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State-of-the-Art Review

Thrombopoietin as a Drug: Biologic Expectations, Clinical Realities, and Future Directions

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Summary: After the cloning of thrombopoietin (*c-mpl* ligand, Tpo) in 1994, 2 recombinant thrombopoietic growth factors, full-length glycosylated recombinant human Tpo (reHuTPO) and polyethylene glycol conjugated megakaryocyte growth and development factor (PEG-reHuMGDF), have been studied in humans in a variety of clinical settings. Both thrombopoietins are generally well tolerated if administered intravenously (IV). The *c-mpl* ligands produce a dose-related enhancement of platelet levels, reduce nonmyeloablative chemotherapy-induced mild thrombocytopenia, and mobilize hematopoietic progenitors. On September 11, 1998, the development of PEG-reHuMGDF was suspended in the U.S., due to formation of the neutralizing anti-Tpo antibody. Those neutralizing antibodies lead to thrombocytopenia and pancytopenia in some patients receiving subcutaneous (SC) PEG-reHuMGDF. Japanese investigators indicate that the probability of antibody formation against PEG-reHuMGDF is low when the drug is administered IV instead of SC. reHuTPO has a more favorable safety profile from the point of antibody production. The *c-mpl* ligands can improve apheresis yields when administered to normal platelet donors. Preliminary data about the use of PEG-reHuMGDF in myelodysplasia, aplastic anemia, and immune thrombocytopenic purpura are promising. Tpo is usually not effective in myeloablative thrombocytopenia when bone marrow hematopoietic progenitors are not present. The major obstacle for the thrombopoietins is their delayed action for managing clinical thrombocytopenia. This review will focus on the biologic basis, current clinical experience, and future directions for the use of thrombopoietic molecules as drugs. The identification of a safe, effective, and potent pharmacologic platelet growth factor could significantly improve the management of thrombocytopenia-induced bleeding.
Key Words: Thrombopoietin—Clinical trials—PEG-reHuMGDF—reHuTpo—Thrombocytopenia—Megakaryocytopoiesis.

Circulating blood thrombocytes (platelets) have a fundamental role in maintaining primary hemostasis and blood clotting. Thrombocytopenia induced by myelotoxic chemotherapy and a wide variety of diseases represents a significant cause of morbidity and mortality (1). Low platelet count has become an increasingly important issue in the treatment of cancer, requiring dose reduction in chemotherapy (1,2). The current approach for the prevention and treatment of thrombocytopenia-induced bleeding is platelet transfusions. However, this approach is far less than ideal because of the availability, cost, serious infectious side effects, disease transmission, and ineffectiveness due to antibody formation against platelets. The incidence of refractoriness to platelet transfusion therapy can be as high as 30% to 40% in patients receiving multiple platelet transfusions (3,4). Although the increased use of hematopoietic stem cells to support high-dose chemotherapy procedures and reduced thresholds for platelet transfusions seem to promise a solution, thrombocytopenia is still a difficult clinical problem. The management of granulocytopenia and anemia with recombinant growth factors (G-CSF and erythropoietin, respectively) is currently in clinical use (5). The availability of thrombopoietin (Tpo) formulations as medicines introduced great hope for the treatment of thrombocytopenia, which is a horri-

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ble problem in clinical medicine (2,6). However, the place of the current recombinant thrombopoietic preparations needs to be elucidated in the prophylaxis and therapy of thrombocytopenia. This review will focus on the biologic basis, current clinical experience, and future directions for the use of thrombopoietic molecules as drugs. The identification of a safe and effective pharmacologic platelet growth factor could significantly improve the management of thrombocytopenia.

HISTORY OF Tpo

Megakaryocytopoiesis and thrombopoiesis have remained the least understood aspect of blood cell production for a long time. In the second half of the 20th century, significant advances were made in exploitation of the platelet production control mechanisms. The term *thrombopoietin* was first proposed for a hypothetical humoral regulator controlling circulating platelet levels and megakaryocytopoiesis by Kelemen in 1958 (7). Then a long hunt for the Tpo molecule began for almost 40 years and several molecules appeared during that time as 'would-be Tpos,' such as gp130 cytokines including interleukin (IL)-6 and IL-11 (8). After the discovery and cloning of Tpo in 1994, thrombopoietic growth factors are used in clinical medicine (9).

As stated by Kaushansky, sometimes in the history of scientific development, studies in 1 research field may lead to a significant catalytic spark in a presumably irrelevant area (6,8,10). In 1986, Wendling and associates (11) isolated the murine myeloproliferative leukemia virus (MPLV), a replication-defective component of Friend leukemia retrovirus, which incites a lethal myeloproliferative leukemic syndrome in mice characterized by the outrageous growth of multiple hematopoietic lineages. Studies of MPLV provided the critical key breakthrough for the discovery of the long-sought thrombopoietin. The transforming sequence of the MPLV, oncogene *v-mpl,* was subsequently identified in its envelope region (12), and the corresponding cellular homolog *c-mpl* proto-oncogene was cloned in 1992 (13). Sequence analysis of human and murine *c-mpl* showed that the encoded protein was an orphan cytokine receptor, a novel member of the hematopoietic growth factor receptor family (9).

Later on, *c-mpl* was demonstrated to be expressed almost solely in hematopoietic cells, predominantly in CD34+ stem cells, megakaryocytes (MKs), and platelets (13). In an experiment, the treatment of CD34+ progenitor cells in vitro with *c-mpl* antisense oligodeoxynucleotides meticulously inhibited the development of MK colonies without any effect on erythroid or myeloid colonies (14). *c-mpl* expression in MKs, their precursors and progeny, and its antisense elimination leading to reductions in megakaryocytic colony formation led investigators working in the field to predict that the *mpl* protein might correspond to the Tpo receptor. The demonstration of a profound decrease in MK and platelet counts in *c-mpl*–deficient mice further perpetuated the theory (15–18). However, the *c-mpl* receptor remained an orphan for a long time until the discovery of its ligand. At last, using 3 different strategies, 5 groups independently purified and cloned the ligand for *c-mpl* in 1994 (16,19–24). Scientists from Genentech Inc. and the Mayo Clinic (16) and from Amgen Inc. (19) purified Tpo from the aplastic plasma of irradiated pigs and dogs, respectively, by using immobilized recombinant *c-mpl* receptor and affinity chromatography. The amino-terminal amino acid sequence was then obtained and the *c-mpl* ligand was cloned via standard molecular strategies. The group at ZymoGenetics in collaboration with the scientists from Washington University (21,22) isolated a mutant hematopoietic cell line after chemical mutagenesis, capable of autonomous transformation due to an autocrine stimulation by the produced *c-mpl* ligand. Specific complementary DNA was then sequenced by functional expression cloning methods. The Kirin group (20,24) and Kuter and associates (23) used the Tpo activity assay combined with direct purification by conventional chromatography methods to purify Tpo from the plasma of thrombocytopenic rats and sheep, respectively. All of the groups have ended up with the same Tpo molecule, except for species-specific differences. Subsequent characterization of the recombinant protein established that the ligand for *c-mpl* is identical to that for Tpo.

Treatment of *c-mpl*–deficient mice and normal mice with Tpo has been demonstrated to substantially increase platelet counts due to the increased numbers of marrow MKs and their progenitor cells. The role of Tpo in early hematopoiesis has been identified in murine and human stem cell populations with respect to *c-mpl* expression and the potential advantage of *c-mpl*–positive hematopoietic stem cells for engraftment (25). Tpo appeared initially to act as a 'lineage-specific' or 'lineage-dominant' late-acting hematopoietic growth factor. However, Tpo has

broader actions from the regulation of early hematopoiesis at the hematopoietic stem cell level to the release of platelets (9,25–27). Tpo is considered a pan-hematopoietic cytokine (27). Moreover, pancytopenia due to neutralizing antibodies against Tpo described in a patient implicated the pan-hematopoietic state of the cytokine (28).

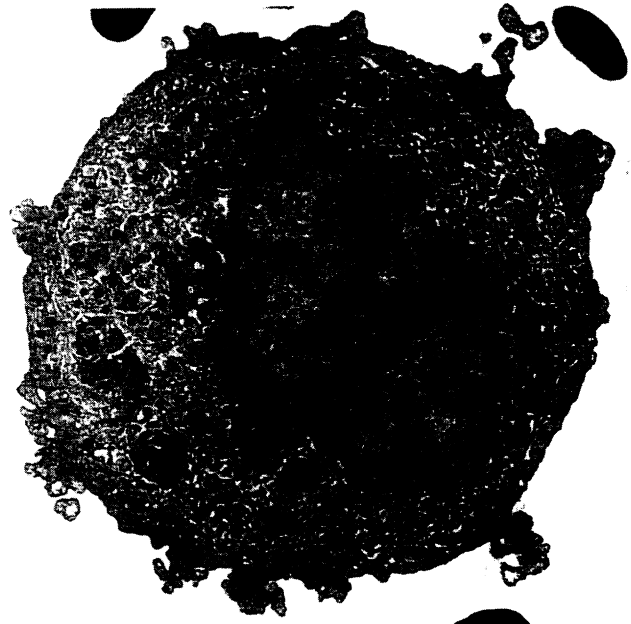


FIG. 1. The microstructure of a mature megakaryocyte, the precursor cell of the platelets. The cytoplasm of the megakaryocyte includes clearly demarcated platelet territories (×5200). (Courtesy of Dr. Dorothea Zucker-Franklin. This photograph was presented in Dr. Zucker-Franklin's lecture at the International Society of Hematology meeting, which was held in Istanbul in 1995.)

BIOLOGY OF MEGAKARYOCYTE AND PLATELET FORMATION

Platelets, the small anucleated blood cells, are the end products of the cytoplasmic fragmentation of mature megakaryocytes. Megakaryocytopoiesis, the development of megakaryocytes from hematopoietic stem cells, is a complex biologic process of cell division, endoreplication, abortive mitosis, and maturation, eventually resulting in the biogenesis of the thrombocytes. The megakaryocyte is the biggest and the most enigmatic cell of the bone marrow (Fig. 1). Megakaryocyte and platelet production is dependent on the interactions of hematopoietic stem cells, bone marrow microenvironment, intracellular events, and many cytokines (8,9). The major steps of thrombopoiesis are under the control of Tpo, which is the primary regulator of megakaryocyte and platelet production (6,8) (Fig. 2).

Cytokine network regulation of megakaryocyte and platelet formation is a crucial biologic mechanism in multiple developmental stages of this process. A wide variety of non-lineage–specific megakaryocytopoietic cytokines and Tpo interact in the regulation of physiologic and pathologic megakaryocytopoiesis (29–31). Many of these regulatory proteins predominantly act on the MK progenitor cells and stimulate proliferation of these early committed CFU-Meg cells. Some of the hematopoietic cytokines control the maturation stage of MK lineage cells by enhancing endoreplication, polyploidization, cytoplasmic maturation, development of the specific cel-

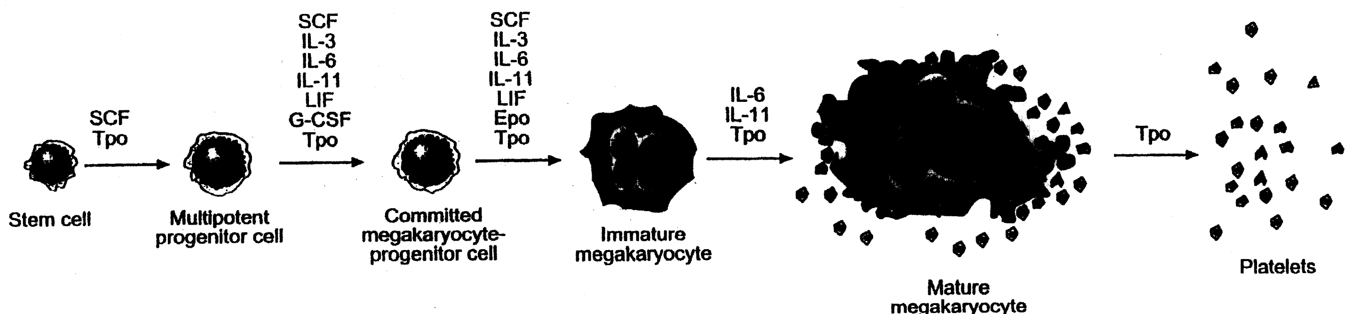


FIG. 2. The major developmental stages of platelets, from the hematopoietic stem cell to their shedding from the megakaryocyte cytoplasm. (Modified from Kaushansky K. Thrombopoietin. *N Engl J Med* 1998;339:746, with permission from the publishing division of the Massachusetts Medical Society.)

lular organelles, and the synthesis of demarcation membrane system and platelet release. Tpo acts at both early and late stages of MK development. Hematopoietic stem cell survival and expansion are also supported by Tpo. Tpo is essential for full megakaryocyte development (6,9,25,27,32,33).

A large number of pleiotropic hematopoietic growth factors have been reported to positively or negatively influence various phases of development in the megakaryocytic lineage, including granulocyte-macrophage colony stimulating factor (GM-CSF); stem cell factor (SCF; also known as steel factor or *c-kit* ligand); IL-3, IL-6, IL-11; leukemia inhibitory factor (LIF); and Tpo (8,9,29). Historically, these cytokines had been divided into early acting MK colony-stimulating factors and late-acting MK potentiators (8). IL-3, GM-CSF, and SCF have been shown by several investigators to display MK colony-stimulating activity and support the proliferation of MK progenitors. MK potentiators, defined by the capacity to augment MK colony numbers, include gp130 cytokines namely, IL-6, IL-11, LIF, and oncostatin M (OSM). These cytokines share gp130 as a signal transducing system to exert their biologic effects (8,9,17,26,34,35). The division between proliferation and maturation factors, however, is not clearcut and these factors often acting in synergistic combinations have overlapping roles influencing megakaryocytopoiesis at various differentiation stages (Fig. 2).

The paramount role of Tpo in the regulation of megakaryopoiesis has been clearly demonstrated by animal studies of genetically engineered mice lacking Tpo or its receptor, *c-mpl*, which displayed severe thrombocytopenia (15–17). Tpo is required to sustain 85% of basal levels of platelet production. The *mpl-/-* mice have a 85% decrease in their platelet numbers with 100% penetrance and with no significant alteration in erythrocyte, leukocyte, and differential cell counts. The causes of thrombocytopenia in gene-targeted animals are reduced numbers of MKs and decreased MK ploidy. Analysis of lymphoid organs and cell populations for B and T cell surface markers was similar in mutant and wild type animals. Ultrastructural and functional analyses revealed that the MKs and platelets produced in mpl-/- mice were similar to those of wild-type mice and were fully functional (34).

Because Tpo-deficient mice retain the capacity to produce sufficient platelets to prevent bleeding, an important contribution to thrombopoiesis in vivo is likely to be made by other megakaryocytopoietic growth factors. Tpo is not the sole regulator of megakaryocytopoiesis. Cytokines with thrombopoietic activity, other than Tpo, affect megakaryocytic proliferation and maturation. When injected into *mpl*-knockout mice, IL-6 and LIF are able to promote MK and their progeny in bone marrow and increase circulating platelet counts, suggesting alternative megakaryocytopoietic stimuli can function in the absence of Tpo signaling (17,36). The increase in platelet numbers in mpl-/- mice injected with IL-6 was also accompanied by significant increases in the numbers of mature MKs and their progenitors in hematopoietic organs, a response consistent with that in wild-type animals. IL-6/IL-6R complex in the presence of SCF has been suggested to play a role in Tpo-independent megakaryopoiesis (37). IL-6, IL-11, or other cytokines that signal via gp130 may be responsible for residual thrombopoiesis in the absence of Tpo or *c-mpl* receptor. However, in a study of double mutant mice deficient in *c-mpl-/*-IL-6-/-, *c-mpl-/-*IL-11-/- and *c-mpl-/*-LIF-/-, these cytokines have been reported to have no role as single regulators in residual megakaryopoiesis seen in *c-mpl*–deficient mice (37,38). Similarly, IL-3 appears not to contribute significantly to megakaryocytopoiesis, in *c-mpl-/-* mice (36). Although, gene targeting studies have shown that in the presence of Tpo, the loss of such cytokines does not significantly alter platelet levels, subtle actions of these factors may exist and be shown or amplified in mice lacking the dominant Tpo signaling system. Ultrastructural analyses indicated that platelets and megakaryocytes present in the Tpo-knockout mice are morphologically and functionally normal (34). Currently, however, there is no direct evidence that any of the non-Tpo thrombopoietic cytokines control the residual steady-state MK and platelet development in *c-mpl-/-* mice. Non-Tpo cytokines with clinical potential in stimulating thrombopoiesis were triad to be developed clinically. The developments of IL-1, IL-3, and IL-6 as platelet-enhancing drugs were suspended because of the higher incidence of side effects and a lesser degree of thrombopoietic activity (4). Recombinant human IL-11 (reHuIL-11, Oprelvekin, Neumega) has been approved by U.S. Food and Drug Administration for the treatment of chemotherapy-induced thrombocytopenia (39). However, hypervolemic complications, fluid retention, edema, pleural effusion, cardiac arrhythmias, and modest efficacy for the improvement of thrombocytopenia represent the challenges to the recombinant IL-11 (4).

MOLECULAR BIOLOGY OF Tpo

Tpo Gene

The Tpo gene is comprised of 5 coding and 2 non-coding upstream exons and 6 introns distributed over 8 kb (40,41). The structural similarity to the erythropoietin (EPO) gene suggests the possibility that the 2 genes might have developed from a common ancestral sequence by gene duplication. Southern blotting analysis revealed that a single copy of the *c-mpl* ligand gene exists in humans. The gene encoding the human *c-mpl* ligand is localized on the long arm of chromosome 3q26-q27 region (42). Structural abnormalities of the long arm of chromosome 3 have been reported to be associated with abnormal thrombocytopoiesis in patients with acute myeloblastic leukemia. However, because the Tpo locus is not so close to the breakpoint cluster area, the Tpo gene does not seem to be directly involved in the "3q21-q26 syndrome" (42).

Tpo Receptor

The *c-mpl* receptor is a member of the cytokine receptor superfamily (12,13). The receptor is a transmembrane protein with an amino-terminal extracellular domain of 463 amino acids, a transmembrane domain of 22 amino acids and a carboxyl-terminal intracellular domain consisting of 122 amino acids. Like other hematopoietic growth factor receptors, its extracellular domain contains 2 copies of the characteristic hematopoietin receptor domain (HRD) sequence defined by a peculiar 200 amino acid module with two pairs of evenly spaced cysteine residues and the hallmark WSXWS motif (Trp-Ser-Xaa-Trp-Ser) (6,43). The cytoplasmic domain of the receptor is competent for proliferative signalling in hematopoietic cells. Human and murine *c-mpl* receptors share 86% homology (43). Two major isoforms of the receptor have been isolated, namely mplP and mplK, with a sole difference in their intracellular cytoplasmic domain after a common 9 amino acid juxtamembrane sequence (13,44). mplP is the functional predominant form of the receptor in human tissues capable of Tpo signalling. The precise function of mplK receptors is still unknown. The receptor expression is largely confined to the hematopoietic tissues, such as bone marrow, spleen and fetal liver (12–14,45,46). It is mostly expressed on primitive CD34+ cells, cells of megakaryocytic family, and platelets (45). The gene for *c-mpl* receptor is mapped to chromosome band 1p34 in humans (47) and chromosome 4 band D in mice (46). The human gene contains 12 coding exons spanning over 17 kb of DNA. The characterized promoter sequence of the *c-mpl* gene possesses putative binding sites for MK-associated transcription factors, ETS, GATA, and Sp1 (44,48).

Tpo Signaling

The thrombopoietic activity of Tpo following receptor dimerization depends on tyrosine phosphorylation and a complex series of signaling events including activation of JAK/STAT, Shc/Ras/MAPK and PI3K/Akt pathways (27). The binding of *c-mpl* receptor to its ligand, Tpo, results in proliferation and differentiation of MK ancestors and progeny. The Janus family of tyrosine kinases (JAKs) and the signal transducers and activators of transcription (STATs) are involved in these processes (49,50,50–52). Tpo activates the Janus family of tyrosine kinases, JAK2 and TYK2 (but not JAK1 and JAK3), and STAT families of transcription factors by tyrosine phosphorylation. Upon activation of *c-mpl* receptor, both JAK2 and TYK2 kinases are tyrosine phosphorylated to induce MK proliferation (50,52), however, TYK2 may not be involved in the final stages of Tpo-induced differentiation (50). The second signalling pathway involves the STAT family of transcription factors. STATs are seven in number and located in cytoplasm. STAT proteins dimerize after tyrosine phosphorylation by JAKs upon stimulation by the cytokine, and translocate to the nucleus to direct transcriptional responses. Phosphorylation of STAT1, STAT3, and STAT5 have been reported upon stimulation by Tpo. STAT5 is essential for early proliferation of MK progenitors, and STAT3 is involved in terminal differentiation (49,52,53). RAS/mitogen-activated protein kinase (MAPK) signal transduction pathway activation may also take place in Tpo-induced megakaryocytopoiesis, besides JAK/STAT pathway (54). Consequently, an increase in MK number, increase in nuclear mass, increased ploidy, and cytoplasmic maturation and platelet release follow.

Tpo Molecular Structure

The human *c-mpl* ligand is a 353 amino acid precursor protein with a predicted molecular weight of 36 kDa, including a 21 amino acid signal polypeptide at the amino-terminal and a 332 amino acid mature protein composed of two domains (Fig. 3) (16,19,22,55). The potential dibasic proteolytic cleavage site between the two domains of Tpo contains a pair of arginine residues

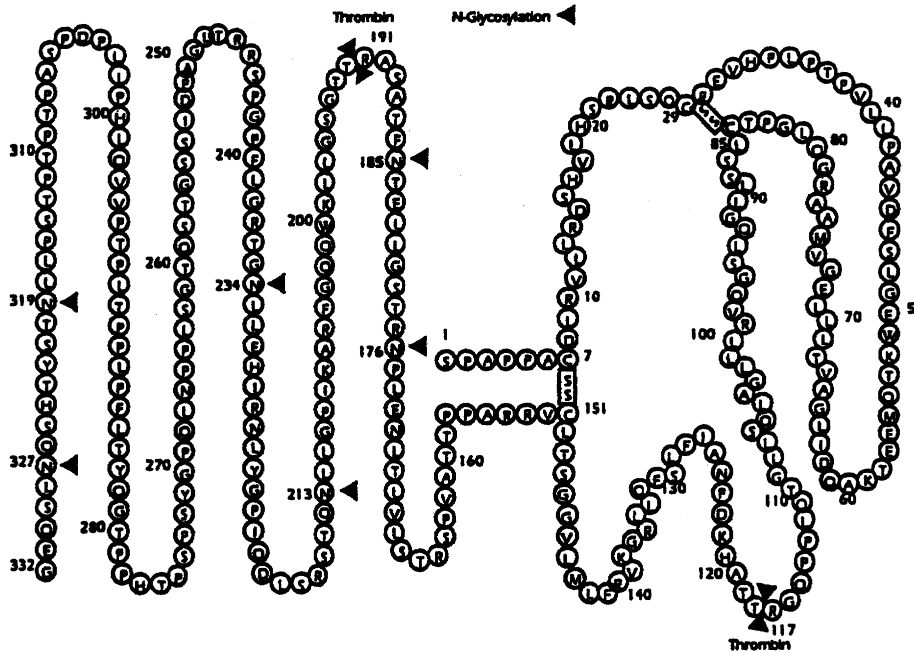


FIG. 3. The molecular structure of the thrombopoietin molecule. (Modified from Kato T, Matsumoto A, Ogami K, Tahara T, Morita H, Miyazaki H. Native thrombopoietin: Structure and function. *Stem Cells* 1998;16(suppl. 2):11, with permission from AlphaMed Press.)

at positions 153-154. The highly conserved amino-terminal with a 153 amino acid "EPO-like" domain shares 23% sequence identity to EPO and a nearly 50% similarity when conservative amino acid substitutions are included, and contains 4 cysteine residues, 3 of which are conserved with EPO. A weak homology of EPO-like domain with interferons is also evident (6,20,56,57). Amino-terminal EPO-like domain confers thrombopoietic biologic activity and binds to the *c-mpl* receptor. Two disulfide bonds between the cysteine residues located in the amino-terminus appear to be essential for function. The unique 179 amino acid carboxyl-terminus displays no homology to other known protein domains (6,20,56,58). The carboxyl domain is rich in serine, threonine, and proline residues and serves exclusively to furnish the molecule with necessary glycolysation. Human Tpo has 6 potential amino-linked glycolysation sites and several O-linked glycolysation sites in its carboxyl-terminal half. These glycolysation sites are not conserved with EPO. The glycosylated carboxyl-terminus is thought to be required for secretion and enhancing the stability or half-life of the molecule in the circulation (56). Glycolysation yields a longer half-life, and the glycosylated form may be more potent in vivo than the non-glycolysated form. The carboxyl-terminal domain of Tpo serves the important role of enhancing secretion of the protein (59). Full-length Tpo molecule is the principal form in the bloodstream of normal subjects and thrombocytopenic patients, although small amounts of truncated species have been detected (55).

Tpo Generated Pharmacologic Forms

Clinical development of *mpl*-ligand has commenced using two distinguished preparations, which differ in structure in the carboxyl terminal domain (2,6,60–64). Genentech (San Francisco, CA) produced a full-length, glycosylated recombinant form of human *c-mpl* ligand and termed as recombinant human thrombopoietin (reHuTPO). On the other hand, Amgen (Thousand Oaks, CA) developed a modified form of Tpo that includes a 163-amino acid receptor-binding EPO-like amino terminal domain of *c-mpl* ligand in which the carbohydrate-rich carboxyl-terminal domain is truncated. This nonglycosylated, *Escherichia coli*–produced molecule, recombinant human MK growth and development factor (reHuMGDF), was then

covalently conjugated to polyethylene glycol (PEG) for in vivo stability, and named as "pegylated reHuMGDF" (PEG-reHuMGDF). Tpo, when truncated, has a decreased circulatory half-life compared to the glycosylated native molecule. Although the truncated molecule, reHuMGDF, consisting of the amino-terminus retains full activity in vitro with only little activity in vivo, the addition of PEG moiety increases the circulatory half-life tenfold with a corresponding increase in biologic activity (61,62).

Another recombinant cytokine, the IL-3-Tpo chimeric fusion protein, promegapoietin (PMP), activates the IL-3 and *c-mpl* receptors (65). The initial experimental studies resulted in promising improvements in platelet-related parameters. Promegapoietin-1a (PMP-1a), the multifunctional agonist for the human IL-3 and *c-mpl* receptors, was given to enhance hematopoietic reconstitution in nonhuman primates following severe radiation-induced myelosuppression. In this study, PMP-1a, irrespective of administration schedule, significantly improved thrombopoietic regeneration in this setting. Thrombocytopenia was eliminated, the severity of platelet nadirs was significantly improved, and recovery of platelet counts to greater than 20,000/μL was significantly reduced in all PMP-1a-treated animals (65). However, the progress of PMP molecule was discontinued by Searle, Inc due to concerns about antibody development (4). The terminology of Tpo and thrombopoiesis is depicted in Table 1.

Peptide agonists with no apparent sequence similarity to Tpo molecule have been synthesized, which are capable to bind and activate Tpo receptor in vitro (66–69). The suggested mechanism of action of these novel Tpo-mimetic peptides is the induction of covalent dimerization of the receptor resulting in a 4,000-fold increased potency compared to the monomeric ligands. The discovery of these miniature peptides may enable to the development of less antigenic, therapeutically useful agents that activate the *c-mpl* receptor (70,71).

PHYSIOLOGY OF Tpo

Synthesis of Tpo: Liver and Kidney

Tpo is produced mainly by liver and kidney as a major 1.8 kb mRNA, and lesser amounts are transcribed in other organs, such as the brain, muscle, bone marrow, and spleen. Tpo is secret-

TABLE 1. Tpo and Thrombopoiesis: Terminology and Abbreviations

Term	Abbreviation	Description
(Endogenous) Thrombopoietin	Tpo, *c-mpl* ligand	Major regulator of the megakaryocyte and platelet production. Tpo named and hypothesised in 1958 as the substance in blood responsible for platelet recovery following thrombocytopenic stress and discovered (cloned) in 1994
v-mpl proto-oncogene	*v-mpl*	Viral oncogene causing a myeloproliferative disorder in mice
c-mpl (proto-onco)gen	—	Cellular homologue of the *v-mpl* encoding the *c-mpl* receptor
c-mpl receptor	*c-mpl*	A specific cytokine receptor, which the *c-mpl* ligand (Tpo) binds, expressed mainly in hematopoietic stem cells, megakaryocytic cells and platelets
c-mpl ligand	Thrombopoietin, Tpo (endogenous or recombinant)	The lineage-dominant cytokine of the megakaryocytic lineage, principal regulator of the megakaryocytopoiesis/thrombocytopoiesis
Megakaryocyte growth and development factor	MGDF	A truncated protein with homology to the erythropoietin-like amino-terminus of the *c-mpl* ligand (Tpo)
Recombinant Human pegylated-Megakaryocyte growth and development factor	PEGReHuMGDF	A truncated recombinant version of *c-mpl* ligand (Tpo) produced in *E. coli,* and derivatized with polyethylene glycol (PEG) by covalent linkage
Recombinant human thrombopietin	ReHuTpo	A recombinant full-length glycosylated form of *c-mpl* ligand (Tpo)
Promegapoietin	PMP	A chimeric fusion protein consisted of *c-mpl* ligand and IL-3

ed as a glycoprotein immediately after the synthesis with no storage pool. Levels of Tpo mRNA in liver and kidney are found to be constant, regardless of the thrombocytopenic state (9,72,73). Tpo production is therefore constitutive and invariant in these organs. The primary site of Tpo production is hepatocytes in the liver at all developmental stages from fetus to adult. Liver sinusoidal endothelial cells were also shown to express Tpo and *c-mpl* (74,75). Impaired production of Tpo in the degenerated liver was speculated to be the main causative factor for thrombocytopenia in patients with cirrhosis (72,76). Low platelet counts associated with liver cirrhosis were recovered following orthotopic liver transplantation (72). On the other hand, cavernous transformation of the portal vein leads to high Tpo concentrations in peripheral blood possibly due to the alterations in the portal hemodynamics due to the thrombosis and endothelial activation (77). Serum Tpo levels are also low in patients with end-stage renal disease undergoing hemodialysis and renal transplant recipients (78,79). Decreased serum Tpo levels despite low platelet counts in hemodialysis patients suggested that the proposed feedback mechanism of platelet uptake of Tpo is not fully operative in these patients. Moreover, arteriovenous fistulas of those patients might affect the local production and/or catabolism of Tpo (78). Therefore, intricate mutifactorial relations between liver, kidney, and bloodstream occur during the regulation of circulating Tpo levels (74–78,80,81) (Fig. 4A) .

REGULATION OF Tpo

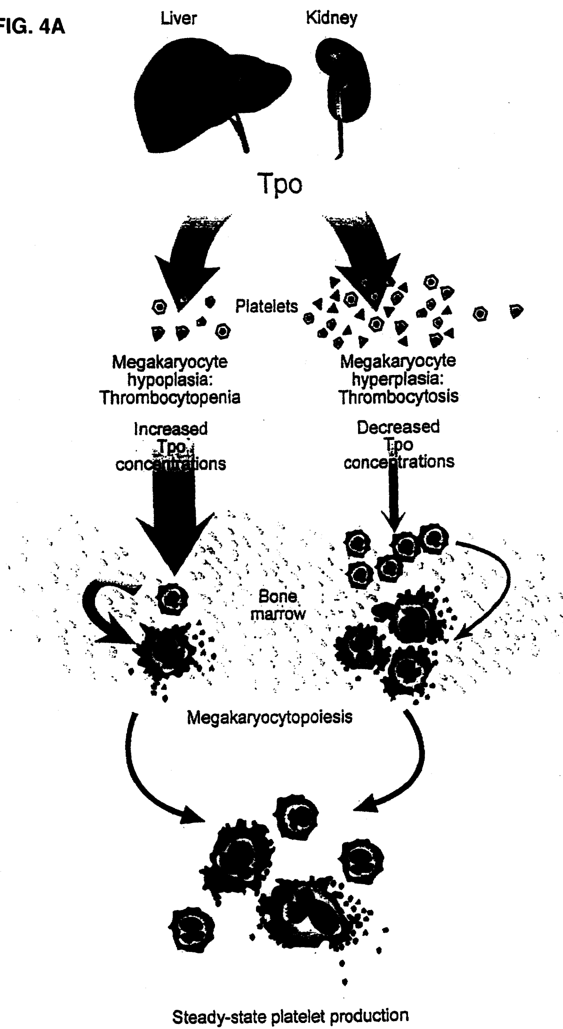
Transcriptional Regulation of Tpo

Tpo is the major physiologic regulator of platelet production. Hence, it is of utmost importance to delineate the precise regulatory mechanisms of Tpo concentration. Circulating levels of Tpo are inversely proportional to the platelet number and elevated in the plasma of thrombocytopenic animals (81–84). A feedback mechanism for Tpo was initially proposed as analogous to EPO. In the regulation of EPO production, a sensing mechanism in the kidney tubules detects any decrease in oxygen delivery due to anemia and upregulates the transcription rate of EPO gene. Increased serum EPO levels stimulate erythrocyte production subsequently. However, no sensing mechanism or organ/tissue were recognized to sense a yet unidentified platelet product or effect. Unlike EPO, Tpo does not appear to be regulated at the transcriptional level (81–84). Tpo mRNA expression in the liver and kidney was not upregulated in mice rendered thrombocytopenic by antiplatelet antibodies or irradiation (81,82) and in *c-mpl-/-* mice (85), despite a significant increase in the serum Tpo levels. Additionally, the gene dosage effect in heterozygous *c-mpl+/-* mice, which have peripheral platelet numbers intermediate between normal and knockout mice, strongly suggests that Tpo is not transcriptionaly regulated (86). The role of the platelets in the clearance of Tpo in humans was studied via the in vitro specific binding of reHuTPO to human platelet-rich plasma, washed platelets, and cloned *c-mpl*. This study indicated that human platelets specifically bind reHuTPO with high affinity, internalize, and then degrade the reHuTPO (87).

Kuter-Rosenberg 'Sponge' Model of Tpo Regulation

A model has been proposed in which circulating Tpo is wasted by *c-mpl* expressed on platelets. Platelet-mediated clearance of Tpo is suggested to be the predominant mechanism in the regulation of Tpo levels. According to this model originally described by De Gabriele and Pennington (88) and further supported by Kuter and Rosenberg (84), Tpo is released into the circulation at a constant rate and the plasma level of Tpo is determined by the platelets themselves. The *c-mpl* is expressed on the surface of human platelets which specifically bind Tpo with high affinity, internalize, adsorb, and degrade the protein (89,90). Circulating platelet numbers directly control Tpo production. In normal circumstances, platelets bind and remove Tpo from the circulation. In thrombocytopenic conditions, the plasma concentration of Tpo increases with the resultant induction of platelet production from the MKs. Fielder and associates provided further evidence to this model in *c-mpl-/-* mice and in human platelets (85,87). The *c-mpl* knockout mice have low platelet numbers and increased Tpo levels in plasma. Platelets from wild-type mice were able to bind, internalize, and degrade Tpo in contrast to *c-mpl*-deficient mice lacking receptors for Tpo (85). The transfusion of normal purified platelets to *c-mpl-/-* mice rapidly decreased elevated Tpo concentration. Altogether, these results provide evidence that Tpo is constitutively synthesized by the liver and kidney and Tpo plasma clearance is mainly mediated by platelet-Tpo binding via *c-mpl* present on the surface of circulating platelets (9).

FIG. 4A



NF-E2–Deficient Mouse Model

MK mass also contributes to the regulation of circulating Tpo levels. In thrombocytopenic subjects, serum Tpo concentrations correlate with both platelet and MK mass rather than with platelet numbers alone. This theory is supported by studies reporting that both the NF-E2 transcription factor–deficient mice (91,92) and patients with idiopathic thrombocytopenic purpura (93,94) are highly thrombocytopenic despite normal or low circulating Tpo levels.

NF-E2 is a lineage-restricted nuclear transcription factor expressed exclusively on erythroid cells, mast cells, the cells of megakaryocytic lineage and multipotential progenitors. NF-E2 target genes are required in the terminal phase of MK cytoplasmic maturation leading to platelet production and release. Genetically engineered NF-E2 p45 subunit knockout mice man-

FIG. 4. **A:** The regulation of platelet production and circulating thrombopoietin concentrations. **B:** Circulating thrombopoietin levels in normal steady-state and in benign and malign diseases. AA: aplastic anemia, chemo: chemotherapy-induced thrombocytopenia, AMT: amegakaryocytic thrombocytopenia, AML: acute myeloid leukemia, ITP: immune thrombocytopenic purpura. (Modified from **A:** Kaushansky K. Thrombopoietin. *N Engl J Med* 1998;339:746, with permission from the publishing division of the Massachusetts Medical Society; **B:** From Nichol JL. Endogenous Tpo (eTpo) levels in health and disease: Possible clues for therapeutic intervention. *Stem Cells* 1998;16(suppl 2):165, with permission.)

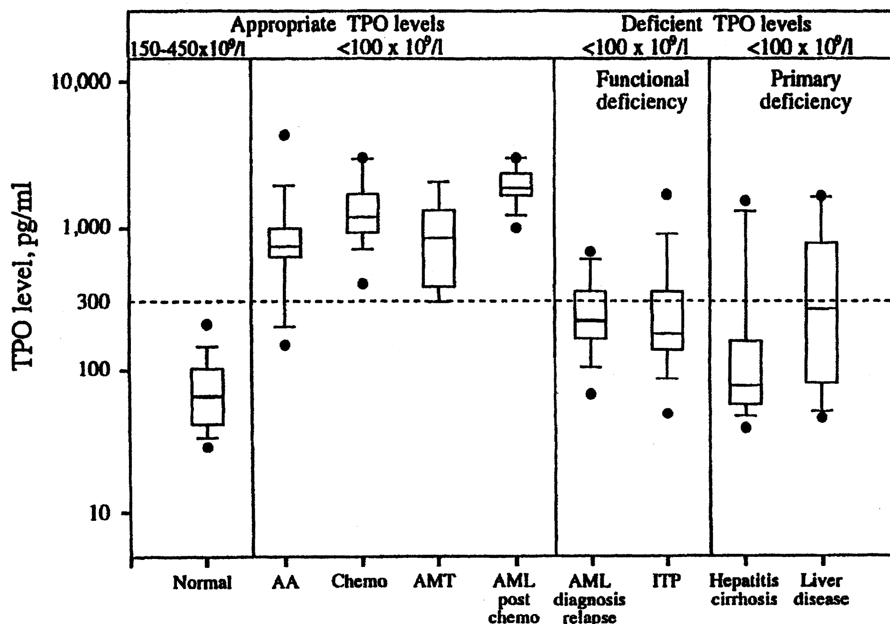


FIG. 4B

ifest a subtle erythroid cell defect and profound thrombocytopenia leading to death in the neonatal period due to hemorrhage (91,92). These mice are deeply thrombocytopenic resulting from a late maturation arrest of MKs with a high number of MKs in the bone marrow, but serum Tpo levels are not as elevated as expected for the degree of thrombocytopenia. Marrow MKs in NF-E2-/- mice are demonstrated to bind significant amounts of radioiodinated Tpo (91,92). Radiolabeled Tpo is also shown to be bound to *c-mpl* receptors expressed on human megakaryocytic cell lines and primary human MKs (95). These observations indicated that MK mass could also be involved in the regulation of bloodstream Tpo levels (Fig. 4A).

Regulation of Tpo Synthesis: Bone Marrow and Spleen

There exist some additional mechanisms regulating megakaryocytopoiesis and serum Tpo levels. Tpo synthesis in kidney and liver is constant, whereas an increase in Tpo mRNA expression occur in the spleen and bone marrow stroma, but not in the liver and kidney, under thrombocytopenic conditions in response to platelet demand (9,73). Tpo production by bone marrow stromal cells and spleen are modulated by platelet counts by feedback mechanisms. Tpo may also upregulate its own receptors. Increments in Tpo concentration could be attributed to Tpo release by activated platelets, which might lead to subsequent stimulation of platelet formation (96).

Physiologic hematopoiesis, including megakaryocytopoiesis, depends on the balance between cellular proliferation and differentiation. Excessive proliferation of MKs or their progenitors result in overproduction of platelets, that is, clonal or reactive thrombocytosis. On the other hand, stem cell differentiation without proliferation eventuates as megakaryocytic aplasia and thrombocytopenia. Normal cytokine network is disrupted in MK and platelet disorders (29–31). Because Tpo is the major cytokine to maintain platelet counts, there is a close relationship between Tpo and pathologic thrombocytopoiesis, that is, Tpo in thrombocytosis and thrombocytopenia (94,97). Circulating Tpo levels in health and disease may serve us as a clue for possible future use of recombinant Tpo preparations as pharmacologic interventions in certain clinical settings (98) (Figs. 4A, 4B).

THROMBOPOIETINS IN CLINICAL MEDICINE

After the cloning of Tpo in 1994, the pharmacologic versions of the molecule moved from the bench to the clinical trials in 2 short years. The effects of the pegylated recombinant human megakaryocyte growth and development factor (PEG-reHuMGDF) on platelet production in humans were described in 1996. Patients with advanced cancer received PEG-reHuMGDF daily for up to 10 days. The doses of 0.3 and 1.0 μg/kg/d of PEG-reHuMGDF provided a threefold median increase (maximum tenfold) in platelet counts by day 16 (99). The most widely studied Tpo molecule in clinical trials is PEG-reHuMGDF. PEG-reHuMGDF has been administered to humans in a variety of clinical settings since that time.

Another 2 years with PEG-reHuMGDF, however, brought a major disappointment for this thrombopoietic medicine. On September 11, 1998, Amgen declared that the development of recombinant truncated human Tpo (the PEG-reHuMGDF) had been suspended due to the neutralizing antibody formation against the molecule leading to thrombocytopenia in some patients participating in cancer clinical trials, and in some healthy volunteer donors in platelet donation trials (www.amgen.com/ Corporate Center/ Amgen News).

The rationale, current data, and future prospects for the use of Tpo preparations, namely PEG-reHuMGDF and full-length recombinant human thrombopoietin (reHuTpo), will herein be summarized in a variety of clinical conditions.

Effects of Thrombopoietin on Normal Healthy Adults

The thrombopoietic potential of MGDF has been tested in normal humans. Harker and colleagues analyzed the effects of PEG-reHuMGDF on megakaryo-thrombocytopoiesis in normal healthy adult humans (62). In that randomized, placebo-controlled, blinded study, 3 μg/kg PEG-reHuMGDF was administered subcutaneously to 16 normal healthy human volunteers. PEG-reHuMGDF transiently doubled circulating normal functional platelet counts, from $237 \pm 41 \times 10^9/L$ to $522 \pm 90 \times 10^9/L$ ($p<0.0001$). Platelets hit the highest point on day 12 (10–14) of the PEG-reHuMGDF. Concurrently, bone marrow megakaryocytopoiesis expanded twofold ($p=0.015$). Platelet counts normalized by day 28. PEG-reHuMGDF-induced perturbation in steady state thrombopoiesis resolved by 4 weeks (62).

Development of neutralizing antibodies to endogenous Tpo after administration of PEG-reHuMGDF is a great concern. In 1997, Dale and coworkers injected a recombinant human MpL ligand subcutaneously for 8 weeks to 3 normal dogs. They determined cross-reacting antibodies to Tpo causing thrombocytopenia in those animals (100). As previously mentioned, the development of PEG-reHuMGDF has been suspended by Amgen since 1998, because of the neutralizing antibody production against both PEG-reHuMGDF and endogenous native human Tpo leading to thrombocytopenia in clinical trials. Three of the subjects in whom severe thrombocytopenia developed in those trials were evaluated thoroughly by Li and associates (101). All 3 thrombocytopenic subjects had antibody to PEG-reHuMGDF that cross-reacted with endogenous Tpo and neutralized its biologic activity. Thrombocytopenia was together with reduced bone marrow megakaryocytes. Easy bruising and heavy menses were present in these cases. Anti-Tpo antibodies were the type of immunoglobulin G4. Anti-Tpo antibody had recognized epitopes located in the first 163 amino acids of Tpo and prevented Tpo from binding to its receptor. In two subjects, endogenous Tpo had found to be circulated as a biologically inactive immune complex with anti-Tpo IgG (101). Recently, pancytopenia and aplastic anemia-like syndrome due to the neutralizing antibodies against Tpo associated with the SC use of PEG-reHuMGDF have been reported in a cancer patient (28). This interesting observation suggests another potential obstacle for the clinical use of Tpo. Kirin (Japan) is still performing clinical trials with PEG-reHuMGDF. Neither antibodies against PEG-reHuMGDF nor neutralizing antibodies against endogenous thrombopoietin were observed in those Japan trials (102). Japanese investigators indicate that the probability of antibody formation against PEG-reHuMGDF is low when the drug is administered intravenously instead of subcutaneous route. Clinical experience with full-length glycosylated Tpo molecule (reHuTPO) so far indicated a more favorable safety profile from the point of antibody production (64). Although anti-Tpo antibodies were detected in the initial studies with reHuTPO, they were non-neutralizing and transient (103).

Thrombopoietin in Transfusion Medicine

Platelet transfusions are crucial to manage thrombocytopenia. More than 60% of the platelet products are obtained by single-donor apheresis. Pre-apheresis donor platelet count is important for this aim (104). PEG-reHuMGDF has been tested to increase platelet counts and consequently platelet yields from apheresis in healthy platelet donors. In a recent blinded, two-cycle, crossover study by Kuter and coworkers (105), 59 platelet donors were randomized to receive a single subcutaneous injection of PEG-reHuMGDF (1 μg/kg or 3 μg/kg) or placebo and 15 days later undergo platelet apheresis. Donors treated with placebo had a median peak platelet count after PEG-reHuMGDF injection of 248×10^9/L compared with 366×10^9/L in donors treated with 1 μg/kg PEG-reHuMGDF and 602×10^9/L in donors treated with 3 μg/kg PEG-reHuMGDF. A direct relationship between the platelet yield and the pre-apheresis platelet count was observed. No serious adverse events were reported in this recent trial. The authors concluded that PEG-reHuMGDF can increase platelet counts in healthy donors to provide a median threefold increase in apheresis platelets compared with untreated donors (105). A companion study investigated the platelets harvested from healthy donors treated with PEG-reHuMGDF whether those platelets could provide larger increases in platelet counts compared to routinely available platelets (106). In that study, 166 of those platelet concentrates were transfused to 120 thrombocytopenic patients. Early after transfusion, the median platelet count increment was higher in patients receiving PEG-reHuMGDF–derived platelets than placebo-derived platelets. The difference was maintained 18 to 24 hours after transfusion. Thus, in that trial, it has been shown that platelets collected from healthy donors undergoing PEG-reHuMGDF therapy resulted in significantly greater platelet count increments and longer transfusion-free intervals than platelets obtained from donors treated with placebo (106).

Thrombopoietin in Stem Cell Transplantation

The administration of PEG-reHuMGDF can result in the mobilization of hematopoietic progenitor cells of multiple lineages into the peripheral blood. Rasko and coworkers examined the effects of PEG-reHuMGDF at doses of 0.03, 0.1, 0.3, or 1.0 μg/kg in a placebo-controlled double-blinded randomized study (107). They observed a dose-dependent increment in peripheral platelet counts with twofold enhanced marrow megakaryocytes, and no alteration in white and red blood cells. The authors also demonstrated a dose-dependent mobilization of multiple

hematopoietic progenitors into the peripheral blood. Maximum blood levels of progenitor cells occurred at day 12 in that trial. Increased levels of Meg-CFC (maximum increase 30-fold), day 7 and day 14 GM-CFC and BFU-E were established at doses of 0.3 and 1.0 μg/kg PEG-reHuMGDF (107). MGDF was suggested as a potent agent to augment progenitor cell mobilization after successful induction or consolidation chemotherapy in patients with acute myelogenous leukemia (AML). Recruitment of CD34+ cells after chemotherapy was also potentiated by MGDF. The median increases of progenitor cell numbers by chemotherapy alone and chemotherapy plus 5 μg/kg/d MGDF over that in normal individuals with steady-state haemopoiesis were tenfold and 45-fold for CFU-GM, threefold and 17-fold for BFU-E, and twofold and 18-fold for CFU-mix. CFU-Mk levels were not increased above normal by chemotherapy alone but were 15-fold enhanced by chemotherapy plus MGDF (108).

A multicenter, phase I dose-escalation trial examined the effects of full-length Tpo (reHuTPO) in patients with persistent severe thrombocytopenia (<20,000/μL) for more than 35 days after hematopoietic stem cell transplantation (HSCT) (109). Thirty-seven patients were evaluated in that trial. In 1 patient group, a single dose of reHuTPO was administered at doses of 0.6, 1.2, and 2.4 μg/kg. Multiple doses of reHuTPO every 3 days for a total of 5 doses were administered to another group in the same trial. Ten patients had recovery of platelet counts during the 28-day study period; 3 of these 10 had an increase in marrow megakaryocyte content 7 days after completing treatment with reHuTPO. There was no correlation between the dose of reHuTPO and the recovery of platelet counts. No significant adverse effects were observed. It remains to be answered whether platelet recovery in those patients was due to the administration of reHuTpo or spontaneous (109).

Tpo may also be used for ex vivo expansion of hematopoietic progenitors and increasing the number of megakaryocyte progenitors in HSCT (35,110–112). The numbers of clonogenic megakaryocyte progenitors have been shown to be increased by cytokine combinations including MGDF, SCF, IL-3, IL-6, IL-11, and FLT3 ligand (35). The effects of different combinations of Tpo, SCF, IL-3, IL-6, and IL-11 on stroma-free liquid cultures of purified human CD34+ cells mobilized by G-CSF were evaluated. Among those thrombopoietic cytokines, the use of Tpo, SCF, IL-6, and IL-11 seems to be rational for ex vivo expansion of the hematopoietic progenitor cell population (35,110). The expanded CD34+ cells have been sustained most of the in vitro characteristics of the unmanipulated CD34+ cells, including clonogenic efficiency (112). The availability of Tpo preparations and synergistic combinations of cytokines seems to develop the strategies of ex vivo expansion of MK progenitor cells and mature MKs. The clinical applications of the re-infusion of the ex vivo–generated MK cells have been investigated successfully in cancer patients following high-dose chemotherapy (111). Megakaryocytic cells can also be effectively produced ex vivo with the combination of stem cell factor (SCF) and promegapoietin (PMP), the fusion protein of IL-3-*c-mpl* ligand (113).

Thrombopoietin for Acute Leukemia–Associated Thrombocytopenia

Thrombocytopenia is a major challenge in the management of acute leukemia. The use of platelet transfusions has the risks of alloimmunization and transmitting blood-borne diseases. Therefore, a thrombopoietic drug that potentially improve the thrombocytopenia due to leukemia or chemotherapy is clinically required (114). PEG-reHuMGDF has been tested for thrombocytopenia after myelosuppressive chemotherapy of AML. PEG-reHuMGDF administration starting 1 day after the last dose of chemotherapy failed to shorten the time of transfusion-dependent thrombocytopenia following AML therapy (115). At this AML study, 108 adult patients with AML were randomized to receive either PEG-reHuMGDF (2.5 μg/kg/d or 5 μg/kg/d) for up to 21 doses, a single dose of 2.5 μg/kg PEG-reHuMGDF, 7 daily doses of 2.5 μg/kg PEG-reHuMGDF, or placebo. There was no effect on median time to transfusion-independent platelet recovery in AML patients at any dose schedule. The median time to neutrophil recovery and red blood cell transfusion requirements were similar in all groups, and there was no apparent stimulation of leukemia (115). In a very recent multicenter, randomized, placebo-controlled, double-blind study (116), the effects of the administration of PEG-reHuMGDF on platelet recovery and transfusion requirements were tested before, concurrent to, and 1 day after chemotherapy in patients receiving consolidation chemotherapy for AML. Patients were randomized to receive either 30 μg/kg PEG-reHuMGDF as a single dose on day -6 (n=37), 30 μg/kg PEG-reHuMGDF administered on day

-6 followed by 10 μg/kg on days -5 to day 6 (through chemotherapy and including the day after chemotherapy, n=35), or placebo (n=18) administered in the same way as in either PEG-reHuMGDF arm. There were no significant differences in the number of days of platelet transfusions between either PEG-reHuMGDF schedule or placebo. The PEG-reHuMGDF day -6 to 6 group had a delayed neutrophil recovery compared to either placebo or PEG-reHuMGDF day -6–treated patients. The authors concluded that earlier dosing of PEG-reHuMGDF before and during chemotherapy did not improve platelet recovery but delayed neutrophil reconstitution (116). Because Tpo could increase the number of hematopoietic progenitor cells in S-phase and their mobilization into the peripheral blood, administration of PEG-reHuMGDF could explain the delayed myeloid recovery in those patients. Schiffer and coworkers randomized newly diagnosed AML patients to receive either 2.5 or 5 μg/kg/day of PEG-reHuMGDF or a placebo administered subcutaneously after completion of chemotherapy. Patients receiving PEG-reHuMGDF achieved higher platelet counts after remission. However, there was no significant difference in the number of days on which platelet transfusions were administered among the 3 groups. Postremission consolidation chemotherapy with either placebo or PEG-reHuMGDF was given to 28 patients beginning the day after completion of chemotherapy. PEG-reHuMGDF had no impact on the duration of severe thrombocytopenia or the platelet transfusion requirement following induction and consolidation therapy in AML. There were no side effects that could be attributed to the use of PEG-reHuMGDF in that study (117). Leukemic blast cells express functional Tpo receptors and proliferate in response to Tpo (118); however, there are no clinical data regarding the adverse neoplastic growth due to the administration of Tpo in patients with acute leukemias. The complete remission rate was 71% for patients younger than 60 years and 64% for those 60 years or older, with no significant difference among the 3 groups in Schiffer study (117).

Thrombopoietin for Myelodysplasia-Associated Thrombocytopenia and Aplastic Anemia

Pancytopenia and defective platelet function are present during the complicated clinical course of myelodysplastic syndrome (MDS). Supportive care including platelet transfusions requires for the therapeutic management of MDS (119). A few preliminary clinical trials with thrombopoietin (PEG-reHuMGDF) have been performed for managing the thrombocytopenia of MDS. Komatsu and coworkers tested the efficacy of PEG-reHuMGDF in patients with aplastic anemia (AA) and myelodysplastic syndrome (MDS) complicated with severe thrombocytopenia in a phase I/II, open-label, cohort sequential dose escalation study (120). The patients had AA (n=16) or MDS (n=21) (refractory anemia [RA] or RA with ringed sideroblasts [RARS]) whose average platelet counts were below 30×10^9/L. Intravenous (IV) PEG-reHuMGDF was administrated at the initial dose of 0.5 μg/kg/d. The dose of PEG-reHuMGDF was escalated up to 5.0 μg/kg/d. Komatsu and associates demonstrated that platelet counts were increased after 5 weeks of PEG-reHuMGDF treatment in AA and MDS. Increments in platelet counts were observed from 2.5 and 1.25 μg/kg/d in AA and MDS patients, respectively. The peak platelet level has been observed after 5 to 6 weeks of PEG-reHuMGDF administration. No serious side effect was observed due to PEG-reHuMGDF. No neutralizing antibody formation was observed associated with IV PEG-reHuMGDF. This preliminary study is an important initial step for the safety and efficacy of IV PEG-reHuMGDF in the therapy of AA or MDS-associated thrombocytopenia (120). The same research group has updated their data of PEG-reHuMGDF in 44 patients (18 AA patients, 26 MDS patients (24 RA and 2 RARS). They indicated that IV administration of PEG-reHuMGDF was effective with no antibody production in the thrombocytopenia of AA and MDS (121). Furthermore, Iki and associates demonstrated trilineage hematopoietic response via 5.0 μg/kg/d PEG-reHuMGDF for 14 days in a 54-year-old patient with RA. Response durations in the red blood cell and platelets were 5 and 17 weeks, respectively (122). There is a concern based on in vitro studies that Tpo may activate the growth of neoplastic progenitors in chronic myeloproliferative disorders and MDS (123). Mice induced to overexpress Tpo develop myelofibrosis and osteosclerosis. Circulating transforming growth factor-beta 1 and platelet-derived growth factor are also increased in Tpo overexpressing mice. Those effects might be secondary to Tpo, resulting from high levels of megakaryocytes and platelets (124). However, there are no clinical human data that Tpo could induce leukemic cell proliferation and/or fibrosis in acute and chronic hematological malignancies.

Thrombopoietin for Immune Thrombocytopenic Purpura

A recent cohort sequential dose escalation study evaluated the safety and efficacy of PEG-reHuMGDF in four patients with chronic ITP (102). Patients with chronic ITP received daily intravenous PEG-reHuMGDF administration for up to seven days. An increase in platelet counts was observed in 3 patients. In 2 patients, platelet counts were elevated over 700×10^9/L after 7 or 6 consecutive doses. In one patient platelet counts increased greater than 100×10^9/L with a single PEG-reHuMGDF injection. Bleeding episodes observed before PEG-reHuMGDF treatment improved in all of the patients. Adverse events related to PEG-reHuMGDF as hypertension, vomiting, and headache were reported in 2 patients. The authors suggested that PEG-reHuMGDF is a promising treatment in ITP patients. PEG-reHuMGDF could increase platelet counts and improve bleeding episodes in severely chronic ITP patients (102). A variant form of chronic resistant ITP presenting as cyclic thrombocytopenia and anti-gpIb-specific antiplatelet antibodies has been shown to responded to PEG-reHuMGDF (125). The pathobiologic basis of ITP includes inappropriately normal or low Tpo levels despite thrombocytopenia (93,97). Therefore, a state of relative Tpo deficiency may be present in some patients with ITP that may respond to recombinant thrombopoietins (126). The preliminary clinical data denote a rationale for this hypothesis.

Thrombopoietin for Solid Tumor-Associated Thrombocytopenia

Thrombocytopenia is a frequent complication during the pathobiological course and the therapy of malignancy. PEG-reHuMGDF and reHuTpo have been tested in solid tumor-associated thrombocytopenia in several clinical trials (103,127–130).

Basser and coworkers investigated PEG-reHuMGDF in advanced cancer before chemotherapy (127). In this placebo-controlled study, PEG-reHuMGDF was given to 13 patients with cancer subcutaneously at a dose of 0.03, 0.1, 0.3, or 1.0 μg/kg up to 10 days. PEG-reHuMGDF produced a dose-dependent increase in platelet counts. Platelets increased from day 6 of PEG-reHuMGDF administration and peaked between days 12 and 18 (127). Basser and associates also administered PEG-rHuMGDF with filgrastim after dose-intensive chemotherapy to 41 patients with advanced cancers to test the effects on hematologic recovery (128). PEG-rHuMGDF was given at doses of 0.03, 0.1, 0.3, 1.0, 3.0, and 5.0 μg/kg by daily subcutaneous injection for between 7 and 20 days. All patients received concurrent filgrastim 5 μg/kg/d until neutrophil recovery. Recovery to baseline platelet count was achieved significantly earlier following PEG-reHuMGDF administration compared with placebo (median, 17 days for PEG-reHuMGDF 0.3 to 5.0 μg/kg vs. 22 days for placebo, $p=0.014$). Levels of peripheral blood progenitor cells on day 15 after chemotherapy were significantly greater in patients administered PEG- reHuMGDF 0.3 to 5.0 μg/kg and filgrastim compared with those given placebo plus filgrastim. The authors suggested that PEG-reHuMGDF accelerates platelet recovery after moderately dose-intensive chemotherapy (128). Fanucchi and associates conducted a randomized, double-blind, placebo-controlled dose-escalation study of reHuMGDF in 53 patients with lung cancer who were treated with carboplatin and paclitaxel (129). reHuMGDF doses were 0.03, 0.1, 0.3, 1.0, 3.0, or 5.0 μg/ kg administered subcutaneously. In the 38 patients who received reHuMGDF after chemotherapy, the median nadir platelet count was 188×10^9/L per cubic millimeter (68×10^9/L -373×10^9/L), as compared with 111,000 per cubic millimeter (21×10^9/L -307×10^9/L) in 12 patients receiving placebo ($p=0.013$). The platelet count recovered to baseline levels in 14 days in the treated patients as compared with more than 21 days in those receiving placebo ($p<0.001$) (129). These studies (127–129) had demonstrated the biologic effect of reHuMGDF in cancer patients. However, the cancer patients in those trials were not severely thrombocytopenic.

Phase I/II clinical trials of full-length glycosylated recombinant human thrombopoietin (reHuTpo) were conducted in sarcoma and gynecologic malignancy. Vadhan Raj and colleagues administered reHuTpo to patients with advanced cancer and severe thrombocytopenia (103,130). At the initial investigation, a single dose of reHuTpo increased circulating platelet counts (mean increase from baseline, 61% to 213%) and bone marrow megakaryocytes (as much as fourfold) in a dose-related manner (103). That increase began by day 4 and peaked on day 12. reHuTpo also mobilized hematopoietic progenitors (maximum, 5.7-fold to tenfold) into peripheral blood. The authors concluded that a single dose of reHuTpo is a potent stimulus for thrombopoiesis. (103). In a further investigation, Vadhan Raj and associates again as-

sessed the ability of reHuTPO to ameliorate chemotherapy-induced severe thrombocytopenia in solid tumors (130). At this phase I/II clinical cohort study, 29 patients with gynecological cancer were given reHuTpo before chemotherapy and after a second cycle of carboplatin therapy. Administration of reHuTPO after chemotherapy significantly reduced the degree and duration of thrombocytopenia and enhanced platelet recovery. In patients who received the optimal biologic dose of reHuTPO (1.2 μg/kg) in cycle 2 (carboplatin plus reHuTPO), the mean platelet count nadir was higher (44×10^9/L and 20×10^9/L; $p=0.002$) and the duration of thrombocytopenia was shorter ($p=0.002$). The need for platelet transfusion in this group was reduced from 75% of patients in cycle 1 to 25% of patients in cycle 2 ($p=0.013$). The authors concluded that reHuTPO could attenuate chemotherapy-induced severe thrombocytopenia and reduce the need for platelet transfusions in thrombocytopenic patients with solid tumors (130). Although reHuTPO administered before chemotherapy (starting from day –5) and after chemotherapy (day 4) appears to reduce the degree of platelet nadir in solid tumor patients, the effect is not uniform and the standard dosage and schedule of the cytokine has not been fully elucidated (64).

Future Perspectives of Thrombopoietic Growth Factors

After the cloning of Tpo in 1994, 2 recombinant thrombopoietic growth factors, namely PEG-reHuMGDF and reHuTPO, have been studied in humans in a variety of clinical settings. Synthetic *c-mpl* ligands have also been developed subsequently. Both thrombopoietins are generally well tolerated if administered intravenously. They produce a dose-related enhancement of platelet levels and may reduce chemotherapy-induced mild thrombocytopenia (4,103,127, 129,130). A single dose of reHuTPO effectively increases platelet counts and reHuTPO has clinical activity as secondary prophylaxis. reHuTPO reduced the depth of platelet nadir and the need for platelet transfusions in patients with advanced gynecologic malignancies (64,103,130). Although recombinant Tpos seem to be effective in nonmyeloablative chemotherapy–associated thrombocytopenia, the optimal dosing and administration schedule are still an unresolved important issue. Tpo as a drug could have the potential to increase the patients' ability to maintain dose-intensive chemotherapy schedules. Tpo appears to be generally not effective in the thrombocytopenia of myeloablative setting especially when hematopoietic/ megakaryocytic stem and progenitor cells are not present in the bone marrow (109,115). The pharmacologic use of Tpo has no augmentation effect on white cell count or hematocrit in clinical trials (131); however, neutralizing antibodies against Tpo (PEG-reHuMGDF) may cause pancytopenia and aplastic anemia (28). The immunogenicity of MGDF may be related to either substantial modification of the variant molecule or subcutaneous (SC) route of administration. The SC route may be detrimental due to antibody formation against Tpo as illustrated in animal studies (100) and human trials (28,101). Therefore, SC administration of thrombopoietins should best be avoided in any clinical setting for the prevention of chronic thrombocytopenia (101) and even pancytopenia (28). If a relatively large dose of PEG-reHuMGDF is administered, a single intravenous (IV) injection may be effective in improving thrombocytopenia possibly due to the stability of PEG-reHuMGDF in the circulation (132). The *c-mpl* ligands can improve apheresis yields when administered to normal platelet donors (105,106). The data on the use PEG-reHuMGDF in the management of thrombocytopenia due to MDS, aplastic anemia, and idiopathic thrombocytopenic purpura are very preliminary but promising (120,122,133). Studies are needed about the use of thrombopoietins in thrombocytopenia of chronic liver diseases. The efficacy of PEG-reHuMGDF in human immunodeficiency virus–infected chimpanzees (134) and postbypass thrombocytopenia in dogs (135) cast hope for the management of thrombocytopenia for those indications in humans. Small peptide agonists mimicking Tpo can stimulate thrombopoiesis with less antigenic stimuli (71,136,137). The clinical development of the fusion protein of IL-3-*c-mpl* ligand (65) was also suspended due to concerns about antibody formation (4). A chimeric dual G-CSF and IL-3 receptor agonist (138) is under investigation for the management of cytopenias including the thrombocytopenia. Recombinant human IL-11 (reHuIL-11, Oprelvekin, Neumega) has been approved by FDA for the treatment of chemotherapy-induced thrombocytopenia (39). Fluid retention, hypervolemic complications, cardiac arrhythmias, and modest efficacy for the improvement of thrombocytopenia are the fundamental problems with recombinant IL-11 (4). The major obstacle for the clinical use of thrombopoietic growth factors is the failure to match the clinical

need for managing thrombocytopenia and the intrinsic biologic functions of thrombopoietins. The effects of Tpo require the presence of hematopoietic and megakaryopoietic progenitors. Furthermore the platelet augmentation of physiologic and pharmacological thrombopoietins are delayed as a consequence of inherent kinetics (139). However, aplastic thrombocytopenia–induced bleeding is a medical emergency and requires urgent intervention. Further experimental and clinical studies with thrombopoietic cytokines will better delineate the place of those recombinant growth factors in the pharmacologic management of thrombocytopenia as well as developing new molecules to reach the aim of a clinically effective and safe 'platelet-augmentation drug.'

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ERRATUM

In the January 2002 issue of *Clinical and Applied Thrombosis/Hemostasis,* "Effects of Extracorporeal Circulation on Thrombin-Antithrombin III and Prothrombin Fragment 1+2 Levels" (Yilmaz M, Haznedaroglu IC, Kirazli S, and Pasaoglu I. *Clin Appl Thrombosis/ Hemostasis* 2002;8:61-63).

On p. 62 under the "Materials and Methods" section, in the first line of the first sentence, the word "sixteen" should be "twenty." The authors regret the error.