

Theme Issue Article

Haematopoietic growth factors and their therapeutic use

Meenu Wadhwa, Robin Thorpe

Biotherapeutics Group, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, United Kingdom

Summary

Haematopoietic growth factors constitute an important group of proteins that predominantly regulate the process of haematopoiesis. While some of these proteins have a very broad array of action on very early haematopoietic progenitors leading to multi-lineage increases in haematopoietic cell production and differentiation, others act in a restricted manner on specific committed terminally differentiated cell types. On the basis of their unique spectrum of activities, several factors are approved for clinical use in various indications while others are under investigation in the clinic either alone or as combination therapy.

Keywords

Haematopoiesis, second-generation, biosimilars, stem cells, adverse

In this review, we have described factors which directly and in some cases indirectly influence haematopoiesis with particular focus on those factors which are either approved or show potential for clinical use. A brief description of the products that are currently available for clinical use is also provided. At present, several new products which include fusion proteins, peptide mimetics are either at the pre-clinical stage or in clinical development for various indications and these are also briefly described.

Thromb Haemost 2008; 99: 863–873

Introduction

The haematopoietic system is a hierarchical structure, with multipotent stem cells eventually becoming non-replicating mature haematopoietic cells that circulate in the peripheral blood. Early haematopoietic cells, known as stem cells, replicate and produce progeny that develop and differentiate along the different haematopoietic lineages. Haematopoietic progenitor cells appear to express multiple cytokine receptors and selectively lose the ability to respond to all but a few selected growth factors as they become mature haematopoietic cells (Fig. 1).

Although the human stem cell has not been fully characterized, some cell surface receptors (such as CD34) are used as markers for cells that appear to be the “least committed” haematopoietic progenitors. Except for these early cells, all the major haematopoietic progenitors have been identified and require specific haematopoietic growth factors for differentiation along specific lineages (Fig. 1).

Haematopoietic growth factors (HGFs) are a family of cytokines that are required for the survival, proliferation, and differentiation of haematopoietic progenitors. The isolation and cloning

of these growth factors over the past two decades was heralded as a significant advance in the treatment of patients with anaemia, malignancy and cancer-related problems. Consequently, a number of these factors were extensively characterised in terms of their biological activity and later assessed in clinical trials, including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), erythropoietin (EPO), interleukin-3 (IL-3), stem cell factor (SCF), interleukin-11 (IL-11) and thrombopoietin (TPO).

Despite sharing a number of properties, including redundancy, pleiotropy, autocrine and paracrine effects, receptor subunit oligomerisation and signal transduction mechanisms, each haematopoietic growth factor has a unique spectrum of haematopoietic activity. Data from numerous studies has elucidated previously unrecognised functions for haematopoietic growth factors in innate immunity, pulmonary physiology, cardioprotection, neuroregulation and bone metabolism. In addition, the important role of these proteins in regulation of stem cells within the niches of the bone marrow and other tissues is now increasingly recognised. Such effects are mainly mediated by signal

Correspondence to:
Dr. Meenu Wadhwa
Cytokine and Growth Factor Section
Biotherapeutics Group
National Institute for Biological Standards and Control
Blanche Lane, South Mimms, Potters Bar
Hertfordshire, EN6 3QG, UK
Tel.: +44 1707 641472, Fax: +44 1707 641057
E-mail: mwadhwa@nibsc.ac.uk

Received November 27, 2007
Accepted after minor revision January 25, 2008

Prepublished online April 9, 2008
doi:10.1160/TH07-11-0703

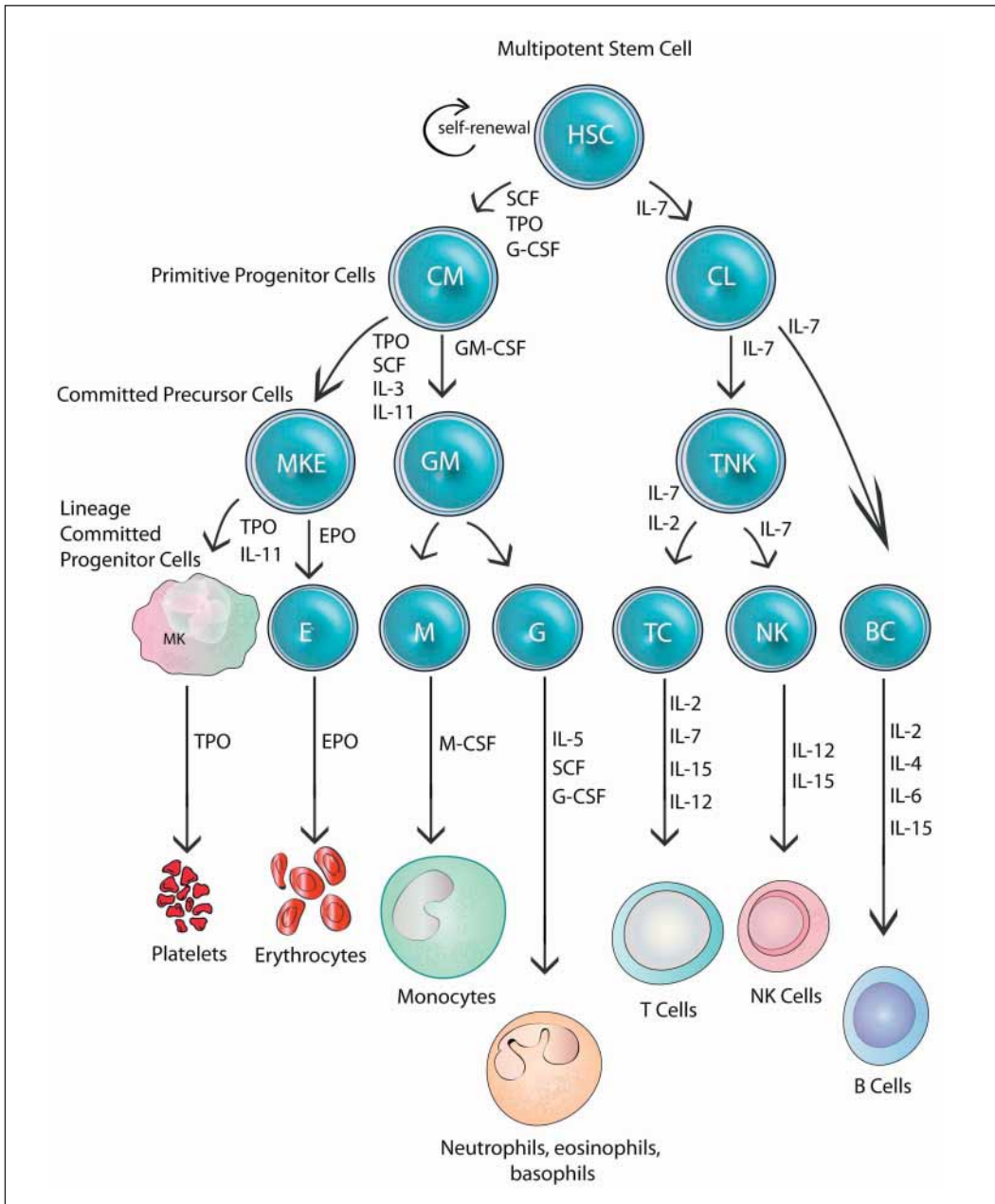


Figure 1: A diagrammatic representation of the haematopoietic system showing predominant cell types, differentiation pathway and the different haematopoietic growth factors which positively influence the process of haematopoiesis.

The abbreviations for the different cell types are denoted as follows: HSC – human stem cell, CM – common myeloid cell, CL- common lymphoid cell, MKE – megakaryocyte erythroid, GM- granulocyte macrophage, TNK – T and natural killer cell, MK- megakaryocyte, E – erythroid, M-macrophage, G- granulocyte, TC – T cell, NK- natural killer cell, BC – B cell.

transduction mechanisms involving tyrosine kinase activation and phosphotyrosine cascades although other signalling mechanisms are also likely to operate resulting in distinct activities of haematopoietic growth factors, e.g. lineage-determining signals during the process of haematopoiesis. Therefore, while some growth factors (e.g. GM-CSF) have a rather broad array of action on very early haematopoietic progenitors, leading to multi-lineage increases in haematopoietic cell production/differentiation, others (e.g. M-CSF) seem to act mainly on more terminally differentiated cell types, producing fairly incisive changes in specific committed populations, such as monocytes/macrophages (Fig. 1).

Based on their unique activities, some haematopoietic growth factors have been successfully approved for clinical use,

in particular, EPO and G-CSF and have generated significant income for the pharmaceutical industry and benefit to patients. Others such as GM-CSF, SCF and IL-11 have been approved for certain clinical indications in a few countries. Unfortunately, TPO despite its potential in enhancing platelet numbers, proved to be non viable as a product on the basis of its safety profile. However, recent trials involving analogues of TPO seem promising and may improve the treatment of chronic thrombocytopenia in the future.

In this article, we describe most of the factors which directly and in some cases indirectly influence haematopoiesis. However, we have placed most emphasis on those factors which are either approved or show most potential for clinical use.

Factors controlling haematopoiesis

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) is a protein that mainly acts on the neutrophil lineage to stimulate the proliferation, differentiation, and activation of committed progenitor cells and functionally active neutrophils (1). It induces the release of neutrophils from the bone marrow and enhances the phagocytic capacity, generation of superoxide anions and bacterial killing by these cells. Purified from bladder (5637) and squamous (CHU-2) carcinoma cell-lines with an approximate molecular weight of 18 kD or 19 kD, respectively, two different cDNA clones coding for mature human G-CSF proteins of 174 and 177 amino acid residues were originally identified in 1987 (2–5). The 174 amino acid form is the most abundant and biologically active form (as opposed to the other form), bears significant structural similarity with interleukin-6 and is O-glycosylated on Thr133. The glycoprotein has abundant α -helices with a small amount of β -sheet pleating. The sugar moiety accounts for approximately 4% of the total weight and has little, if any impact on the three dimensional structure of the molecule or binding to its receptor.

G-CSF is predominantly produced by endothelial cells, monocytes, macrophages, stromal cells and fibroblasts. Inflammatory cytokines such as TNF- α , IL-1, and IL-6 often produced by activated monocytes stimulate G-CSF production. G-CSF acts on target cell types directly by binding to single, high-affinity receptors, G-CSFR, which are specific to G-CSF although G-CSF may also impart significant indirect effects (6, 7). The receptor is expressed by all neutrophils and their precursors including myelocytes, promyelocytes, myeloblasts, myeloid progenitor cells and primitive haematopoietic stem cells. This accounts for the biological effects of G-CSF on the early haematopoietic stem cell up to the developmental stage of the mature neutrophil and helps to explain the potent clinical effects of this protein (8, 9). Other cell types, e.g. endothelial cells, placental cells and activated T-lymphocytes that are not usually considered to be targets of G-CSF action also express functional G-CSFRs. Although the relevance of receptor expression on these cell types remains to be elucidated, recent evidence suggests that G-CSF exposure *in vivo* induces changes in T-cell function, including polarization towards a Th2 phenotype and reduced proliferation in response to alloantigens. G-CSF also induces the production of regulatory dendritic cells which in turn influence T-cell development and function (10).

Clinical use and available products

G-CSF was initially tested in clinical trials for its ability to prevent or reduce severe neutropenia and its complications (11, 12). Randomised trials demonstrated that G-CSF accelerates recovery of neutrophils and reduces the duration of neutropenia after chemotherapy (13). As a consequence of accelerated neutrophil recovery, G-CSF administration reduced the incidence of inflammation, duration of fever and duration of hospitalization. Therefore, the clinical use of G-CSF includes several indications relating to neutropenia (14, 15). For example, it is used for decreasing the incidence of infection, reducing the duration of neutropenia and related clinical sequelae, e.g. febrile neutropenia in patients with nonmyeloid malignancies undergoing mye-

loablative chemotherapy followed by marrow transplantation and for reducing the time to neutrophil recovery and the duration of fever subsequent to chemotherapy in acute myeloid leukaemia patients. In addition, it is used chronically in symptomatic patients with severe neutropenia (congenital, cyclic, or idiopathic) for reducing the incidence and duration of sequelae of neutropenia, e.g. fever, infections, oropharyngeal ulcers (14–16).

Increasingly, G-CSF is being used for mobilization of haematopoietic progenitor cells into the peripheral blood for collection by leukapheresis and subsequent haematopoietic stem-cell transplantation (17–19). Such mobilization is not a direct, receptor-mediated effect on the stem and progenitor cells but rather an indirect effect of G-CSF. Activation of neutrophils in the bone marrow by G-CSF causes them to release matrix metalloproteases, which in turn facilitate the migration of progenitor cells from the bone marrow microenvironment.

Various recombinant G-CSF products which differ in glycosylation and/or protein sequence are available for clinical use (15, 16, 18, 19). Most commonly used are granulocyte (WHO International Non-Proprietary name, INN Lenograstim) which is produced in Chinese hamster ovary (CHO) cells and neupogen (INN Filgrastim), a product expressed in *E. coli*. The former is glycosylated on the same site as the native protein, the sugar contributing to the increased stability of the product. The latter differs from the natural protein as it is not glycosylated and has an additional N-terminal methionine. Filgrastim has an elimination half-life, in both normal subjects and cancer patients of approximately 3.5 hours (h) after either subcutaneous or intravenous dosing. To increase the half-life, a second-generation G-CSF product has been produced by conjugating a 20 kD monomethoxypolyethylene glycol molecule to the N-terminal methionyl residue of Filgrastim. This recently approved Neulasta[®] (INN pegfilgrastim) has a reduced renal clearance and persists longer *in vivo* (a half-life of 15 to 80 h) in cancer patients after subcutaneous administration and is therefore administered only once per chemotherapy cycle (18). For stem cell mobilization, however, the advantages and disadvantages of the utility of pegylated G-CSF instead of G-CSF are not clear and the pegylated form is not approved for use as a stem cell-mobilizing agent. However, pegfilgrastim (Neulastim) is used for the treatment of patients with Hodgkin's lymphoma.

It should be noted that the indications for use of the different products may vary between countries where it is approved. For example, in the UK, Filgrastim is also approved for treatment of neutropenia in patients with advanced HIV infection as well as the indications given above.

Adverse effects

G-CSF is usually very well-tolerated although very rare allergic-type reactions may occur. G-CSF treatment in patients with severe chronic neutropenia and in cancer patients after myelosuppressive chemotherapy has been associated with mild to moderate bone pain (15). Adult respiratory distress syndrome has occurred in neutropenic patients receiving G-CSF. Cytogenetic abnormalities, transformation to myelodysplastic syndrome with the eventual development of myeloid leukaemia have been observed in patients treated with G-CSF for severe chronic neutropenia. In healthy donors and patients given G-CSF for periph-

eral blood progenitor cell collection and therapy, rare cases of splenic rupture have been reported (21, 22).

For G-CSF, the incidence of antibody development in patients has not been adequately determined or studied. It has been reported that the incidence of binding antibodies in patients treated with *E. coli*-expressed G-CSF and pegylated G-CSF is 3% and 6%, respectively, with no evidence of any neutralizing antibodies using a cell-based bioassay. Detection of antibodies is highly dependent on the sensitivity and specificity of the assays used, and the observed incidence of antibody positivity in an assay may be influenced by several factors including timing of sampling, sample handling, concomitant medications, and underlying disease.

Erythropoietin

Erythropoietin (EPO) is a hormone-like glycoprotein which promotes survival and proliferation of erythroid precursors (23). It stimulates red cell production, differentiation and maturation. It is primarily produced by renal peritubular interstitial cells where it is induced by hypoxia, but it is also constitutively produced by the liver.

Erythropoietin is normally present at very low concentrations in blood, but is secreted in the urine from which it was originally isolated. The human molecule was cloned in 1985, which has allowed production of therapeutic products using rDNA procedures (24). The mature protein has 165 amino acids, contains about 40% carbohydrate and has a molecular weight of 30.4 k. The EPO tertiary structure contains four antiparallel alpha helices.

A single type of receptor for erythropoietin is expressed on a wide range of cell types (25). Binding of a single EPO molecule to two adjacent receptors causes homodimerization of the receptors which triggers signalling processes which are responsible for the biological effects of EPO. The responding target cells in erythropoiesis are late stage erythroid burst-forming unit (BFU-E) cells, erythroid colony forming unit (CFU-E) cells and erythroblasts.

Unlike most cytokines and hormones, glycosylation is very important for activity *in vivo* (26). Although the non-glycosylated and glycosylated forms show very similar activity *in vitro*, non-glycosylated erythropoietin is almost completely inactive *in vivo* (26, 27). The glycosylation is very important for maintaining the half-life of the molecule, and sialic acid terminal residues on the carbohydrate chains are particularly important for this. The half-life seems to be proportional to the number of such residues present on the molecule. The precise mechanism(s) by which the carbohydrate moieties prolong half-life is not entirely clear, but the hepatic asialoglycoprotein binding lectin (or receptor) may play a role in this. Sialic acid residues protect against binding to this lectin. The importance of this mechanism for maintaining half-life is supported by the finding that asialated erythropoietin has virtually no biological activity *in vivo* (27).

Clinical use and available products

The clinical uses of erythropoietin are essentially to induce erythropoiesis to increase erythrocyte numbers. Thus patients suffering with clinically significant anaemia can benefit from EPO therapy. EPO is approved for treatment of anaemia associated

with chronic kidney disease (CKD) and various cancers (especially in patients receiving chemotherapy) in many parts of the world (28–30). However, it can also be used to treat other anaemias, e.g. those due to myelodysplasia, infections or other conditions associated with anaemia of chronic disease (31). Some other clinical indications for EPO have been proposed, e.g. for treating anaemia associated with surgery.

A number of EPO products are available for clinical use throughout the world. Most have identifying trademarks, but several also have WHO INNs which are also used to identify them. To date all INNs for EPO products consist of Epoetin plus a Greek letter suffix, which is intended to reflect a possible difference in glycan sequences. The most widely used products in the developed world are the epoetin α Eprex[®] (known as Erypo in Germany) and Epogen[®] (also marketed as Procrit) and the epoetin α Neorecormon[®]. Another product, epoetin delta (marketed as Dynepo[®]) is approved in Europe. Recently an EPO α product has been approved in the EU via the biosimilar regulatory process; this is distributed by different pharmaceutical companies under the trade names Binocrit[®], Asbseamed[®] and Epoetin α hexal[®]. Very recently a second biosimilar (epoetin zeta) has been approved in the EU (Silapo[®] and Retacrit[®]).

Attempts have been made to produce altered EPO products which show increased half-life *in vivo*. This is clearly advantageous as it reduces the frequency of injections of EPO required to achieve the desired clinical effects. Two such products are approved for use in the EU. One of them (Aranesp[®], INN darbopoietin alfa) has an altered amino acid sequence which results in the number of N-linked glycan chains being increased to five (natural sequence EPO has 3 N-linked and 1 O-linked carbohydrate chains) (32, 33). This molecule consists of 51% carbohydrate with a maximum of 22 sialic acid residues. Darbopoietin has biological activity *in vitro* which is very similar to EPO, but approximately three times the half-life (32). Aranesp is also approved for clinical use in the US and many other countries. Another approach to increasing half life is to pegylate the EPO molecule. One such product (Micera[®]) is approved for use in the EU and USA for the treatment of anaemia associated with CKD.

A number of EPO mimetics are also being investigated, including peptides/other small molecules which are related or unrelated to the EPO structure. Hematide, a non-EPO sequence related peptide is in phase II clinical trials at present. However, none are yet approved for clinical use.

Adverse effects

The most common adverse effect of EPO is hypertension, caused by increased hematocrit and resultant blood viscosity. This can be controlled by dosing and the use of anti-hypertensive drugs and/or fluid removal. Until the late 1990s there were few other adverse effects noted with EPO therapy.

However, following 1998, rare but often clustered cases of pure red cell aplasia (PRCA) were noted to be largely associated with use of one EPO product (Eprex/Erypo). Patients who developed PRCA were found to produce neutralizing antibodies against EPO protein (34). Some changes to the formulation of Eprex had been made prior to 1998, but the precise cause of the enhanced immunogenicity of the Eprex is still unclear. Patients developing antibody-mediated PRCA are managed by ceasing

treatment with erythropoietic agents (the antibodies cross-react with all forms of EPO including aranesp) and sometimes other therapeutic intervention.

Granulocyte-macrophage colony-stimulating factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) causes the differentiation and proliferation of granulocyte/macrophage progenitor haematopoietic cells. It also regulates the survival and function of several mature cell lineages, e.g. neutrophils, macrophages and dendritic cells (35–38). In addition, GM-CSF functions as an inflammatory mediator, acting on a number of different cells types and modulates antigen-presenting cell function.

GM-CSF was discovered in the 1960s and cloned from the Mo leukaemia line and separately from a T-cell line in 1985 (39, 40). The mature protein contains 127 amino acids, is glycosylated and if isolated from natural sources has a molecular weight of around 23 kD. Glycosylation is not necessary for biological activity as GM-CSF expressed in *E. coli* has comparable or even higher activity than the glycosylated form. GM-CSF made in CHO cell expression systems is variably glycosylated on N and O linked sites and shows a molecular weight distribution of 18–30 kD. Structural analysis shows that the molecule consists of two pairs of antiparallel α -helices. GM-CSF binds to a single heterodimeric receptor and this results in phosphorylation of a number of signalling proteins (41, 42). Receptors are found on haemopoietic cells, but also on some non-haemopoietic cell types.

A wide range of cell types can produce GM-CSF, but this often requires a stimulus. Activated T and B cells, macrophages, endothelial cells, fibroblasts and mast cells are examples of cells which secrete GM-CSF following stimulation.

Clinical use and available products

GM-CSF is approved for use for reversing neutropenia after chemotherapy or bone marrow transplantation. This results in a reduction of infectious complications in GM-CSF treated patients (43, 45). Trials have also been conducted to assess the use of GM-CSF in numerous other indications, e.g. sepsis, drug-induced neutropenia, HIV infection, acute myeloid leukaemia, aplastic anaemia and myelodysplasia, but with variable results. It enhances the anti-viral effects of azidothymidine, AZT and is used clinically for this in HIV infected patients.

In general, the enhancement of haematopoietic effects induced by GM-CSF are no better (and possibly inferior) to G-CSF. However, GM-CSF is associated with a much higher rate of adverse effects and so its clinical use has declined over the past few years. But, in view of its potent immunostimulatory activity and activation effects on dendritic cells, it is being assessed for use as an 'adjuvant' for prophylactic and therapeutic vaccines for use in protecting against infection and treating cancer (44).

An *E. coli*-expressed GM-CSF has been approved for use in the EU (Molgramostim-Leucomax[®]) since 1991. A yeast derived, glycosylated product (Sargramostim-Leukine[®]) is approved in the US and elsewhere, but is not used in the EU. Several other GM-CSF products are used in other parts of the world, some of which are considered biosimilars.

Adverse effects

GM-CSF is known to cause adverse effects at high doses. These include bone pain, erythroderma, weight gain oedema and other inflammatory problems (45). Both the immunomodulatory activity and haematopoietic effects of GM-CSF are probably involved in these adverse reactions. GM-CSF can induce unwanted immunogenicity in some patients, particularly if they receive long term multi-dose treatment and have relatively intact immune systems. This can compromise therapy if the antibodies induced neutralise the biological activity of GM-CSF (46, 47).

Stem cell factor

Stem cell factor (SCF) is an early-acting haematopoietic cytokine that supports the proliferation and survival of pluripotent progenitor cells. It also facilitates lineage commitment and differentiation of distinct progenitor cells in response to lineage specific cytokines. Originally purified and cloned by three different groups as an activity in medium conditioned by different cells (e.g. buffalo rat liver, BALB/c 3T3 and a bone marrow stromal cell-line) which stimulated in vitro growth of mast cells, the protein was named mast cell growth factor, stem cell factor or kit ligand (48–50).

SCF is normally found in both soluble and transmembrane forms (generated by alternative splicing that includes or excludes a proteolytic cleavage site). The transmembrane form contains 220 amino acids. Full length SCF comprises 248 amino acids, which after proteolytic cleavage yields the soluble biologically active form which has 165 amino acids and a molecular weight of approximately 18.5 kD (51). Soluble SCF is glycosylated and exists as a non-covalently bonded dimer linked by two intramolecular disulphide bonds (Cys4-Cys89 and Cys43-Cys138) which are critical for maintaining its biological activity. SCF has considerable secondary structure, including regions of α -helices and β -sheets (50, 52).

SCF is produced by fibroblasts, endothelial cells, keratinocytes and epithelial cells. It has been detected in the thymus and other tissues including lung, heart, brain and liver. Inflammatory stimuli such as TNF- α or IL-1 may modestly enhance SCF production by marrow stromal cells. SCF is normally found in human serum; circulating levels are in range of 1–8 ng/ml.

The receptor for SCF, c-kit is broadly distributed within the hierarchy of haematopoietic cells and other tissues therefore explaining the pleiotropic effects of SCF on not only haematopoietic and stem cells, but also mast cells, germ cells and melanocytes (53). However, SCF has so far mainly been exploited for stem cell mobilization in leukaemic patients (18, 54, 55). SCF synergises with other cytokines such as EPO, IL-3, GM-CSF, G-CSF to support direct growth of erythroid, granulocyte macrophage and erythroid/macrophage/megakaryocyte colonies (53). In addition to promoting progenitor cell survival, SCF can accelerate stem cell entry into cell cycle and function as a chemotactic and chemokinetic factor for these cells. SCF in combination with thrombopoietin or IL-3 causes synergistic proliferative effects on megakaryocytic progenitor cells.

Since SCF plays an essential role in mast cell biology and is necessary for mast cell development, proliferation, survival and degranulation, several potential therapeutic antagonists for targeting SCF and its receptor are currently being investigated for their anti-allergic properties (56).

Clinical use, available products and adverse effects

SCF has largely been used in the clinic in combination with G-CSF for stem cell mobilization (18, 54, 55). Despite the benefits of this mobilization strategy seen in initial studies, severe toxicity, e.g. allergic reactions, respiratory symptoms due to the actions of SCF on mast cells caused concern and retarded further clinical development. Ancestim[®] (Stemgen, Amgen, Thousand Oaks, CA, USA), an *E. coli*-expressed product which has 166 amino acids in a sequence identical to the natural sequence (except for an additional N-terminal methionine) is the only available licensed product. It is approved for use in combination with G-CSF (Filgrastim) in some countries (e.g. Canada, New Zealand, Australia) but not in USA or Europe in the setting of peripheral blood progenitor cell (PBPC) transplantation for patients at risk of poor PBPC mobilization.

Interleukin-11

Interleukin-11 (IL-11) is a pleiotropic cytokine which stimulates proliferation of primitive haematopoietic progenitor cells as well as a range of other effects (57, 58). It synergizes with other cytokines e.g. IL-3, IL-4, SCF, EPO, which results in stimulation of megakaryocytopoiesis, erythropoiesis, lymphopoiesis and myelopoiesis. It induces secretion of some cytokines, e.g. IL-6, which also contributes to its pleiotropic effects (59–61). IL-11 acts on a wide range of cell types. It is produced by several types of cells, e.g. bone marrow stromal fibroblasts, lung fibroblasts and numerous cell lines. It usually requires stimulation of these cells with IL-1, phorbol esters or transforming growth factor β (TGF- β) for secretion. It was first observed as a factor produced by IL-1 stimulated stromal cells in 1990 and was cloned in the same year (57). The mature IL-11 protein contains 178 amino acids and has no N-linked glycosylation sites. The protein is made up of four α -helical bundles and two β -sheets. The IL-11 receptor is a heterodimer consisting of an IL-11-specific binding chain and the gp130 signalling chain which is also found in receptors for some other cytokines, e.g. IL-6, LIF and oncostatin M.

Clinical use and available products

IL-11 has been trialled for use in a range of clinical indications with limited success. Only one product is approved for clinical use in the USA and no products are approved in the EU. The approved product, Neumega[®] (INN, Oprevelekin) is used for treatment of chemotherapy induced thrombocytopenia, as it increases megakaryocytopoiesis and thrombopoiesis (59, 62, 63).

Adverse effects

IL-11 has been associated with serious and mild adverse effects. These include pulmonary peripheral oedema, dyspnea, capillary leak syndrome, dilutional anaemia, atrial arrhythmia, tachycardia, fluid retention and papilledema (59, 62)

Thrombopoietin

Thrombopoietin (TPO) is a major regulator of megakaryopoiesis and thrombopoiesis, leading to platelet production (64, 65). TPO promotes the viability of pluripotential stem cells and early progenitors of all lineages although its major effect is to support the survival and proliferation of megakaryocytic lineage cells. De-

spite being cloned in 1994, the protein is not approved for clinical use (65–67). TPO, a 332 amino acid glycoprotein with a molecular weight of 95 kD contains two domains; an amino domain (residues 1–153) that shows considerable homology with human EPO and binds to its receptor and a carbohydrate-rich domain (residues 154–332) which is important for maintaining protein stability. Deletion of this region does not affect the activity of the protein *in vitro* but substantially reduces its bioavailability after parental administration. TPO has an antiparallel 4-helix bundle fold structure.

TPO is predominantly produced in the liver. It binds to its receptor, c-mpl expressed on haematopoietic stem cells, progenitor cells and platelets (68). In normal physiology, TPO, enters the circulation (after being produced) and is removed rapidly as it binds to its receptors on megakaryocytes and platelets, undergoes internalisation and subsequent degradation (68, 69). Thus normal or elevated levels of platelets inhibit the action of TPO on target cells (bone marrow) by binding to circulating TPO.

In situations where platelet production or megakaryocyte mass is reduced, circulating levels of TPO increase, peaking by 24 h such that platelet production can be stimulated. In conditions associated with marrow failure (e.g. aplastic anaemia), TPO levels are high whereas in idiopathic thrombocytopenic purpura (ITP), TPO levels are low.

While TPO is the main regulator of megakaryocytopoiesis, it is not exclusive in this activity. Other factors such as IL-1, IL-3, IL-6, IL-11 and SCF also stimulate megakaryocyte growth alone or in combination with TPO (70–72). This ability of TPO to synergise with other cytokines has been utilised effectively to expand haematopoietic stem cells and megakaryocyte progenitor cells *in vitro* (73, 74).

Clinical use and adverse effects

Based on the biological activity of TPO, two recombinant forms of TPO, were extensively studied in clinical trials in various thrombocytopenic disorders and in healthy humans for platelet apheresis and use in transfusions (75, 76). One was a full-length polypeptide, TPO while the other was a truncated form, pegylated megakaryocyte growth and development factor, peg-MGDF. The latter comprising the amino terminal region (163 amino acids) of TPO which binds to the TPO receptor was chemically modified by the addition of polyethylene glycol. Produced in CHO cells, recombinant TPO had the same amino acid sequence as the native protein but a slightly lower molecular weight (90 kD) than native TPO (95 kD), possibly due to differences in glycosylation. When administered intravenously to cancer patients, this molecule dramatically enhanced platelet production and had a half-life of 30–40 h (77).

Peg-MGDF, in contrast, was produced in *E. coli*, was non-glycosylated and had a molecular weight of 60 kD and a half-life of 25–30 h after subcutaneous administration (76). Although structurally different from the native protein, the truncated version showed similar biologic activities as the full-length protein; the platelet count rise began five days after injection and peaked 10 to 14 days later. However, the development of these products was curtailed in 1998 when some patients developed autoantibodies against Peg-MGDF which cross-reacted with and neutralized endogenous TPO, causing thrombocytopenia in healthy

human subjects (78, 79). Development of Peg-MGDF was immediately terminated and, although the full-length TPO was not directly associated with these problems, there was no further clinical development with this molecule. However, alternative approaches have been pursued to overcome this immunogenicity problem and develop a desirable thrombopoietic candidate. One of the approaches has been to generate anti-mpl antibodies (and convert whole IgG to minibodies agonist antibodies) to serve as TPO mimics without any risk of generating neutralizing antibodies against endogenous TPO (76).

Based on clinical studies showing the effectiveness of the above proteins in several thrombocytopenic disorders, several second-generation thrombopoietic growth factors with unique pharmacological properties have been developed. These include peptide mimetics, non-peptide mimetics and agonist antibodies bind to and activate the TPO receptor in different ways (Table 1); some are currently undergoing investigation in the clinic (76, 80). Among these are AMG-531 and eltrombopag. Both molecules bear no structural resemblance to thrombopoietin, but still bind and activate the thrombopoietin receptor. AMG-531 is a "peptibody" made of two disulphide-bonded immunoglobulin Fc fragments each of which is covalently bound at residue 228 with two identical peptide sequences linked via polyglycine. The carrier Fc component of the molecule binds to the FcRn salvage receptor and undergoes endothelial recirculation which results in a substantially longer half-life than the peptide alone. In a small clinical study, AMG-531 caused no major adverse events and increased platelet counts in patients with ITP (81). Eltrombopag (SB-497 115) an oral, small-molecule, non-peptide agonist of the thrombopoietin receptor in clinical trials for thrombocytopenia of various etiologies also seems promising. Initial results have indicated its safety and ability to increase platelet counts in a dose-dependent manner (82, 83). Details on clinical trials currently being pursued with these molecules and treatment options available for management of idiopathic thrombocytopenic purpura are provided in a recent article (84).

Interleukin-3

Interleukin-3 (IL-3) is a haematopoietic growth factor which stimulates the formation of colonies for erythroid, megakaryocytic, granulocytic and monocytic lineages, and in addition, stimulates the renewal of pluripotent haematopoietic stem cells (85, 86). Alternatively termed multiclonal-stimulating factor (multi-CSF), mast cell growth factor (MCGF), megakaryocyte growth factor, eosinophil CSF, IL-3 was discovered independently by several groups because of its diverse biological effects on a variety of cells (87–89).

IL-3 is a relatively small monomeric protein of 133-amino-acids with a molecular weight of approximately 15 kD. It is heavily N-glycosylated and has an α -helical bundle structure.

IL-3 is predominantly produced by activated T cells, natural killer (NK) cells and mast cells stimulated with a combination of phorbol ester and calcium ionophore, and activated eosinophils also produce IL-3.

IL-3 has the broadest target specificity of any of the classic HGFs and acts on target cells types such as monocytes, eosinophils, basophils, megakaryocytes and mast cells via IL-3 receptors (IL-3R) which are heterodimeric and consist of a spe-

cific IL-3R α chain (CD123) that binds IL-3, and non-binding 'converter' IL-3R β chain (β c) that also functions as the high-affinity 'converter' subunit of both the IL-5 and GM-CSF receptors (90, 91).

IL-3 acts on early stages of haematopoiesis, rather than the processes of terminal differentiation and maturation, and its biological activity is species restricted. It acts in synergy with other cytokines to induce progenitors of various lineages (89, 92). Together with EPO, it induces erythroid lineages, and with GM-CSF or G-CSF it induces the granulocyte-macrophage lineages. IL-3 synergizes with TNF for short-term proliferation of CD34 cells as well as causing their development into dendritic or Langerhans cells. It supports the growth-promoting effects of SCF on mast cell precursors and enhances the activity of IL-2 in the proliferation and differentiation of B lymphocytes. Consequently, IL-3 is included in a majority of protocols to culture haematopoietic stem cells and early progenitor cells (93).

Additionally, IL-3 modulates the activity of a number of mature cell types. It enhances histamine release from basophils, causes eosinophil activation, influences monocyte function and regulates macrophage cytotoxicity. IL-3 and GM-CSF induce monocyte adhesiveness by induction of the integrin CD18 as well as stimulating expression of human leucocyte antigen (HLA)-DR, CD14 and IL-1 α .

Despite the broad range of biological activities *in vitro*, the clinical utility of IL-3 has been elusive. In several trials including patients with MDS, AML, aplastic anemia, IL-3 has shown very limited efficacy but considerable side-effects (93–95). Pre-clinical studies have suggested that IL-3 in combination with an additional growth factor may elicit profound effects on erythropoiesis and thrombopoiesis. Therefore, receptor agonists and hybrid agonists which have shown significant pre-clinical activity are currently being pursued (96), some of these are listed in Table 1.

Macrophage colony-stimulating factor

Macrophage colony-stimulating factor (M-CSF) or colony-stimulating factor-1 (CSF-1) is the primary regulator of the survival, proliferation, differentiation and function of mononuclear phagocytes (97). M-CSF was originally identified by its ability to selectively support macrophage colony formation in semisolid medium by progenitor cells in bone marrow (98). M-CSF is a 70 kD type I transmembrane protein that is cleaved to produce a circulating disulfide-linked homodimeric growth factor. The molecule is heavily glycosylated and contains two bundles of four α -helices.

M-CSF is produced by various cells including monocytes, endothelial cells, smooth muscle cells, bone marrow stromal cells, uterine cells, hepatocytes, cells of the monocyte/macrophage lineage and fibroblasts. It is found in measurable concentrations in various tissues including urine, plasma, cerebrospinal fluid and fetal amniotic fluid. Elevated levels have been reported in trauma, autoimmune diseases, infections and various malignancies including breast cancer, ovarian cancer and endometrial carcinoma (99, 100).

The actions of M-CSF are induced by binding to a single high-affinity site on the extracellular domain of the M-CSF receptor which is a transmembrane protein encoded by the *c-fms* protooncogene (101).

Growth factor	Type of product
Thrombopoietin	Peptide mimetics Fab 59 – 2 TPO receptor-activating peptides inserted into complementarity- –determining regions of a fully human Fab AMG 531 – A peptide agonist or 'peptibody' containing 2 disulphide-bonded human IgG1 heavy chain constant regions (an Fc fragment) each covalently bound to identical peptide sequences linked via polyglycine Peg-TPOmp – A pegylated TPO peptide agonist
	Non-peptide mimetics SB-497115 – Orally available hydrazone small molecule which has an acidic group (COOH) at one end, lipophilic (CH ₃) groups at the other end and a metal chelate group in the middle creating a potent TPO receptor agonist AKR-501 – Orally active TPO non-peptide agonist identified by screening of small molecule libraries NIP-004 – A TPO receptor activator identified by screening compounds.
	Agonist antibodies VB22B sc (Fv) ₂ – Small bivalent antibody fragment created by isolation and –genetic engineering of heavy chain and light chain variable regions of an antibody that binds c-mpl receptor
	Receptor agonist Daniplestim – High affinity IL-3 receptor agonist which has a 21 amino acid deletion from N- and C-terminal regions and changed positions of 27 amino acids compared with native IL-3 Promegapoeitin-1 alpha – Chimeric dual IL-3 and mpl-L receptor agonist Leridistim – Chimeric dual G-CSF and IL-3 receptor agonist
Interleukin-3	Fusion protein PIXY321 – GM-CSF and IL-3 linked by a short amino acid sequence DT388IL3 – Truncated Diphtheria toxin linked to IL-3

Table 1: Some products with potential thrombopoietin activity in development.

M-CSF is a pleiotropic cytokine with diverse biological activities. For example, it enhances cytotoxicity, superoxide production, phagocytosis, chemotaxis and cytokine production in monocytes and macrophages, modulates the development and immune functions of dendritic cells, has anti-tumor activity and a role in inflammatory diseases including arthritis, systemic lupus erythematosus, atherosclerosis and obesity (102).

Clinical trials involving M-CSF, both urinary and recombinant forms have shown mixed response although a greater efficacy has been seen when combined with other anticancer therapies. So far there is no clinically licensed product.

Other factors which can influence haematopoiesis

A number of cytokines and growth factors can influence aspects of haematopoiesis although they are not normally considered haematopoietic growth factors. Often their effects are indirect via induction or inhibition of other substances.

IL-1 is a multifunctional cytokine which mediates direct and indirect stimulatory effects on haematopoiesis (103, 104). There are two forms of IL-1, known as IL-1 α and IL-1 β which are both about 17 kD in size, but share little structural homology. They

both bind to the same receptors and mediate the same functions. They are predominantly produced by cells of monocyte/macrophage lineage and act on many cell types. Effects on haematopoiesis *in vitro* and *in vivo* are significant and the factor originally described as haemopoietin-1 is now known to be IL-1 α (105). Although IL-1 could theoretically be used clinically to enhance haematopoiesis, its severe toxicity, mainly due to its inflammatory and pyrogenic properties precludes this. No products are approved for clinical use.

Interleukin-2 (IL-2) is a potent T- and B-lymphocyte growth factor and promotes lymphopoiesis (106, 107). It is a 15 kD glycoprotein produced predominantly by activated T cells and acts on activated T and B cells as well as NK cells and large granular lymphocytes (108, 109). Although it is used clinically to treat cancer patients, its clinical function is mediated via immunostimulatory mechanisms rather than haematopoiesis.

Interleukin-4 (IL-4) and interleukin-6 (IL-6) are 18–19 kD and 19–30 kD glycoproteins which are important for lymphopoiesis of B lymphocytes and B-cell proliferation and development (110–113). IL-6 also synergizes with other factors involved in haematopoiesis (e.g. IL-3) to enhance several haematopoietic effects. IL-4 is predominantly produced by activated T lymphocytes, whereas IL-6 is produced by a variety of cell types especially activated monocytes/macrophages. The haemato-

poietic effects of IL-4 and IL-6 are not sufficient for them to be used clinically for stimulation of haematopoiesis and no products are approved for clinical use.

Interleukin-5 (IL-5) is a 45–50 kD glycoprotein which specifically promotes the growth, activation and differentiation of eosinophils in human systems (114, 115). It also primes basophils. It is primarily derived from T cells. There is no clear clinical role for IL-5 as a product, but IL-5 antagonists can be used to suppress eosinophilia and asthma.

Interleukin-7 (IL-7) is a 20–28 kD glycoprotein which acts early in haematopoiesis and specifically mediates lymphopoiesis (116, 117). It is essential for the earlier steps in lymphopoiesis including activities on stem cells, production and stimulation of pre-B lymphocytes, pre-T lymphocytes and pre-NK cells, and other effects e.g. stimulation of mature T lymphocytes. It is produced by stromal cells from bone marrow, other sources and several stromal cell lines. Although IL-7 shows considerable potential for clinical use in stimulating lymphopoiesis and enhancing immune responses in patients with infections and particularly cancer and several clinical trials have been conducted, no products have been approved for use.

Interleukin-9 (IL-9) is a 30–40 kD glycoprotein which acts on various cells involved in haematopoiesis and especially the immune system (118). It mediates a range of effects on T cells, B cells and erythroid precursors. Many of its effects require synergy with other cytokines, e.g. EPO and IL-4. It is produced mainly by Th₂ lymphocytes. There seems to be little evidence to support clinical use of IL-9 and no products are approved for use.

Interleukin-12 (IL-12) is a potent modulator of NK-cell and T-cell functions (119, 120). It is a heterodimer with subunits of 35 kD and 40 kD and is produced primarily by monocytes/macrophages. It mediates potent activation effects on NK cells which appears to be its principal function *in vivo*. However it also stimulates proliferation of activated T lymphocytes. It is a potent inducer of interferon- γ from both NK and T cells. Although a clinical role for IL-12, especially in inducing NK cells, e.g. in cancer patients, seems promising and clinical trials have been conducted, its very significant toxicity, largely due to induction of high levels of interferon- γ seriously limits clinical use and no products have been approved for use.

Interleukin-15 (IL-15) is a 14–18 kD cytokine with lymphopoiesis stimulating properties similar to IL-2 (121, 122). It is produced by a range of cell types including monocytes, muscle, placental and skeletal tissues and peripheral blood mononuclear cells. Although it shows the same clinical potential as IL-2, it has not been used clinically to stimulate haematopoiesis and no products are approved for use.

Some factors negatively regulate at least some haematopoietic systems. For example some members of the TGF- β family, e.g. TGF- β_1 , inhibit haematopoiesis and other systems (123). The α and β interferons (IFN- α and IFN- β) inhibit the growth of haematopoietic progenitor cells *in vitro*, and it seems likely that this also occurs *in vivo*, although the pleiotropic effects of IFN- α/β s can cloud this issue (124). No direct clinical use of TGF- β or IFN- α/β for inhibiting haematopoiesis has been proposed, although both IFN- α and IFN- β products are approved widely for treatment of other indications.

Conclusion

As described above, the haematopoietic system is controlled by a complex array of factors which directly, indirectly or synergistically influence the development, proliferation and function of haematopoietic cells. Several of these substances show considerable potential for clinical use and some are approved for such use. It seems most likely that scientific progress will identify further cytokines, growth factors and hormones which play a role in haematopoiesis and some of these are likely to be assessed for clinical use. Some of these will succeed as products whereas others will not. It is also possible that combination therapy with haematopoietic growth factors may improve clinical responses.

Second generation products, mutated in amino acid sequences, with altered glycosylation or PEGylation are already used and approved for therapy and there are likely to be further developments with this approach. Mimetics, often bearing no chemical similarity with growth factors or sequences contained within growth factors show considerable potential for clinical use and can be advantageous in some situations (67, 125). Haematopoietic growth factor antagonists can also be used clinically for some indications, and development of these is likely in the future (56).

Fusion proteins comprising a haematopoietic growth factor and other proteins or two haematopoietic growth factors could show enhanced clinical effects compared to the use of a natural sequence haematopoietic growth factor. A GM-CSF-IL-3 fusion protein (PIXY321) has been evaluated in clinical trials in cancer patients, but showed unacceptable immunogenicity and limited development.

The expiry of patents on some of these haematopoietic growth factor products allows production and marketing (following regulatory approval) of similar products produced by competing manufacturers. Often the “biosimilar” (termed “follow-on biologics” in USA) approach as in Europe is taken for regulatory approval of such products (126–129). For this, the manufacturer provides sufficient comparative data to prove that the new product is sufficiently similar to the innovator product to be considered biosimilar. It is therefore expected to show comparable safety and efficacy to the innovator product. However, as it is impossible to prove complete identity between the two products for biologicals (unlike for generic chemicals), it is anticipated that some clinical assessment, e.g. bridging studies, will be necessary for biosimilars (which is not the case for generics). Biosimilar EPOs have already been approved in the EU and elsewhere, and biosimilar versions of other haematopoietic growth factors are being pursued world-wide. Another potentially promising approach which is currently being evaluated is the gene transfer of different haematopoietic growth factors for various clinical indications but whether this is beneficial and offers any advantages over current treatment modalities remains to be seen.

Acknowledgement

We thank Katie-Lee Loveitt for the figure.

References

1. Nicola NA, et al. Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor. *J Biol Chem* 1983; 258: 9017–9023.
2. Welte K, et al. Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor. *Proc Natl Acad Sci USA* 1985; 82: 1526–1530.
3. Nomura H, et al. Purification and characterization of human granulocyte colony-stimulating factor (G-CSF). *EMBO J* 1986; 5: 871–876.
4. Nagata S, et al. Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature* 1986; 319: 415–418.
5. Souza LM, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science* 1986; 232: 61–65.
6. Nicola NA. Hemopoietic cell growth factors and their receptors. *Annu Rev Biochem* 1989; 58: 45–77.
7. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. *Blood* 1991; 78: 2791–2808.
8. Platzer E. Human hemopoietic growth factors. *Eur J Haematol* 1989; 42: 1–15.
9. Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood* 1993; 81: 2844–2853.
10. Rutella S, et al. Granulocyte colony-stimulating factor promotes the generation of regulatory DC through induction of IL-10 and IFN-alpha. *Eur J Immunol* 2004; 34: 1291–1302.
11. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (1). *N Engl J Med* 1992; 327: 28–35.
12. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (2). *N Engl J Med* 1992; 327: 99–106.
13. Ozer H, et al. Update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. American Society of Clinical Oncology Growth Factors Expert Panel. *J Clin Oncol* 2000; 18: 3558–3585.
14. Roberts AW. G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors* 2005; 23: 33–41.
15. Heuser M, et al. Use of colony-stimulating factors for chemotherapy-associated neutropenia: review of current guidelines. *Semin Hematol* 2007; 44: 148–156.
16. Ottmann OG, et al. Current status of growth factors in the treatment of acute myeloid and lymphoblastic leukemia. *Semin Hematol* 2007; 44: 183–192.
17. Dührsen U, et al. Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 1988; 72: 2074–2081.
18. Möhle R, Kanz L. Hematopoietic growth factors for hematopoietic stem cell mobilization and expansion. *Semin Hematol* 2007; 44: 193–202.
19. Ings SJ, et al. Peripheral blood stem cell yield in 400 normal donors mobilised with granulocyte colony-stimulating factor (G-CSF): impact of age, sex, donor weight and type of G-CSF used. *Br J Haematol* 2006; 134: 517–525.
20. Oh-eda M, et al. O-Linked sugar chain of human granulocyte stimulating factor protects it against polymerisation and denaturation allowing it to retain its biological activity. *J Biol Chem* 1990; 265: 11432–11435.
21. Balaguer H, et al. Splenic rupture after granulocyte-colony-stimulating factor mobilization in a peripheral blood progenitor cell donor. *Transfusion* 2004; 44: 1260–1261.
22. Tighe CC, et al. Granulocyte-colony stimulating factor administration to healthy individuals and persons with chronic neutropenia or cancer: an overview of safety considerations from the Research on Adverse Drug Events and Reports project. *Bone Marrow Transplant* 2007; 40: 185–192.
23. Graber SE, Krantz SB. Erythropoietin and the control of red cell production. *Annu Rev Med* 1978; 29: 51–66.
24. Lin FK, et al. Cloning and expression of the human erythropoietin gene. *Proc Natl Acad Sci USA* 1985; 82: 7580–7584.
25. Constantinescu SN, et al. The erythropoietin receptor: Structure, activation and intracellular signal transduction. *Trends Endocrinol Metab* 1999; 10: 18–23.
26. Takeuchi M, et al. Role of sugar chains in the in vitro biological activity of human erythropoietin produced in recombinant Chinese hamster ovary cells. *J Biol Chem* 1990; 265: 12127–12130.
27. Lukowsky WA, Painter RH. Studies on the role of sialic acid in the physical and biological properties of erythropoietin. *Can J Biochem* 1972; 50: 909–917.
28. Eschbach JW, et al. 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: 73–78.
29. de Campos E, et al. Clinical and in vitro effects of recombinant human erythropoietin in patients receiving intensive chemotherapy for small-cell lung cancer. *J Clin Oncol* 1995; 13: 1623–1631.
30. Osterborg A, et al. Recombinant human erythropoietin in transfusion-dependent anemic patients with multiple myeloma and non-Hodgkin's lymphoma—a randomized multicenter study. The European Study Group of Erythropoietin (Epoetin Beta) Treatment in Multiple Myeloma and Non-Hodgkin's Lymphoma. *Blood* 1996; 87: 2675–2682.
31. Means RT Jr, et al. Treatment of the anemia of rheumatoid arthritis with recombinant human erythropoietin: clinical and in vitro studies. *Arthritis Rheum* 1989; 32: 638–642.
32. Egrie JC, Browne JK. Development and characterization of novel erythropoiesis stimulating protein (NESP). *Br J Cancer* 2001; 84 (Suppl 1): 3–10.
33. Elliott S, et al. Enhancement of therapeutic protein in vivo activities through glycoengineering. *Nat Biotechnol* 2003; 21: 414–421.
34. Casadevall N, et al. Pure red-cell aplasia and anti-erythropoietin antibodies in patients treated with recombinant erythropoietin. *N Engl J Med* 2002; 346: 469–475.
35. Metcalf D, et al. Biologic properties in vitro of a recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1986; 67: 37–45.
36. Ruef C, Coleman DL. Granulocyte-macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. *Rev Infect Dis* 1990; 12: 41–62.
37. Jones TC. The effect of granulocyte-macrophage colony stimulating factor (rGM-CSF) on macrophage function in microbial disease. *Med Oncol* 1996; 13: 141–147.
38. Caux C, et al. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. *Nature* 1992; 360: 258–261.
39. Wong GG, et al. Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. *Science* 1985; 228: 810–815.
40. Lee F, et al. Isolation of cDNA for a human granulocyte-macrophage colony-stimulating factor by functional expression in mammalian cells. *Proc Natl Acad Sci USA* 1985; 82: 4360–4364.
41. Gearing DP, et al. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. *EMBO J* 1989; 8: 3667–3676.
42. Hayashida K, et al. Molecular cloning of a second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF): reconstitution of a high-affinity GM-CSF receptor. *Proc Natl Acad Sci USA* 1990; 87: 9655–9659.
43. Gerhartz HH, et al. Randomized, double-blind, placebo-controlled, phase III study of recombinant human granulocyte-macrophage colony-stimulating factor as adjunct to induction treatment of high-grade malignant non-Hodgkin's lymphomas. *Blood* 1993; 82: 2329–2339.
44. Mellstedt H, et al. Augmentation of the immune response with granulocyte-macrophage colony-stimulating factor and other hematopoietic growth factors. *Curr Opin Hematol* 1999; 6: 169–175.
45. Lieschke GJ, et al. Effects of bacterially synthesized recombinant human granulocyte-macrophage colony-stimulating factor in patients with advanced malignancy. *Ann Intern Med* 1989; 110: 357–364.
46. Wadhwa M, et al. Production of neutralizing granulocyte-macrophage colony-stimulating factor (GM-CSF) antibodies in carcinoma patients following GM-CSF combination therapy. *Clin Exp Immunol* 1996; 104: 351–358.
47. Wadhwa M, et al. Immunogenicity of granulocyte-macrophage colony-stimulating factor (GM-CSF) products in patients undergoing combination therapy with GM-CSF. *Clin Cancer Res* 1999; 5: 1353–1361.
48. Huang E, et al. The hematopoietic growth factor KL is encoded by the S1 locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell* 1990; 63: 225–233.
49. Williams DE, et al. Identification of a ligand for the c-kit proto-oncogene. *Cell* 1990; 63: 167–174.
50. Zsebo KM, et al. Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver-conditioned medium. *Cell* 1990; 63: 195–201.
51. Huang EJ, et al. Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. *Mol Biol Cell* 1992; 3: 349–362.
52. Arakawa T, et al. Glycosylated and unglycosylated recombinant-derived human stem cell factors are dimeric and have extensive regular secondary structure. *J Biol Chem* 1991; 266: 18942–18948.
53. Broudy VC. Stem cell factor and hematopoiesis. *Blood* 1997; 90: 1345–1364.
54. Glaspy JA, et al. Peripheral blood progenitor cell mobilization using stem cell factor in combination with filgrastim in breast cancer patients. *Blood* 1997; 9: 2939–2951.
55. To LB, et al. Successful mobilization of peripheral blood stem cells after addition of ancestim (stem cell factor) in patients who had failed a prior mobilization with filgrastim (granulocyte colony-stimulating factor) alone or with chemotherapy plus filgrastim. *Bone Marrow Transplant* 2003; 31: 371–378.
56. Jensen BM, et al. Targeting kit activation: a potential therapeutic approach in the treatment of allergic inflammation. *Inflamm Allergy Drug Targets* 2007; 6: 57–62.
57. Paul SR, et al. Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci USA* 1990; 87: 7512–7516.
58. Turner KJ, Clark SC. Interleukin-11; biological and clinical perspectives. In: *Hematopoietic growth factors in clinical applications*. Marcel Dekker 1995: 315–336.
59. Du XX, Williams DA. Interleukin-11: a multifunctional growth factor derived from the hematopoietic microenvironment. *Blood* 1994; 83: 2023–2030.
60. Paul SR, Schendel P. The cloning and biological

- characterization of recombinant human interleukin 11. *Int J Cell Cloning* 1992; 10: 135–143.
61. Quesniaux VF, et al. Interleukin-11 stimulates multiple phases of erythropoiesis in vitro. *Blood* 1992; 80: 1218–1223.
62. Gordon MS, et al. A phase I trial of recombinant human interleukin-11 (neumega rhIL-11 growth factor) in women with breast cancer receiving chemotherapy. *Blood* 1996; 87: 3615–3624.
63. Kaye JA. FDA licensure of NEUMEGA to prevent severe chemotherapy-induced thrombocytopenia. *Stem Cells* 1998; 16 (Suppl 2): 207–223.
64. Kaushansky K. Thrombopoietin. *N Engl J Med* 1998; 339: 746–754.
65. de Sauvage FJ, et al. Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. *Nature* 1994; 369: 533–538.
66. Kaushansky K, et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature* 1994; 369: 568–571.
67. Lok S, et al. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. *Nature* 1994; 369: 565–58.
68. Broudy VC, et al. Human platelets display high-affinity receptors for thrombopoietin. *Blood* 1997; 89: 1896–1904.
69. Fielder PJ, et al. Human platelets as a model for the binding and degradation of thrombopoietin. *Blood* 1997; 89: 2782–2788.
70. Deutsch VR, Tomer A. Megakaryocyte development and platelet production. *Br J Haematol* 2006; 134: 453–466.
71. Kaushansky K. Lineage-specific hematopoietic growth factors. *N Engl J Med* 2006; 354: 2034–2045.
72. Yang M, et al. Expression of interleukin (IL) 1 type I and type II receptors in megakaryocytic cells and enhancing effects of IL-1beta on megakaryocytopoiesis and NF-E2 expression. *Br J Haematol* 2000; 111: 371–380.
73. Kaushansky K. Thrombopoietin and the hematopoietic stem cell. *Ann NY Acad Sci* 2005; 1044: 139–141.
74. Ivanovic Z, et al. A clinical-scale expansion of mobilized CD 34+ hematopoietic stem and progenitor cells by use of a new serum-free medium. *Transfusion* 2006; 46: 126–131.
75. Begley CG, Bassler RL. Biologic and structural differences of thrombopoietic growth factors. *Semin Hematol* 2000; 37: 19–27.
76. Kuter DJ. New thrombopoietic growth factors. *Blood* 2007; 109: 4607–4616.
77. Vadhan-Raj S, 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: 673–681.
78. Li J, et al. Thrombocytopenia caused by the development of antibodies to thrombopoietin. *Blood* 2001; 98: 3241–3248.
79. Bassler RL, et al. Development of pancytopenia with neutralizing antibodies to thrombopoietin after multicycle chemotherapy supported by megakaryocyte growth and development factor. *Blood* 2002; 99: 2599–2602.
80. Ciurea SO, Hoffman R. Cytokines for the treatment of thrombocytopenia. *Semin Hematol* 2007; 44: 166–182.
81. Bussel JB, et al. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. *N Engl J Med* 2006; 355: 1672–1681.
82. Bussel JB, et al. Eltrombopag for the treatment of chronic idiopathic thrombocytopenic purpura. *N Engl J Med* 2007; 357: 2237–2247.
83. Jenkins JM, et al. Phase I clinical study of eltrombopag, an oral, nonpeptide thrombopoietin receptor agonist. *Blood* 2007; 109: 4739–4741.
84. Stasi R, et al. Idiopathic thrombocytopenic purpura: Current concepts in pathophysiology and management. *Thromb Haemost* 2008; 99: 4–13.
85. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. *Science* 1987; 236: 1229–1237.
86. Metcalf D. Control of granulocytes and macrophages: molecular, cellular, and clinical aspects. *Science* 1991; 254: 529–533.
87. Clark-Lewis I, et al. Purification to apparent homogeneity of a factor stimulating the growth of multiple lineages of hemopoietic cells. *J Biol Chem* 1984; 259: 7488–7494.
88. Yang YC, et al. Human IL-3 (multi-CSF): identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. *Cell* 1986; 47: 3–10.
89. Leary AG, et al. Recombinant gibbon interleukin 3 supports formation of human multilineage colonies and blast cell colonies in culture: comparison with recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1987; 70: 1343–1348.
90. Kitamura T, et al. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. *Cell* 1991; 66: 1165–1174.
91. Miyajima A, et al. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. *Blood* 1993; 82: 1960–1974.
92. Platzer E, et al. Biological activities of a human pluripotent hemopoietic colony stimulating factor on normal and leukemic cells. *J Exp Med* 1985; 162: 1788–1801.
93. Eder M, et al. IL-3 in the clinic. *Stem Cells* 1997; 15: 327–333.
94. Ganser A, et al. Effects of recombinant human interleukin-3 in patients with myelodysplastic syndromes. *Blood* 1990; 76: 455–462.
95. Ganser A, et al. Effects of recombinant human interleukin-3 in patients with normal hematopoiesis and in patients with bone marrow failure. *Blood* 1990; 76: 666–676.
96. Kurzrock R. Thrombopoietic factors in chronic bone marrow failure states: the platelet problem revisited. *Clin Cancer Res* 2005; 11: 1361–1367.
97. Tushinski RJ, et al. Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell* 1982; 28: 71–81.
98. Stanley ER, et al. CSF-1--a mononuclear phagocyte lineage-specific hemopoietic growth factor. *J Cell Biochem* 1983; 21: 151–159.
99. Ralph P, Sampson-Johannes A. Macrophage growth and stimulating factor, M-CSF. *Prog Clin Biol Res* 1990; 338: 43–63.
100. Kirma N, et al. Elevated expression of the oncogene c-fms and its ligand, the macrophage colony-stimulating factor-1, in cervical cancer and the role of transforming growth factor-beta 1 in inducing c-fms expression. *Cancer Res* 2007; 67: 1918–1926.
101. Sherr CJ. Colony-stimulating factor-1 receptor. *Blood* 1990; 75: 1–12.
102. Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. *Curr Opin Immunol* 2006; 18: 39–48.
103. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991; 77: 1627–1652.
104. Oppenheim JJ, Gery I. Interleukin 1 is more than an interleukin. *Immunol Today* 1982; 3: 113–119.
105. Bagby GC Jr. Interleukin-1 and hematopoiesis. *Blood Rev* 1989; 3: 152–161.
106. Smith KA. T-cell growth factor. *Immunol Rev* 1980; 51: 337–357.
107. Gearing A, et al. Human B cell proliferation is stimulated by interleukin 2. *Immunol Lett* 1985; 9: 105–108.
108. Robb R. Interleukin-2: the molecule and its function. *Immunol Today* 1984; 5: 203–209.
109. Trinchieri G, et al. Response of resting human peripheral blood natural killer cells to interleukin 2. *J Exp Med* 1984; 160: 1147–1169.
110. Callard RE. Immunoregulation by interleukin-4 in man. *Br J Haematol* 1991; 78: 293–299.
111. Yokota T, et al. Molecular biology of interleukin 4 and interleukin 5 genes and biology of their products that stimulate B cells, T cells and hemopoietic cells. *Immunol Rev* 1988; 102: 137–187.
112. Kishimoto T. The biology of interleukin-6. *Blood* 1989; 74: 1–10.
113. Rennick D, et al. Interleukin-6 interacts with interleukin-4 and other hematopoietic growth factors to selectively enhance the growth of megakaryocytic, erythroid, myeloid, and multipotential progenitor cells. *Blood* 1989; 73: 1828–1835.
114. Clutterbuck E, et al. Recombinant human interleukin 5 is an eosinophil differentiation factor but has no activity in standard human B cell growth factor assays. *Eur J Immunol* 1987; 17: 1743–1750.
115. Clutterbuck EJ, et al. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989; 73: 1504–1512.
116. Namen AE, et al. B cell precursor growth-promoting activity. Purification and characterization of a growth factor active on lymphocyte precursors. *J Exp Med* 1988; 167: 988–1002.
117. Conlon PJ, et al. Murine thymocytes proliferate in direct response to interleukin-7. *Blood* 1989; 74: 1368–1373.
118. Yang YC, et al. Expression cloning of cDNA encoding a novel human hematopoietic growth factor: human homologue of murine T-cell growth factor P40. *Blood* 1989; 74: 1880–1884.
119. Kobayashi M, et al. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J Exp Med* 1989; 170: 827–845.
120. Lieberman MD, et al. Natural killer cell stimulatory factor (NKSF) augments natural killer cell and antibody-dependent tumoricidal response against colon carcinoma cell lines. *J Surg Res* 1991; 50: 410–415.
121. Carson WE, et al. Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor. *J Exp Med* 1994; 180: 1395–1403.
122. Grabstein KH, et al. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science* 1994; 264: 965–968.
123. Goey H, et al. Inhibition of early murine hemopoietic progenitor cell proliferation after in vivo locoregional administration of transforming growth factor-beta 1. *J Immunol* 1989; 143: 877–880.
124. Rigby WF, et al. The effects of recombinant-DNA-derived interferons on the growth of myeloid progenitor cells. *Blood* 1985; 65: 858–861.
125. Naranda T, et al. Activation of erythropoietin receptor through a novel extracellular binding site. *Endocrinology* 2002; 143: 2293–2302.
126. Thorpe R, Wadhwa M. Protein therapeutics and their immunogenicity. *Eur J Hosp Pharm Pract* 2006; 12: 17–18.
127. Wadhwa M, Thorpe R. Unwanted immunogenicity: Is it a problem for biosimilar products? *BIOforum Europe* 2006; 10: 46–48.
128. Wadhwa M, Thorpe R. Unwanted immunogenicity: Implications for follow-on biologicals. *Drug Inf J* 2007; 41: 1–10.
129. Mellstedt H, et al. The challenge of biosimilars. *Ann Oncol* 2007; Epub ahead of print.