24 Hematopoietic Growth Factors

Juan Jose Pérez-Ruixo

INTRODUCTION

Hematopoiesis is an intricate, well-regulated, and homeostatic multistep process that allows immature precursor cells in the bone marrow to proliferate, differentiate, mature, and become functional blood cells that transport oxygen and carbon dioxide; contribute to host immunity; and facilitate blood clotting. In the early 1900s, scientists recognized the presence of circulating factors that regulate hematopoiesis. It took approximately 50 years to develop in vitro cell culture systems in order to definitively prove that the growth and survival of early blood cells require the presence of specific circulating factors, called hematopoietic growth factors (HGF). The presence of many HGF with different targets at extremely small amounts in blood, bone marrow, and urine confounded the search for a single HGF with a specific activity. Scientific progress was slow until it became possible to purify sufficient quantities to evaluate the characteristics and biologic potential of the isolated materials. The introduction of recombinant DNA technology triggered a flurry of studies and an information explosion, which confirmed hematopoiesis is mediated by a series of HGF that acts individually and in various combinations involving complex feedback mechanisms. Today, many HGF have been isolated; some have been studied extensively, and a few have been manufactured for clinical use.

Different mature blood cells have been identified, each derived from primitive hematopoietic stem cells in the bone marrow. The most primitive pool of pluripotent stem cells comprises approximately 0.1% of the nucleated cells of the bone marrow, and 5% of these cells may be actively cycling at a given time. The stem cell pool maintains itself, seemingly without extensive depletion, by asymmetrical cell division. When a stem cell divides, one daughter cell remains in the stem cell pool and the other becomes a committed colony-forming unit (CFU). The CFU proliferates at a greater rate than the other stem cells and are more limited in self-renewal than pluripotent hematopoietic stem cells. Proliferation and differentiation are regulated by different mechanisms that necessarily involve HGF, which eventually convert the dividing cells into a population of terminally differentiated functional cells committed to the myeloid or the lymphoid pathway. Functional hematopoietic-derived blood cells from the myeloid pathway are red blood cells (erythrocytes), granulocytes (neutrophils, eosinophils, and basophils), monocytes and macrophages, tissue mast cells, and platelets (thrombocytes). Cells committed to the lymphoid pathway give rise to B- or T-lymphocytes and plasma cells.

Most HGF are glycosylated single-chain polypeptides encoded by a specific gene. Production of a recombinant HGF protein is accomplished by first identifying and isolating the particular HGF gene coding region, inserting the HGF DNA into a plasmid, and then expressing the recombinant growth factor protein in a biologic system (e.g., bacteria, yeast, or mammalian cells). The carbohydrate content of HGF varies by the particular protein and production method, which affects not only the molecular weight of the glycoprotein, but potentially the specific biologic activity and the circulating half-life as well. For these reasons why the recombinant copies of HGF proteins cannot be exactly identical to the original HGF protein, however, they might become biosimilars of the original HGF protein. An extensive review of the characteristics of the biosimilar products has been recentaly published (Schellekens et al. 2016). In addition, a summary of the HGF and their activities is provided in Table 24.1.

This chapter focus on reviewing the molecular structure, mechanism of action, pharmacokinetics and pharmacodynamics, clinical indications, and adverse events of HGF proteins stimulating erythropoiesis, granulopoiesis and thrombopoiesis. The common phar-

J. J. Pérez-Ruixo (✉)
Department of Clinical Pharmacology, Janssen Research & Development, Beerse, Belgium
e-mail: jjperezr@its.jnj.com

© Springer Nature Switzerland AG 2019
D. J. A. Crommelin et al. (eds.), *Pharmaceutical Biotechnology*, https://doi.org/10.1007/978-3-030-00710-2_24

Factor	Molecular weight (kDa)	Target cells	Actions
Erythropoietin (EPO)	34–39	Erythroid progenitors	Increase red blood cell counts
Granulocyte colony-stimulating factor (G-CSF)	18	Granulocyte progenitors and mature neutrophils	Increase neutrophil counts
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	14–35	Granulocyte-macrophage progenitors and eosinophil progenitors	Increase neutrophil, eosinophil, and monocyte counts
Stem cell factor (SCF)	18	Granulocyte-erythroid progenitors, lymphoid progenitors, and natural killer cells	Increase pluripotent stem cells and progenitor cells for all other cell types
Thrombopoietin (TPO)	35	Stem cells, megakaryocytes, and erythroid progenitors	Increase platelet counts

Table 24.1 ■ Hematopoietic growth factors and their activities

macokinetic and pharmacodynamic features across the HGF are presented in detail for erythropoietin-stimulating agents (ESA), and then briefly discussed for other HGF. In this context, the existence of flip-flop pharmacokinetics justifying efficiency of the subcutaneous administration relative to the intravenous administration, as well as the concentration-dependent disposition mediated by its binding to the target receptor and the time-dependent pharmacokinetics, consequence of its pharmacodynamic action extending the receptor pool over time, are common features of the recombinant proteins targeting the receptors for erythropoietin, G-CSF and thrombopoietin.

ERYTHROPOIESIS-STIMULATING AGENTS

Erythropoietin (EPO) is a 30.4 kDa glycoprotein hormone secreted by the kidneys in response to tissue hypoxia, which stimulates red blood cell (RBC) production. EPO requires glycosylation to regulate erythrocyte production by activating the EPO receptor (EPOR) and stimulating the proliferation and differentiation of erythrocytic progenitors in the bone marrow, which leads to reticulocytosis, erythrocytosis and the increase of hemoglobin concentration in the blood. The gene that encodes EPO is located on chromosome 7. The cloning of the *EPO* gene in the early 1980s allowed for the development of recombinant erythropoietins and analogs (erythropoiesis-stimulating agents [ESAs]), offering an alternative to transfusion as a method of raising hemoglobin levels that has been successfully used for over 25 years to treat anemia in millions of anemic patients.

Epoetin alfa (Epogen®), the first commercial form of recombinant human erythropoietin (rHuEPO) marketed in the USA, EU, Japan, and China, and epoetin beta (Recormon®, NeoRecormon®), marketed outside of the USA, are both expressed in Chinese hamster ovary cells. Both have the same 165 amino acid sequence, which is identical to human EPO, and contain two disulfide bonds and three N-linked and one O-linked sialic acid-containing carbohydrate chains (Halstenson et al. 1991) and lead to the same biological effects as endogenous EPO (Egrie et al. 1986). No important differences in clinical efficacy are apparent between epoetin alfa and beta (Jelkmann 2000). Darbepoetin alfa (Aranesp®) is a hyperglycosylated erythropoietin analog with five amino acid changes and two additional N-linked carbohydrate chains, which has the same mechanism of action as EPO (Elliott et al. 2004a). However, darbepoetin alfa has a threefold increased serum half-life (Macdougall et al. 1999; Elliott et al. 2003; Sinclair and Elliott 2005) and increased in vivo potency (Egrie et al. 2003), allowing for more convenient modes of administration, including extended dosing intervals (Vansteenkiste et al. 2002; Nissenson et al. 2002) up to monthly dosing as described in the US label. It is marketed globally and is indicated to treat the anemia of patients with chronic kidney disease and chemotherapy-induced anemia in cancer patients.

A large methoxy polyethylene glycol (PEG) polymer chain was integrated into the epoetin beta molecule via amide bonds between either the N-terminal amino group or the ε-amino group of lysine by means of a succinimidyl butanoic acid linker (Macdougall 2005). The resulting pegylated epoetin beta molecule has been marketed as Mircera® to treat the anemia of patients with chronic kidney disease (CKD), but its clinical development as treatment for chemotherapy-induced anemia was stopped (Gascon et al. 2010). The pegylated epoetin beta stimulates erythropoiesis by binding to EPOR; however, the EPOR binding affinity is reduced (Jarsch et al. 2008). This biologic disadvantage is counterbalanced with an extended half-life in humans, which allows for extended dosing intervals in CKD patients (Chanu et al. 2010), similar to the dosing interval of darbepoetin.

Five rHuEPO biosimilars manufactured by two companies have been approved in the EU. Abseamed®, Binocrit® and Epoetin alfa HEXAL® all produced by Rentschler Biotechnologie GmbH, but marketed by three different companies, are epoetin alfa biosimilars of the reference product Eprex®. Comparable safety and efficacy between these three biosimilars and Eprex® was demonstrated in randomized controlled trials in hemodialysis patients with renal anemia. Although the EMA regulatory guidelines for rHuEPO biosimilars recommend that comparable efficacy and safety are demonstrated with two randomized trials in the nephrology setting, these biosimilars were approved based on a single nephrology trial. Two additional biosimilar versions of Eprex®, Retacrit® and Silapo® are manufactured by Norbitec GmbH, under the international nonproprietary name (INN) of epoetin zeta. The comparability of epoetin zeta to Eprex® was demonstrated in two randomized clinical trials, a correction phase study and a maintenance phase study, involving hemodialysis patients with renal anemia. In the correction phase study, the comparability between epoetin zeta and Eprex® over the evaluation period was demonstrated for mean hemoglobin levels, but not for mean dose. Similar results were reported in the maintenance phase study, suggesting a possible difference in the bioactivity of epoetin zeta and Eprex®. Data from studies in cancer patients receiving chemotherapy and treated with epoetin alfa biosimilars and epoetin zeta were also submitted for approval, but these studies were not adequately powered to demonstrate therapeutic equivalence to the reference product in this patient population. However, epoetin alfa biosimilars and epoetin zeta were approved in EU for indications in renal anemia, chemotherapy-induced anemia and for pre-donation of blood prior to surgery for autologous transfusion (Schellekens and Moors 2010). Retacrit® was approved in May 2018 in the US for the treatment of anemia caused by chronic kidney disease, chemotherapy or use of zidovudine in patients with HIV infection.

■ Regulation of Erythropoietin

The primary site of EPO synthesis in adults is the peritubular cells of the kidney (Jelkmann 2000; Jelkmann 1992). The liver is a secondary site of EPO production, with synthesis occurring in both hepatocytes and fibroblastoid interstitial cells (Spivak 1998). No preformed stores of EPO exist, and serum EPO concentrations are maintained at a constant concentration by homeostatic turnover, which consists of the basal production and elimination of the hormone (Fisher 2003). Within a healthy individual, the serum EPO concentration tends to be controlled tightly; however, large interindividual variability is evident from the normal range, 5–35 IU/L (Fisher 2003). Maintenance of normal serum concentrations of endogenous EPO requires the synthesis of about 2–3 IU/kg/day, or approx. 1000–1500 IU/week for a 70-kg man. Sex differences and regular-to-moderate athletic training do not appear to affect endogenous EPO serum concentrations. The blood flow in the kidney has a circadian rhythm in normal individuals; therefore, the endogenous production of EPO has diurnal variations with the highest levels in the evening and at night (Wide et al. 1989).

The overexpression of EPO occurs in a number of adaptive and pathologic conditions. In response to acute hypoxic stress, such as severe blood loss or severe anemia, EPO production rate can increase 100- to 1000-fold. Numerous studies have shown an exponential increase in serum EPO, with increasing degrees of anemia, although the maximal bone marrow response to such stimulation is only a four to sixfold increase in RBC production rate (Jelkmann 2000). Overproduction of EPO with accompanying erythrocytosis may be an adaptive response to conditions that produce chronic tissue hypoxia, such as living at high altitude, chronic respiratory diseases, cyanotic heart disease, sleep apnea, smoking, localized renal hypoxia, radiotherapy, or hemoglobinopathies with increased oxygen affinity. Paraneoplastic production of EPO from some tumors and cysts can also result in high serum concentrations of EPO. Following bone marrow ablation, aplastic anemia, or anemia in patients with hypoplastic marrows, serum EPO levels are disproportionately increased relative to slightly decreased hemoglobin levels. Conversely, individuals with hyperactive marrow owing to hemolytic anemia had disproportionately low serum EPO levels and rapid EPO serum disappearance.

In chronic kidney disease, up to 60% of patients have hemoglobin concentrations below 11 g/dL before beginning dialysis (Jungers et al. 2002). Multiple mechanisms contribute to the low hemoglobin levels (Fisher 2003), but the most important is the inability of the diseased kidneys to produce an appropriate EPO response for the given degree of anemia or an inability to meet the increased RBC demands of uremic patients (Adamson and Eschbach 1990). In addition, the uremic state itself appears to blunt the bone marrow response to EPO (Fisher 2003). It is of interest that serum EPO concentrations in chronically anemic dialysis patients increase to some extent in response to acute hypoxic stress (from either acute bleeding or systemic hypoxemia), suggesting that kidney failure does not result in a complete inability to produce EPO (Kato et al. 1994).

In cancer patients, anemia is of multifactorial etiology (Fisher 2003), and there are three distinct types of anemia: cancer-related anemia (nontreatment related), anemia related to myelosuppressive chemotherapy,

and anemia related to other causes such as bleeding, nutritional deficiency, or iron deficiency, among others. As with other anemias of chronic disease, including those associated with chronic infection and inflammatory disorders, the anemia of cancer is characterized by a decreased production of endogenous EPO (Miller et al. 1990), cytokine-induced suppression of bone marrow function, disordered iron absorption and metabolism (Bron et al. 2001), and decreased erythrocyte survival. In the anemia related to chemotherapy treatment, the amount of endogenous EPO transiently increases up to sixfold within the 48 h after the administration of chemotherapy and returns to baseline within a week (Glaspy et al. 2005). After myeloablative chemotherapy, severe thrombocytopenia and bleeding might contribute to a significant loss of RBC. Finally, the anemias associated with infant prematurity, pregnancy, allogeneic bone marrow transplantation, and HIV infection are often characterized by inappropriately low EPO concentrations (Spivak 1998).

■ Pharmacokinetics

Absorption

After subcutaneous (s.c.) dosing of rHuEPO, its absorption is slow, leading to peak serum concentrations at 5–30 h and a longer terminal half-life (24–79 h) than that obtained after intravenous (i.v.) administration (McMahon et al. 1990). These results indicate the presence of flip-flop pharmacokinetics, where the rate of absorption is slower than the rate of elimination. Thus, the absorption process is the rate limiting process for its disposition, and the observed terminal half-life after s.c. dosing reflects the absorption rate rather than elimination rate.

Following s.c. administration, protein therapeutics, including the marketed recombinant HGF proteins, typically enter into the systemic circulation via the blood capillaries or the lymphatic system (Porter and Charman 2000; McLennan et al. 2006). The lymphatic system is considered to be the primary route of absorption from the s.c. injection site for protein therapeutics greater than 16 kD due to the restricted vascular access afforded by the continuous endothelial layer of blood capillaries (Supersaxo et al. 1990). In both healthy subjects and cancer patients, the fraction of dose absorbed via the lymphatics is about 80–90% and increases at doses higher than 300 IU/kg (Olsson-Gisleskog et al. 2007; Ait-Oudhia et al. 2011; Krzyzanski et al. 2005; Ramakrishnan et al. 2004). The s.c. absorption rates of rHuEPO vary according to the administration site, with a more rapid and extensive absorption when injected into the thigh compared with the abdomen or arm (Jensen et al. 1994). This relatively small difference is most likely reflecting regional differences in blood and lymph flow, and not considered to be clinically relevant as the pharmacodynamic profile (i.e., reticulocytes time course) did not evidence any difference across the site of administration. Small differences in the absorption due to the administration site has been also observed for other HGF, such as G-CSF and romiplostim, but they are of limited clinical relevance.

The s.c. absorption of darbepoetin alfa in humans is also slow, with peak concentrations reached at 34–58 h post-dose, followed by a generally monophasic decline. Similarly to rHuEPO, darbepoetin alfa also displays flip-flop pharmacokinetics, with a longer terminal half-life after s.c. dosing than after i.v. dosing (Agoram et al. 2007). The mean terminal half-life of darbepoetin alfa, 73 h, is associated with large variability between patients, consistent with the variability observed for other ESAs (Glaspy et al. 2005). The mean absorption time of darbepoetin alpha is 56 h, substantially longer than the mean absorption time reported for rHuEPO (Olsson-Gisleskog et al. 2007).

The reported 20–30% reduction in the darbepoetin alfa absorption rate per decade of age (Agoram et al. 2007) is consistent with the estimated effect of age on the rHuEPO absorption rate in healthy subjects (Olsson-Gisleskog et al. 2007) and cancer patients (Ait-Oudhia et al. 2011) and reflects the longer terminal half-life and the larger exposure to both drugs in older patients. It has been hypothesized (Agoram et al. 2007) that the age-dependent reduction in lymphatic flow rate could be the physiological reason behind this relationship, as it has also been reported for monoclonal antibodies administered by s.c. route (Sutjandra et al. 2011; Kakkar et al. 2011). The data available also suggest that the pharmacokinetic profile of rHuEPO and darbepoetin alfa after s.c. administration is similar in adults and children; however, s.c. absorption in children may be more rapid than in adults for both drugs (Heatherington 2003).

Bioavailability

Initial bioavailability estimates for rHuEPO after s.c. administration range from about 15 to 40% and are similar for epoetin alfa and beta (Deicher and Horl 2004). When the pharmacokinetics of s.c. rHuEPO and darbepoetin alfa were studied over a wider dose range in healthy volunteers and the rHuEPO nonlinear clearance was accounted for, exposure was found to increase more than proportional with dose (Olsson-Gisleskog et al. 2007; Agoram et al. 2007; Cheung et al. 1998, 2001). The s.c. bioavailability of darbepoetin alfa increases from 57 to 69% when the 200 μg dose is increased up to 400 μg, while the s.c. bioavailability of rHuEPO increases from 54 to 65% when the 40 kIU dose is increased up to 80 kIU. The apparent increase in s.c. bioavailability with dose of ESA might indicate saturable pre-systemic processes. Nevertheless, despite the

apparent low bioavailability, s.c. administration of ESA has been reported to produce equivalent or better efficacy relative to i.v. administration, although there is a wide range of inter-patient variability (Kaufman et al. 1998). The flip-flop kinetics, together with the increase in absolute bioavailabitlity following s.c. dosing, results in a substantial increase in the efficiency of the ESA s.c. administration relative to i.v. administration. This phenomenon has been also reported for the G-CSF agonists (filgrastim, lenograstim and pegfilgrastim) as well as the c-Mpl agonist (romiplostim).

Distribution

During i.v. infusion, serum rHuEPO and darbepoetin alfa concentrations rise rapidly and then decline in a bi-exponential manner (Olsson-Gisleskog et al. 2007; Doshi et al. 2010). The peak serum rHuEPO and darbepoetin alfa concentrations correlate linearly with dose. A rHuEPO dose of 50 IU/kg produces concentrations of about 1000 mIU/mL 15 min after the end of the infusion, while a darbepoetin alfa dose of 0.75 μg/kg generates serum concentrations of about 10–20 ng/mL after the end of the infusion (Doshi et al. 2010). As expected from its large molecular weight, the volume of distribution of rHuEPO is similar to the plasma volume (40–60 mL/kg), suggesting confinement of rHuEPO within the plasma circulation (McMahon et al. 1990; Olsson-Gisleskog et al. 2007). The data available also suggest that the volume of distribution, normalized by body weight, in adults and children is similar after i.v. administration of rHuEPO and darbepoetin alfa. These results are also consistent with the findings observed for the G-CSF agonists (filgrastim, lenograstim and pegfilgrastim) as well as the c-Mpl agonist (romiplostim).

Elimination

Despite the long clinical experience with ESAs, the mechanism(s) of their clearance have not been fully elucidated, and there is a paucity of information regarding which organ(s) and tissue(s) are important in the metabolism of these drugs. Two ESA clearance pathways have been suggested to explain ESA elimination: (1) a capacity-limited clearance pathway utilizing EPO receptor-mediated endocytosis by erythroid progenitor cells and (2) a EPOR-independent linear clearance reflecting other mechanism(s). In vivo studies demonstrate that the kidney, liver, and lymph exert a negligible effect on in vivo EPOR-independent clearance. Clearly our understanding of the nature of the EPOR-independent clearance pathways is incomplete. However, it is important to recognize that renal excretion and hepatic metabolism of ESAs plays a minor role in their elimination and altered renal or hepatic function does not warrant dose adjustments. Notably, the presence of two clearance pathways also determines the elimination of the G-CSF agonists (filgrastim and lenograstim) as well as the c-Mpl agonist (romiplostim).

An investigation of the trafficking and degradation of rHuEPO by EPOR-expressing cells (BsF3) in cell culture found that rHuEPO was subjected to EPO receptor-mediated endocytosis followed by degradation in lysosomes (Gross and Lodish 2006). The rHuEPO receptor-binding, dissociation, and trafficking properties affected the relative rate of rHuEPO cellular uptake and intracellular degradation (Walrafen et al. 2005). About 57% of surface-bound rHuEPO was internalized ($k_{in} = 0.06\ min^{-1}$) and, after internalization, 60% of the ligand was recycled intact to the cell surface, while 40% was degraded. In spite of the in vitro results suggesting the role of EPOR on ESA clearance, the in vivo evidence is indirect and mostly arises from chemotherapy studies in patients treated with rHuEPO and darbepoetin alfa (Chapel et al. 2001). Chemotherapy-based approaches may also affect EPOR-independent clearance mechanisms, due to destruction of macrophages or neutrophils. The reduction in the number of these cells may explain, or at least contribute to, the decrease in ESA clearance observed after chemotherapy treatment.

Studies investigating the pharmacokinetics of rHuEPO analogs with different EPOR binding activity, suggested that EPOR-independent pathway plays a major role in the ESA clearance since decreasing the number of receptors with chemotherapy or, blocking the EPOR pathway with analogs without binding activity, were unable to completely shut down the elimination of rHuEPO. In addition, since pegylation has been shown to mainly affect the EPOR-independent clearance pathway, EPOR-mediated clearance may not be the dominant route of ESA elimination (Agoram et al. 2009).

It has been shown that carbohydrate side chains of EPO are necessary for persistence and in vivo biologic activity of the molecule, but not for in vitro receptor binding or stimulation of proliferation. Indeed rHuEPO molecules with increased sialic acid content have less affinity for the EPOR (Sinclair and Elliott 2005; Elliott et al. 2004b). Darbepoetin alfa is a hyperglycosylated analog of rHuEPO, with three to fivefold lower affinity for the EPOR compared to rHuEPO (Gross and Lodish 2006; Elliott et al. 2004b), but has three to fourfold longer serum half-life and greater in vivo activity than rHuEPO (Egrie et al. 2003). Surface-bound darbepoetin alfa was internalized at the same rate than rHuEPO, and after internalization, 60% of each ligand was re-secreted intact and 40% degraded (Gross and Lodish 2006). While in vitro experiments suggested that relative to rHuEPO, darbepoetin may

have reduced clearance in vivo because of reduced EPOR-mediated endocytosis and degradation, darbepoetin alfa has other biophysical characteristics, such as increased molecular size and decreased isoelectric point, suggesting that the reduced clearance might be better explained by other mechanisms. In this context, studies investigating the pharmacokinetics of rHuEPO analogs with different EPOR binding activity suggest that hyperglycosylation mainly impacts the EPOR-independent clearance pathway, which also supports the hypothesis that EPOR-mediated clearance may not play a dominant role in ESA elimination (Agoram et al. 2009).

A population pharmacokinetic meta-analysis of rHuEPO in 533 healthy subjects enrolled in 16 clinical studies, where a wide range of i.v. and s.c. rHuEPO doses were administered, has helped in quantifying the two separate elimination pathways and understanding the influence of demographic characteristics and other covariates on the pharmacokinetic parameters of rHuEPO (Olsson-Gisleskog et al. 2007). At low concentrations, including the endogenous EPO concentrations observed at baseline or in ESA-untreated states, the nonlinear clearance operates at full capacity, giving a total clearance of about 0.9 L/h. As concentrations increase, the nonlinearity of pharmacokinetics becomes more important and, at the concentration of 394 IU/L, the clearance is 0.6 L/h. When the concentration are above 3546 IU/L, the nonlinear clearance of rHuEPO was fully (>90%) saturated and the total clearance decreased to almost one third, being mainly represented by the linear component. At concentrations higher than 3546 IU/L, rHuEPO pharmacokinetics is approximately linear. The concentration-dependent clearance appears to be independent of the type of rHuEPO (epoetin alfa vs. epoetin beta) or population (healthy subjects or patients with chronic renal failure).

A further indication of the possible involvement of EPOR binding in the disposition of rHuEPO can be found when investigating the rHuEPO pharmacokinetics after multiple dosing. A rHuEPO time-dependent clearance, with a 10–30% increase after several weeks of treatments with no subsequent changes (McMahon et al. 1990; Cheung et al. 1998; Yan et al. 2012) has been attributed to the limited number of EPOR located on the finite, but expandable, number of bone marrow erythroid progenitors. The pharmacodynamic action of rHuEPO increases BFU-E and CFU-E cell expansion and, consequently, the number of EPOR, which in turn results in an increase in rHuEPO clearance, a decrease in the apparent volume of distribution and a reduction in terminal half-life. The term pharmacodynamic-mediated drug disposition (PDMDD) has been coined to describe these types of TMDD models where pharmacodynamics affects the size of the target pool and influences the drug clearance, as has been described for ESAs. Consequently, the pharmacokinetics of rHuEPO is considered nonlinear because it is concentration dependent and nonstationary (time-dependent) (Yan et al. 2012).

The rHuEPO pharmacokinetic models for healthy subjects can be applied to patients with anemia due to renal insufficiency; however, it may have limited predictive value when applied to patients receiving chemotherapy. The consequences of the chemotherapy effect on the pharmacokinetics of rHuEPO in oncology patients are derived from the reduced number of EPOR available to clear rHuEPO in progenitor cells and the reduction of non-EPOR-mediated clearance (Olsson-Gisleskog et al. 2007). In cancer patients treated with chemotherapy, a correlation between the decline in the absolute reticulocyte count and the decrease in the clearance of rHuEPO over time has been observed (Ait-Oudhia et al. 2011). As a consequence, the rHuEPO elimination process becomes slower than the absorption process, and the flip-flop phenomenon observed in healthy subjects disappears when rHuEPO is given s.c. to cancer patients receiving chemotherapy (Olsson-Gisleskog et al. 2007; Ait-Oudhia et al. 2011). Furthermore, this phenomenon has clinical implications with respect to the synchronicity of ESA and chemotherapy administration, suggesting asynchronous dosing might be superior (Glaspy et al. 2005).

■ Pharmacodynamics

After rHuEPO is administered, it binds to the EPOR on the surface of the BFU-E, CFU-E, and proerythroblast and activates the signal transduction pathways. CFU-E cells have the highest EPOR density (1000 receptors per cell) and are the most sensitive to EPO. Experimental data suggest that approximately only 5–10% of EPOR must be continuously occupied with rHuEPO in order to prevent apoptosis and stimulate proliferation and differentiation of erythroid precursors. Then, CFU-Es will differentiate into normoblasts (including proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, and orthochromatic erythroblast) and, upon normoblast denucleation, reticulocytes will be formed and reside in the bone marrow for 1 day before they are released into the bloodstream, where they circulate for about 1 day before maturing to erythrocytes. In healthy adults, the RBC life span is about 120 days and shows a relatively narrow distribution. The RBC life span is similar in cancer patients but markedly reduced in patients with chronic kidney disease, 60–65 days in dialysis patients and 82 days in nondialysis patients, with a moderate interindividual variability (Uehlinger et al. 1992; Chanu et al. 2010).

Previous studies have demonstrated that highly glycosylated rHuEPO has increased in vivo biological activity and serum half-life, but decreased receptor binding affinity (Egrie and Browne 2001). Given these relationships, a comparison of clearance among different ESAs has to be interpreted in conjunction with EPOR binding affinity and/or in vivo activity. Darbepoetin alfa stimulates erythropoiesis by the same mechanisms as those previously discussed for endogenous EPO and rHuEPO. In vitro, the affinity of darbepoetin alfa for the EPOR is one third to one fifth of the rHuEPO affinity (Gross and Lodish 2006); however, the increase in mean residence time of darbepoetin alfa results in a prolonged period above an erythropoietic threshold that more than compensates for the reduced receptor affinity, yielding an increased in vivo activity (Elliott et al. 2003; Egrie et al. 2003; Krzyzanski et al 2005).

Different mechanisms have been proposed to explain the pharmacodynamic tolerance of the rHuEPO effect. Besides the increase in rHuEPO clearance over time due to the increase in the number of EPOR, an oxygen-mediated feedback mechanism, erythroid precursor pool depletion, and iron-restricted erythropoiesis have been also proposed as tolerance mechanisms (Krzyzanski et al. 2005; Ramakrishnan et al. 2004). The oxygen feedback mechanism is regulated through an oxygen-sensing system: a high hemoglobin level leads to an increased oxygen level and eventually inhibits the production of endogenous EPO. Erythroid progenitor cells are EPO dependent; they cannot survive without EPO. On the other hand, extensive rHuEPO treatment results in anemia due to depletion of the erythroid precursor pool (Piron et al. 2001). This anemia is not due to low endogenous EPO levels but rather exhaustion of erythroid progenitors (Krzyzanski et al. 2005; Perez-Ruixo et al. 2009). Furthermore, iron-restricted erythropoiesis occurs in the presence of absolute iron deficiency, functional iron deficiency, and/or iron sequestration. Absolute iron deficiency is a common nutritional deficiency in women's health, pediatrics, and the elderly. Functional iron deficiency occurs in patients with significant EPO-mediated erythropoiesis or therapy with ESAs, even when storage iron is present. Iron sequestration, mediated by hepcidin, is an underappreciated but common cause of iron-restricted erythropoiesis in patients with chronic inflammatory disease. It has been shown that iron supplementation improves the hematopoietic response of ESAs used for chemotherapy-induced anemia. In multiple-dosing regimens, even though the endogenous EPO production might be suppressed, the total concentration of EPO is still high, and tolerance may occur due to precursor pool depletion and/or iron-restricted erythropoiesis. However, the oxygen feedback mechanism might be present, especially at the end of dosing intervals in regimens that extend longer than four rHuEPO half-lives.

■ Indications for Cancer Patients and Potential Adverse Events

Unless otherwise indicated, the information pertaining to ESA indications in cancer patients provided in this section is derived from the product prescribing information package inserts as well as the National Comprehensive Cancer Network for cancer- and chemotherapy-induced anemia. ESAs are indicated for the treatment of anemia due to the effects of concomitantly administered chemotherapy for a duration of ≥2 months in patients with metastatic, nonmyeloid malignancies. However, ESA treatment is not indicated for patients receiving hormonal agents, biologics, or radiotherapy, unless they are receiving concomitant myelosuppressive chemotherapy. Notably, ESA therapy should not be used to treat anemia associated with malignancy or anemia of cancer in patients with either solid or nonmyeloid hematological malignancies who are not receiving concurrent chemotherapy (Rizzo et al. 2008). Furthermore, ESA treatment is not indicated for patients receiving myelosuppressive therapy when the anticipated outcome is cure, due to the absence of studies that adequately characterize the impact of ESA therapy on progression-free and overall survival. ESA therapy is also not indicated for the treatment of anemia in cancer patients due to other factors such as absolute or functional iron deficiency, folate deficiencies, hemolysis, or gastrointestinal bleeding. ESA use in cancer patients has not been demonstrated in controlled clinical trials to improve symptoms of anemia, quality of life, fatigue, or patient well-being.

Depending on the clinical situation and the severity of anemia, red blood cell transfusion could be an alternative option to ESA therapy (Rizzo et al. 2008). Otherwise, a s.c. rHuEPO dose of 150 IU/kg three times in a week (TIW) or 40 kIU weekly (QW) is recommended to increase hemoglobin and decrease transfusions in patients with chemotherapy-associated anemia when the hemoglobin concentration is approaching, or has fallen below, 10 g/dL. Alternatively, s.c. rHuEPO dose of 80 kIU biweekly (Q2W) or 120 kIU every 3 weeks (Q3W) can be used as initial dosing because these two dosage schedules have not been found to have any differences in efficacy with respect to the approved TIW and QW dosing schedules. The dose of ESA therapy should be titrated for each patient to achieve and maintain the lowest hemoglobin level sufficient to avoid the need for blood transfusion. Therefore, the TIW s.c. dose of rHuEPO should be increased to 300 IU/kg if no reduction in transfusion

requirements or rise in hemoglobin after 8 weeks of treatment has been observed. Similarly, the QW dose should increase to 60 kIU if no increase in hemoglobin by at least 1 g/dL after 4 weeks of treatment is observed. In addition, if hemoglobin exceeds 11 g/dL, but not 12 g/dL, the dose should be reduced by 25%. However, if hemoglobin exceeds 12 g/dL, therapy should be held until hemoglobin falls below 11 g/dL and then restarted at a 25% dose reduction. The pediatric dosing guidance is based on an initial i.v. dose of 600 IU/kg QW (maximum 40 kIU). If there is no increase in hemoglobin by at least 1 g/dL after 4 weeks of treatment (in the absence of RBC transfusion), the rHuEPO dose should be increased to 900 IU/kg (maximum 60 kIU) in order to maintain the lowest hemoglobin level sufficient to avoid RBC transfusion.

The recommended initial s.c. dose of darbepoetin alfa is 2.25 μg/kg QW or 500 μg once every 3 weeks (Q3W). The initial darbepoetin alfa s.c. dose of 2.25 μg/kg QW should be increased to 4.5 μg/kg QW if hemoglobin increase is less than 1 g/dL after 6 weeks of treatment. In addition, if hemoglobin increases by more than 1 g/dL in any 2-week period or when the hemoglobin reaches a level needed to avoid transfusion, the dose should be reduced by 40%. If hemoglobin exceeds a level needed to avoid transfusion, therapy should be held until hemoglobin approaches a level where transfusions may be required then restarted at a 40% dose reduction. A s.c. darbepoetin alfa dose of 100 μg QW, 200 μg Q2W, or 300 μg Q3W can be used as alternative initial dosing since differences in efficacy have not been found. If needed, these initial dose levels should be increased to 150–200 μg QW, 300 μg Q2W, or 500 μg Q3W, respectively. At this time the safety and efficacy of darbepoetin alfa in children receiving chemotherapy has not been established.

Although no specific serum rHuEPO level has been established which predicts which patients would be unlikely to respond to epoetin alfa therapy, treatment is not recommended for patients with grossly elevated serum rHuEPO levels (e.g., greater than 200 mUnits/mL). The hemoglobin should be monitored on a weekly basis in patients receiving ESA therapy until hemoglobin becomes stable and then at regular intervals thereafter.

Patients with multiple myeloma, especially those with renal failure, may benefit from adjunctive ESA therapy to treat anemia. Endogenous EPO levels should be monitored in order to assist in planning ESA therapy. No high-quality, published studies support the exclusive use of epoetin or darbepoetin in anemic myeloma, non-Hodgkin's lymphoma, or chronic lymphocytic leukemia in the absence of chemotherapy. Treatment with chemotherapy and/or corticosteroids should be initiated first. If a rise in hemoglobin does not result, treatment with epoetin or darbepoetin may begin in patients with particular caution exercised with chemotherapeutic agents and disease states where the risk of thromboembolism is increased. Blood transfusion is also an option (Rizzo et al. 2008). The current standard of care for symptomatic anemia in patients with myelodysplastic syndrome (MDS) is supportive care with RBC transfusion. Patients with serum EPO levels less than or equal to 500 IU/L, normal cytogenetics, and less than 15% marrow-ringed sideroblasts may respond to relatively high doses of rHuEPO (40–60 kIU s.c.TIW) or darbepoetin alfa (150–300 μg QW s.c.). Evidence supports the use of epoetin or darbepoetin in patients with anemia associated with low-risk myelodysplasia (Rizzo et al. 2008). Supportive care with RBC transfusion is the standard of care for symptomatic anemia in patients with hematologic malignancies (non-Hodgkin's lymphoma, chronic lymphocytic leukemia). There is insufficient data to recommend ESA therapy for patients responding to treatment with good prognosis and persistent transfusion-dependent anemia.

Iron supplementation improves the hematopoietic response of ESAs used for chemotherapy-induced anemia. A recent meta-analysis of randomized, controlled trials, comparing parenteral or oral iron and no iron, when added to ESAs in anemic cancer patients, evidenced that overall parenteral iron reduces the risk of transfusions by 23% and increases the chance of hematopoietic response by 29% when compared with ESAs alone. On the contrary, oral iron does not increase hematopoietic response or transfusion rate. The significance of these results is that the proportion of nonresponders to ESAs treated with parenteral iron will have strongly improved quality of life and cost ameliorated (Petrelli et al. 2012).

Several studies have reported a possible decreased survival rate in cancer patients receiving ESA for correction of anemia. Analyses of eight randomized studies in patients with cancer found a decrease in overall survival and/or locoregional disease control associated with ESA therapy for correction of anemia with an off-label target hemoglobin level greater than 12 g/dL. These results were confirmed in three recent meta-analyses (Bennett et al. 2008; Bohlius et al. 2009; Tonelli et al. 2009) and refuted in other two meta-analyses (Ludwig et al. 2009; Glaspy et al. 2010). There are also observational data and data from randomized studies that show no increase in mortality with ESA use according to prescribing label specifically in patients receiving chemotherapy. In addition, an increased risk for thromboembolic events has been reported with ESA therapy in cancer patients. Besides the intrinsic risk associated with the malignancy itself, the chemotherapy, and other concomitant factors, results from sev-

eral meta-analyses established a significant association between the increased risk for thrombotic events and ESA use, with relative risk point estimates ranging from 1.48 to 1.69 (Bennett et al. 2008; Tonelli et al. 2009; Ludwig et al. 2009; Glaspy et al. 2010). The increased risk for mortality and thrombotic events in cancer patients receiving ESA therapy is specified in the black box warning included in the FDA label. Seizures and antibody-associated pure red cell aplasia (PRCA) have occurred in chronic renal failure patients receiving ESA therapy. While it is unclear whether cancer patients receiving ESA therapy are at risk of seizures and/or PRCA, ESA treatments should be closely monitored.

MYELOID HEMATOPOIETIC GROWTH FACTORS

■ Granulocyte Colony-Stimulating Factor (G-CSF)

The chemical properties of the myeloid hematopoietic growth factors, G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF), have been characterized (Table 24.2) and extensively reviewed (Armitage 1998). The gene that encodes G-CSF is located on chromosome 17; the mature G-CSF polypeptide has 174 amino acids and is produced in monocytes, fibroblasts, endothelial cells, and bone marrow stromal cells. Filgrastim, a non-glycosylated r-metHuG-CSF, is marketed by several companies under various trade names throughout the world, and several filgrastim biosimilars have also been approved. Lenograstim, a glycosylated rHuG-CSF, is not marketed in the United States but is marketed in other countries under several trade names. Pegfilgrastim, a sustained-duration form of filgrastim to which a 20 kDa polyethylene glycol molecule is covalently bound to the N-terminal methionine residue, is marketed as Neulasta® in the European Union, the United States, and other countries, and several pegfilgrastim biosimilars are in development. Although not all indications are approved in every country, filgrastim, lenograstim, and pegfilgrastim are indicated for the prevention and treatment of chemotherapy-induced febrile neutropenia in cancer patients receiving chemotherapy, mobilization of stem cells for transplantation in oncology patients, and support of induction/consolidation chemotherapy for AML and hematopoiesis after bone marrow transplantation, among others (Aapro et al. 2011).

Filgrastim is primarily eliminated by glomerular filtration in the kidney and binding to the G-CSF receptor on the cell surface of neutrophils and neutrophil precursors, with subsequent internalization of the growth factor-receptor complexes via endocytosis and degradation inside the cells. Pegylation of filgrastim renders renal clearance insignificant, and neutrophil-mediated clearance becomes the predominant elimination pathway. After subcutaneous administration, both filgrastim and pegfilgrastim exhibits flip-flop phenomenon, justifying efficiency of the s.c. administration relative to the i.v. dosing, as well as nonlinear and nonstationary pharmacokinetics due to pharmacodynamic-mediated drug disposition. These findings were quantitatively characterized in a recent population pharmacokinetic and pharmacodynamic meta-analysis using data from 10 phase I-III clinical studies, conducted in 110 healthy adults, and 618 adult and 52 paediatric patients on chemotherapy, following administration of a wide range of i.v. and s.c. doses of filgrastim or pegfilgrastim (Melhem et al. 2018).

Filgrastim and pegfilgrastim increases the proliferation and differentiation of neutrophils from committed progenitor cells, induces maturation, and enhances the survival and function of mature neutrophils, resulting in dose-dependent increases in neutrophils counts. Although similar dissociation constant for filgrastim and pegfilgrastim were found *in vitro*, a four-fold increase in the pegfilgrastim dissociation constant, relative to that from filgrastim, have been observed in human, which suggest pegfilgrastim had lower affinity for the G-CSF receptor than filgrastim. However, the longer half-life of pegfilgrastim, relative to filgrastim, counter balance the lower receptor affin-

	G-CSF	GM-CSF
Nonproprietary name	Filgrastim, lenograstim, and pegfilgrastim	Molgramostim and sargramostim
Chromosome location	17	4
Amino acids	174[a]	127 or 128[b]
Glycosylation	*O*-linked (lenograstim)	*N*-linked (sargramostim)
Pegylation	Pegfilgrastim	None
Source of gene	Bladder carcinoma cell line (filgrastim, pegfilgrastim) and squamous carcinoma cell line (lenograstim)	Human monocyte cell line (molgramostim) and mouse T-lymphoma cell line (sargramostim)
Expression system	*E. coli* (bacteria): filgrastim and pegfilgrastim	*E. coli* (bacteria): molgramostim
	Chinese hamster ovary cell line (mammalian): lenograstim	*Saccharomyces cerevisiae* (yeast): sargramostim

[a]Native G-CSF has two forms, one with 177, which is less active than the other form with 174 amino acids; filgrastim has an N-terminal methionine
[b]Molgramostim has 128 amino acids; sargramostim 127

Table 24.2 ■ Characteristics of the marketed myeloid growth factors, rhG-CSF, and rhGM-CSF

ity to the point that, in humans, the net stimulatory effects of pegfilgrastim were significantly greater than those of filgrastim. Actually, during chemotherapy-induced neutropenia, the clearance of pegfilgrastim is significantly reduced, and the concentration of pegfilgrastim is sustained until the onset of neutrophil recovery. Data from a pivotal study confirmed that a once-per-chemotherapy-cycle injection of pegfilgrastim at 6 mg was as safe and effective as 11 daily injections of filgrastim at 5 μg/kg in reducing neutropenia and its complications in patients with breast cancer receiving four cycles of doxorubicin/docetaxel chemotherapy (Green et al. 2003). Because of the highly efficient regulation of pegfilgrastim clearance via neutrophils and neutrophil precursors, a single fixed dose of pegfilgrastim can be given once per chemotherapy cycle in conjunction with a variety of myelosuppressive chemotherapy regimens (Yang and Kido 2011). Extensive clinical reviews on the myeloid growth factors have been published elsewhere (Keating 2011; Crawford et al. 2009).

■ Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Stem Cell Factor (SCF)

The granulocyte-macrophage colony-stimulating factor (GM-CSF) is a polypeptide of 128 amino acids encoded by a gene located on chromosome 4, secreted by macrophages, T cells, mast cells, NK cells, endothelial cells, and fibroblasts. Molgramostim (marketed in the EU) and sargramostim (marketed in the USA) are two versions of rHuGM-CSF rarely used today. rHuGM-CSF is indicated for neutropenia associated with bone marrow transplantation and antiviral therapy for AIDS-related cytomegalovirus. rHuGM-CSF is also indicated for failed bone marrow transplantation or delayed engraftment and for use in mobilization and after transplantation of autologous PBPCs.

Similarly to G-CSF and GM-CSF, stem cell factor (SCF), encoded on chromosome 12, is a membrane-bound polypeptide of 248 amino acids that proteolytically release a soluble SCF containing 165 amino acids. SCF is an early-acting hematopoietic growth factor that stimulates the proliferation of primitive hematopoietic and non-hematopoietic cells. In vitro, SCF alone has minimal colony-stimulating activity on hematopoietic progenitor cells; however, it synergistically increases colony-forming or stimulatory activity of other HGF. Unlike most hematopoietic growth factors, SCF circulates in relatively high concentrations in normal human plasma. Ancestim® is a non-glycosylated version of the soluble r-metHuSCF marketed in Canada, Australia, and New Zealand and is rarely used in combination with G-CSF to increase the mobilization of peripheral blood progenitor cells (PBPC) for harvesting and support of autologous transplantation after myeloablative chemotherapy in patients with cancer. Comprehensive reviews of r-metHuSCF have been published (Langley 2004).

■ Megakaryocyte Hematopoietic Growth Factors

Megakaryocytopoiesis is a continuous developmental process of platelet production regulated by a complex network of HGF. In this process, hematopoietic stem cells undergo proliferation, differentiation, and maturation, generating megakaryocytes and platelets. Platelet production is controlled by signaling through the hematopoietic c-Mpl receptor. The ligand for this receptor, thrombopoietin (TPO) is the primary regulator of megakaryocyte development and subsequent platelet formation. TPO is a HGF encoded on chromosome 3 and produced in the liver and bone marrow stroma. Depending on the source, the mature polypeptide has between 305 and 355 amino acids, which may undergo cleavage to a smaller polypeptide that retains biologic activity. Upon binding to the c-Mpl receptor, TPO triggers several cellular signal transduction processes, which involve the FOLLOWING pathways: JAK-STAT and TYK2 tyrosine kinase, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and nuclear factor kappa B (NF-κB).

Early recombinant forms of TPO, rHu-TPO and the pegylated megakaryocyte growth and development factor (Peg-MGDF), showed promising results in clinical trials. However, later studies failed to meet their clinical endpoints, because the recombinant proteins generated antibodies that cross-reacted with c-Mpl ligands and resulted in paradoxical thrombocytopenia (Li et al. 2001). Further clinical development of these molecules was therefore suspended. An extensive compilation of the biology of rHu-TPO and Peg-MGDF has been published elsewhere (Kuter et al. 1997).

Romiplostim (Nplate®), previously known as AMG 531, is a novel biological thrombopoiesis-stimulating agent that was developed to overcome the problem of cross-reacting autoantibodies by use of a peptide sequence with no homology to endogenous TPO to activate the c-Mpl receptor. Structurally, romiplostim is a 59 kDa fusion protein that consists of two identical subunits, each containing a human IgG_1 Fc domain covalently linked at the C-terminus to a peptide consisting of two c-Mpl binding domains. The four copies of the TPO mimetic peptide stimulate megakaryocytopoiesis by binding the TPO receptor, yet because they bear no sequence homology with TPO, there is a reduced potential for the generation of anti-TPO antibodies. In vitro, romiplostim competes with TPO for binding to the c-Mpl receptor on normal platelets and Mpl-transfected cells (BaF3-Mpl cells). Upon binding to the c-Mpl receptor, romiplostim activates

the Janus kinase/signal transducers and activators of transcription (JAK-STAT) and other pathways in the same way as endogenous TPO (Broudy and Lin 2004). When cocultured with murine bone marrow cells, romiplostim promotes the growth of CFU-megakaryocytes and promotes the proliferation as well as the maturation of megakaryocytes. During preclinical development, romiplostim led to robust dose-dependent platelet responses in mice, rats, rabbits, and monkeys. The pharmacokinetics and pharmacodynamics of romiplostim in animals, healthy subjects, and patients with immune thrombocytopenia purpura (ITP) have been extensively investigated during clinical development (Wang et al. 2010, 2011; Yan and Krzyzanski 2013; Perez-Ruixo et al. 2012). Similar to erythropoietin stimulating agents and G-CSF analogs, romiplostim exhibits flip-flop phenomenon after s.c. administration, justifying efficiency of the s.c. route relative to the i.v. dosing, as well as nonlinear and non-stationary pharmacokinetics due to pharmacodynamic-mediated drug disposition (Wang et al. 2010). Similar to rHu-EPO, clinical data suggest that approximately only 20–30% of c-Mpl receptors must be occupied with thrombopoietin receptor agonist in order to have 50% of the maximal effect in stimulating the proliferation and differentiation of precursors cells (Wang et al. 2010, Samtani et al. 2009).

Currently, romiplostim has been approved in the USA and the EU and is indicated for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP) who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy (Bussel et al. 2006). An extensive review of the use of romiplostim in ITP patients has been published (Keating 2012). At this time, romiplostim or other protein-based c-Mpl ligands are not approved for clinical use in cancer patients; however, clinical trial data in oncology patients have been recently reported (Kantarjian et al. 2010a, b).

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What do hematopoietic factors do?
2. What are the major lineages or types of mature blood cells?
3. In general, describe chemically the hematopoietic growth factors.
4. How do hematopoietic growth factors function?
5. What are the in vivo actions of rhG-CSF and rhGM-CSF in patients with advanced cancer?
6. What is the physiologic role of EPO?
7. What are the currently commercially available hematopoietic growth factors?
8. What are the indications for rhG-CSF?
9. What are the indications for rhEPO?
10. What is the indication for romiplostim?
11. What are the relevant and common pharmacokinetic and pharmacodynamic properties of the HGF?

■ Answers

1. Hematopoietic growth factors regulate both hematopoiesis and the functional activity of blood cells (including proliferation, differentiation, and maturation). Some hematopoietic growth factors mobilize progenitor cells to move from the bone marrow to the peripheral blood.
2. The myeloid pathway gives rise to red blood cells (erythrocytes), platelets, monocytes/macrophages, and granulocytes (neutrophils, eosinophils, and basophils). The lymphoid pathway gives rise to lymphocytes.
3. They are glycoproteins, which can be distinguished by their amino acid sequence and glycosylation (carbohydrate linkages). Hematopoietic growth factors have folding patterns that are dictated by physical interactions and covalent cysteine-cysteine disulfide bridges. Correct folding is necessary for biologic activity. Most hematopoietic growth factors are single-chain polypeptides weighing approximately 14–35 kDa. The carbohydrate content varies depending on the growth factor and production method, which in turn affects the molecular weight but not necessarily the biologic activity.
4. HGF act by binding to specific cell surface receptors. The resultant complex sends a signal to the cell to express genes, which in turn induce cellular proliferation, differentiation, or activation. A hematopoietic growth factor may also act indirectly if the cell expresses a gene that causes the production of a different hematopoietic growth factor or another cytokine, which in turn binds to and stimulates a different cell.
5. Both HGF cause a transient leucopenia that is followed by a dose-dependent increase in the number of circulating mature and immature neutrophils. Both HGF enhance the in vitro function of neutrophils obtained from treated patients. rhGM-CSF, but not rhG-CSF, also increases the number of circulating monocytes/macrophages and eosinophils, as well as in vitro monocyte cytotoxicity and cytokine production.
6. EPO maintains a normal red blood cell count by causing committed erythroid progenitor cells to proliferate and differentiate into normoblasts.

EPO also shifts marrow reticulocytes into circulation.

7. Besides the biosimilars, five HGF are commercially available, rhG-CSF (filgrastim, lenograstim, pegfilgrastim), rhGM-CSF (molgramostim and sargramostim), rhEPO (epoetin alfa, epoetin beta, darbepoetin alfa), rhSCF (ancestim), and rhIL-11 (oprelvekin).
8. Approval for marketing varies by country and not all countries have all labeled uses. rhG-CSF is indicated for neutropenia associated with myelosuppressive cancer chemotherapy, bone marrow transplantation, and severe chronic neutropenia; rhG-CSF is also indicated to mobilize peripheral blood progenitor cells (PBPC) for PBPC transplantation; and rhG-CSF is indicated for the reversal of clinically significant neutropenia and subsequent maintenance or adequate neutrophil counts in patients with HIV infection during treatment with antiviral and/or other myelosuppressive medications.
9. rhEPO is indicated to treat anemia associated with chronic renal failure, zidovudine-induced anemia in HIV-infected patients, and chemotherapy-induced anemia. rhEPO is also indicated to reduce allogeneic blood transfusions and hasten erythroid recovery in surgery patients.
10. Romiplostim is indicated for the treatment of thrombocytopenia in patients with chronic immune thrombocytopenia (ITP) who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy.
11. There are two main characteristics that are common to erythropoietin stimulating agents, G-CSF analogs and thrombopoietin receptor agonist. The first one is the presence of the flip-flop pharmacokinetics that justifies the efficiency of the s.c. administration relative to the i.v. dosing. The second is the nonlinear (concentration-dependent) and nonstationary pharmacokinetics due to pharmacodynamic-mediated drug disposition, which justify the dose approved since they achieve the level of receptor coverage needed to achieve clinically relevant endpoints.

Acknowledgements Parts of this chapter are updated versions of previously published portions of several other chapters and/or review manuscripts, that include:

1. Heatherington AC (2003) Clinical pharmacokinetic properties of rHuEPO: a review. In: Molineux G, Foote MA, Elliott S (eds) Erythropoietins and erythropoiesis: molecular, cellular, preclinical, and clinical biology. Birkhauser, Basel, pp 87–112
2. Elliot S, Heatherington AC, Foote MA (2004) Erythropoietic factors: clinical pharmacology and pharmacokinetics. In: Morstyn G, Foote MA, and Lieschke GJ (eds) Hematopoietic growths factors in oncology. Humana Press, Totowa, pp 97–123
3. Foote AN (2008) Hematopoietic growth factors. In: Crommelin DJA, Sindelar RD, Meibohm B (eds) Pharmaceutical biotechnology. Fundamentals and applications, 3rd edn. Informa Healthcare USA, New York, pp 225–242
4. Doshi S, Perez-Ruixo JJ, Jang GR, Chow A, Elliot S (2008) Pharmacocinétique de les agents stimulant l'érythropoïèse. In: Rossert J, Casadevall N, Gisselbrecht C (eds) Les agents stimulant l'érythropoïèse. Paris, France
5. Doshi S, Perez-Ruixo JJ, Jang GR, Chow AT (2009) Pharmacokinetics of erythropoiesis-stimulating agents. In: Molineux G, Foote MA, Elliott S (eds) Erythropoietins and erythropoiesis: molecular, cellular, preclinical, and clinical biology. 2nd edn. Birkhäuser Verlag AG, Basel, pp 195–224.
6. Doshi S, Krzyzanski W, Yue S, Elliott S, Chow A, Pérez-Ruixo JJ. Clinical pharmacokinetics and pharmacodynamics of erythropoiesis-stimulating agents. Clin Pharmacokinet. 2013 Dec;52(12):1063-83.

REFERENCES

Aapro MS, Bohlius J, Cameron DA et al (2011) 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours. Eur J Cancer 47:8–32

Adamson JW, Eschbach JW (1990) Treatment of the anemia of chronic renal failure with recombinant human erythropoietin. Annu Rev Med 41:349–360

Agoram B, Sutjandra L, Sullivan JT (2007) Population pharmacokinetics of darbepoetin alfa in healthy subjects. Br J Clin Pharmacol 63:41–52

Agoram B, Aoki K, Doshi S et al (2009) Investigation of the effects of altered receptor binding activity on the clearance of erythropoiesis-stimulating proteins: nonerythropoietin receptor-mediated pathways may play a major role. J Pharm Sci 98:2198–2211

Ait-Oudhia S, Vermeulen A, Krzyzanski W (2011) Non-linear mixed effect modeling of the time-variant disposition of erythropoietin in anemic cancer patients. Biopharm Drug Dispos 32:1–15

Armitage JO (1998) Emerging applications of recombinant human granulocyte colony-stimulating factor. Blood 92:4491–4508

Bennett CL, Silver SM, Djulbegovic B et al (2008) Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. JAMA 299:914–924

Bohlius J, Schmidlin K, Brillant C et al (2009) Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. Lancet 373:1532–1542

Bron D, Meuleman N, Mascaux C (2001) Biological basis of anemia. Semin Oncol 28:1–6

Broudy VC, Lin NL (2004) AMG531 stimulates megakaryopoiesis in vitro by binding to Mpl. Cytokine 25:52–60

Bussel JB, Kuter DJ, George JN et al (2006) AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. N Engl J Med 355:1672–1681

Chanu P, Gieschke R, Charoin JE, Pannier A, Reigner B (2010) Population pharmacokinetic – pharmacodynamic model for a C.E.R.A. in both ESA-naive and ESA-treated chronic kidney disease patients with renal anemia. J Clin Pharmacol 50:507–520

Chapel S, Veng-Pedersen P, Hohl RJ, Schmidt RL, McGuire EM, Widness JA (2001) Changes in erythropoietin pharmacokinetics following busulfan-induced bone marrow ablation in sheep: evidence for bone marrow as a major erythropoietin elimination pathway. J Pharmacol Exp Ther 298:820–824

Cheung WK, Goon BL, Guilfoyle MC, Wacholtz MC (1998) Pharmacokinetics and pharmacodynamics of recombinant human erythropoietin after single and multiple subcutaneous doses to healthy subjects. Clin Pharmacol Ther 64:412–423

Cheung WK, Minton N, Gunawardena K (2001) Pharmacokinetics and pharmacodynamics of epoetin alfa once weekly and three times weekly. Eur J Clin Pharmacol 57:411–418

Crawford J, Armitage J, Balducci L et al (2009) Myeloid growth factors. J Natl Compr Cancer Netw 7:64–83

Deicher R, Horl WH (2004) Differentiating factors between erythropoiesis-stimulating agents: a guide to selection for anemia of chronic kidney disease. Drugs 64:499–509

Doshi S, Chow A, Pérez Ruixo JJ (2010) Exposure-response modeling of darbepoetin alfa in anemic patients with chronic kidney disease not receiving dialysis. J Clin Pharmacol 50(9 Suppl):75S–90S

Egrie JC, Browne JK (2001) Development and characterization of novel erythropoiesis stimulating protein (NESP). Br J Cancer 84(Suppl 1):3–10

Egrie JC, Strickland TW, Lane J et al (1986) Characterization and biological effects of recombinant human erythropoietin. Immunobiology 72:213–224

Egrie JC, Dwyer E, Browne JK, Hitz A, Lykos MA (2003) Darbepoetin alfa has a longer circulating half-life and greater in vivo potency than recombinant human erythropoietin. Exp Hematol 31:290–299

Elliott S, Lorenzini T, Asher S et al (2003) Enhancement of therapeutic protein in vivo activities through glycoengineering. Nat Biotechnol 21:414–421

Elliott S, Heatherington AC, Foote MA (2004a) Erythropoietic factors. In: Morstyn G, Foote MA, Lieschke GJ (eds) Hematopoietic growth factors in oncology: basic science and clinical therapeutics. Humana Press Inc, Totowa, pp 97–123

Elliott S, Egrie J, Browne J et al (2004b) Control of rHuEPO biological activity: the role of carbohydrate. Exp Hematol 32:1146–1155

Fisher JW (2003) Erythropoietin: physiology and pharmacology update. Exp Biol Med 228:1–14

Gascon P, Pirker R, Del Mastro L, Durrwell L (2010) Effects of CERA (continuous erythropoietin receptor activator) in patients with advanced non-small-cell lung cancer (NSCLC) receiving chemotherapy: results of a phase II study. Ann Oncol 21:2029–2039

Glaspy J, Henry D, Patel R et al (2005) The effects of chemotherapy on endogenous erythropoietin levels and the pharmacokinetics and erythropoietic response of darbepoetin alfa: a randomised clinical trial of synchronous versus asynchronous dosing of darbepoetin alfa. Eur J Cancer 41:1140–1149

Glaspy J, Crawford J, Vansteenkiste J et al (2010) Erythropoiesis stimulating agents in oncology: a study-level meta-analysis of survival and other safety outcomes. Br J Cancer 102:301–315

Green MD, Koelbl H, Baselga J et al (2003) A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol 14:29–35

Gross AW, Lodish HF (2006) Cellular trafficking and degradation of erythropoietin and novel erythropoiesis stimulating protein (NESP). J Biol Chem 281:2024–2032

Halstenson CE, Macres M, Katz SA et al (1991) Comparative pharmacokinetics and pharmacodynamics of epoetin alfa and epoetin beta. Clin Pharmacol Ther 50:702–712

Heatherington AC (2003) Clinical pharmacokinetic properties of rHuEPO: a review. In: Molineux G, Foote MA, Elliott S (eds) Erythropoietins and erythropoiesis: molecular, cellular, preclinical, and clinical biology. Birkhauser, Basel, pp 87–112

Jarsch M, Brandt M, Lanzendörfer M, Haselbeck A (2008) Comparative erythropoietin receptor binding kinetics of C.E.R.A. and epoetin-beta determined by surface plasmon resonance and competition binding assay. Pharmacology 81:63–69

Jelkmann W (1992) Erythropoietin: structure, control of production, and function. Physiol Rev 72:449–489

Jelkmann W (2000) Use of recombinant human erythropoietin as an antianemic and performance enhancing drug. Curr Pharm Biotechnol 1:11–31

Jensen JD, Jensen LW, Madsen JK (1994) The pharmacokinetics of recombinant human erythropoietin after subcutaneous injection at different sites. Eur J Clin Pharmacol 46:333–337

Jungers PY, Robino C, Choukroun G, Nguyen-Khoa T, Massy ZA, Jungers P (2002) Incidence of anaemia, and use

of Epoetin therapy in pre-dialysis patients: a prospective study in 403 patients. Nephrol Dial Transplant 17:1621–1627

Kakkar T, Sung C, Gibiansky L et al (2011) Population PK and IgE pharmacodynamic analysis of a fully human monoclonal antibody against IL4 receptor. Pharm Res 28:2530–2542

Kantarjian H, Fenaux P, Sekeres MA et al (2010a) Safety and efficacy of romiplostim in patients with lower-risk myelodysplastic syndrome and thrombocytopenia. J Clin Oncol 28:437–444

Kantarjian HM, Giles FJ, Greenberg PL et al (2010b) Phase 2 study of romiplostim in patients with low- or intermediate-risk myelodysplastic syndrome receiving azacitidine therapy. Blood 116:3163–3170

Kato A, Hishida A, Kumagai H, Furuya R, Nakajima T, Honda N (1994) Erythropoietin production in patients with chronic renal failure. Ren Fail 16:645–651

Kaufman JS, Reda DJ, Fye CL et al (1998) Subcutaneous compared with intravenous epoetin in patients receiving hemodialysis. Department of Veterans Affairs Cooperative Study Group on erythropoietin in hemodialysis patients. N Engl J Med 339:578–583

Keating GM (2011) Lenograstim: a review of its use in chemotherapy-induced neutropenia, for acceleration of neutrophil recovery following haematopoietic stem cell transplantation and in peripheral blood stem cell mobilization. Drugs 71:679–707

Keating GM (2012) Romiplostim. Drugs 72:415–435

Krzyzanski W, Jusko WJ, Wacholtz MC, Minton N, Cheung WK (2005) Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after multiple subcutaneous doses in healthy subjects. Eur J Pharm Sci 26:295–306

Kuter DJ, Hunt P, Sheridan W, Zucker-Franklin D (eds) (1997) Thrombopoiesis and thrombopoeitin. Humana Press Inc, Totowa, p 412

Langley KE (2004) Stem cell factor and its receptor, c-Kit. In: Morstyn G, Foote MA, Lieschke GJ (eds) Hematopoietic growth factors in oncology. Humana Press Inc, Totowa, pp 153–184

Li J, Yang C, Xia Y et al (2001) Thrombocytopenia caused by the development of antibodies to thrombopoietin. Blood 98:3241–3248

Ludwig H, Crawford J, Osterborg A et al (2009) Pooled analysis of individual patient-level data from all randomized, double-blind, placebo controlled trials of darbepoetin alfa in the treatment of patients with chemotherapy-induced anemia. J Clin Oncol 27:2838–2847

Macdougall IC (2005) CERA (continuous erythropoietin receptor activator): a new erythropoiesis-stimulating agent for the treatment of anemia. Curr Hematol Rep 4:436–440

Macdougall IC, Gray SJ, Elston O et al (1999) Pharmacokinetics of novel erythropoiesis stimulating protein compared with epoetin alfa in dialysis patients. J Am Soc Nephrol 10:2392–2395

McLennan DN, Porter CJ, Edwards GA, Heatherington AC, Martin SW, Charman SA (2006) The absorption of darbepoetin alfa occurs predominantly via the lymphatics following subcutaneous administration to sheep. Pharm Res 23:2060–2066

McMahon FG, Vargas R, Ryan M et al (1990) Pharmacokinetics and effects of recombinant human erythropoietin after intravenous and subcutaneous injections in healthy volunteers. Blood 76:1718–1722

Melhem M, Delor I, Pérez-Ruixo JJ et al (2018) Pharmacokinetic-pharmacodynamic modelling of neutrophil response to G-CSF in healthy subjects and patients with chemotherapy-induced neutropenia. Br J Clin Pharmacol 84:911–925

Miller CB, Jones RJ, Piantadosi S, Abeloff MD, Spivak JL (1990) Decreased erythropoietin response in patients with the anemia of cancer. N Engl J Med 322:1689–1692

Nissenson AR, Swan SK, Lindberg JS et al (2002) Randomized, controlled trial of darbepoetin alfa for the treatment of anemia in hemodialysis patients. Am J Kidney Dis 40:110–118

Olsson-Gisleskog P, Jacqmin P, Perez-Ruixo JJ (2007) Population pharmacokinetics meta-analysis of recombinant human erythropoietin in healthy subjects. Clin Pharmacokinet 46:159–173

Perez-Ruixo JJ, Krzyzanski W, Bouman-Thio E et al (2009) Pharmacokinetics and pharmacodynamics of the erythropoietin mimetibody construct CNTO 528 in healthy subjects. Clin Pharmacokinet 48:601–613

Perez-Ruixo JJ, Green B, Doshi S, Wang YM, Mould DR (2012) Romiplostim dose-response in patients with immune thrombocytopenia. J Clin Pharmacol 52:1540–1551

Petrelli F, Borgonovo K, Cabiddu M, Lonati V, Barni S (2012) Addition of iron to erythropoiesis-stimulating agents in cancer patients: a meta-analysis of randomized trials. J Cancer Res Clin Oncol 138:179–187

Piron M, Loo M, Gothot A, Tassin F, Fillet G, Beguin Y (2001) Cessation of intensive treatment with recombinant human erythropoietin is followed by secondary anemia. Blood 97:442–448

Porter CJ, Charman SA (2000) Lymphatic transport of proteins after subcutaneous administration. J Pharm Sci 89:297–310

Ramakrishnan R, Cheung WK, Wacholtz MC, Minton N, Jusko WJ (2004) Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. J Clin Pharmacol 44:991–1002

Rizzo JD, Somerfield MR, Hagerty KL et al (2008) Use of epoetin and darbepoetin in patients with cancer: 2007 American Society of Clinical Oncology/American Society of Hematology clinical practice guideline update. J Clin Oncol 26:132–149

Samtani MN, Perez-Ruixo JJ, Brown KH et al (2009) Pharmacokinetic and pharmacodynamic modeling of pegylated thrombopoietin mimetic peptide (PEG-TPOm) after single intravenous dose administration in healthy subjects. J Clin Pharmacol 49:336–350

Schellekens H, Moors E (2010) Clinical comparability and European biosimilar regulations. Nat Biotechnol 28:28–31

Schellekens H, Smolen JS, Dicato M, Rifkin RM (2016) Safety and efficacy of biosimilars in oncology. Lancet Oncol 17:e502–e509

Sinclair AM, Elliott S (2005) Glycoengineering: the effect of glycosylation on the properties of therapeutic proteins. J Pharm Sci 94:1626–1635

Spivak JL (1998) The biology and clinical applications of recombinant erythropoietin. Semin Oncol 25(3 suppl 7):7–11

Supersaxo A, Hein WR, Steffen H (1990) Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. Pharm Res 7:167–169

Sutjandra L, Rodriguez RD, Doshi S et al (2011) Population pharmacokinetic meta-analysis of denosumab in healthy subjects and postmenopausal women with osteopenia or osteoporosis. Clin Pharmacokinet 50:793–807

Tonelli M, Hemmelgarn B, Reiman T et al (2009) Benefits and harms of erythropoiesis-stimulating agents for anemia related to cancer: a meta-analysis. CMAJ 180:E62–E71

Uehlinger DE, Goth FA, Sheiner LB (1992) A pharmacodynamic model of erythropoietin therapy for uremic anemia. Clin Pharmacol Ther 51:76–89

Vansteenkiste J, Pirker R, Massuti B et al (2002) Double-blind, placebo-controlled, randomized phase III trial of darbepoetin alfa in lung cancer patients receiving chemotherapy. J Natl Cancer Inst 94:1211–1220

Walrafen P, Verdier F, Kadri Z, Chrétien S, Lacombe C, Mayeux P (2005) Both proteasomes and lysosomes degrade the activated erythropoietin receptor. Blood 105:600–608

Wang YM, Krzyzanski W, Doshi S, Xiao JJ, Pérez-Ruixo JJ, Chow AT (2010) Pharmacodynamics-mediated drug disposition (PDMDD) and precursor pool lifespan model for single dose of romiplostim in healthy subjects. AAPS J 12:729–740

Wang YM, Sloey B, Wong T, Khandelwal P, Melara R, Sun YN (2011) Investigation of the pharmacokinetics of romiplostim in rodents with a focus on the clearance mechanism. Pharm Res 28:1931–1938

Wide L, Bengtsson C, Birgegard G (1989) Circadian rhythm of erythropoietin in human serum. Br J Haematol 72:85–90

Yan X, Lowe PJ, Fink M, Berghout A, Balser S, Krzyzanski W (2012) Population pharmacokinetic and pharmacodynamic model-based comparability assessment of a recombinant human epoetin alfa and the biosimilar HX575. J Clin Pharmacol 52:1624–1644

Yan X, Krzyzanski W (2013) Quantitative assessment of minimal effective concentration of erythropoiesis-stimulating agents. CPT Pharmacometrics Syst Pharmacol 2:e62. https://doi.org/10.1038/psp.2013.39

Yang BB, Kido A (2011) Pharmacokinetics and pharmacodynamics of pegfilgrastim. Clin Pharmacokinet 50:295–306