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The development of romiplostim for patients with immune thrombocytopenia

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Comprised of peptide and carrier components in an overall structure superficially resembling an antibody, romiplostim is the prototype of a new class of protein therapeutics called peptibodies. Romiplostim (AMG 531, Nplate™) was designed to evade the immune response to recombinant thrombopoietin and offer a new method of treatment for patients with immune thrombocytopenia (ITP), an "orphan" disease. In contrast with agents designed to suppress immune function or hinder the processes of platelet destruction, romiplostim works by stimulating the production of new platelets. It was proven to increase platelet counts, reduce the need for other ITP therapies and emergency treatments, and demonstrate an acceptable safety profile. In addition, romiplostim improves patient-reported outcomes and quality of life in those suffering from this rare disease.

Keywords: romiplostim; platelets; ITP; peptibody

Introduction

Platelets, or thrombocytes, are small blood cells that were first recognized in the 1840s and are involved in blood clotting. The condition of low platelet numbers (thrombocytopenia) is linked to an inability to appropriately clot blood and may manifest itself as bleeding either from wounds, in organs, or under the skin. The "I" of ITP at one time indicated "idiopathic" (of uncertain or unknown origin), the "T" referred to thrombocytopenia or lack of platelets, and the "P" indicated "purpura;" the word is derived from the Greek "porphyra," meaning purple, and originates with a gastropod mollusc from which a purple dye was extracted.[1] More recently, the nomenclature has been updated so that primary Immune ThrombocytoPenia now derives the same acronym (ITP) from a slightly different source.[2]

The clinical manifestations of primary ITP relate to the severity of thrombocytopenia and include purpura, petechiae, ecchymoses, and potentially life-threatening bleeding episodes. For many years, ITP was considered a relatively benign disease with a minor impact on life expectancy. However, the minority of patients refractory to multiple interventions can suffer significant risks for fatal events and reduced overall quality-adjusted and absolute life expectancy.[3]

The diagnosis is often one of exclusion, though acute ITP occurs more often in children (as many as five cases per 100,000 children), usually following a viral infection, and the chronic form is more common in adults, predominantly women 18–40 years of age at around two cases per 100,000 adults.[4]

In chronic ITP, the main treatment goal is to prevent bleeding episodes by raising platelet counts sufficiently for symptomatic relief, not necessarily into the normal range. Management is adapted to the individual to account for symptomotology and lifestyle. In general, platelet counts $>30 \times 10^9$/L do not require treatment unless high-risk activity is foreseen that may result in blood loss (e.g., dental extraction or childbirth), in which case a minimum of 50×10^9/L has been recommended.

ITP is an autoimmune disorder where low platelet counts are due to both antibody-mediated destruction and suboptimal platelet production. The inhibition of platelet production is likely mediated by suppression of megakaryocyte progenitors, in

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which decreased proliferation and increased apoptosis have been seen. This is probably due to a combination of factors such as a direct effect of antibodies on progenitor growth and indirect effects such as insufficiently elevated TPO levels. To date, these processes have not been subject to pharmacologic intervention. Most treatments attempt to control excessive platelet destruction by either suppressing the production of antiplatelet antibodies or controlling platelet consumption. Corticosteroids like prednisone suppress the immune system and are usually the first choice of treatment, effective in about two-thirds of patients. However, particularly in the long term, corticosteroids are associated with intolerable side effects, including hypertension, glaucoma, Cushing's syndrome, and promotion of osteoporosis and diabetes mellitus. In addition, immunosuppression can predispose patients to infection, a major cause of death in ITP.[5,6] An armamentarium of treatments has evolved for patients who are either refractory to steroids or require unacceptably high doses to maintain a safe platelet count. These include immunosuppressants such as danazol, azathioprine, cyclosporine, mycofenolate mofetil, and rituximab and agents such as vinca alkaloids and dapsone (more familiar as cancer and leprosy medications, respectively). Other medications such as anti-D antibodies and platelet transfusions may also be necessary for acute bleeding episodes. Splenectomy removes the major site of platelet destruction and is often considered for patients who are refractory to corticosteroid treatment or who have relapsed after an initial response. Splenectomy can improve platelet counts in approximately two-thirds of patients. However, it has recently become less popular, or at least delayed, due to the risks inherent in the procedure and the potential for spontaneous remission.[7,8] Thus, most therapeutic options are associated with significant adverse events and 20–30% of patients remain refractory or are intolerant to most interventions.[9]

ITP has a major impact on health and well-being. McMillan *et al.*[10] concluded that the quality of life (QoL) of ITP patients was substantially worse than the general U.S. population in measures of both physical and psychological impact. The QoL scores in ITP were broadly aligned with individuals with diabetes and were midway between the general population and those suffering from chronic heart failure or who were missing a limb.

It was apparent as early as the 1990s that something new was required for the treatment of ITP. The majority of treatment efforts were, and still are, focused on various methods of controlling platelet destruction. Kosugi *et al*. showed in 1996[11] that patients with ITP had low levels of thrombopoietin (TPO) compared to patients with other thrombocytopenias, suggesting there was a relative TPO shortfall in ITP. However, at that time, there were no agents available that might be used to replace the shortfall and thus stimulate the production of new platelets. In addition to the lack of such reagents, it was still unknown whether the strategy of stimulating platelet production as opposed to controlling consumption was a valid approach. Testing the hypothesis would have to await the long-sought TPO, and as it was cloned a year or two earlier the opportunity to test the hypothesis was at hand.

The first generation thrombopoietins

The existence of a humoral factor that stimulated platelet production was suggested in 1958,[12] and the term "thrombopoietin" was first coined at this time. However, there were many failures to isolate it during the 1980s,[13] though a number of accessory factors were found.

The critical step in isolating TPO was the discovery of *v-mpl*[14] and its normal homolog, c-*mpl* in 1992.[15] *v-mpl* is the transforming oncogene of the myeloproliferative leukemia virus, and *c-mpl*, the proto-oncogene, was shown to encode a polypeptide product that resembled a growth factor receptor.[16]

Almost 40 years after its existence was first proposed, the c-mpl ligand was identified simultaneously in 1994 by several independent groups.[17–20] Soon the effects of TPO on megakaryocyte growth and development were demonstrated both *in vitro* and *in vivo*.[18,19,21,22] Genetic disruption of the genes for either *TPO* or *c-mpl* caused a reduction in platelet number and little else.[23,24] This confirmed that c-mpl and TPO were the nonredundant ligand/receptor pair responsible for lineage-specific control of platelet production.

TPO is a glycoprotein of 332 amino acids comprising two domains—one similar to erythropoietin and another of largely unknown function.[17,18] TPO is produced primarily in the liver,[19] and serum levels are regulated by cells bearing c-mpl (CD110).[25] Upon binding to c-mpl, TPO stimulates a cascade of

intracellular signaling processes involving Janus kinase (JAK)-2 and signal transducers and activators of transcription (STAT)-5. TPO is involved in all stages of megakaryocyte development, although the terminal steps of platelet formation are independent or possibly even antagonized by TPO.[26]

TPO is present at low concentrations in the serum (normally <200 pg/mL), and under most circumstances there is an inverse relationship between platelet numbers and TPO levels.[27] This is because after TPO binds to and agonizes c-mpl, the receptor/ligand complex is internalized and destroyed. Thus, megakaryocytes and platelets control TPO levels, and thereby regulate their own production. However, in ITP this relationship is lost and inappropriately low TPO levels have been reported.[11,28]

Clinical development of first generation thrombopoietins

Two forms of TPO were developed for testing in clinical trials. These were full-length glycosylated recombinant human thrombopoietin (rHuTPO) and a truncated form (the first 163 amino acids) with the second domain replaced with a 20 kDa polyethylene glycol moiety (pegylated recombinant human megakaryocyte growth and development factor, PEG-rHuMGDF).

Both agents increased platelet counts in healthy animals and disease models.[29–32] Trials in humans showed they were effective in improving platelet recovery after cancer chemotherapy. PEG-rHuMGDF also increased platelet counts in 3/4 ITP patients.[33] Bleeding events were controlled in these patients, two of whom had platelet counts increase from $<30 \times 10^9$/L to over 700×10^9/L after the administration of PEG-rHuMGDF. Counts returned to pretreatment levels within 4 to 6 weeks.

These studies showed that platelet counts could indeed be manipulated, and there was hope for clinical efficacy.[34] However, all studies were abruptly halted when it was discovered that some subjects treated with PEG-rHuMGDF developed antibodies to the drug that then cross-reacted with and neutralized endogenous TPO.

This was a serious setback for patients, physicians, and researchers who had seen the potential to treat diseases such as ITP. It was apparent that a new class of agents was required if this therapeutic approach was to be made available. Research efforts resulted in the development of a new generation of agents with

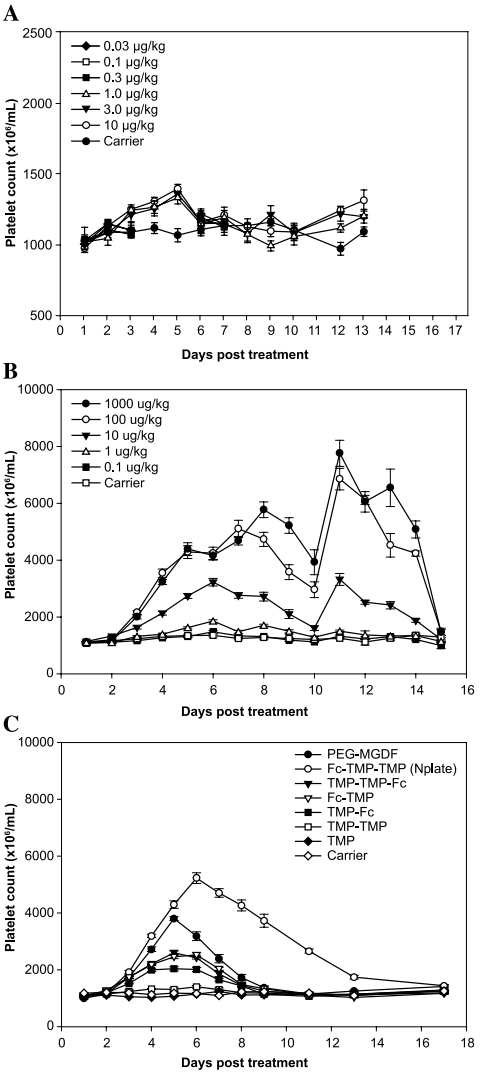

Figure 1. Platelet counts in BDF_1 mice ($n = 5$ per treatment point, data are expressed as mean ± SEM) in response to administration of (A) a single s.c. dose of thrombopoietin mimetic peptide (TMP) [peptide only]; doses are micrograms of peptide per kilogram body weight and administration was on day 0; (B) various doses of TMP administered by continuous subcutaneous infusion; pumps were implanted on day 0; doses indicated are dose-delivered per day, and though the design life of the pump is 7 days, the reservoir of the pump contained enough material for up to 8.3 days of delivery; (C) a single s.c. injection of each TMP construct at 100 µg/kg on day 0; PEG-rHuMGDF was administered as a positive control. The naming convention is that TMP is the peptide; Fc preceding TMP indicates C-terminal conjugation; and succeeding TMP indicates N-terminal conjugation. TMP–TMP indicates TMP dimer spaced by polyglycine. Carrier is phosphate buffered saline supplemented with 0.1% bovine serum albumin as stabilizer. Platelet counts were measured daily post-treatment on an ADVIA automated blood cell analyzer using species-appropriate software.

diverse molecular structures and mechanisms of action (reviewed in Ref. 35). Of these, romiplostim was the first to reach the clinic.

Romiplostim

Romiplostim represents a new class of therapeutics called "peptibodies." This name represents the component peptide (the "pepti-") and the Fc portion of an immunoglobulin in an overall structure that resembles an antibody (the "-body"). In this format, the peptide "warhead" interacts with c-mpl and transduces a signal indistinguishable from that resulting from TPO. The second domain, the Fc component, stabilizes the complex in the body, extending residence time into a therapeutically useful range.

The optimized peptide sequence Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala was shown to displace TPO from c-mpl after it had been iteratively derived from recombinant phage libraries.[36] This peptide could also stimulate the proliferation of a cell line engineered to express c-mpl. The peptide, however, had a marginal effect at best (Fig. 1A) when administered to mice; it was not a drug, though it was perhaps a new pharmacophore. In contrast, when the peptide was administered for continuous exposure using small-automated pumps, the platelet response was spectacular (Fig. 1B). Where a single injection had increased platelets by a maximum of 30%, continuous exposure increased platelet counts sixfold above baseline values. In an additional step to optimally bridge the two c-mpl receptors of the homodimeric receptor pair,[37] the peptide was dimerized with an 8 glycine linker. The definition of this bridge sequence was informed by the analysis of the EPO/EPO-R interaction[38] and extrapolated to c-mpl. It was shown that glycine or glycine/proline sequences could be used to provide a flexible interpeptide bridge that could be tuned (based upon receptor activation experiments with peptides bridged with G ($n = 2$) to G ($n = 12$)) to optimize the receptor monomer orientations in the ligand:receptor dimer complex. A similar approach for romiplostim yielded the 8-glycine bridge of the final molecule (see Table 1).

Table 1. Interpeptide polyglycine bridge length influences cellular activity of dipeptides

Glycine bridge length[a]	Activity[b]
0	4.5
1	4.0
2	4.0
3	4.0
4	4.0
5	4.0
6	4.0
7	4.0
8	4.5
9	4.0
10	4.0
14	4.0

Proliferation (as measured by reduction of MTS) of the mouse cell line 32D (modified to express human c-mpl) was used to test the activity of various dipeptide constructs *in vitro*.

[a]Two identical peptides were connected with polyglycine bridges of various lengths between 0 and 14.

[b]Optical density of reduced MTS was assessed against a standard curve produced with the same cells growing in the presence of TPO. The scale is logarithmic, meaning the difference between 1 and 4 is 1,000-fold.

Development of the peptide into a drug relied on conjugating it to a partner molecule that would extend its persistence in the body. The choice of partner for romiplostim was driven by several factors including the desire to produce a fully recombinant drug, as opposed to a semi-synthetic construct chemically modified after expression and purification. The selection of Fc conjugation was based upon the desire to increase the molecular mass above the threshold for renal clearance and gain the ability to engage the neonatal Fc receptor (FcRN), both of which contribute to the longevity of antibodies. Human Fc was selected because the final application was in humans, and IgG1 was chosen in particular because of its increased flexibility in the hinge region (between the Fc and Fab regions of an antibody) and the relative simplicity of this region for folding purposes after expression in *Escherichia coli*. Expression in *E. coli* also removed the risk of effector function normally associated with IgG1s since this function is dependent upon glycosylation of the Fc, which is impossible in prokaryotes such as *E. coli*. The 5-glycine bridge between the carboxy-terminus of the Fc and the active peptide dimer was selected based on computer modeling studies to ensure the peptide moiety would not be hindered by the Fc in

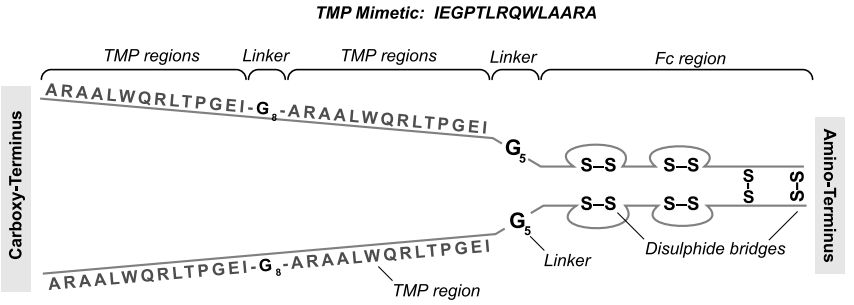

Figure 2. **Structure of romiplostim.**

binding to c-mpl. Using the key peptide, numerous complexes of Fc and peptide were made and tested empirically to make the final selection. The most active candidate was a C-terminal conjugation of Fc-peptide-peptide with five glycines bridging the Fc to peptide and eight glycines between the peptides (Figs. 1C and 2).

Romiplostim is made as a recombinant protein in *E. coli* as a 269 amino acid monomer that is then refolded into covalent homodimers of 50,096 Da. The Fc fragment is derived from the human IgG1 heavy chain. *In vitro* experiments showed that romiplostim bound to and activated c-mpl and stimulated the growth and maturation of colony-forming units-megakaryocyte (CFU-Meg) in a concentration-dependent manner.[39]

Early pharmacodynamic/pharmacokinetic studies showed that romiplostim was active in mice, rats, rabbits, dogs, and monkeys. Romiplostim was well tolerated and induced dose-dependent increases in platelets in all species, though nonhuman primates (NHP) were less responsive compared with other tested species; however, as these were presumed to be more predictive of the human response, they were used as the basis for first-in-human dosing.

Forty-eight normal volunteers were the first humans to receive romiplostim.[40] The first person received 10 μg/kg i.v., which, based on NHP data, should have been a no-effect dose. Surprisingly, platelet counts in this subject increased a remarkable sixfold above baseline. Further volunteers were treated with lower doses while a more complete understanding of the dose–response relationship was obtained. These data revealed that the dose to double platelet counts in normal humans was between 1 and 3 μg/kg—comparable to the dose in rodents, but substantially less than that required in NHP.

Drug exposure in these volunteers was nonlinear after administration of 0.3–10 μg/kg. A dose of 1 μg/kg administered i.v. gave a C_{max} of 12,900 pg/mL, but the same dose administered s.c. resulted in no detectable serum levels of romiplostim probably due to slow absorption (the limit of detection was 18 pg/mL). Given by either route, this dose resulted in platelet counts approximately doubling, broadly in line with rodent data. Also in line with prior experience, the platelet response took between 3 and 5 days to become evident, and peak counts occurred 12–16 days after administration.

A second double-blind, phase 1 study in 30 healthy Japanese subjects evaluated the safety, platelet response, and pharmacokinetics of a single s.c. dose of romiplostim (0.3, 1, or 2 μg/kg[41]). Romiplostim was well tolerated, though headache, "feeling hot," and malaise were reported by 5/24 romiplostim-treated volunteers and were considered drug related. Platelet counts increased to ≥150% of baseline in 4/8 subjects who received 1 μg/kg and in 7/8 who received 2 μg/kg. In only 2 volunteers was serum romiplostim above the assay detection limit.

The target cell-mediated clearance exhibited by romiplostim (i.e., clearance from the body by platelets) introduces some complexity when attempting to determine the optimal drug dose and schedule, and the dynamics of the disease process in ITP are a further complication. For instance, platelets are consumed at an accelerated rate in ITP patients, and as platelets consume TPO and possibly romiplostim, serum drug levels may be subject to altered clearance via platelets. In addition, the contribution of megakaryocytes to TPO and romiplostim clearance is not fully understood. Undoubtedly, megakaryocyte mass (or, more

precisely, the quantity of surface c-mpl on megakaryocytes) varies between patients. Romiplostim has also been shown to displace TPO both *in vitro* and *in vivo*, questioning whether the pharmacodynamic response to romiplostim may be driven by a combination of both endogenous TPO and romiplostim. It is also unknown whether TPO influences romiplostim clearance or vice versa. All of these questions remained unanswered after the initial normal volunteer trials. So even though it was known that romiplostim could stimulate platelet production, it was unknown how the drug would perform in patients with ITP.

One further observation made in these normal volunteer trials related to antibody formation. A sensitive assay was developed to detect antibodies to romiplostim, the peptide component, the Fc domain, or endogenous TPO. Using this assay, no antibodies were detected in this study or in any of the subsequent phase 1–2 studies.

Early clinical development

As befits "orphan" designation (fewer than 200,000 patients in the U.S.), a limited number of subjects were treated with romiplostim before it was approved. In total, 78 normal volunteers and 73 ITP patients received romiplostim before the phase 3 trials. The phase 3 trials recruited 125 patients (of whom two-thirds received active drug).

Two phase 1–2 clinical trials in patients with chronic ITP were completed. In the U.S. trial, patients received romiplostim dosed by body weight at 0.2–10 μg/kg.[42] In the European trial, romiplostim was administered as a fixed dose between 30 and 500 μg on days 1 and 15 (or day 22 if the platelet count was $>50 \times 10^9$/L on day 15).[43] A platelet response was defined in these studies as a doubling of the baseline platelet count to between 50 and 450×10^9/L.

Romiplostim was well tolerated and produced a dose-related increase in platelets. In the per body weight study, 7/12 patients treated at 3, 6, or 10 μg/kg achieved a platelet count $>50 \times 10^9$/L; in 4 patients the counts were within the target range and 3 were above the upper limit (i.e., $>450 \times 10^9$/L). Continuation of the trial for 6 weeks at 1 or 3 μg/kg/week resulted in platelet responses within the target range in 10/16 patients, and an additional 2 patients at 3 μg/kg were above the target range. Four patients showed a transient rebound thrombocytopenia when treatment was stopped.

Sixteen patients were enrolled in the fixed-dose study, and platelet responses were seen in all patients receiving 30, 100, or 300 μg. One patient treated at 500 μg had an excessive platelet count and so this dose was not explored further. Eight of the 11 patients who received $>\sim 1$ μg/kg showed a platelet response and so this starting dose was chosen for phase 3 studies.

Late clinical development

Two pivotal studies were completed with romiplostim in adult chronic ITP. Both were double-blind, placebo-controlled, randomized trials conducted in multiple centers internationally—one in splenectomized and the other in nonsplenectomized patients.[44] All patients had severe thrombocytopenia ($<30 \times 10^9$/L platelets) and were randomized 2:1 (romiplostim:placebo) to receive a starting dose of 1 μg/kg weekly s.c., with dose adjustments to target a platelet count of $50–250 \times 10^9$/L. The primary endpoint was to maintain a platelet count of $>50 \times 10^9$/L without rescue medication for 6 of the last 8 weeks of the 24-week study ("durable platelet response"). A "transient platelet response" was recorded where, in the absence of rescue medication, four or more weekly platelet counts throughout the study period were within the target range. A total of 125 patients enrolled. At the time of study entry, the average duration of ITP was 2 years for nonsplenectomized patients and 8 years for splenectomized patients; one-third of patients were receiving one or more ITP medications; and two-thirds had at some point received medication for their disease. Despite this, the median platelet count at entry was 16×10^9/L.

Romiplostim sustained a durable platelet response in 38% (16/42) of splenectomized and 61% (25/41) of nonsplenectomized patients. Including transient responses brought the final tally to 33/42 (79%) responders in the splenectomy group and 36/41 (88%) in nonsplenectomized group. In the placebo groups, 0/21 splenectomized patients and 3/21 nonsplenectomized patients responded.

Eighty-seven percent of romiplostim-treated patients who were taking ITP medications upon enrollment were able to either stop or reduce the dose by more than 25%. In the placebo group, 38% were able to stop or reduce ITP medications.

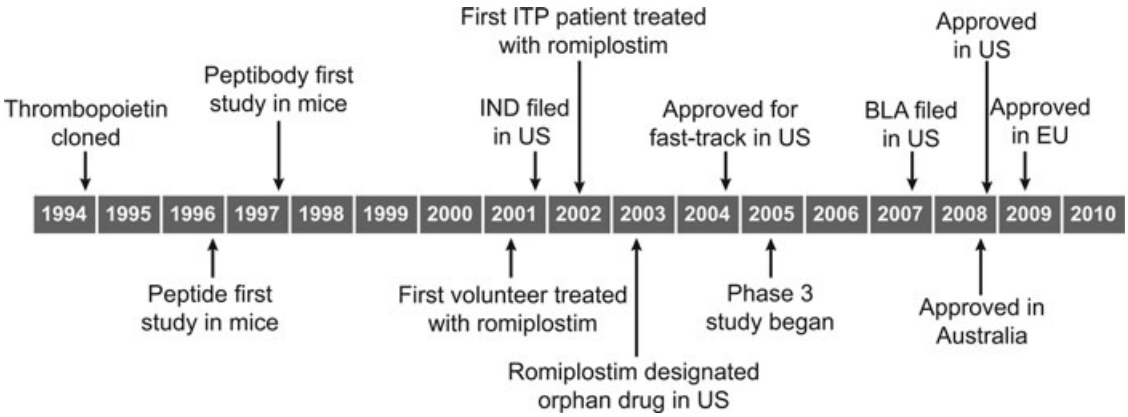

Figure 3. **Timeline of romiplostim development.**

Romiplostim recipients also required rescue medications (steroids, IVIg, anti-D Ig, platelet transfusions, or increased dose of baseline ITP medications) less often; 26% versus 57% of placebo recipients in splenectomized subjects and 17% versus 62% in the group with intact spleens.

Almost all patients reported adverse events; 100% and 95% of patients receiving romiplostim or placebo, respectively. Romiplostim-related events occurred infrequently; 2% of patients reported arterial embolism or reticulin deposition in the bone marrow, but only 3% discontinued use due to an adverse event. Serious bleeding was reduced in the romiplostim patients from 12% incidence to 7%. Also of great relief to patients and physicians (and no less the scientists who designed romiplostim), no neutralizing antibodies to thrombopoietin were found in any patient.

A long-term extension study was created for ITP patients who had completed a previous romiplostim study to monitor the long-term safety and efficacy of continued romiplostim treatment.[45] Some patients have received romiplostim for almost 5 years, and have sustained increases in platelet counts with an acceptable safety profile.[46] Thrombotic events in romiplostim clinical trials have occurred at the same rate in patients receiving romiplostim as those receiving placebo,[47] and occur mostly in patients with preexisting risk factors such as cardiovascular disease. Reticulin has been reported in the bone marrow of a small number of ITP patients who received romiplostim; increases in bone marrow reticulin tended to be reversible upon romiplostim withdrawal.[48] Two patients have developed antibodies that neutralized romiplostim, but resolved in each case after drug withdrawal and the antibodies did not cross-react with TPO nor affect the platelet count.

Quality of life analyses

In addition to the mortal risk presented by the disease, patients with ITP report adversely affected QoL linked to fatigue, concerns over appearance, and impaired ability to conduct routine daily activities.[49,50] Romiplostim improved QoL in the two romiplostim phase 3 trials and long-term extension study, as determined by the ITP Patient Assessment Questionnaire (ITP-PAQ).[51,52]

Due to the seriousness of the disease and the lack of recent developments in the treatment of ITP, authorities in several countries granted accelerated review of romiplostim. It has now been approved for treatment of adult chronic ITP in several countries and in a phase 3b trial versus standard of care, has recently been shown to reduce the requirement for splenectomy and incidence of treatment failure in nonsplenectomized patients.[53] In addition, romiplostim is being tested in children with ITP.

Summary

Thrombopoietin was one of the last growth factors to be tracked down in the hematopoietic halcyon days of the 1980s and 1990s. It was seized upon as the therapeutic for platelets and it was a tremendous disappointment when development stopped due to the apparently intractable problem of antibody-mediated neutralization of both the administered therapeutic and the endogenous hormone. The disappointment was particularly acute for those involved with ITP who had glimpsed

all too briefly the promise held by this kind of therapy. That a c-mpl ligand should have worked in this disease was perhaps counterintuitive, but the proof was there in those few patients who had received the drug. It was to be more than a dozen years (Fig. 3) before romiplostim emerged as the prototype of a new therapeutic class developed specifically with ITP patients in mind.

Acknowledgments

I would like to acknowledge the many scientists at Amgen who touched romiplostim over its long development history. In particular, Janet Nichol, who remained a staunch advocate over many years, and without whom romiplostim would not exist. I would like to thank all the physicians who administered this novel therapeutic agent hoping to help their patients, and most importantly, every patient who selflessly volunteered to receive an experimental drug. This drug, and any relief it brings, is dedicated to the memory of Dr. Dora Menchaca, a lead scientist on MGDF, a wife and mother, who died returning from a meeting with the FDA in the tragic events of 9/11/2001.

Conflicts of interest

The author is an employee of and owns equity in Amgen Inc., the manufacturer of romiplostim. James O'Kelly, an employee of Amgen Inc., provided editorial assistance with the manuscript.

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