REVIEW ARTICLE

MECHANISMS OF DISEASE

Lineage-Specific Hematopoietic Growth Factors

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Hematopoiesis is the process that generates blood cells of all lineages. Calculations based on the blood volume and the level and half-life of each type of blood cell in the circulation indicate that each day an adult produces approximately 200 billion erythrocytes, 100 billion leukocytes, and 100 billion platelets. Moreover, these rates can increase by a factor or 10 or more when the demand for blood cells increases.

In 1906, Carnot[1] found that injecting healthy rabbits with serum from anemic animals prompted a rapid increase of erythrocytes in the recipients. The responsible humoral substance was first termed "hemopoietine" and was later named "erythropoietin." In 1957, Jacobson and colleagues[2] identified the kidney as the source of erythropoietin; in 1985, the factor was purified and its gene was cloned, thereby making it available for physiological study and therapeutic use.[3] In a similar fashion, humoral substances that support the production of leukocytes[4] and platelets[5] were defined, cloned, and studied. Simultaneously with these advances, a model of blood-cell production was constructed in which hematopoiesis was envisioned as a hierarchical progression of multipotential hematopoietic stem cells that gradually lose one or more developmental options, becoming progenitor cells committed to a single lineage; these progenitors then mature into the corresponding types of peripheral-blood cells (Fig. 1).

Lineage-Specific Hematopoietic Growth Factors

Growth factors are required for the survival and proliferation of hematopoietic cells at all stages of development (Fig. 1). Of the factors that affect multipotential cells, steel factor, Fms-like tyrosine kinase 3 (FLT3) ligand, granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin-2, interleukin-3, and interleukin-7 are the best characterized. Each of these proteins supports the survival and proliferation of a number of distinct target cells, and with the exception of interleukin-7 and steel factor, the elimination of any one of them does little harm because of the redundancy in the functions of these early-acting growth factors.

Erythropoietin

Blood levels of erythropoietin (normal, 20 mU per milliliter) are inversely related to tissue oxygenation — the level can increase up to 20,000 mU per milliliter in response to anemia or arterial hypoxemia.[6] The juxtatubular interstitial cells of the renal cortex, which produce approximately 90 percent of the erythropoietin in blood (Fig. 2), sense oxygen levels through a newly identified, oxygen-dependent prolyl hydroxylase that regulates the stability of the primary transcription factor for erythropoietin, hypoxia-inducible factor 1α (HIF-1α). Once hydroxylated, HIF-1α binds the von Hippel–Lindau (VHL) protein, thereby targeting it for ubiquitin-mediated de-

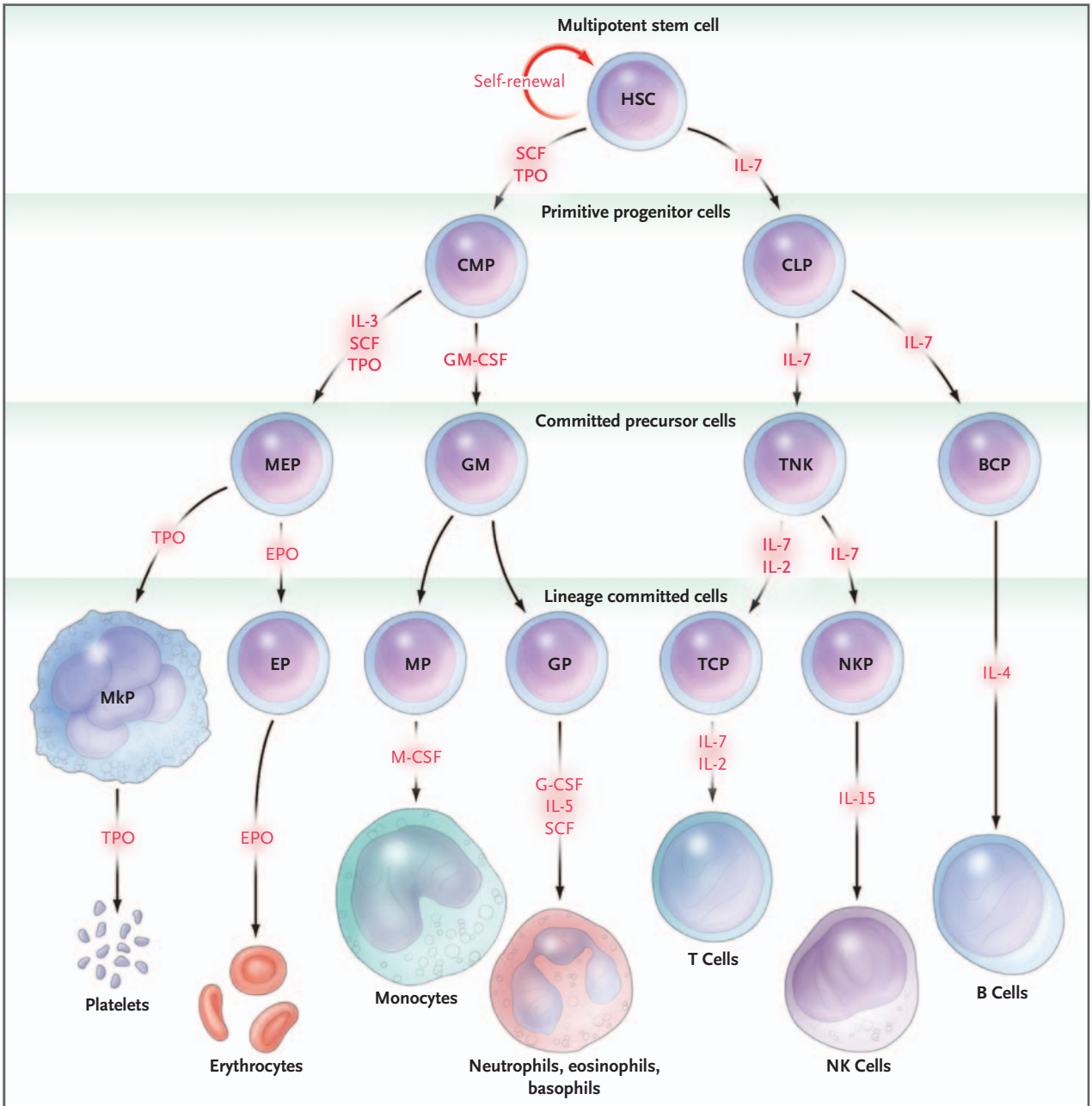

Figure 1. A General Model of Hematopoiesis.

Blood-cell development progresses from a hematopoietic stem cell (HSC), which can undergo either self-renewal or differentiation into a multilineage committed progenitor cell: a common lymphoid progenitor (CLP) or a common myeloid progenitor (CMP). These cells then give rise to more-differentiated progenitors, comprising those committed to two lineages that include T cells and natural killer cells (TNKs), granulocytes and macrophages (GMs), and megakaryocytes and erythroid cells (MEPs). Ultimately, these cells give rise to unilineage committed progenitors for B cells (BCPs), NK cells (NKPs), T cells (TCPs), granulocytes (GPs), monocytes (MPs), erythrocytes (EPs), and megakaryocytes (MkPs). Cytokines and growth factors that support the survival, proliferation, or differentiation of each type of cell are shown in red. For simplicity, the three types of granulocyte progenitor cells are not shown; in reality, distinct progenitors of neutrophils, eosinophils, and basophils or mast cells exist and are supported by distinct transcription factors and cytokines (e.g., interleukin-5 in the case of eosinophils, stem-cell factor [SCF] in the case of basophils or mast cells, and G-CSF in the case of neutrophils). IL denotes interleukin, TPO thrombopoietin, M-CSF macrophage colony-stimulating factor, GM-CSF granulocyte-macrophage CSF, and EPO erythropoietin.

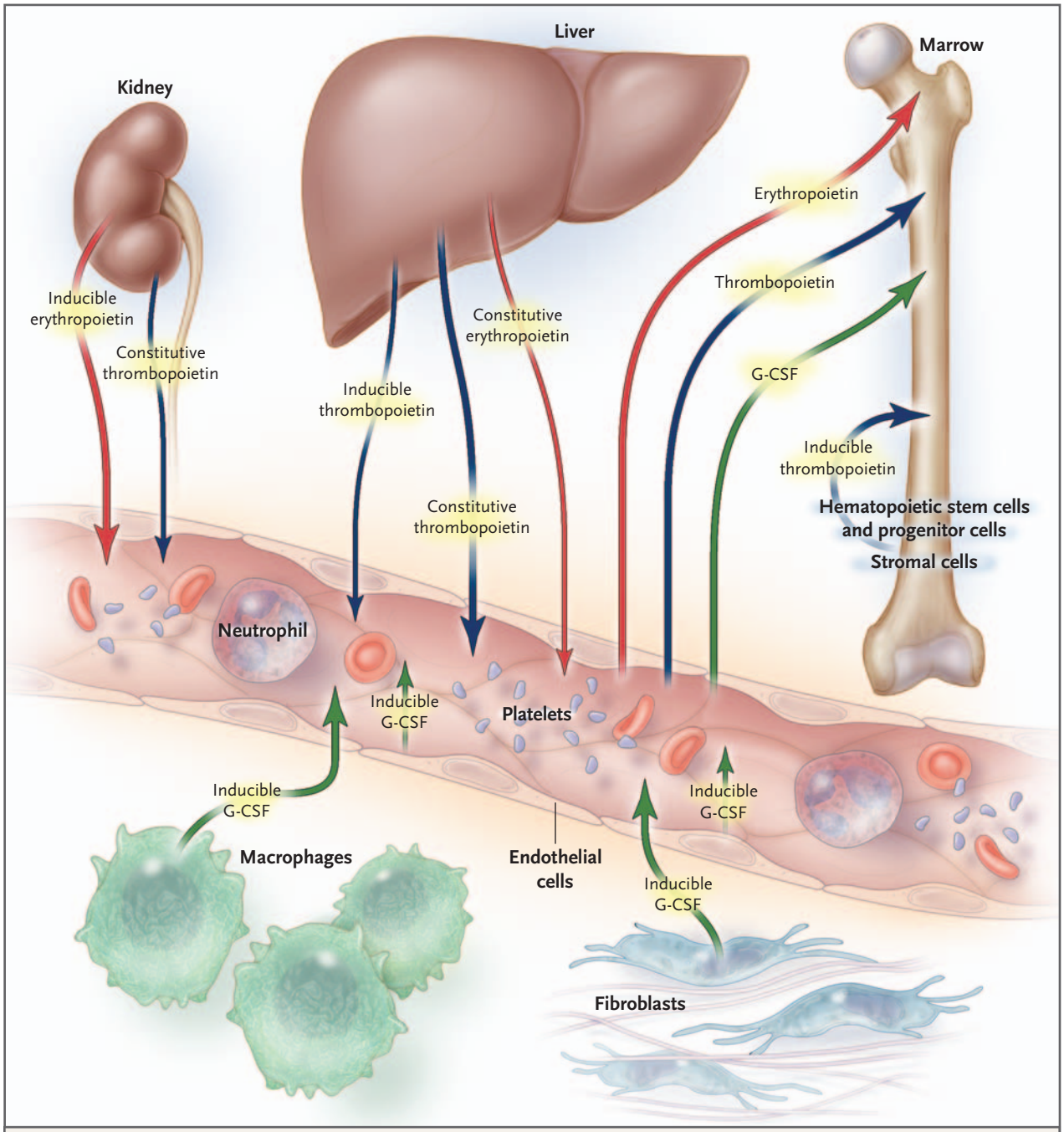

Figure 2. Regulation of the Production of Hematopoietic Growth Factors.

The production of growth factors by various tissues and cells (arrows) is both constitutive and inducible and subject to adsorption by receptor-bearing cells. Production of erythropoietin (red arrows) is inducible by hypoxia in the kidney and constitutive in the liver (10 percent of total body erythropoietin), and it is excreted in the urine. G-CSF (green arrows) is mostly inducible in tissues from fibroblasts and macrophages and from endothelial cells by inflammatory mediators (interleukin-1, interleukin-6, and tumor necrosis factor α. Thrombopoietin (blue arrows) is produced constitutively by the liver (accounting for approximately 50 percent of baseline thrombopoietin levels) and kidney (accounting for a minority of the total amount) and is inducible in the liver by inflammatory mediators (especially interleukin-6) and from bone marrow stromal cells by thrombocytopenia. In addition, both G-CSF and thrombopoietin are removed from the circulation by receptor-mediated uptake and destruction by the mature cells that bear the corresponding receptors on neutrophils and platelets. Arrows indicate the production and secretion of hematopoietic growth factors.

struction.[7] Under conditions of low oxygen tension, the hydroxylase is inactive; consequently, HIF-1α is not hydroxylated and cannot bind VHL, thereby maintaining its stability and capacity to drive the production of erythropoietin. This mechanism accounts for the erythrocytosis associated with high-altitude, heart or lung disease leading to hypoxemia and hemoglobin variants with a low affinity for oxygen. The discovery of a familial form of erythrocytosis in the Chuvashian population of the capital of the Chuvash Republic, Shupashkar (in Russian, Chebokshary), is of considerable interest because affected members of these families have a missense mutation in the *VHL* gene and high erythropoietin levels.[8]

Erythropoietin promotes the proliferation of erythroid progenitor cells by reducing the level of cell-cycle inhibitors and augmenting transcription of cyclins and supports their survival by increasing the antiapoptosis protein $BCLX_L$.[6] Elimination of the erythropoietin gene or its receptor in mice causes severe anemia and death.[9] The administration of erythropoietin to animals or humans increases the number of erythroid progenitor cells, which differentiate into normoblasts, enucleate, and leave the bone marrow.[6] Within one or two weeks after the administration of the hormone, the reticulocyte count rises because of a large increase in red-cell production and the premature exit of erythrocytes from the bone marrow.

Erythropoietin was initially assumed to be an erythroid-specific hormone, but messenger RNA for erythropoietin and its receptor is readily detectable in neuronal and glial cells of the central nervous system and in the retina.[10,11] Moreover, the hormone protects neurons from noxious stimuli[12] and induces the proliferation of neurons after neuronal injury.[13] Preclinical trials have shown that exogenously administered erythropoietin crosses the blood–brain barrier and exerts a protective effect on ischemic neurons. As compared with control treatment, erythropoietin was associated with an improvement in outcome scales and infarct size in experimental stroke[14,15] and in a single clinical trial.[16] Should additional clinical studies confirm these results, an important new application for an "erythroid-specific" hormone will become available.

GRANULOCYTE COLONY-STIMULATING FACTOR

Granulocyte colony-stimulating factor (G-CSF) has been identified as a factor that stimulates the growth of neutrophil colonies when added to cultures of murine or human marrow cells in a semisolid medium. In contrast, GM-CSF stimulates the production of neutrophils, eosinophils, basophils, monocytes, and dendritic cells in culture and activates most of these types of mature cells. The gene for G-CSF was cloned in 1987, and the recombinant protein was assessed in clinical trials soon thereafter.[4] Genetic studies in knockout mice demonstrated that G-CSF is essential for normal neutrophil production and for the leukocytosis that occurs in inflammation,[17] whereas elimination of GM-CSF affected only the function and number of alveolar macrophages.

G-CSF supports the survival and stimulates the proliferation of neutrophil progenitors and promotes their differentiation into mature neutrophils.[4,18] In addition, the cytokine causes premature release of neutrophils from the bone marrow and enhances the phagocytic capacity, generation of superoxide anions, and bacterial killing by these cells. The administration of G-CSF causes toxic granulation of neutrophils in the peripheral blood, which is a morphologic correlate of their heightened functional state, and a leftward shift (immaturity) in the leukocyte differential count. Activation of neutrophils in the bone marrow by G-CSF causes them to release matrix metalloproteases. This effect helps to explain the mobilization of hematopoietic stem cells out of the bone marrow in response to the administration of G-CSF,[19] a finding that has greatly facilitated the collection of hematopoietic stem cells for transplantation.

Endothelial cells, fibroblasts, and macrophages in virtually all organs produce G-CSF (Fig. 2). Inflammatory cytokines such as tumor necrosis factor α, interleukin-1, and interleukin-6, derived from activated monocytes, stimulate G-CSF production, helping to explain the leukocytosis in patients with infection or inflammation.[20]

THROMBOPOIETIN

Thrombopoietin is the primary regulator of platelet production.[5] It supports the survival and proliferation of megakaryocyte progenitors by initiating many of the mechanisms that are triggered by erythropoietin and G-CSF, including increased expression of the cell-cycle regulator cyclin D and the antiapoptosis molecule $BCLX_L$ and suppression of the cell-cycle inhibitor $p27^{Kip}$. In vitro, thrombopoietin induces the differentiation of progenitor cells into large megakaryocytes, each one capable

of producing thousands of platelets. Thrombopoietin, a potent stimulator of platelet production, causes thrombocytosis when administered to animals or humans. It also primes platelets to aggregate in response to otherwise subthreshold levels of thrombin, collagen, or adenosine diphosphate by activating the phosphoinositol 3-kinase signaling pathway in platelets.[21] In mice, elimination of the thrombopoietin gene or its receptor reduces the level of production of megakaryocytes and platelets to approximately 10 percent of normal.[22]

In addition to stimulating megakaryocytopoiesis, thrombopoietin has an important and nonredundant role in the survival and expansion of hematopoietic stem cells. When tested alone or in combination with other cytokines in vitro, thrombopoietin supports the survival and proliferation of hematopoietic stem cells.[23] Moreover, genetic elimination of thrombopoietin or its receptor in mice reduces by a factor of 7 to 8 the number of stem cells with the capacity to repopulate the bone marrow and decreases by a factor of 17 the expansion in the number of hematopoietic stem cells that occurs after transplantation.[24,25] The effects of thrombopoietin on stem cells are mediated by enhanced expression or nuclear localization of several transcription factors — signal transducers and activators of transcription, homeobox B4, and homeobox A9 — and by autocrine production of vascular endothelial growth factor.[26] As a result of these studies, virtually every current attempt in the laboratory to expand hematopoietic stem cells includes thrombopoietin or a thrombopoietin mimetic in the culture medium, and current clinical trials of thrombopoietic agents are designed to detect the panhematopoietic effects that were seen in the preclinical trials of the hormone.

The cause of congenital amegakaryocytic thrombocytopenia has been traced to a deficiency of the thrombopoietin receptor.[27] In children with this disorder, aplastic anemia develops within one to five years after birth because of stem-cell exhaustion, consistent with the effects of thrombopoietin on hematopoietic stem cells. Thrombopoietin levels in blood are inversely related to the number of platelets in the blood and megakaryocytes in the bone marrow[5]; unlike erythropoietin production, however, the regulation of thrombopoietin levels is multifaceted (Fig. 2). At least half the production of thrombopoietin occurs in the liver,[28] but the kidney and skeletal muscles also produce the hormone. Mature platelets and megakaryocytes display thrombopoietin receptors and are capable of removing the hormone from the circulation.[29] Thus, one mechanism of the inverse relationship between the platelet count and the thrombopoietin level is adsorption of the hormone from the circulation, a finding that can explain how platelet transfusions delay the rate of recovery from chemotherapy-related thrombocytopenia.[30] In addition to these steady-state mechanisms of thrombopoietin production and catabolism, stromal cells in the bone marrow increase production of the hormone substantially in states of severe thrombocytopenia.[31,32] Furthermore, hepatic thrombopoietin production can be enhanced by inflammatory mediators such as interleukin-6,[33,34] providing a molecular explanation for the common finding of reactive thrombocytosis in rheumatoid arthritis, Crohn's disease, and several other inflammatory and infectious conditions.[35]

HEMATOPOIETIC GROWTH FACTOR RECEPTORS

The cytokine-receptor superfamily consists of the cell-surface receptors for erythropoietin, thrombopoietin, several colony-stimulating factors, most interleukins, and several structurally related cytokines and hormones (e.g., growth hormone).[36] Each of these transmembrane proteins includes one or two extracellular cytokine-binding domains containing approximately 200 amino acids, a transmembrane domain of 20 to 25 residues, and an intracellular domain of approximately 100 to 500 amino acids with the box 1 and box 2 motifs that recruit kinases of the Janus kinase (JAK) family (Fig. 3). The functional unit of the erythropoietin, G-CSF, and thrombopoietin receptors is a homodimer[37]; the engagement of a single molecule of hematopoietic growth factor by a homodimeric receptor induces a major conformational shift in the receptor, bringing the two tethered cytoplasmic JAKs into close juxtaposition, thereby triggering activation of the kinases by mutual cross-phosphorylation. Mutations of the thrombopoietin receptor that affect its transmembrane region have been identified in patients with congenital thrombocytosis.[38] These mutations probably cause conformational shifts in receptor geometry similar to those triggered by thrombopoietin binding.

Once the JAKs are activated by the lineage-specific hematopoietic growth factors, a number of secondary signaling molecules are phosphorylated, and these initiate numerous events that

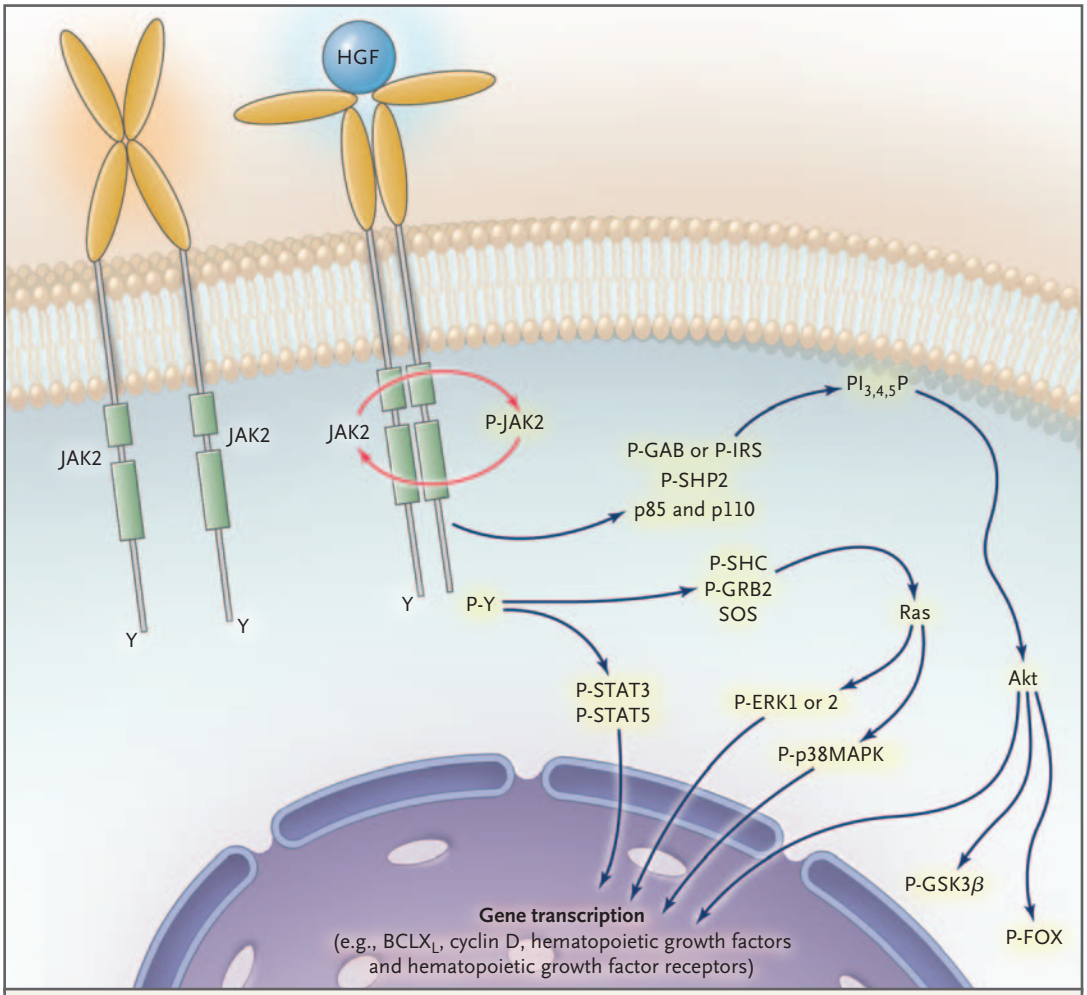

Figure 3. Hematopoietic Growth Factor Signaling.

Each hematopoietic growth factor–receptor dimer is composed of two subunits consisting of one or two 200-amino-acid motifs (each oval represents 100 amino acids), which bind two molecules of Janus kinase 2 (JAK2) but assume an open conformation (left) in the absence of the cognate growth factor. Ligand binding substantially changes the conformation of the receptor, bringing the cytoplasmic domains into close juxtaposition and allowing the tethered kinases to cross-phosphorylate (activate) each other. One of the first substrates for activated JAK2 is receptor tyrosine (Y) residues; when these are phosphorylated (P), they serve as docking sites for adaptors (SHC [Src homology containing], GRB2 [growth factor receptor–bound protein 2], GAB [Grb binding], and IRS [insulin-receptor substrate]), phosphatases (SHP2 [Shc homology domain containing phosphatase]), G-protein activators (SOS [son of seven-less]), and lipid kinases (p85 and p110 and phosphoinositol 3-kinase). Additional effectors of growth factor signaling include signal transducer and activator of transcription 3 and 5 (STAT3 and STAT5, respectively), membrane phospholipids (phosphoinositol 3,4,5-phosphate [$PI_{3,4,5}P$]), the small guanine nucleotide–binding protein Ras, and the kinases AKT, p38 mitogen-activated protein kinase (MAPK), extracellular response–stimulated kinase (ERK) 1 and 2, phosphatase forkhead (P-FOX), and phosphatase glycogen synthase kinase 3β (P-GSK3β). Eventually, most such signals affect nuclear transcription of antiapoptosis molecules ($BCLX_L$), cell-cycle regulators (cyclins), and additional growth factors and their receptors. Arrows indicate signaling pathways.

promote cell survival, proliferation, and differentiation. The cytokine-signaling pathways we know most about entail activation of the signal transducer and activator of transcription factors,[39] phosphoinositol-3 kinase,[40] and mitogen-activated protein kinase,[41] each of which activates an overlapping subgroup of tertiary signaling molecules (Fig. 3). Of these, transcription factors (homeobox, Ets, and forkhead box O[42-44]), cell-cycle activators (cyclins[45]) and inhibitors ($p27^{Kip}$),[46] anti-

apoptosis molecules ($BCLX_L$ and inhibitor of apoptosis[47]), and other growth factors (transforming growth factor β[48] and vascular endothelial growth factor[49]) are ultimately responsible for the effects induced by the binding of a hematopoietic growth factor to its receptor. In addition to these mediators of cell proliferation, the activation of hematopoietic growth factor receptors instigates three events that terminate the growth signal: internalization of receptors, activation of phosphatases, and production of suppressors of cytokine signaling.[50] An interesting finding is that a form of familial erythrocytosis that differs from Chuvash erythrocytosis is due to truncation of the erythropoietin receptor, which eliminates an important, phosphatase-binding negative signaling region.[51]

Numerous signaling kinases in hematopoietic cells have been implicated in pathologic processes. The ABL kinase is persistently activated in patients with chronic myelogenous leukemia[52]; continual activation is also a feature of the FLT3 receptor tyrosine kinase or the JAK2 in patients with acute myelogenous leukemia, the platelet-derived growth factor receptor α tyrosine kinase in those with chronic eosinophilic leukemia, and the platelet-derived growth factor receptor β kinase in patients with chronic myelomonocytic leukemia.[53] In most cases, the aberrantly activated kinase mimics a kinase that is activated by a hematopoietic growth factor; many of the signaling pathways triggered by erythropoietin, G-CSF, and thrombopoietin are also constitutively active in the leukemic cells.

Recently, a change in a single amino acid in the regulatory domain of *JAK2* (in which phenylalanine replaces valine at position 617 [V617F]) was found in hematopoietic cells from most patients with polycythemia vera and from approximately half the patients with essential thrombocythemia or idiopathic myelofibrosis.[54] Molecular modeling predicts that the mutation destabilizes the inactive form of enzyme, thereby causing constitutive activity and signaling. Consistent with this model are the observations that several signaling molecules that mediate the actions of hematopoietic growth factors are constitutively active in hematopoietic cells from patients with polycythemia vera and essential thrombocythemia and that bone marrow cells from such patients are hypersensitive to growth factors in vitro. This work on the *JAK2* mutation has led to improved diagnostic tests for these myeloproliferative disorders. But there is much more work to do, including identifying the cell-surface receptors with which the mutant kinase associates, determining the mechanism by which the same point mutation leads to three phenotypically distinct diseases, deciding whether patients with polycythemia vera or essential thrombocythemia and the mutant kinase differ from those who do not, and developing a specific inhibitor of the mutant JAK2.

FORMS OF AND CLINICAL USES FOR HEMATOPOIETIC GROWTH FACTORS

In principle, there are four general indications for the administration of a hematopoietic growth factor: correction of cytopenia owing to the deficiency of a growth factor, stimulation of the recovery of hematopoietic stem and progenitor cells in iatrogenic and naturally occurring deficiency states, augmentation of hematopoiesis to compensate for pathologically rapid blood-cell destruction, and activation of mature hematopoietic cells. Although the use of these agents in states of growth-factor deficiency (e.g., erythropoietin in the anemia of renal insufficiency) is widely accepted, the efficacy of hematopoietic growth factors in the other three conditions was, a priori, uncertain. However, it is now clear from the results of controlled clinical trials that this group of agents is effective in a variety of clinical states.

ERYTHROPOIETIN

Erythropoietin was approved for use by the Food and Drug Administration in 1989. The initial clinical trial leading to its approval was for treatment of anemia due to chronic renal insufficiency.[55] Thereafter, its use quickly spread to the treatment of anemia owing to many other causes, making it one of the most frequently prescribed products of recombinant DNA technology. Erythropoietin or its derivatives have been administered to treat anemia in over 3 million patients worldwide, including those in intensive care units, those about to undergo surgery, and those undergoing therapy for infection with the human immunodeficiency virus or hepatitis C virus.

Anemia is often multifactorial in patients with cancer. Its causes include suppression of hematopoiesis by the tumor, therapy-induced myelosuppression, and the anemia of chronic inflammation. For these reasons, the effectiveness of epoetin

therapy in patients with cancer and the degree of anemia at which the hormone could be of benefit were uncertain. These questions have been addressed in several controlled trials encompassing nearly 2000 patients with cancer-related anemia. The trials have established that patients with a hemoglobin level of 10 g per deciliter or less benefit from epoetin therapy; they require fewer blood transfusions and have an enhanced sense of well-being. Evidence-based guidelines on the use of epoetin in patients with cancer have been published by the American Society of Hematology and the American Society of Clinical Oncology[56] and by the European Organisation for the Research and Treatment of Cancer,[57] and recent studies have reinforced the validity of a treatment threshold of 10 g of hemoglobin per deciliter. Treatment with epoetin is not risk-free, however. Intensive therapy in patients with head and neck or breast cancer has been associated with hypertension, bleeding, and an increased risk of thrombotic complications.[58,59]

The response to exogenous epoetin is often determined by the patient's ability to produce erythropoietin in response to anemia; one reason that patients with anemia associated with cancer have a response to exogenous epoetin is that they are producing an insufficient amount of endogenous erythropoietin.[60] Conversely, administration of the hormone does not ameliorate anemia in patients with hematologic cancer in whom the blood level of erythropoietin exceeds 500 mIU per milliliter.[61] Administration of epoetin is effective in patients undergoing myelosuppressive chemotherapy because chemotherapy induces apoptosis in both cancer cells and erythroid progenitors and epoetin blocks apoptosis of the erythroid progenitors.[6]

Eliciting a clinical response to a recombinant protein depends on the body's ability to avoid inducing an immune response to the protein. There had been almost no reports of adverse immunologic responses to epoetin for more than a decade, but between 1998 and 2003, pure red-cell aplasia due to antibodies against erythropoietin developed in over 200 patients with end-stage renal disease who were treated with one preparation of recombinant erythropoietin.[62] Immunosuppressive therapy restored responsiveness to the recombinant hormone in about two thirds of the patients. Although the cause of the antierythropoietin antibodies in this condition continues to be explored, partial denaturation of the erythropoietin probably provoked the immune response.

G-CSF

Neutropenia does not in itself cause symptoms, but it predisposes patients to infection, especially if the neutrophil count falls below 500 per cubic millimeter and persists longer than 10 to 14 days. In patients receiving chemotherapy, the administration of G-CSF can lessen the incidence and severity of neutropenia, and in some settings, it can reduce the mortality rate. Moreover, with the recognition that the cytokine mobilizes hematopoietic stem cells from the bone marrow into the peripheral blood,[63] the use of G-CSF to aid in the collection of stem cells for transplantation has now made harvesting of bone marrow for this indication nearly obsolete.

The use of G-CSF in patients receiving myelosuppressive chemotherapy has been evaluated by several expert panels. It is recommended for primary prophylaxis against neutropenia only if the risk of febrile neutropenia historically exceeds 40 percent for the planned chemotherapeutic protocol.[64] Of the 200 most commonly used chemotherapeutic protocols, only 12 are associated with a risk of febrile neutropenia of at least 20 percent.[65] In populations in which the risk is at least 40 percent, such as patients 65 years of age or older who are undergoing aggressive chemotherapy for lymphoma, patients with small-cell carcinoma of the lung or uroepithelial tumors, and patients of any age who have received chemotherapy for acute myelogenous leukemia, G-CSF therapy has proved effective in avoiding the risk of severe neutropenia.[66,67] It is also recommended as secondary prophylaxis in patients with solid tumors who have had chemotherapy-induced febrile neutropenia and who continue to receive intensive chemotherapy, if a dose reduction will impair the antitumor response.

The use of G-CSF in patients undergoing chemotherapy has been linked to an increased risk of treatment-related myeloid leukemia. Initially, the increased risk was seen only after therapy with topoisomerase II inhibitors,[68] but more recent studies indicate that patients treated for breast cancer with cyclophosphamide and doxorubicin may have an increased risk of myelodysplasia or acute myelogenous leukemia if they have also received G-CSF.[69] What might explain this association? Chemotherapy given for a specific cancer

may induce otherwise lethal mutations in a myeloid stem cell or progenitor cell, but the antiapoptotic effect of G-CSF saves the mutant cell from destruction, thereby allowing it to develop into a myeloid cancer. Clearly, additional study is necessary to clarify the risk of acute myelogenous leukemia in patients who receive G-CSF chemotherapy and to determine the mechanism of the association.

The ability to use G-CSF to mobilize hematopoietic stem cells from the bone marrow into the peripheral blood has represented an important advance in hematopoietic stem-cell transplantation.[63] As compared with transplantation of bone marrow cells, the use of G-CSF–mobilized stem cells that originate in the peripheral blood reduces the duration of neutropenia after the administration of the myeloablative preparatory regimen and the need for platelet and red-cell transfusions, reducing both the need for hospitalization and costs. However, the value of the use of the cytokine in recipients of stem-cell transplants to further shorten the duration of neutropenia has not been established.[70]

The administration of G-CSF to patients with congenital neutropenia or cyclic neutropenia has revolutionized the care of children with these disorders, since these conditions were usually fatal before the advent of recombinant G-CSF. The number of neutrophilic progenitor cells is severely reduced in both conditions as a result of mutations of the neutrophil elastase gene.[71] Administration of G-CSF increases neutrophil levels substantially, allowing these children to remain free of life-threatening infections.

Despite this progress, G-CSF has been found to increase the risk of acute myelogenous leukemia in patients with congenital neutropenia. Before recombinant G-CSF was available for clinical use, there were occasional reports of acute myelogenous leukemia or myelodysplasia in patients with congenital neutropenia who survived infancy. Since the introduction of G-CSF, patients with congenital neutropenia live longer, but a malignant myeloid disorder develops in approximately 10 percent.[72] The gene for the G-CSF receptor in the malignant bone marrow cells from these patients usually displays a somatic mutation that eliminates one or more growth inhibitory signals,[73] akin to the truncation in the erythropoietin receptor in patients with familial erythrocytosis.[51] Therefore, it is likely that the administration of G-CSF to patients with congenital neutropenia selects for cells with a mutation in the G-CSF receptor that enhances the proliferation of myeloid cells.

THROMBOPOIETIN

The gene for thrombopoietin was cloned 12 years ago, but the protein has not yet been approved for clinical use. Nevertheless, results of a number of clinical trials point to its eventual clinical utility. It is likely that a thrombopoietic agent will be useful to accelerate platelet recovery after moderately intensive chemotherapy,[74] but not after the intensive chemotherapy necessary for treating acute myelogenous leukemia, or at least with the use of the dosing strategies tested thus far.[75] It can also augment the number of hematopoietic stem cells mobilized by G-CSF[76] and increase the number of platelets available for apheresis from platelet donors.

The potential for the clinical use of recombinant thrombopoietin was irreversibly altered by a study to determine whether the hormone could improve platelet yields from platelet donors. The thrombopoietin used in most of the clinical trials that were conducted between 1995 and 1998 was a truncated form of the protein produced in *Escherichia coli* and modified with polyethylene glycol. Termed "megakaryocyte growth and differentiation factor" (MGDF), the recombinant protein and native thrombopoietin have important differences that probably explain an important adverse effect. Native thrombopoietin contains three sites of serine glycosylation, which are not present in the truncated recombinant protein produced in bacteria. Moreover, because MGDF is a truncated version of thrombopoietin, the C-terminal amino acid of MGDF is not normally a terminal residue. Hence, several nonnative structures (potential neoepitopes) were created in MGDF. When administered subcutaneously to platelet donors, some of the donors produced antibodies against MGDF that cross-reacted with endogenous thrombopoietin, thereby causing severe thrombocytopenia.[77] This adverse event — an important lesson in the engineering of recombinant proteins meant for clinical use — led to the abandonment of the use of MGDF and full-length forms of thrombopoietin as therapeutic proteins.

The experience with MGDF instigated a search for molecules that bind to and stimulate the thrombopoietin receptor on hematopoietic cells. Sophisticated screening techniques have led to the dis-

covery of several small-molecule thrombopoietin mimics that bind and stimulate the thrombopoietin receptor. These agents are undergoing clinical testing. The results of a recent trial of a molecule carrying four copies of a thrombopoietin receptor–binding peptide on an immunoglobulin scaffold point to the potential clinical utility of a thrombopoietin-receptor agonist.[78]

The bone marrow of patients with immune thrombocytopenic purpura usually contains numerous megakaryocytes, reflecting an attempt to compensate for the thrombocytopenia. The abundance of megakaryocytes in these patients argued against an approach involving stimulation of the bone marrow to raise platelet levels. However, thrombopoietin levels are not elevated in patients with immune thrombocytopenic purpura, which raises the possibility that thrombopoietin therapy could be advantageous in such patients. In a recent phase 2 trial,[78] nearly 80 percent of patients with refractory immune thrombocytopenic purpura had a response to the thrombopoietin agonist AMG531, a peptide mimic of the hormone. This observation is evidence that pharmacologic levels of growth factors can increase hematopoiesis above the levels attained in a maximal physiologic response.

CONCLUSIONS

The clinical use of hematopoietic growth factors has improved the care of patients with inadequate blood-cell production, but these agents are costly, require parenteral administration, and can occasionally induce untoward effects. Recent work has focused on understanding and exploiting unexpected biologic activities of hematopoietic growth factors, identifying small-molecule mimics of these proteins, reducing their costs, and eliminating adverse effects. In the clinical setting, we need to be careful to restrict their use to indications that emerge from controlled clinical trials. On the basis of current knowledge, we may soon enter an era in which oral agents that stimulate hematopoiesis become part of routine care in patients with anemia, neutropenia, thrombocytopenia, or all these conditions as a result of natural or iatrogenic causes.

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REFERENCES

1. Carnot P, Deflandre C. Sur l'activité hematopoiëtique du sérum au cours de la régénération du sang. C R Acad Sci Med 1906;3:384-6.
2. Jacobson LO, Goldwasser E, Fried W, Plzak L. Role of the kidney in erythropoiesis. Nature 1957;179:633-4.
3. Jacobs K, Shoemaker C, Rudersdorf R, et al. Isolation and characterization of genomic and cDNA clones of human erythropoietin. Nature 1985;313:806-10.
4. Welte K, Gabrilove J, Bronchud MH, Platzer E, Morstyn G. Filgrastim (r-metHuG-CSF): the first 10 years. Blood 1996;88:1907-29.
5. Kaushansky K. Thrombopoietin. N Engl J Med 1998;339:746-54.
6. Krantz SB. Erythropoietin. Blood 1991;77:419-34.
7. Ratcliffe PJ, O'Rourke JF, Maxwell PH, Pugh CW. Oxygen sensing, hypoxia-inducible factor-1 and the regulation of mammalian gene expression. J Exp Biol 1998;201:1153-62.
8. Pastore YD, Jelinek J, Ang S, et al. Mutations in the VHL gene in sporadic apparently congenital polycythemia. Blood 2003;101:1591-5.
9. Wu H, Liu X, Jaenisch R, Lodish HF. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell 1995;83:59-67.
10. Digicaylioglu M, Bichet S, Marti HH, et al. Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc Natl Acad Sci U S A 1995;92:3717-20.
11. Juul SE, Yachnis AT, Christensen RD. Tissue distribution of erythropoietin and erythropoietin receptor in the developing human fetus. Early Hum Dev 1998;52:235-49.
12. Brines ML, Ghezzi P, Keenan S, et al. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci U S A 2000;97:10526-31.
13. Kretz A, Happold CJ, Marticke JK, Isenmann S. Erythropoietin promotes regeneration of adult CNS neurons via Jak2/Stat3 and PI3K/AKT pathway activation. Mol Cell Neurosci 2005;29:569-79.
14. Lu D, Mahmood A, Qu C, Goussev A, Schallert T, Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. J Neurotrauma 2005;22:1011-7.
15. Chang YS, Mu D, Wendland M, et al. Erythropoietin improves functional and histological outcome in neonatal stroke. Pediatr Res 2005;58:106-11.
16. Ehrenreich H, Hasselblatt M, Dembowski C, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 2002;8:495-505.
17. Lieschke GJ, Grail D, Hodgson G, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood 1994;84:1737-46.
18. Spiekermann K, Roesler J, Emmendoerffer A, Elsner J, Welte K. Functional features of neutrophils induced by G-CSF and GM-CSF treatment: differential effects and clinical implications. Leukemia 1997;11:466-78.
19. van Os R, van Schie ML, Willemze R, Fibbe WE. Proteolytic enzyme levels are increased during granulocyte colony-stimulating factor-induced hematopoietic stem cell mobilization in human donors but do not predict the number of mobilized stem cells. J Hematother Stem Cell Res 2002;11:513-21.
20. Hareng L, Hartung T. Induction and regulation of endogenous granulocyte colony-stimulating factor formation. Biol Chem 2002;383:1501-17.
21. Kojima H, Shinagawa A, Shimizu S, et al. Role of phosphatidylinositol-3 kinase and its association with Gab1 in throm-

bopoietin-mediated up-regulation of platelet function. Exp Hematol 2001;29:616-22.
22. 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.
23. 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.
24. Solar GP, Kerr WG, Zeigler FC, et al. Role of c-mpl in early hematopoiesis. Blood 1998;92:4-10.
25. Fox N, Priestley GV, Papayannopoulou T, Kaushansky K. Thrombopoietin expands hematopoietic stem cells after transplantation. J Clin Invest 2002;110:389-94.
26. Kaushansky K. Thrombopoietin and the hematopoietic stem cell. Ann N Y Acad Sci 2005;1044:139-41.
27. Ballmaier M, Germeshausen M, Schulze H, et al. c-mpl Mutations are the cause of congenital amegakaryocytic thrombocytopenia. Blood 2001;97:139-46.
28. Qian S, Fu F, Li W, Chen Q, deSauvage FJ. Primary role of the liver in thrombopoietin production shown by tissue-specific knockout. Blood 1998;92:2189-91.
29. Yang C, Li YC, Kuter DJ. The physiological response of thrombopoietin (c-Mpl ligand) to thrombocytopenia in the rat. Br J Haematol 1999;105:478-85.
30. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. Transfus Med Rev 2004;18:153-67.
31. McCarty JM, Sprugel KH, Fox NE, Sabath DE, Kaushansky K. Murine thrombopoietin mRNA levels are modulated by platelet count. Blood 1995;86:3668-75.
32. 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 1997;89:101-7.
33. Wolber EM, Jelkmann W. Interleukin-6 increases thrombopoietin production in human hepatoma cells HepG2 and Hep3B. J Interferon Cytokine Res 2000;20:499-506.
34. Kaser A, Brandacher G, Steurer W, et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. Blood 2001;98:2720-5.
35. Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. J Intern Med 1999;245:295-300.
36. Cosman D. The hematopoietin receptor superfamily. Cytokine 1993;5:95-106.
37. Livnah O, Stura EA, Middleton SA, Johnson DL, Jolliffe LK, Wilson IA. Crystallographic evidence for preformed dimers of erythropoietin receptor before ligand activation. Science 1999;283:987-90.
38. Ding J, Komatsu H, Wakita A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood 2004;103:4198-200.
39. Ihle JN, Kerr IM. JAKs and STATs in signaling by the cytokine receptor superfamily. Trends Genet 1995;11:69-74.
40. Miyakawa Y, Rojnuckarin P, Habib T, Kaushansky K. Thrombopoietin induces phosphoinositol 3-kinase activation through SHP2, Gab, and insulin receptor substrate proteins in BAF3 cells and primary murine megakaryocytes. J Biol Chem 2001;276:2494-502.
41. Miura Y, Miura O, Ihle JN, Aoki N. Activation of the mitogen-activated protein kinase pathway by the erythropoietin receptor. J Biol Chem 1994;269:29962-9.
42. Kirito K, Fox NE, Kaushansky K. Thrombopoietin stimulates Hoxb4 expression: an explanation for the favorable effects of TPO on hematopoietic stem cells. Blood 2003;102:3172-8.
43. Mora-Garcia P, Sakamoto KM. Granulocyte colony-stimulating factor induces Egr-1 up-regulation through interaction of serum response element-binding proteins. J Biol Chem 2000;275:22418-26.
44. Dijkers PF, Medema RH, Pals C, et al. Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27(KIP1). Mol Cell Biol 2000;20:9138-48.
45. Henry MK, Nimbalkar D, Hohl RJ, Quelle FW. Cytokine-induced phosphoinositide 3-kinase activity promotes Cdk2 activation in factor-dependent hematopoietic cells. Exp Cell Res 2004;299:257-66.
46. Bouscary D, Pene F, Claessens YE, et al. Critical role for PI 3-kinase in the control of erythropoietin-induced erythroid progenitor proliferation. Blood 2003;101:3436-43.
47. Socolovsky M, Fallon AE, Wang S, Brugnara C, Lodish HF. Fetal anemia and apoptosis of red cell progenitors in Stat5a−/−5b−/− mice: a direct role for Stat5 in Bcl-X(L) induction. Cell 1999;98:181-91.
48. Yanagida M, Ide Y, Imai A, et al. The role of transforming growth factr-beta in PEG-rHuMGDF-induced reversible myelofibrosis in rats. Br J Haematol 1997;99:739-45.
49. Kirito K, Fox NE, Komatsu N, Kaushansky K. Thrombopoietin enhances expression of vascular endothelial cell growth factor (VEGF) in primitive hematopoietic cells through induction of HIF-1α. Blood 2005;105:4258-63.
50. Starr R, Willson TA, Viney EM, et al. A family of cytokine-inducible inhibitors of signalling. Nature 1997;387:917-21.
51. de la Chapelle A, Traskelin AL, Juvonen E. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. Proc Natl Acad Sci U S A 1993;90:4495-9.
52. Mauro MJ, Druker BJ. Chronic myelogenous leukemia. Curr Opin Oncol 2001;13:3-7.
53. Chan IT, Gilliland DG. Oncogenic K-ras in mouse models of myeloproliferative disease and acute myeloid leukemia. Cell Cycle 2004;3:536-7.
54. Kaushansky K. On the molecular origins of the chronic myeloproliferative disorders: it all makes sense. Blood 2005;105:4187-90.
55. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin: results of a combined phase I and II clinical trial. N Engl J Med 1987;316:73-8.
56. Rizzo JD, Lichtin AE, Woolf SH, et al. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. Blood 2002;100:2303-20.
57. Bokemeyer C, Aapro MS, Courdi A, et al. EORTC guidelines for the use of erythropoietic proteins in anaemic patients with cancer. Eur J Cancer 2004;40:2201-16.
58. Henke M, Laszig R, Rübe C, et al. Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. Lancet 2003;362:1255-60.
59. Leyland-Jones B. Breast cancer trial with erythropoietin terminated unexpectedly. Lancet Oncol 2003;4:459-60.
60. Miller CB, Jones RJ, Piantadosi S, Abeloff MD, Spivak JL. Decreased erythropoietin response in patients with the anemia of cancer. N Engl J Med 1990;322:1689-92.
61. Cazzola M, Messinger D, Battistel V, et al. Recombinant human erythropoietin in the anemia associated with multiple myeloma or non-Hodgkin's lymphoma: dose finding and identification of predictors of response. Blood 1995;86:4446-53.
62. Bennett CL, Cournoyer D, Carson KR, et al. Long-term outcome of individuals with pure red cell aplasia and antierythropoietin antibodies in patients treated with recombinant epoetin: a follow-up report from the Research on Adverse Drug Events and Reports (RADAR) Project. Blood 2005;106:3343-7.
63. Sheridan WP, Begley CG, Juttner CA, et al. Effect of peripheral-blood progenitor cells mobilised by filgrastim (G-CSF) on platelet recovery after high-dose chemotherapy. Lancet 1992;339:640-4.
64. Calhoun EA, Schumock GT, McKoy JM, et al. Granulocyte colony–stimulating factor for chemotherapy-induced neutropenia in patients with small cell lung cancer: the 40% rule revisited. Pharmacoeconomics 2005;23:767-75.
65. Heuser M, Ganser A. Colony-stimulat-

ing factors in the management of neutropenia and its complications. Ann Hematol 2005;84:697-708.
66. Repetto L, Biganzoli L, Koehne CH, et al. EORTC Cancer in the Elderly Task Force guidelines for the use of colony-stimulating factors in elderly patients with cancer. Eur J Cancer 2003;39:2264-72.
67. Ohno R, Tomonaga M, Kobayashi T, et al. Effect of granulocyte colony-stimulating factor after intensive induction therapy in relapsed or refractory acute leukemia. N Engl J Med 1990;323:871-7.
68. Relling MV, Boyett JM, Blanco JG, et al. Granulocyte colony-stimulating factor and the risk of secondary myeloid malignancy after etoposide treatment. Blood 2003;101:3862-7.
69. Smith RE, Bryant J, DeCillis A, Anderson S. Acute myeloid leukemia and myelodysplastic syndrome after doxorubicin-cyclophosphamide adjuvant therapy for operable breast cancer: the National Surgical Adjuvant Breast and Bowel Project Experience. J Clin Oncol 2003;21:1195-204.
70. Ringden O, Labopin M, Gorin NC, et al. Treatment with granulocyte colony-stimulating factor after allogeneic bone marrow transplantation for acute leukemia increases the risk of graft-versus-host disease and death: a study from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol 2004;22:416-23.
71. Dale DC, Cottle TE, Fier CJ, et al. Severe chronic neutropenia: treatment and follow-up of patients in the Severe Chronic Neutropenia International Registry. Am J Hematol 2003;72:82-93.
72. Dong F, Brynes RK, Tidow N, Welte K, Löwenberg B, Touw IP. Mutations in the gene for the granulocyte colony-stimulating–factor receptor in patients with acute myeloid leukemia preceded by severe congenital neutropenia. N Engl J Med 1995;333:487-93.
73. Hunter MG, Jacob A, O'Donnell LC, et al. Loss of SHIP and CIS recruitment to the granulocyte colony-stimulating factor receptor contribute to hyperproliferative responses in severe congenital neutropenia/acute myelogenous leukemia. J Immunol 2004;173:5036-45.
74. Vadhan-Raj S, Verschraegen CF, Bueso-Ramos C, et al. Recombinant human thrombopoietin attenuates carboplatin-induced severe thrombocytopenia and the need for platelet transfusions in patients with gynecologic cancer. Ann Intern Med 2000;132:364-8.
75. Archimbaud E, Ottmann OG, Yin JA, et al. A randomized, double-blind, placebo-controlled study with pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) as an adjunct to chemotherapy for adults with de novo acute myeloid leukemia. Blood 1999;94:3694-701.
76. Somlo G, Sniecinski I, ter Veer A, et al. Recombinant human thrombopoietin in combination with granulocyte colony-stimulating factor enhances mobilization of peripheral blood progenitor cells, increases peripheral blood platelet concentration, and accelerates hematopoietic recovery following high-dose chemotherapy. Blood 1999;93:2798-806.
77. Li J, Yang C, Xia Y, et al. Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood 2001;98:3241-8.
78. Bussel JB, Kuter DJ, George JN, et al. Long-term dosing of AMG 531 is effective and well tolerated in thrombocytopenic patients with immune thrombocytopenic purpura. Blood 2005;106:68a. abstract.