Haematologica 1999; 84:158-166 trends in hematology

Recent Advances in Growth Factors

Thrombopoietin: its role from early hematopoiesis to platelet production

FRANÇOISE WENDLING
INSERM U 362, Institut Gustave Roussy, Pavillon de Recherche 1, Villejuif-Cedex, France

ABSTRACT

Background and Objective. Thrombopoietin (TPO), also referred to as Mpl ligand, is the most potent cytokine that physiologically regulates platelet production. With the availability of sufficient amounts of recombinant forms of the protein, the biological *in vitro* and *in vivo* activities of this cytokine have been extensively studied. The objective of this review is to summarize the published data focusing on TPO production and regulation and to discuss the pleiotropic biological action of this hormone. The review also highlights the results so far obtained in preclinical and clinical trials.

Evidence and Information Sources. The material examined in this review includes data published by the author and articles or abstracts published in journals covered by Medline®. The author has contributed to the isolation of TPO, has been working in the field for several years and has contributed original papers on the TPO/Mpl system in normal and pathologic situations.

State of the Art. TPO is a hormone constitutively produced by the liver and kidneys. Plasma levels of TPO are regulated through receptor-mediated uptake, internalization and catabolism. First thought to be a lineage dominant factor promoting megakaryocytopoiesis, several lines of evidence indicate that TPO has pleiotropic effects on hematopoiesis. *In vitro* studies show that TPO alone, or in combination with early acting cytokines, stimulates the proliferation and enhances the expansion of primitive $CD34^+$ $CD38^-$ hematopoietic progenitor cells. *In vivo* studies with *c-mpl*- and *TPO*-null mice reveal that the molecule sustains the survival and proliferation of early committed progenitor cells of various type. Preclinical and clinical trials indicate that recombinant TPO molecules increase platelet counts and megakaryocyte numbers in normal or mildly thrombocytopenic states. However, no significant effects of TPO administration on platelet recovery have so far been reported in patients subjected to intensive chemotherapy regimens. Recombinant molecules appear to be safe to administer and very little toxicity is reported. TPO augments the number of erythroid and myeloid committed progenitor cells in marrow, and mobilized stem cells in peripheral blood.

Perspectives. The potential clinical use of TPO is still unclear. With the increased knowledge of the multiple effects of TPO on hematopoiesis, it is expected that future carefully monitored clinical trials will provide more information regarding the eventual benefits of this cytokine in the treatment of thrombocytopenia. At present, one successful application of TPO appears to be its addition in cytokine cocktails used to expand hematopoietic stem cells *ex vivo*.

Correspondence: Françoise Wendling, Ph.D., INSERM U 362, Institut Gustave Roussy, Pavillon de Recherche 1, 39 rue Camille Desmoulins, 94805 Villejuif-Cedex, France.
Phone: international +33-1-42114671 – Fax: international +33-1-42115240 – E-mail: frog@igr.fr

Key words: thrombopoietin, Mpl ligand, megakaryopoiesis, hematopoiesis, stem cells

Megakaryocytopoiesis is the cellular process of proliferation and differentiation leading to the production of platelets. Back in 1958, it was suggested that this hematopoietic cellular lineage was regulated by a humoral growth factor, termed thrombopoietin,[1] present in plasma from severely thrombocytopenic animals and patients. However, all attempts to purify this molecule remained frustrated. With the isolation of several recombinant cytokines and data obtained mainly from cultures, it was thought that megakaryocytopoiesis was regulated at multiple cellular levels because certain pleiotropic cytokines were able to promote the proliferation of committed megakaryocyte (BFU- and CFU-MK) progenitors, while others primarily induced the differentiation of megakaryoblasts (for reviews, see refs. #2-4). These cytokines are classically divided into 3 groups. Interleukin-3 (IL-3) and granulocyte macrophage-colony stimulating factor (GM-CSF) used alone in culture systems are potent stimulators of MK progenitor proliferation, but have little effect on maturation. The interleukin-6 family members (IL-6, IL-11, oncostatin M and LIF) have virtually no effect on proliferation of MK progenitors, but potentiate the action of IL-3 and GM-CSF by acting predominantly on MK maturation. Stem cell factor (SCF, also known as *c-kit* ligand or *steel factor*) and Flk2/Flt3 ligand (fetal liver kinase) alone have minor effects on MK colony formation, but synergize the proliferative action of several cytokines. Although these cytokines lack specificity for the megakaryocytic lineage, their thrombopoietic effects have been evaluated in clinical trials (for a review see ref. #5).

Isolation of thrombopoietin, the ligand for the proto-oncogene c-mpl

The isolation of the mutant murine myeloproliferative leukemia virus (MPLV) opened a way to the identification of the physiologic regulator of platelet production.[6] This retrovirus has naturally transduced in its genome the *v-mpl* oncogene which corresponds to a truncated version of a cellular gene named *c-mpl*.[7] Sequencing of the human and murine *c-mpl* cDNAs revealed that the polypeptide was a transmembrane receptor, structurally and functionally related to members of the hematopoietic receptor superfamily devoided of intrinsic catalytic domains.[8-11] Subsequently, *c-mpl* expression was shown to be restricted to MK, platelets and $CD34^+$ cells in human marrow populations.[12,13] Exposure of $CD34^+$ purified cells to antisense oligodeoxynucleotides blocking *c-mpl* mRNA expression resulted in a significant inhibition of development of CFU-MK-derived colonies without affecting erythroid or granulocyte/macrophage colony formation.[12] In addition, mice lacking *c-mpl* expression were generated by homologous recombination. The homozygous animals exhibited an 80-90% reduction in platelet counts, but no decrease in the numbers of the other mature blood cells.[14] Together, these observations strongly implicated the Mpl receptor and its putative ligand in the regulation of megakaryocytopoiesis and platelet production.

The isolation of a ligand capable of binding and activating Mpl was reported by five independent groups in 1994.[15-19] This ligand, present in minute amounts in the serum from severely thrombocytopenic animals, received different names: Mpl ligand (ML or Mpl-L), thrombopoietin (TPO), megakaryocyte growth and development factor (MGDF) or megapoietin. The molecule stimulates both the proliferation and differentiation of CFU-MK progenitors arguing against the theory that megakaryopoiesis was regulated at multiple cellular levels.[20] Different recombinant Mpl ligands are used for experimental or clinical investigations. To clarify the terms, TPO should be the name of the physiologic native form of the protein. Mpl-L should be the scientific name for the recombinant polypeptide. A recombinant full-length glycosylated form of the human polypeptide (termed rHuTPO) is produced in mammalian cells by Genentech-Pharmacia-Upjohn.[16] A recombinant non-glycosylated truncated version of the human Mpl-L is produced in *Escherichia coli* by Amgen-Kirin-ZymoGenetics. This protein is termed rHuMGDF.[15] A polyethylene glycol tail is coupled to the polypeptide (PEG-rHuMGDF) to increase its potency and *in vivo* half-life.[21,22]

Structure of the TPO protein and TPO gene

The native human TPO protein is a 60-70 kDa heavily glycosylated polypeptide comprising 332 amino acids. The molecule can be structurally and functionally divided into 2 domains. The amino-terminal domain is highly conserved across species. It contains 153 amino acids showing 23% sequence identity and 50% similarity with erythropoietin (EPO), including 4 conserved cysteine residues (EPO-like domain). The carboxy-terminal domain shows wide species divergence. It contains six potential N-linked and several O-linked glycosylation sites. This domain has no homology with other known proteins.[15-17,23-26] The *EPO-like domain* is sufficient to induce the full spectrum of biological responses *in vitro* and *in vivo*. The carbohydrate domain is required for efficient biosynthesis and secretion, and to increase stability and potency of the protein.[24, 26-28] A crystal structure of native TPO is not yet available. Nevertheless, computer analysis predicts that the N-terminus domain (aa 22-153) forms a four-α-helix bundle similarly to the other members of the cytokine family.[29,30] Library screening of random peptides displayed on filamentous phage has allowed the identification of several agonists for Mpl. A 14-mer peptide covalently dimerized is as potent as recombinant Mpl ligand in promoting megakaryopoiesis *in vitro* and in increasing platelet production in animals.[31,32]

The human TPO gene is mapped to chromosome 3q27-28.[18,24,27,33,34] The gene spans over 8 kb and consists of 5 coding exons and 1 or 2 additional upstream non-coding exons.[14,18,27,35] The promoter region contains no TATA- or CAAT-box motifs and multiple sites for initiation of transcription have been identified.[18,27,36] The molecular mechanisms controlling the expression of the TPO gene are still unknown, although it has been reported that the Ets family member E4TF1/GABP is required for high expression in liver cells.[36]

Because thrombocytosis and dysmegakaryocytopoiesis are usual hematologic features found in AML patients with structural abnormalities of the chromosome 3q26 region, a possible involvement of the TPO gene was searched for. No deregulation was found suggesting that the stimulated thrombocytopoiesis was not due to overexpression of the molecule.[33,34,37]

Physiologic regulation of TPO levels

TPO transcripts are detected in several organs throughout the body, but expression predominates in the liver and kidney.[16,23] *In situ* hybridization techniques have detected TPO mRNA in liver parenchymal and sinusoidal endothelial cells[38,39] and kidney proximal convoluted tubular cells.[40] Biologically active TPO can be demonstrated in culture supernatants from rat primary hepatocytes, mouse sinusoidal endothelial cells, hepatoma cell lines, the human embryonic kidney HEK cell line and bone marrow-derived stroma cells.[39,41-43]

The endogenous TPO levels in plasma are inversely correlated with the platelet counts.[44] By analogy to the transcriptional regulation of *EPO* mRNA by anemia,[45] it was suggested that transcription of the TPO

gene might be upregulated in response to the platelet demand. Alternatively, TPO production might be constant and activity in plasma could be regulated by the binding to platelets and catabolism.[46] Several experimental models were analyzed to discriminate between these possibilities. Mice were made severely thrombocytopenic by radiation, chemotherapy, antiplatelet antibodies injections or combined treatments, or markedly thrombocythemic by platelet transfusions. No transcriptional regulation of the TPO gene was observed in the liver or kidney from these animals.[40,47-49] In addition, no variation in *TPO* alternative splice forms encoding non-secreted proteins was detected.[24,47,48,50,51] Moreover, while homozygous *TPO*-deficient mice show a 90% reduction in their platelet counts, the number of platelets in heterozygous animals is half that of normal littermates.[52] This gene dosage effect on platelet counts strongly argues against a control occurring at a transcriptional level.[52] Nevertheless, it is reported that *TPO* upregulation might occur in marrow stromal cells in response to a decrease in platelet counts.[53,54]

Platelets and MK display a single class of high affinity Mpl receptors (approximately 30 receptors/platelet with a kd of 100 to 200 pmol/L[55] and 2,000-12,140 receptors/MK with a kd of 749 pmol/L[56,57]). Platelets exposed *in vitro* to iodinated Mpl-L actively bind, internalize and degrade the protein.[51,55,58] *C-mpl*-deficient mice have low platelet numbers and elevated TPO levels in plasma.[14] When these mice are transfused with platelets from normal donors, TPO levels rapidly decrease.[51] Surprisingly, no elevation of TPO levels is seen in the plasma of profoundly thrombocytopenic homozygous NF-E2-deficient mice.[59] The marrow from these mice contains numerous mature MK with a well developed demarcation membrane system, but a total absence of platelet shedding. These observations strongly suggest that the MK mass could also be involved in the regulation of the plasma concentration of TPO.[60] ELISA assays[61,62] indicate that serum TPO levels are more correlated with the combined MK and platelet mass than with platelet numbers. In patients with aplastic anemia, amegakaryocytic thrombocytopenia or after bone marrow transplantation, where thrombocytopenia is associated with MK hypoplasia, TPO levels are extremely high.[63,64] In contrast, patients with immune thrombocytopenic purpura (ITP) exhibit normal or only mildly elevated TPO levels.[63-69] Collectively, these data are consistent with a model in which no sensing system controls the production of TPO. From the published data, it appears that TPO is constitutively synthesized by the liver and kidney. It is the binding and degradation by Mpl receptors present on the surface of platelets and MKs that regulate the circulating levels of TPO. However, it has not yet been excluded that TPO might upregulate its own receptors, as is the case for IL-2 and G-CSF receptors.[70,71]

Pleiotropic action of Mpl ligand/TPO

Studies performed *in vitro* demonstrate that Mpl-L is a central cytokine for full development of MK and platelets.[72-80] MKs and platelets produced in culture are morphologically and functionally identical to bone marrow MK and blood-derived platelets[77,79,81-83] However, Mpl-L may be dispensable for the late steps of MK maturation. Indeed, proplatelet formation occurs *in vitro* after Mpl-L deprivation[81,84] and human $CD34^+$ progenitor cells stimulated with a combination of IL-3+IL-6+SCF produce platelets.[83] Furthermore, homozygous *TPO*-deficient mice still produce a low number of functionally normal platelets.[85] The effects of Mpl-L on platelet activation was a major concern. Mpl-L induces phosphorylation of several proteins in platelets,[86-88] but it does not induce spontaneous platelet aggregation. However, platelets stimulated with non-physiologic doses of Mpl-L become more sensitive to activation agonists.[86,87, 89-92]

The obtainment of *c-mpl*- and *TPO*-null mice allowed clear demonstration of the lineage-dominant action of TPO on platelet production.[14,52] The homozygous animals display an identical phenotype with a 80-90% reduction in platelet counts and a markedly decreased number of MKs with a low ploidy in marrow and spleen. Red blood cell and leukocyte counts of homozygous animals are similar to those of wild-type littermates. Nevertheless, *c-mpl*$^{-/-}$ and *TPO*$^{-/-}$ mice show a 60% reduction in the absolute numbers of all myeloid progenitors, including primitive progenitors forming CFU-blast-derived colonies.[93,94] In addition, recently reported results show that progenitor cells exhibiting long-term repopulating ability are significantly reduced in *c-mpl*-null mice and that all the long-term repopulating activity of wild type murine fetal liver progenitor population ($AA4^+$ Sca^+) segregates with Mpl expression.[95] Furthermore, a direct comparison of the reconstituting ability of human $CD34^+$ $CD38^-$ *c-mpl*$^+$ and $CD34^+$ $CD38^-$ *c-mpl*- into NOD/SCID mice shows that *c-mpl* expression correlates with significantly better donor-derived engraftment.[95] This indicates that TPO not only stimulates megakaryopoiesis and platelet production, but has a pleiotropic range of action in hematopoiesis.[96]

Action of TPO on early stem cells

Several *in vitro* studies demonstrate that TPO acts synergistically with the early acting growth factors, Flt3 ligand, c-kit ligand or interleukin-3, to stimulate the proliferation of primitive hematopoietic stem cells directly .[75,97-105] Of potential interest for *ex vivo* expansion of $CD34^+$ cells, it has been shown that progenitors expanded in medium containing TPO in combination with c-kit ligand or Flt3 ligand retain a primitive phenotype, and maintain the capacity for multilineage colony formation.[103,104] In addition, TPO enhances the expansion and survival of $CD34^+$ $CD38^-$ progenitors in culture.[105]

Preclinical and clinical studies

The *in vivo* biological activities of recombinant Mpl-L have been extensively reviewed.[26,74,106-108] In normal animals receiving repeated injections of Mpl-L, platelet numbers increase several-fold but the numbers of red blood cells and leukocytes are not significantly affected. The increment in platelet numbers is preceded by an increase in MK progenitors, and an increase in MK number, volume and ploidy.[22,72] Comparable results are obtained after a single intravenous injection.[109,110]

In mildly thrombocytopenic mice treated with a single injection of carboplatin, rHuMGDF reverses thrombocytopenia.[111] In more severe models of thrombocytopenia induced by a combination of sublethal irradiation and carboplatin, daily administration of PEG-rHuMGDF reduced the severity of the platelet nadir, accelerated platelet recovery and reduced mortality.[21,112] No effect[113] or an accelerated platelet recovery[114] were reported when Mpl-L/PEG-rHuMGDF was given after bone marrow transplantation. Both group of investigators observed that the rate of platelet reconstitution was highly accelerated when donors were pretreated with Mpl-L/PEG-rHuMGDF prior to graft harvest.[113,115] Similar results are obtained in myelosuppressed primates.[22,116-119] In addition to the major effect on platelet recovery, Mpl ligands also dramatically accelerated the recovery of all progenitor classes, improved neutrophil and reticulocyte recovery and mobilized progenitor cells in myelosuppressed mice and monkeys.[21,111, 113,117,120,121]

Results of phase I/II clinical trials with Mpl ligands have been extensively reviewed.[108,122] PEG-rHuMGDF was injected subcutaneously at escalating doses ranging from 0.03 to 1 µg/kg/day during 10 days to patients with advanced cancer before chemotherapy.[123,124] Platelet counts started to increase on day 6, peaked between day 12 and 18 and remained elevated until day 26 for the highest doses of 0.3 and 1 µg/kg. There was no drug-related toxicity and no evidence of ischemia or thromboembolism even in one patient who developed a very high number of platelets (> $1,800 \times 10^9$/L). In another study, patients with sarcoma were given a single intravenous dose of rHuTPO at doses ranging from 0.3 to 2.4 µg/kg. Platelets started to increase in a dose-dependent manner on day 4 and peaked at day 12. No major side effects were reported.[125] In addition, Mpl ligand-treatment expanded marrow myeloid, erythroid and multipotential progenitors and markedly mobilized progenitor cells in blood, but these expansions did not translate into an increment in peripheral leukocytes or red blood cells.[123-126] After chemotherapy, PEG-rHuMGDF was injected daily for up to 16 days to patients with non-small cell lung cancer receiving carboplatin and paclitaxel. Patients experienced a more rapid platelet recovery and a higher nadir platelet count than the placebo group.[127,128] However, in a series of patients with *de novo* AML receiving up to 21 doses (2.5 and 5 µg/kg/day) of PEG-rHuMGDF after the first cycle of chemotherapy, no significant differences in platelet counts were seen between the PEG-rHuMGDF-treated and the placebo group.[129] Patients with sarcoma received one or two intravenous bolus injections of rHuTPO after the second cycle of high dose chemotherapy. A sustained 4-fold increase in platelet counts was observed accompanied by a significant increase of MK in the bone marrow and a mobilization of progenitor cells of multiple lineages in blood. Anti-TPO antibodies were surveyed in the sera from 12 rHuTPO-treated patients. Not neutralizing antibodies were transitorily detected in one patient indicating the need to monitor the administration of these recombinant hormones carefully.[130] Basser *et al.* reported the combined effects of increasing doses of PEG-rHuMGDF (0.03 to 5.0 µg/kg/day) and G-CSF (filgrastim, 5 µg/kg/day) given to 41 patients treated with carboplatin and cyclophosphamide.[131] No difference in the depth of platelet nadir was noted between PEG-rHuMGDF-treated patients and the placebo group. However, platelet recovery to baseline levels was reached 4 days earlier (median of 17 days versus 22 days) in patients treated with PEG-rHuMGDF. It is noteworthy that, when PEG-rHuMGDF was administered both before and after chemotherapy, platelet recovery was hastened as it was during the second cycle of chemotherapy.

These early clinical data indicate that PEG-rHuMGDF and rHuTPO are well tolerated molecules and powerful agents for increasing platelet counts in normal or mildly thrombocytopenic patients. However, in severely thrombocytopenic states induced by intensive regimens, the therapeutic benefit of treatment with either of the Mpl ligands is modest or insignificant. At the present time, clinical trials with PEG-rHuMGDF are discontinued due to evidence of neutralizing antibodies in a few patients.

Involvement of the TPO/Mpl receptor system in human pathology

The TPO/Mpl system has been examined in myeloproliferative disorders with an excess of MKs to understand whether abnormalities could be involved in the pathogenesis of these diseases. In essential thrombocythemia (ET), it has been reported that serum TPO levels are either normal or slightly elevated when compared to those of normal subjects. Given the regulation of TPO plasma levels by the platelet and MK masses, this observation was quite unexpected. Flow cytometry and Western blot analyses indicate that Mpl expression is markedly reduced in platelets from ET patients as compared to platelets from normal subjects.[132,133] Another study shows that expression of Mpl is markedly reduced in platelets from patients with either polycythemia vera (PV) or idiopathic myelofibrosis (PMF), but not in patients with ET.[134] A study performed on a family with hereditary thrombocy-

themia reports that all affected members have elevated serum TPO levels. Genetic analyses demonstrate that a splice donor mutation in the *TPO* gene is responsible for overexpression of the protein due to increased translational efficiency.[135] More recently, we have investigated a possible involvement of the TPO/Mpl system in CD34+ and MK from patients with ET or PMF. We show that, in both diseases, CD34+ progenitor cells produce autonomously developing MK colonies at a single cell level. We were unable to detect autocrine production of TPO in the CD34+ population or in mature MKs despite using a highly sensitive and quantitative RT-PCR technique. In addition, no missense mutation in the coding region of the *c-mpl* gene was detected. Interestingly, addition of soluble Mpl to cultures specifically inhibited the growth of autonomous MK colonies.[136,137] Our data indicate that it seems unlikely that the primary defect in ET and PMF is at the level of the TPO/Mpl loop. More work is needed to understand whether a molecular defect involving a regulatory protein along the signal transduction cascade is involved in these pathologies, as recently suggested for PV.[138] Together, these data provide new insights into the physiopathology of myeloproliferative disorders.

Acknowledgments and Funding

The author's laboratory is supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Institut Gustave Roussy, la Ligue contre le Cancer, the Association de Recherche contre le Cancer (ARC) and the FEGEFLUC.

Disclosures

Conflict of interest: none.

Redundant publication: yes, < 50%.

Manuscript processing

Manuscript received July 31, 1998; accepted October 30, 1998.

References

1. Kelemen E, Cserhati I, Tanos B. Demonstration and some properties of human thrombopoietin in thrombocythemic sera. Acta Haematol (Basel) 1958; 20: 350-5.
2. Gordon MS, Hoffman R. Growth factors affecting human thrombocytopoiesis: potential agents for the treatment of thrombocytopenia. Blood 1992; 80:302-7.
3. Burstein SA. Platelets and cytokines. Curr Opin Hematol 1994; 1:373-80.
4. Vainchenker W, Debili N, Mouthon MA, Wendling F. Megakaryocytopoiesis: Cellular aspects and regulation. Crit Rev Oncol/Hematol 1995; 20:165-92.
5. Wendling F, Han Z-C. Positive and negative regulation of megakaryocytopoiesis. Ballière's Clin Haematol 1997; 10:29-45.
6. Wendling F, Varlet P, Charon M, Tambourin P. MPLV: a retrovirus complex inducing an acute myeloproliferative disorder in mice. Virology 1986; 149:242-6.
7. Souyri M, Vigon I, Penciolelli J-F, Heard J-M, Tambourin P, Wendling F. A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell 1990; 63:1137-47.
8. Bazan JF. Haematopoietic receptors and helical cytokines. Immunol Today 1990; 11:350-4.
9. Vigon I, Mornon J-P, Cocault L, et al. Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: Identification of a member of the hematopoietic growth factor receptor superfamily. Proc Natl Acad Sci USA 1992; 89:5640-4.
10. Vigon I, Florindo C, Fichelson S, et al. Characterization of the murine Mpl proto-oncogene, a member of the hematopoietic cytokine receptor family: molecular cloning, chromosomal location and evidence for a function in cell growth. Oncogene 1993; 8:2607-15.
11. Skoda RC, Seldin DC, Chiang M-K, Peichel CL, Vogt TF, Leder P. Murine c-mpl: a member of the hematopoietic growth factor receptor superfamily that transduces a proliferative signal. EMBO J 1993; 12:2645-53.
12. Methia N, Louache F, Vainchenker W, Wendling F. Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryocytopoiesis. Blood 1993; 82:1395-401.
13. Debili N, Wendling F, Cosman D, et al. The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. Blood 1995; 85:391-401.
14. Gurney AL, Carver-Moore K, de Sauvage FJ, Moore MW. Thrombocytopenia in c-mpl-deficient mice. Science 1994; 265:1445-7.
15. Bartley TD, Bogenberger J, Hunt P, et al. Identification and cloning of a megakaryocyte growth and development factor that its a ligand for the cytokine receptor Mpl. Cell 1994; 77:1117-24.
16. de Sauvage FJ, Hass PE, Spencer SD, et al. Stimulation of megakaryopoiesis and thrombopoiesis by the c-MPL ligand. Nature 1994; 369:533-8.
17. Lok S, Foster DC. The structure, biology and potential therapeutic applications of recombinant thrombopoietin. Stem Cells 1994; 12:586-98.
18. Sohma Y, Akahori H, Seki N, et al. Molecular cloning and chromosomal localization of the human thrombopoietin gene. FEBS Lett 1994; 353:57-61.
19. Kuter DJ, Rosenberg RD. Appearance of a megakaryocyte growth-promoting activity, megapoietin, during acute thrombocytopenia in the rabbit. Blood 1994; 84:1464-72.
20. Wendling F, Maraskovsky E, Debili N, et al. c-Mpl ligand is a humoral regulator of megakaryocytopoiesis. Nature 1994; 369:571-4.
21. Hokom MM, Lancey D, Kinstler OB, et al. Pegylated megakaryocyte growth and development factor abrogates the lethal thrombopenia associated with carboplatin and irradiation in mice. Blood 1995; 86:4486-92.
22. Harker LA, Marzec UM, Hunt P, et al. Dose-response effects of pegylated human megakaryocyte growth and development factor on platelet production and function in nonhuman primates. Blood 1996; 88:511-21.
23. Lok S, Kaushansky K, Holly RD, et al. Cloning and expression of murine thrombopoietin and stimulation of platelet production in vivo. Nature 1994; 369:564-8.
24. Gurney AL, Kuang W-J, Xie M-H, Malloy BE, Eaton DL, de Sauvage FJ. Genomic structure, chromosomal localization, and conserved alternative splice forms of thrombopoietin. Blood 1995; 85:981-8.
25. Hoffman RC, Andersen H, Walker K, et al. Peptide, disulfide, and glycosylation mapping of recombinant human thrombopoietin from Ser1 to Arg246. Bio-

chemistry 1996; 35:14849-61.
26. Eaton DL, de Sauvage FJ. Thrombopoietin: The primary regulator of megakaryocytopoiesis and thrombopoiesis. Exp Hematol 1997; 25:1-7.
27. Chang M-S, McNinch J, Basu R, et al. Cloning and characterization of the human megakaryocyte growth and development factor (MGDF) gene. J Bioch Chem 1995; 270:511-4.
28. Foster D, Lok S. Biological roles for the second domain of thrombopoietin. Stem Cells 1996; 14:102-7.
29. Drachman JG, Kaushansky K. Structure and function of the cytokine receptor superfamily. Curr Opin Hematol 1995; 2:22-8.
30. Park H, Park SS, Jin EH, et al. Identification of functionally important residues of human thrombopoietin. J Biol Chem 1998; 273:256-61.
31. Cwirla SE, Balasubramanian P, Duffin DJ, et al. Peptide agonist of the thrombopoietin receptor as potent as the natural cytokine. Science 1997; 276:1696-9.
32. Dower WJ, Cwirla SE, Balasubramanian P, Schatz PJ, Baccanari D, Barrett RW. Peptide agonists of the thrombopoietin receptor. Stem Cells 1998; 16 (suppl 2):21-9.
33. Suzukawa K, Satoh H, Taniwaki M, Yokota J, Morishita K. The human thrombopoietin gene is located on chromosome 3q26.33-q27, but is not transcriptionally activated in leukemia cells with 3q21 and 3q26 abnormalities (3q21q26 syndrome). Leukemia 1995; 9:1328-31.
34. Schnittger S, de Sauvage FJ, Le Paslier D, Fonatsch C. Refined chromosomal localization of the human thrombopoietin gene to 3q27-q28 and exclusion as the responsible gene for thrombocytosis in patients with rearrangements of 3q21 and 3q26. Leukemia 1996; 10:1891-6.
35. Foster DC, Sprecher CA, Kramer JM, et al. Human thrombopoietin: gene structure, cDNA sequence, expression and chromosomal localization. Proc Natl Acad Sci USA 1994; 91:13023-7.
36. Kamura T, Handa H, Hamasaki N, Kitajima S. Characterization of the human thrombopoietin gene promoter. J Biol Chem 1997; 272:11361-8.
37. Bouscary D, Fontenay-Roupie M, Chretien S, et al. Thrombopoietin is not responsible for the thrombocytosis observed in patients with acute myeloid leukemias and the 3q21q26 syndrome. Br J Haematol 1995; 91:425-7.
38. Nomura S, Ogami K, Tsukamoto I, et al. Cellular localization of thrombopoietin mRNA in the liver by in situ hybridization. Exp Haemat 1997; 25:565-72.
39. Cardier JE, Dempsey J. Thrombopoietin and its receptor, c-mpl, are constitutively expressed by mouse liver endothelial cells: Evidence of thrombopoietin as a growth factor for liver endothelial cells. Blood 1998; 91:923-9.
40. Sungaran R , Markovic B, Chong BH. Localization and regulation of thrombopoietin mRNA expression in human kidney, liver, bone marrow and spleen using in situ hybridization. Blood 1996; 89:101-7.
41. Shimada Y, Kato T, Ogami K, et al. Production of thrombopoietin (TPO) by rat hepatocytes and hepatoma cell lines. Exp Hematol 1995; 23:1388-96.
42. McDonald TP, Wendling F, Vainchenker W, Mc Carty JM, Jorgensen MJ, Kaushansky K. Thrombopoietin from human embryonic kidney cells is the same factor as c-mpl-Ligand. Blood 1995; 85:292-4.
43. Guerriero A, Worford L, Holland HK, Guo G-R, Sheedan K, Waller EK. Thrombopoietin is synthesized by bone marrow stromal cells. Blood 1997; 90:3444-55.
44. Kuter DJ, Rosenberg RD. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. Blood 1995; 85:2720-30.
45. Bondurant MC, Koury M. Anemia induces accumulation of erythropoietin mRNA in the kidney and liver. Mol Cell Biol 1986; 6:2731-9.
46. de Gabriele G, Pennington DG. Regulation of platelet production. Br J Haematol 1967; 13:210-7.
47. Stoffer R, Wiestner A, Skoda RC. Thrombopoietin in thrombocytopenic mice: Evidence against regulation at the mRNA level and for a direct regulatory role of platelets. Blood 1996; 87:567-73.
48. Cohen-Solal K, Villeval J-L, Titeux M, Lok S, Vainchenker W, Wendling F. Constitutive expression of Mpl ligand transcript during thrombocytopenia and thrombocytosis. Blood 1996; 88:2578-84.
49. Nagata Y, Shozaki Y, Nagahisa H, Nagasawa T, Abe T, Todokaro K. Serum thrombopoietin level is not regulated by transcription but by the total counts of both megakaryocytes and platelets during thrombocytopenia and thrombocytosis. Thromb Haemost 1997; 77:808-14.
50. Chang M-S, Hsu R-Y, McNinch J, Copeland NG, Jenkins NA. The gene for murine megakaryocyte growth and development factor (Thrombopoietin, Thpo) is located on mouse chromosome 16. Genomics 1995; 26:636-7.
51. Fielder PJ, Gurney AL, Stefanich E, et al. Regulation of thrombopoietin levels by c-mpl-mediated binding to platelets. Blood 1996; 87:2154-61.
52. de Sauvage FJ, Carver-Moore K, Luoh S-M, et al. Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. J Exp Med 1996; 183:651-6.
53. McCarthy JM, Sprugel KH, Fox NE, Sabath DE, Kaushansky K. Murine thrombopoietin mRNA levels are modulated by platelet count. Blood 1995; 86: 3668-75.
54. Hirayama Y, Sakamaki T, Matsunaga T, et al. Concentrations of thrombopoietin in bone marrow in normal subjects and in patients with idiopathic thrombocytopenic purpura, aplastic anemia, and essential thrombocythemia correlate with its mRNA expression of bone marrow stromal cells. Blood 1998; 92:46-52.
55. Broudy V, Lin N, Sabath D, Papayannopoulou T, Kaushansky K. Human platelets display high-affinity receptors for thrombopoietin. Blood 1997; 89:1896-904.
56. Kuwaki T, Hagiwara T, Yuki C, Kodama I, Kato T, Miyazaki H. Quantitative analysis of thrombopoietin receptors on human megakaryocytes. FEBS Lett 1998; 427:46-50.
57. Sato T, Fuse A, Niimi H, Fielder PJ, Avraham H. Binding and regulation of thrombopoietin to human megakaryocytes. Br J Haematol 1998; 100:704-11.
58. Fielder P, Hass P, Nagel M, et al. Human platelets as a model for the binding and degradation of thrombopoietin. Blood 1997; 89:2782-8.
59. Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, et al. Transcription factor NF-E2 is required for platelet formation independent of actions of thrombopoietin/ MGDF in megakaryocyte development. Cell 1995; 81:695-704.
60. Shivdasani RA, Fielder P, Keller G-A, Orkin SH, de Sauvage FJ. Regulation of the serum concentration of thrombopoietin in thrombocytopenic NF-E2 knockout mice. Blood 1997; 90:1821-7.
61. Tahara T, Usuki K, Sato H, et al. A sensitive sandwich ELISA for measuring thrombopoietin in human serum: Serum thrombopoietin levels in healthy volunteers and in patients with haematopoietic disorders. Br J Haematol 1996; 93:783-8.

62. Folman CC, von dem Borne AE, Rensink IH, et al. Sensitive measurement of thrombopoietin by a monoclonal antibody based sandwich enzyme-linked immunosorbent assay. Thromb Haemostas 1997; 78:1262-7.
63. Emmons RVB, Reid DM, Cohen RL, et al. Human thrombopoietin levels are high when thrombocytopenia is due to megakaryocyte deficiency and low when due to increased platelet destruction. Blood 1996; 87:4068-71.
64. Mukai HY, Kojima H, Todokoro K, et al. Serum thrombopoietin (TPO) levels in patients with amegakaryocytic thrombocytopenia are much higher than those with immune thrombocytopenic purpura. Thromb Haemost 1996; 76:675-8.
65. Meng YG, Martin TG, Peterson ML, Shuman MA, Cohen RL, Wong WL. Circulating thrombopoietin concentrations in thrombocytopenic patients, including cancer patients following chemotherapy, with or without peripheral blood progenitor cell transplantation. Br J Haematol 1996; 95:535-41.
66. Marsh JCW, Gibson FM, Prue RL, et al. Serum thrombopoietin levels in patients with aplastic anaemia. Br J Haematol 1996; 95:605-10.
67. Kosugi S, Kutara Y, Tomiyama Y, et al. Circulating thrombopoietin level in chronic immune thrombocytopenic purpura. Br J Haematol 1996; 93:704-6.
68. Ichikawa N, Ishida F, Shimodaira S, Tahara T, Kato T, Kitano K. Regulation of serum thrombopoietin levels by platelets and megakaryocytes in patients with aplastic anaemia and idiopathic thrombocytopenia purpura. Thromb Haemost 1996; 76:156-60.
69. Porcelijn L, Folman CC, Bossers B, et al. The diagnostic value of thrombopoietin level measurements in thrombocytopenia. Thromb Haemost 1998; 79:1101-5.
70. Depper JM, Leonard WJ, Drogula C, Kronke M, Waldmann TA, Greene WC. Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. Proc Natl Acad Sci USA 1985; 82:4230-5.
71. Steinman RA, Tweardy DJ. Granulocyte colony-stimulating factor receptor mRNA upregulation is an immediate early marker of myeloid differentiation and exibits dysfunctional regulation in leukemic cells. Blood 1994; 83:119-27.
72. Kaushansky K, Lok S, Holly RD, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand, thrombopoietin. Nature 1994; 369:568-71.
73. Kaushansky K. Thrombopoietin: The primary regulator of platelet production. Blood 1995; 86:419-31.
74. Kaushansky K, Broudy VC, Lin N, et al. Thrombopoietin, the Mpl ligand, is essential for full megakaryocyte development. Proc Natl Acad Sci USA 1995; 92:3234-8.
75. Zeigler FC, de Sauvage F, Widmer HR, et al. In vitro megakaryocytopoietic and thrombopoietic activity of c-mpl ligand (TPO) on purified murine hematopoietic stem cells. Blood 1994; 84:4045-52.
76. Choi ES, Nichol JL, Hokom MM, Hornkohl AC, Hunt P. Platelets generated in vitro from proplatelet-displaying human megakaryocytes are functional. Blood 1995; 85:402-13.
77. Choi ES, Hokom M, Bartley T. Recombinant human megakaryocyte growth and development factor (rHuMGDF), a ligand for c-Mpl, produces functional human platelets in vitro. Stem cells 1995; 13:317-22.
78. Debili N, Wendling F, Katz A, et al. The Mpl-Ligand or thrombopoietin or megakaryocyte growth and development factor has both direct proliferative and differentiative activities on human megakaryocyte progenitors. Blood 1995; 86:2516-25.
79. Choi E. Regulation of proplatelet and platelet formation in vitro. In: Kuter DJ, Hunt P, Sheridan W, Zucker-Franklin D, eds. Thrombopoiesis and thrombopoietins. Totowa, N J Humana Press, 1996. p. 271-84.
80. Zucker-Franklin D, Kaushansky K. Effects of thrombopoietin on the development of megakaryocytes and platelets. Blood 1996; 88:1632-40.
81. Choi ES, Hokom MM, Chen J-L, et al. The role of megakaryocyte growth and development factor in terminal stages of thrombopoiesis. Br J Haematol 1996; 95:227-33.
82. Cramer E, Norol F, Guichard J, et al. Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand. Blood 1997; 89:2326-46.
83. Norol F, Vitrat N, Cramer E, et al. Effects of cytokines on platelet production from blood and marrow CD34+ cells. Blood 1998; 91:830-4.
84. Ito T, Ishida Y, Kuriya S-I. Recombinant human c-mpl ligand is not a direct stimulator of proplatelet formation in mature human megakaryocytes. Br J Haematol 1996; 94:387-90.
85. Bunting S, Widmer R, Lipari T, et al. Normal platelets and megakaryocytes are produced in vivo in the absence of thrombopoietin. Blood 1997; 90:3423-9.
86. Miyakawa Y, Oda A, Druker BJ, et al. Recombinant thrombopoietin induces rapid protein tyrosine phosphorylation of Janus Kinase 2 and Shc in human blood platelets. Blood 1995; 86:23-7.
87. Miyakawa Y, Oda A, Druker BJ, et al. Thrombopoietin induces tyrosine phosphorylation of Stat3 and Stat5 in human blood platelets. Blood 1996; 87:439-46.
88. Miyakawa Y, Oda A, Drucker B, et al. Thrombopoietin and thrombin induce tyrosine phosphorylation of Vav in human blood platelets. Blood 1997; 89:2789-98.
89. Oda A, Miyakawa Y, Druker BJ, et al. Thrombopoietin primes human platelet aggregation induced by shear stress and by multiple agonists. Blood 1996; 87:4664-70.
90. Chen J, Herced-Harjacek L, Groopman JE, Grabarek J. Regulation of platelets activation in vitro by the c-Mpl ligand, thrombopoietin. Blood 1995; 86:4054-62.
91. Toombs CF, Young CH, Glaspy JA, Varnum BC. Recombinant human megakaryocyte growth and development factor (MGDF) moderately enhances ex vivo platelet aggregation by a receptor dependent mechanism. Thromb Res 1995; 80:23-33.
92. Montrucchio G, Brizzi MF, Calosso G, Marengo S, Pegoraro L, Camussi G. Effects of recombinant human megakaryocyte growth and development factor on platelet activation. Blood 1996; 87:2762-8.
93. Alexander WS, Roberts AW, Nicola NA, LI R, Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietin receptor *c-mpl*. Blood 1996; 87:2162-70.
94. Carver-Moore K, Broxmeyer HE, Luoh S-M, et al. Low levels of erythroid and myeloid progenitors in thrombopoietin- and c-mpl-deficient mice. Blood 1996; 88: 803-8.
95. Solar GP, Kerr WG, Ziegler FC, et al. Role of c-mpl in early hematopoiesis. Blood 1998; 92:4-10.
96. Kaushansky K. Thrombopoietin and the hematopoietic stem cell. Blood 1998; 92:1-3.
97. Ku H, Yonemura Y, Kaushansky K, Ogawa M. Thrombopoietin, the ligand for the Mpl receptor, synergizes with Steel Factor and other early acting cytokines in supporting proliferation of primitive hematopoietic progenitors of mice. Blood 1996; 87:4544-51.
98. Sitnicka E, Lin N, Priestley GV, et al. The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells. Blood 1996; 87:4998-5005.

99. Kobayashi M, Laver JH, Kato T, Miyazaki H, Ogawa M. Thrombopoietin supports proliferation of human primitive hematopoietic cells in synergy with steel factor and/or interleukin-3. Blood 1996; 88:429-36.
100. Petzer AL, Zanstra PW, Piret JM, Eaves CJ. Differential cytokine effects on primitive (CD34+ CD38-) human hematopoietic cells: novel response to flt-3 ligand and thrombopoietin. J Exp Med 1996; 183:2551-7.
101. Ramsfjell V, Borge OJ, Veiby OP, et al. Thrombopoietin, but not erythropoietin, directly stimulates multilineage growth of primitive murine bone marrow progenitor cells in synergy with early acting cytokines: Distinct interactions with the ligands for c-kit and FLT3. Blood 1996; 88:4481-92.
102. Ohmizono Y, Sakabe H, Kimura T, et al. Thrombopoietin augments ex vivo expansion of human cord blood-derived hematopoietic progenitors in combination with stem cell factor and flt3 ligand. Leukemia 1997; 11:524-30.
103. Piacibello W, Sanavio SF, Garetto L, et al. Extensive amplification and self-renewal of human primitive hematopoietic stem cells from cord blood. Blood 1997; 89:2644-53.
104. Ramsfjell V, Borge OJ, Cui L, Jacobsen SEW. Thrombopoietin directly and potently stimulates multilineage growth and progenitor cell expansion from primitive (CD34+ CD38-) human bone marrow progenitor cells. J Immunol 1997; 158:5169-74.
105. Borge OJ, Ramsfjell V, Cui L, Jacobsen SEW. Ability of early acting cytokine to directly promote survival and suppress apoptosis of human primitive CD34+ CD38- bone marrow cells with multilineage potential at the single cell level: Key role of thrombopoietin. Blood 1997; 90:2282-9.
106. Alexander WS, Begley CG. Thrombopoietin in vitro and in vivo. Cytokines Cell Mol Ther 1998; 4:25-34.
107. Alexander WS. Cytokines in hematopoiesis. Int Rev Immunol 1998; 16:651-82.
108. Miyazaki H. Update on thrombopoietin in preclinical and clinical trials. Curr Opin Hematol 1998; 5:197-202.
109. Arnold JT, Daw NC, Stenberg PE, Jayawardene D, Srivastava DK, Jackson CW. A single injection of pegylated murine megakaryocyte growth and development factor (MGDF) into mice is sufficient to produce a profound stimulation of megakaryocyte frequency, size and ploidy. Blood 1997; 89:1683-7.
110. Daw NC, Arnold JT, Abushullaih BA, et al. A single intravenous dose of murine megakaryocyte growth and development factor potently stimulates platelet production, challenging the necessity for daily administration. Blood 1998; 91:466-74.
111. Ulich TR, del Castillo J, Yin S, et al. Megakaryocyte growth and development factor ameliorates carboplatin-induced thrombocytopenia in mice. Blood 1995; 86:971-6.
112. Thomas GR, Thibodeaux H, Erret CJ, et al. In vivo biological effects of various forms of thrombopoietin in a murine model of transient thrombocytopenia. Stem Cells 1996; 14:244-51.
113. Fibbe WE, Heemskerk DPM, Laterveer L, et al. Accelerated reconstitution of platelets and erythrocytes after syngeneic transplantation of bone marrow cells derived from thrombopoietin pretreated donor mice. Blood 1995; 86:3308-13.
114. Molineux G, Hartley CA, McElroy P, McCrea C, McNiece IK. Megakaryocyte growth and development factor stimulates enhanced platelet recovery in mice after bone marrow transplantation. Blood 1996; 88:1509-14.
115. Molineux G, Hartley C, McElroy P, McCrea C, McNiece IK. Megakaryocyte growth and development factor accelerates platelet recovery in peripheral blood progenitor cell transplant recipients. Blood 1996; 88:366-76.
116. Farese AM, Hunt P, Boone T, MacVittie TJ. Recombinant human megakaryocyte growth and development factor stimulates thrombocytopoiesis in normal nonhuman primates. Blood 1995; 86:54-9.
117. Farese AM, Hunt P, Grab LB, MacVittie TJ. Combined administration of recombinant human megakaryocyte growth and development factor and granulocyte colony-stimulating factor enhances multilineage hematopoietic reconstitution in nonhuman primates after radiation-induced marrow aplasia. J Clin Invest 1996; 97:2145-51.
118. Harker LA, Hunt P, Marzec UM, et al. Regulation of platelet production and function by megakaryocyte growth and development factor in nonhuman primates. Blood 1996; 87:1833-44.
119. Harker LA, Marzec UM, Kelly AB, et al. Prevention of thrombocytopenia and neutropenia in a nonhuman primate model of marrow suppressive chemotherapy by combining pegylated recombinant human megakaryocyte growth and development factor and recombinant human granulocyte colony-stimulating factor. Blood 1997; 89:155-65.
120. Kaushansky K, Broudy VC, Grossmann A, et al. Thrombopoietin expands erythroid progenitors, increases red cell production, and enhances erythroid recovery after myelosuppressive therapy. J Clin Invest 1995; 96:1683-7.
121. Grossmann A, Lenox J, Deisher TA, et al. Synergistic effects of thrombopoietin and granulocyte colony-stimulating factor on neutrophil recovery in myelosuppressed mice. Blood 1996; 88:3363-70.
122. Begley CG. Clinical studies with megakaryocyte growth and development factor (Mpl-ligand). Thromb Haemostas 1997; 78:42-6.
123. Basser RL, Rasko JEJ, Clarke K, et al. Thrombopoietic effects of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHUMGDF) in patients with advanced cancer. Lancet 1996; 348:1279-81.
124. O'Malley CJ, Rasko RL, Basser RL, et al. Administration of pegylated recombinant human megakaryocyte growth and development factor to humans stimulates the production of functional platelets that show no evidence of in vitro activation. Blood 1996; 88:3288-98.
125. Vadjan-Raj S, Murray LJ, Bueso-Ramos C, et al. Stimulation of megakaryocyte and platelet production by a single dose of recombinant human thrombopoietin in patients with cancer. Ann Intern Med 1997; 126: 673-81.
126. Rasko JE, Basser RL, Boyd J, et al. Multilineage mobilization of peripheral blood progenitor cells in humans following administration of PEG-rHuMGDF. Br J Haematol 1997; 97:871-88.
127. Fanucchi M, Glapsy J, Crawfold J, et al. Safety and biological effects of pegylated megakaryocyte growth and development factor in lung cancer patients receiving carboplatin and paclitaxel: Randomised, placebo-controlled phase I study. Proc Am Soc Clin Oncol 1996; 15:720-6.
128. Fanucchi MG, Glapsy J, Crawford J, et al. Effects of polyethylene glycol-conjugated recombinant human megakaryocyte growth and development factor on platelet counts after chemotherapy for lung cancer. N Engl J Med 1997; 336:404-9.
129. Archimbaud E, Ottmann O, Lui Yin JA, et al. A randomised, double-blind, placebo-controlled study using PEG-rhuMGDF as an adjunct to chemotherapy for adults with de-novo acute myeloid leukemia

(AML): early results. Blood 1996; 88 (Suppl 1):447.
130. Vadhan-Raj S, Patel S, Broxmeyer HE, et al. Phase I-II investigation of recombinant human thrombopoietin (rhTPO) in patients with sarcoma receiving high dose chemotherapy (CT) with adriamycin (A) and ifosfamide (I). Blood 1996; 88(suppl 1):448.
131. Basser RL, Rasko JE, Clarke K, et al. Randomized, blinded, placebo-controlled phase I trial of pegylated recombinant human megakaryoctyte growth and development factor with filgrastim after dose-intensive chemotherapy in patients with advanced cancer. Blood 1997; 89:3118-28.
132. Li J, Xia Y, Kuter DJ. Analysis of the thrombopoietin receptor (Mpl) on platelets from normal and essential thrombocythemic (ET) patients. Blood 1996; 88:545-52.
133. Horikawa Y, Matsumura I, Hashimoto K, et al. Markedly reduced expression of platelet c-mpl receptor in essential thrombocythemia. Blood 1997; 90: 4031-8.
134. Moliterno AR, Hankins WD, Spivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with polycythemia vera. N Engl J Med 1998; 338:572-80.
135. Wiestner A, Schlemper RJ, van der Maas APC, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. Nature Genetics 1998; 18:49-52.
136. Taksin A-L, Cohen-Solal K, Le Couedic J-P, et al. Myelofibrosis: Experimental models and human studies. Stem Cells 1998; 16 (suppl 2):155-64.
137. Taksin A-L, Le Couedic J-P, Massé A, et al. Autonomous megakaryocyte growth in essential thrombocythemia and idiopathic myelofibrosis is not related to a c-mpl mutation or to an autocrine stimulation by Mpl-L. Blood 1999; 93:125-39.
138. Dai CH, Krantz SB, Sawyer ST. Polycythemia vera. V. Enhanced proliferation and phosphorylation due to vanadate are diminished in polycythemia vera erythroid progenitor cells: A possible defect of phosphatase activity in polycythemia vera. Blood 1997; 89:3574-81.