counts rose to > 1000 x 10^9/l (1 patient each from the 300- and 500-μg groups). In the safety evaluation, all 16 patients reported at least one adverse event (AE). Most AEs were mild to moderate; 2 of 4 patients with serious AEs attributed these to romiplostim (worsening thrombocytopenia in 1 patient in the 300-μg dose group and elevated lactate dehydrogenase in a patient in the 500-μg group). The most frequent AEs were headache (50%), arthralgia (31%), fatigue (25%), contusion (25%), epistaxis (25%) and petechiae (25%). A number of other AEs were reported with a lower frequency. In terms of efficacy, platelet responses (as defined by doubling of baseline count and platelets between 50 and 450 x 10^9/l) occurred with all doses, 8 of 11 patients (73%) responding at a dose equivalent to 1 μg/kg or more. Overall, the platelet counts in 80% of patients rose to at least 20 x 10^9/l above baseline and in 8 of 15 (53%) platelet counts were > 100 x 10^9/l. Mean increases of > 20 x 10(9)/l were observed 5 days after the first injection in the 100- and 300-μg groups. There was a median of 10 days between the first dose and the peak platelet count.

In another phase I/II study in patients with ITP, 7 of 12 (58%) in the phase I portion doubled their platelet count with increases above 50 x 10^9/l after two doses of romiplostim of 3, 6 or 10 μg/kg. Peak platelet counts were 163 x 10^9/l, 309 x 10^9/l and 746 x 10^9/l, respectively, for doses of 3, 6 and 10 μg/kg (45) (Fig. 5).

In the phase II component of the above study, patients with ITP treated with romiplostim at 1 or 3 μg/kg were given the drug weekly for 6 weeks. Twelve of 16 (75%) patients had doubling of their platelet counts and achieved platelet counts of > 50 x 10^9/l. The mean platelet counts were 135 x 10^9/l, 241 x 10^9/l and 81 x 10^9/l, respectively, for doses of 1 and 3 μg/kg and placebo (45).

A further study was conducted to determine the long-term safety and efficacy of escalating s.c. doses of romiplostim (53, 54). This study was an extension study and involved ITP patients who had previously taken part in a romiplostim study. The study was designed to assess the safety and efficacy of long-term weekly s.c. romiplostim in adults with ITP. Patients previously treated with romiplostim received the same starting dose as their final dose in the previous study in which they were enrolled. Those patients receiving placebo in a prior romiplostim study were started on the lowest dose (1 μg/kg weekly) of romiplostim on entry to the extension study. Doses of romiplostim could be omitted, decreased, increased or maintained depending on the patient's platelet responses. In the latest report from this study, 104 patients had been enrolled. The longest period of treatment with romiplostim was 2 years (96 weeks). The safety subset involved 36 patients (25 female, 11 male) with a mean age of 50 ± 13 years. Most (83%) had undergone splenectomy. Twelve patients entered the study on concomitant corticosteroids which were later tapered when the platelet count exceeded 50 x 10^9/l. Thirty-one of 36 (86%) remain on romiplostim while 5 patients discontinued treatment (3 of 5 due to AEs). Efficacy as determined by platelet response was 86%. The median time to response was 3.1 weeks and the mean dose of romiplostim at first response was 3.4 μg/kg. The number of patients with platelet counts > 150 x 10^9/l at any time was 29 (81%) and that of patients

with platelet counts > 400 x 10^9/l at any time was 15 (42%).

Conclusions

The first-generation thrombopoietic growth factors, although able to elevate platelet counts, were associated with severe unwanted effects. Recently, a number of new second-generation molecules have been designed. Among these is romiplostim, a genetically engineered peptibody which binds to and activates the TPO receptor. The drug competes with native TPO, binding to the same extracellular domain of the TPO receptor as native TPO. Upon binding, JAK/STAT signaling induces megakaryocyte proliferation and differentiation, with a rise in platelet count after 3-5 days and peaking at 12-16 days. Phase I and II studies have shown that romiplostim is able to promote megakaryocyte proliferation and platelet maturation in healthy volunteers and patients with ITP. The drug is well tolerated and headache was the most common AE reported in clinical studies. No antibodies against romiplostim or TPO were detected in any of the studies. Rebound thrombocytopenia was seen in a small number of patients and reversible increases in bone marrow fibrosis were seen in 2 patients. Romiplostim has been shown to have good efficacy in adult patients with ITP irrespective of their splenectomy status. This would tend to suggest that romiplostim may be a useful treatment for patients who are more refractory to standard therapies.

This second-generation thrombopoietic growth factor has been shown to be effective in adults with chronic ITP, and unlike most of the current conventional therapies it is not immunosuppressive. Romiplostim is in late-stage clinical development and should be approved for use in adults with ITP within the next several months. Romiplostim will doubtless have application much wider than ITP, for example chemotherapy-induced thrombocytopenia, infection-associated thrombocytopenia, myelodysplastic syndrome and other indications, and studies using the drug in other settings are ongoing or planned.

Source

Amgen, Inc. (US).

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