Chapter 2
Early-Acting Hematopoietic Growth Factors: Biology and Clinical Experience

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Abstract Secreted protein growth factors that stimulate the self-renewal, proliferation, and differentiation of the most primitive stem cells are among the most biologically interesting molecules and at least theoretically have diverse applications in the evolving field of regenerative medicine. Among this class of regulators, the early-acting hematopoietic growth factors and their cellular targets are perhaps the best characterized and serve as a paradigm for manipulating other stem cell based tissues. This chapter reviews the preclinical knowledge accumulated over ~40 years, since the discovery of the first such growth factor, and the clinical applications of those that, upon testing in humans, ultimately gained regulatory approval for the treatment of various hematological diseases.

Introduction

Blood comprises many cell types that carry out highly specialized functions such as transporting oxygen to tissues and combating infection via both cell-based and humoral mechanisms. The production of blood, or hematopoiesis, is one of the most well-studied physiological processes and serves as a paradigm for other adult stem cell systems and the regulation of self-renewing tissues. During steady-state hematopoiesis in humans, approximately 200 billion new erythrocytes, 100 billion leukocytes, and 100 billion platelets are produced each day to replace those lost through natural aging processes. In response to hematological stress (e.g., hypoxia, infection), the numbers of a particular type of blood cell required to meet physiological demands can expand rapidly by >tenfold. This remarkable capacity for lineage-specific expansion while maintaining the appropriate balance of blood cell types resides in a hierarchy of hematopoietic stem and progenitor cells that are found

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G.H. Lyman, D.C. Dale (eds.), *Hematopoietic Growth Factors in Oncology*,
Cancer Treatment and Research 157, DOI 10.1007/978-1-4419-7073-2_2,
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mainly in the bone marrow (BM). At the origin of this hierarchy lies a comparatively rare population of ~50 million pluripotent hematopoietic stem cells (HSCs) [1]. HSCs are normally quiescent or cycle very slowly. When stimulated to proliferate, they undergo a series of asymmetric cell divisions and fate decisions during which they gradually lose the potential to execute one or more developmental options. This leads to the generation of a heterogeneous pool of multipotent, tripotent, bipotent, and ultimately unipotent progenitor cells that are committed to differentiate into one of the eight lineages of morphologically identifiable cells in the peripheral blood. In addition to their extensive capacity for proliferation and multilineage differentiation, HSCs have the ability to self-renew thus preventing exhaustion of the stem cell pool and ensuring that an adequate supply of blood cells can be produced for the lifetime of the individual.

At the level of each individual stem or progenitor cell, the probability of executing any one of these developmental options, or of dying by apoptosis, is tightly regulated by a network of glycoprotein hormones known as the hematopoietic growth factors (HGFs). HGFs exhibit a general hierarchical organization in their actions that mirrors that of the cellular elements of the hematopoietic system. However, there is considerable overlap in target cell populations and some cytokines that were originally thought to act only on lineage-committed progenitor cells or their progeny are now known to have multiple levels of activity, including on the most primitive HSCs (e.g., thrombopoietin; TPO). Conversely, some factors that were initially thought to act only on multipotential cells were found to stimulate the proliferation of mature cells (e.g., stem cell factor; SCF). Thus, pleiotropy and redundancy have emerged as dominant themes.

Most HGFs are produced by macrophages, fibroblasts, osteoblasts, and endothelial cells that comprise the BM microenvironment. These so-called stromal cells also express adhesion molecules that serve to physically retain stem and progenitor cells within "niches," thus co-localizing them with factors that regulate the earliest events in their development. The action of HGFs to promote proliferation and differentiation is balanced by that of various inhibitory factors that attenuate the proliferation response once physiological demand is satisfied. The opposing activities of these positive and negative regulators on various cell types can be further modulated in a concentration-dependent manner and depending on the context in which they are presented to the target cell, i.e., either alone or in combination with other cytokines, and whether the growth factor is secreted or bound to the surface of stromal cells.

Since the regulatory approval and commercial launch of the first recombinant human (rHu) HGFs in the early-mid-1990s, several such agents have been administered to millions of patients. Much attention has been focused on the late-acting cytokines such as erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), and TPO because these have proven to be most useful for the treatment of different cytopenias. Comparatively little attention has been given to early-acting factors as these have in general not made a large therapeutic impact or proven to be as commercially successful. Moreover, discovery of the "holy grail" of experimental hematology, the putative stem cell-specific self-renewal factor, has remained elusive (indeed current evidence suggests it may not exist at all). Nevertheless,

early-acting HGFs, defined herein as cytokines with actions on multipotential cells, exhibit an array of interesting activities and their therapeutic utility remains to be fully explored. In this chapter, the biology of the most well-characterized early-acting HGFs will be reviewed with particular emphasis on those that have been investigated in the clinic.

Stem Cell Factor (SCF)

The history of the discovery of SCF originates early in the last century with the description of the dominant White spotting (*W*) locus in mice. Mice bearing any of the ~20 allelic variants of the *W* gene exhibit multiple defects including macrocytic anemia [2]. The identification of another mutation, designated Steel (*Sl*), with a virtually identical phenotype but localized to a different chromosome led to the hypothesis that these loci may represent a receptor/ligand pair. Confirmation of his hypothesis was obtained in 1988, when two groups simultaneously showed that the *W* locus encoded a tyrosine kinase receptor, c-kit, with the same general structure as the receptors for macrophage colony-stimulating factor (M-CSF), Flt3 ligand (Flt3L), and platelet-derived growth factor [3, 4]. This ignited a vigorous race to identify the cognate ligand that culminated with the cloning of the mouse, rat, and human SCF genes simultaneously by three groups in 1990 [5–7]. Though named differently by each team, SCF (c-kit ligand [KL], steel factor, or mast cell growth factor [MGF]) was found to be a 248 amino acid type I transmembrane protein that also undergoes proteolytic cleavage to generate a ~165 amino acid secreted ligand, both of which are biologically active [5, 6, 8]. Alternative mRNA splicing appears to be an additional mechanism for producing soluble or membrane-bound forms of SCF. The ratio of these isoforms differs between tissues, but the physiological relevance of this phenomenon is unknown. Cell-bound SCF is required for normal development since mice bearing the Steel-Dickie (Sl^d) mutation that eliminates only this form of the factor exhibit several developmental abnormalities [9]. Moreover, when cDNAs encoding either form of human SCF were transfected into *Sl/Sl* stromal cells, the membrane-bound form of SCF was better able to support human hematopoietic cells in vitro than secreted SCF [10]. The amino acid sequences of the mouse and human proteins are 82% identical, but while murine SCF is fully active on human cells, human SCF is ~1,000-fold less active on rodent cells than murine SCF [6]. Native SCF is glycosylated [11]. The recombinant human protein produced for clinical studies in *Escherichia coli* (see below) is non-glycosylated but identical to the native amino acid sequence except for the presence of an N-terminal methionine (met).

SCF (together with Flt3L discussed below) exemplifies a distinct group of early-acting HGFs that have little or no growth-promoting activity as single agents, but which selectively promote the survival of primitive hematopoietic cells including stem cells with long-term repopulating ability [12, 13]. However, SCF can synergize with most other HGFs in vitro to directly enhance stem and progenitor cell

proliferation [14–16]. In such cultures, SCF increases the number and size of the colonies produced, but their composition typically reflects the later-acting lineage-associated cytokines that are present. In vivo administration of recombinant SCF to *Sl/Sl*^{d} mice ameliorates their anemia, which reappears upon cessation of treatment. The number of granulocytes, monocytes, platelets, and lymphocytes also increases above normal levels [17]. Early after administration of SCF to normal mice, circulating neutrophil counts increase modestly and primitive clonogenic cells are mobilized to the peripheral blood from which they redistribute to peripheral sites such as the spleen [18]. BM stem and progenitor cells also begin to proliferate so that their numbers may be greatly expanded after ~2 weeks. These effects are much more pronounced when SCF is combined with G-CSF, resulting in greater than additive increases in circulating neutrophils and enhanced mobilization of stem/progenitor cells that are capable of rescuing mice, dogs, and non-human primates from lethal irradiation [19–21]. Mast cells are the most dependent on SCF for their survival, proliferation, maturation, and function. SCF increases mast cell numbers in *Sl* (but not *W*) mutant mice as well as in normal rodents and non-human primates [22].

Based on these preclinical data, clinical development of r-metHuSCF (ancestim, STEMGEN®) focused on its use in combination with r-metHuG-CSF (filgrastim, NEUPOGEN®) to optimally mobilize hematopoietic stem and progenitor cells for transplantation after myeloablative therapy in cancer patients. Results of these clinical studies have been reviewed in detail [23]. Briefly, in phase I/II studies of patients with breast cancer, non-Hodgkin's lymphoma, and ovarian cancer, 5 μg/kg/day rHuSCF as a single agent did not induce significant mobilization. However, when combined with r-metHuG-CSF, r-metHuSCF improved apheresis yields by twofold to threefold. The numbers of circulating $CD34^{+}$ cells and in vitro colony-forming cells (CFCs) then returned to pretreatment levels usually within 4–7 days after cessation of treatment. In the pivotal phase III trial, a greater proportion of breast cancer patients treated with 20 μg/kg/day r-metHuSCF plus 10 μg/kg/day filgrastim achieved the target yield of 5×10^{6} $CD34^{+}$ cells/kg for autologous transplantation than did patients treated with filgrastim alone (63% vs. 47%) [24] (Fig. 2.1). The improved mobilization with r-metHuSCF plus r-metHuG-CSF resulted in a statistically significant reduction in the number of aphereses required to collect the target number of peripheral blood stem/progenitor cells (median 4 vs. ≥6) and increased the number of patients for which a sufficient graft could be collected, compared to mobilization with r-metHuG-CSF alone. These clinical findings led to approval of STEMGEN® in Canada, Australia, and New Zealand as a co-administration with filgrastim for hematopoietic stem and progenitor cell mobilization. Consistent with the collateral effects of SCF in stimulating mast cell proliferation and degranulation, all patients require prophylactic administration of H1 and H2 antihistamines and a bronchodilator to ameliorate systemic anaphylactoid reactions.

In addition to stem cell mobilization, SCF has demonstrated utility in ex vivo expansion. Addition of SCF to cultures containing interleukin (IL)-3, IL-6, IL-11, TPO, granulocyte-macrophage colony-stimulating factor (GM-CSF), and other cytokines enhances the survival, proliferation, and differentiation of $CD34^{+}CD38^{-}$

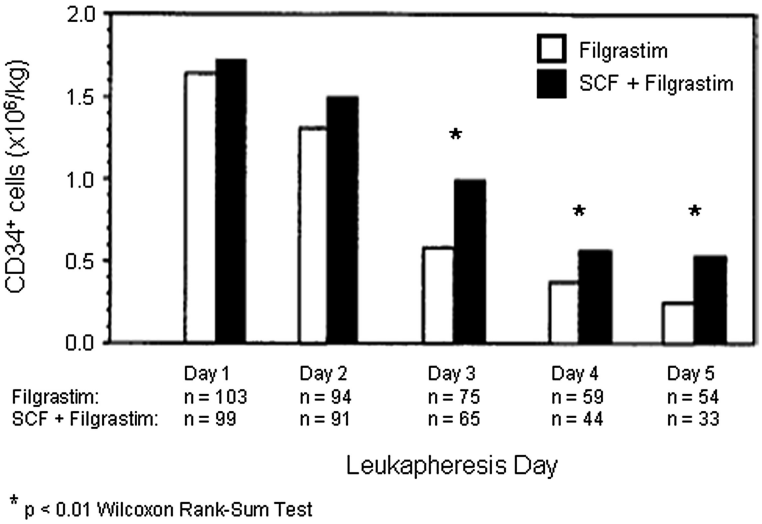


Fig. 2.1 SCF enhances the mobilization of hematopoietic cells by G-CSF. Shown is the median $CD34^+$ cell yield on each day of leukapheresis in patients mobilized with 10 μg/kg/day rHuG-CSF (filgrastim) alone or together with 20 μg/kg/day rHuSCF. Leukapheresis was started on day 5 after growth factor treatment (indicated as day 1) until the cumulative $CD34^+$ cell yield reached $\geq 5 \times 10^6$/kg or a maximum of 5 leukaphereses. Reproduced with permission from Ref. [24]

candidate HSCs, long-term culture-initiating cells (LTC-IC), and stem cells with long-term repopulating ability [25–27]. Expanded hematopoietic cells have been used to accelerate the rate of hematopoietic reconstitution after transplantation [28, 29] and to support repetitive cycles of high-dose chemotherapy thus eliminating the need for additional BM harvests or leukaphereses [30]. Ex vivo expansion may also extend the application of umbilical cord blood stem cell transplantation to adult patients for whom the number of HSCs in a typical cord blood unit is often insufficient to facilitate engraftment or enable the purging from autologous stem cell preparations of contaminating tumor cells that can contribute to disease relapse following transplantation. Finally, SCF is being used extensively in the developing field of gene therapy where it offers an advantage over late-acting HGFs in better preserving the engraftment potential of the targeted stem cells during their infection with viral vectors in vitro.

Flt3 Ligand (Flt3L)

Like SCF, the discovery of Flt3L began with the identification of its receptor, fms-like tyrosine kinase 3 or flt3 [31]. Murine flt3 was used as a probe to identify the murine [32, 33] and human [33, 34] ligands, which were found to be 72% identical at the amino acid level. Flt3L has many features in common with SCF. Like SCF, Flt3L is a type I transmembrane glycoprotein, the mature form

of which is composed of 209 amino acids in man. The membrane-bound isoform is predominant and biologically active, though like SCF, rare alternative isoforms resulting from alternative splicing have been reported and an active soluble protein is produced by proteolytic cleavage [32]. The form of rHuFlt3L used in clinical studies described below is truncated (153 aa) and glycosylated with a molecular weight of 18–29 kDa. Unlike SCF, Flt3L does not exhibit any species specificity and human Flt3L is equally potent on rodent, rabbit, and non-human primate cells.

Flt3L is produced at highest levels in the BM and expression is further upregulated by conditions that damage the HSC compartment (e.g., radiation). Although SCF and Flt3L overlap considerably in their biological actions, there are notable differences. For example, murine long-term repopulating HSCs are c-kit$^+$ but flt3-negative and upregulate the receptor only upon activation and maturation [35]. However, in a rare example of differences between the mouse and human hematopoietic systems, human HSCs capable of engrafting immunodeficient mice are flt3$^+$ [36, 37]. Expression of flt3 is then progressively downregulated during myeloid differentiation and is shut off completely prior to erythroid commitment. Flt3 is also absent on mast cells so it does not exhibit the anaphylactic effects of SCF in vivo. Similar to SCF, as a single agent Flt3L promotes the survival rather than proliferation of HSCs but synergizes with virtually all the other HGFs, including SCF, to enhance their activities [38–40]. However, when directly compared to SCF as a supplement to otherwise identical cytokine combinations, Flt3L is often less potent, generating slightly fewer and smaller colonies than those observed with SCF-containing cocktails. This is consistent with the absence of Flt3 on more primitive clonogenic cells, at least in mice, as discussed above.

In vivo administration of rHuFlt3L results in the expansion of hematopoietic progenitor populations in the BM and their mobilization into the blood [41]. Stem cell mobilization induced by G-CSF is increased ~fivefold by co-administration of Flt3L, and mobilized stem/progenitor cells retain the ability to reconstitute hematopoiesis after transplantation [42]. Injection of Flt3L into mice also increases the number of immature B cells, monocytes, and natural killer (NK) cells in the BM and blood and induces the appearance of dendritic cells (DCs) in the spleen and secondary lymphoid tissues. The expansion of DCs is particularly significant as these cells are among the most efficient at presenting processed antigens to cytotoxic T cells (CTL), resulting in their activation and proliferation to mediate antitumor and antiviral immune responses. DCs have been used as vaccine vectors for cancer and infectious diseases, but success is limited by their low abundance in peripheral blood and lymphoid tissues. Administration of Flt3L has beneficial effects in preclinical cancer models [43, 44], but maximal efficacy is probably limited by the fact that the DCs generated by Flt3L alone are immature [45]. Combination therapy with CD40 ligand (CD40L), a potent inducer of DC maturation that is required to promote the development and expansion of antigen-specific CTL, has been shown to significantly improve antitumor immunity [46] (Fig. 2.2).

Based on these preclinical data, clinical studies were conducted to assess the ability of Flt3L to improve the yield of stem/progenitor cells for stem cell mobilization,

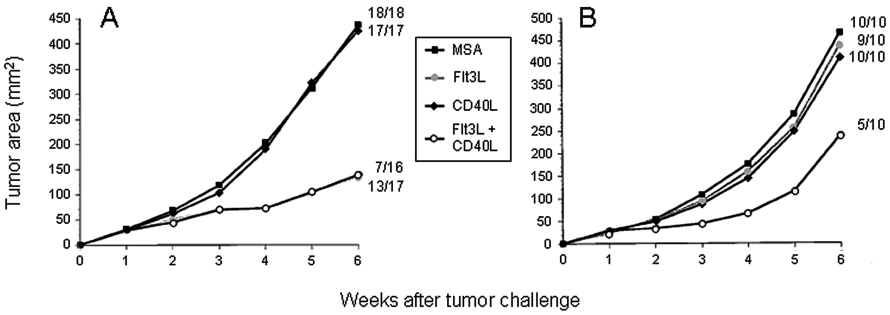


Fig. 2.2 Combination treatment with Flt3L and CD40L inhibits the growth of murine tumors in vivo. **Panel a**: C57BL/6 mice were transplanted 5×10^5 B10.2 sarcoma cells and treated for 19 days with mouse serum albumin (MSA; 0.1 μg/day), Flt3L (10 μg/day), CD40L (10–30 μg/day), or a combination of Flt3L (10 μg/day) and CD40L (10–30 μg/day). Treatments were started on the same day as the tumor cell inoculations. **Panel b**: C3H/HeN mice were transplanted 5×10^5 '87' sarcoma cells and treated with mouse serum albumin (MSA; 0.1 μg/day), Flt3L (10 μg/day), CD40L (10 μg/day), or a combination of Flt3L and CD40L (each at 10 μg/day). Treatments were started on the day after tumor cell inoculation, except for CD40L either alone or in combination with Flt3L, which began 7 days after the initiation of MSA or Flt3L treatment. Mice were treated until day 20. The number of tumor-bearing mice as a fraction of all animals per treatment group is indicated for the last time point. Reproduced with permission from Ref. [46]

as a stand-alone immunotherapeutic agent for cancer patients or as an adjuvant for cancer or infectious diseases. As in animals, administration of Flt3L alone induced dose-dependent increases in circulating neutrophils, monocytes, $CD34^+$ cells, B cells, and plasmacytoid and conventional DCs. Combining Flt3L with r-metHuG-CSF (NEUPOGEN®) or rHuGM-CSF (sargramostim, LEUKINE®) resulted in increased stem cell mobilization in "hard to mobilize" patients with non-Hodgkin's lymphoma (NHL) but had limited effects in "easy to mobilize" patients with breast cancer. When combined with chemotherapy in patients with hormone-refractory prostate cancer, NHL, or metastatic melanoma, Flt3L treatment led to increased numbers of monocytes and DCs in the peripheral blood. However, there was no effect on tumor response rates perhaps, as noted in preclinical studies, because the DCs that were generated in vivo were immature. In numerous studies in which Flt3L was tested in the vaccine adjuvant setting, it did not augment antigenicity when delivered in vivo prior to the vaccine. However, intriguing results were noted in one study when Flt3L was used to stimulate DC expansion in vivo, from which a vaccine was prepared ex vivo [47]. In this study, 12 patients were administered Flt3L at different dosing schedules to expand DCs prior to collection of peripheral blood cells, which were then primed with tumor antigen in culture. Following reinfusion of tumor antigen-primed DCs, seven patients developed tumor-specific CTL and five patients had their tumors regress though they did not receive any other cancer treatment during this time (Fig. 2.3). Although CTL activity after vaccination did not correlate directly with clinical responses, both the percent and fold expansion of vaccine-induced $CD8^+$ T cells did.

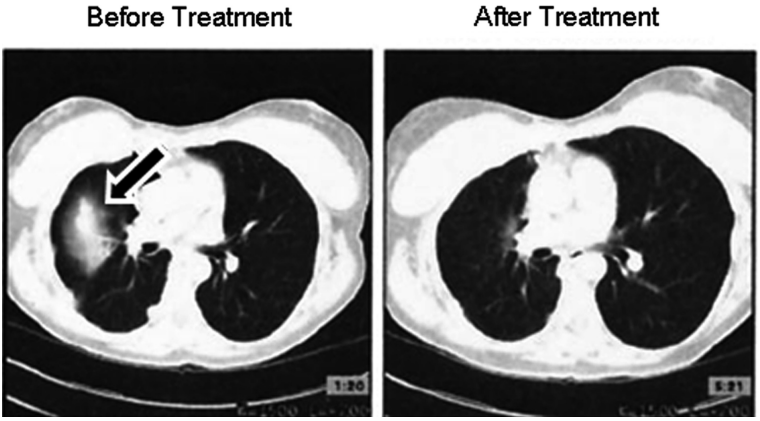


Fig. 2.3 Tumor regression in a cancer patient treated with a DC vaccine generated ex vivo following in vivo treatment with Flt3L. Computed tomography scans were performed within 1 month before the first DC vaccination (*left*) and 4 months after the final DC vaccination (*right*) in a patient with progressive metastatic colorectal cancer. A growing lung metastasis (*black arrow* in *left* scan) and malignant pleural effusion (not shown) that were evident before vaccination completely resolved after treatment. Reproduced with permission from Ref. [47]

For now, clinical development of rHuFlt3L has been discontinued, but preclinical research continues in earnest. Recently, Flt3L was included among a list of 12 agents with the greatest potential to cure cancer by a National Cancer Institute workshop [48]. In addition to the uses described above, other applications of Flt3L (as with SCF) include gene therapy to improve HSC transduction efficiency with viral vectors and ex vivo expansion. The recent finding that common lymphoid progenitors (CLPs), but not HSCs or common myeloid progenitors (CMPs), are reduced ~tenfold in Flt3L knock-out mice [49] suggests its potential utility in driving CLP expansion as a means to accelerate the typically slow rate of lymphoid reconstitution that follows allogeneic transplantation in particular or as a prophylactic therapy for infection in neonates or the elderly who often have dampened immunity. The promise of this agent remains high, and there is an obvious need for further studies.

Thrombopoietin (TPO)

TPO is the seminal regulator of megakaryocyte differentiation and platelet production that is constitutively produced mainly in the liver. TPO was first postulated to exist in 1958 [50], well before the gene was cloned by five groups simultaneously in 1994 [51–55]. Purified recombinant TPO was confirmed to be the ligand for the orphan cytokine receptor, c-mpl (the cellular homolog of the myeloproliferative leukemia virus oncogene, v-mpl), whose expression is restricted to primitive hematopoietic cells, megakaryocytes, and platelets. Human TPO comprises a total of 332 amino acids and nearly half of its ~70 kDa mass is contributed by N-linked

and O-linked carbohydrates [52–54]. Sugars are attached predominantly to the 181 amino acid C-terminal domain that lacks any sequence similarity to other HGFs and serves to enhance secretion of the protein from producer cells [56]. An N-terminal domain of 153 amino acids has some sequence similarity to EPO and is sufficient for biological activity in vitro [53]. The basic biology of TPO, its late-acting effects on megakaryocytopoiesis, and the clinical development of rHuTPO and second-generation mpl ligands (e.g., romiplostim, Nplate®) for the treatment of thrombocytopenia are described in detail elsewhere in this volume. I will focus here on the early actions of TPO on primitive HSCs, which have only recently been described in mechanistic detail and have not as yet been exploited for therapeutic purposes.

The effect of TPO on non-megakaryocytic lineages was obvious from early studies characterizing *TPO*-deficient and *c-mpl*-deficient mice. As expected, both knock-out strains had reduced numbers of megakaryocytic progenitor cells (CFU-Mk) in the BM and spleen, a lower average ploidy of surviving megakaryocytes, and ~85% fewer circulating platelets. Significantly, however, the number of multipotential and committed myeloid and erythroid progenitors in the BM, spleen, and peripheral blood was also reduced [57, 58], suggesting that TPO exerts either direct or indirect actions on multipotential stem cells. This was supported by studies demonstrating that administration of recombinant TPO or megakaryocyte growth and development factor (MGDF, a non-glycosylated and truncated version of TPO that is covalently conjugated to polyethylene glycol [PEG] to enhance the stability of the molecule) to normal or myelosuppressed mice resulted in the expansion of CFU-GM and BFU-E (as well as CFU-Mk) and reduced the severity of chemotherapy-induced or radiation-induced leukopenia and anemia [59, 60]. TPO was shown to act directly in single cell cultures of highly enriched murine long-term repopulating HSCs. In combination with other early-acting HGFs (e.g., SCF, Flt3L, IL-3, IL-6), TPO enhanced HSC survival, accelerated the timing of the first cell division, and synergistically increased the number and size of multilineage colonies [61–64]. Solar et al. [65] found that ~50–70% of murine fetal liver AA4.1$^+$Sca-1$^+$c-kit$^+$ cells, murine BM-derived Sca-1$^+$c-kit$^+$Lin$^-$ cells, and human BM CD34$^+$CD38$^-$ cells expressed the c-mpl protein. As shown in Table 2.1 for human stem cells, when these were fractionated into mpl$^-$ and mpl$^+$ populations, essentially all of the HSCs able to reconstitute hematopoiesis in vivo were recovered in the mpl$^+$ fraction [65].

The molecular mechanisms underlying these actions of TPO on HSCs are beginning to be deciphered (Fig. 2.4). TPO was recently shown to modulate HoxB4 and HoxA9, members of a homeodomain-containing family of transcription factors that are expressed at high levels in HSCs and are critical for self-renewal. The level of HoxB4 mRNA is ~threefold lower in mouse stem cells (Sca-1$^+$c-kit$^+$Gr-1$^-$) from *TPO*$^{-/-}$ vs. wild-type mice. TPO increases HoxB4 mRNA levels in a p38-dependent manner in a cell line engineered to express human *c-mpl* [66]. TPO also induces the importation of HoxA9 into the nucleus, in this case without altering overall levels, where it can physically associate with the co-factor MEIS1 (myeloid ecotropic viral integration site 1) whose expression is also increased by TPO [67].

Table 2.1 Human hematopoietic stem cells with in vivo repopulating ability express the TPO receptor (c-mpl)

Cell phenotype	Study no.	No. of mice engrafted	Donor HLA/CD34 (%)	Donor HLA/CD33 (%)	Donor HLA/CD19 (%)
$CD34^+CD38^-$	1	3/5	8.4 ± 2.8	14.3 ± 5.3	64.2 ± 11.3
$CD34^+CD38^-$ c-mpl^+	1	5/5	22.7 ± 5.7	18.9 ± 3.6	67.6 ± 2.5
$CD34^+CD38^-$ c-mpl^-	1	1/4	4.2	12.5	18.9
$CD34^+CD38^-$	2	3/5	7.9 ± 1.2	17.7 ± 4.2	48.5 ± 3.3
$CD34^+CD38^-$ c-mpl^+	2	4/5	27.8 ± 3.5	12.9 ± 4.9	59.3 ± 9.6
$CD34^+CD38^-$ c-mpl^-	2	1/5	1.8	5.6	5.9

Immunodeficient *scid/scid* mice were implanted with human fetal bone marrow fragments and then injected with 3×10^4 adult bone marrow-derived $CD34^+CD38^-$ hematopoietic stem cells that had been separated into c-mpl^- or c-mpl^+ fractions by fluorescence-activated cell sorting. The progeny of transplanted human HSCs was distinguished from cells comprising the human fetal bone graft and host mouse cells by expression of a donor-specific human leukocyte antigen (HLA). In contrast to c-mpl^- cells that contributed rarely to only low levels to hematopoiesis, c-mpl^+ HSCs could regenerate large numbers of stem/progenitor (HLA/CD34), myeloid (HLA/CD33), and B lymphoid cells in the majority of transplanted recipients. Reproduced with permission from Ref. [65].

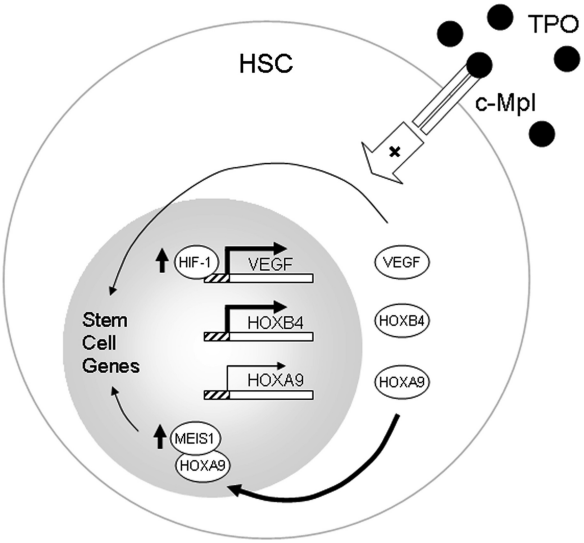


Fig. 2.4 TPO stimulates an intracellular circuit to promote the survival and proliferation of hematopoietic stem cells. TPO binding to its cell surface receptor, c-mpl, initiates signals that increase transcription of VEGF (through enhanced synthesis and stabilization of HIF-α) and HOXB4. Nuclear import of HOXA9 protein is also enhanced facilitating its interaction with MEIS1, transcription of which is also stimulated by TPO. Together these signals increase the expression of diverse "stemness" genes that play key roles in HSC development

Finally, TPO increases the synthesis and stabilization of hypoxia-inducible factor (HIF)-1α, the primary transcription factor responsible for expression of vascular endothelial growth factor (VEGF), which in addition to being the principal regulator of blood vessel formation, also controls HSC survival and repopulating potential through an intracellular autocrine loop [68]. This is consistent with the fact that compared to other tissues, under physiological conditions the marrow is somewhat hypoxic, and exposure of human HSCs to low oxygen tension in vitro leads to an increase in HIF-1 expression and in vivo repopulating ability [69]. Enriched HSCs from $TPO^{-/-}$ mice express ~fivefold lower levels of VEGF mRNA than wild-type mice but VEGF expression can be induced by TPO [70]. Pharmacologic blockade of VEGF receptor kinase activity in cultures of single BM stem cells significantly reduced the ability of TPO to promote their survival and proliferation in vitro [70]. Taken together, these findings paint a complex picture of TPOs action on HSCs and provide an interesting example of how a secreted HGF can modulate stem cell development by influencing the production of an array of transcription factors.

Leukemia Inhibitory Factor (LIF)

LIF was originally named for its ability to induce differentiation and suppress proliferation of a murine myeloid leukemia cell line, M1. However, with the breadth of activities that have now been ascribed to LIF [71], the most interesting of which are arguably outside of the hematopoietic system, it is somewhat misleading to refer to this hormone as a hematopoietic growth factor, at least in the same vane as the other cytokines described herein and elsewhere in this volume. Mice harboring only one or no intact copies of the *LIF* gene do have reduced numbers of hematopoietic progenitor cells, proportional to *LIF* copy number and which can be normalized by administration of recombinant LIF, but the number of circulating erythrocytes, leukocytes, and platelets is normal. Furthermore, $LIF^{-/-}$ BM and spleen cells exhibit normal hematopoietic repopulating ability in vivo, indicating that LIF is not required for the maintenance of primitive HSCs [72]. Thus while LIF can act directly on lineage-committed progenitors and differentiated cells as described below, more primitive HSCs do not express LIF receptors [73] and LIFs action on these cells appears to be indirect.

Structurally, LIF is similar to many other HGFs, comprised of a single 4-α-helix polypeptide chain of 179 amino acids with ~79% sequence identity between mouse and human proteins [74]. Human LIF acts on mouse, non-human primate, and human cells, but murine LIF is species-restricted in its action. The molecule is heavily glycosylated, resulting in a molecular mass ranging from 38 to 67 kDa, but this is not required for biological activity in vivo. LIF mRNA is transcribed in multiple organs, but of most relevance to its hematopoietic actions, LIF protein is produced by a variety of BM mesenchymal and immune system cells, including fibroblasts, monocytes/macrophages, endothelial cells, and activated T cells, among others [75]. It is thought to be produced constitutively in many tissues but can be upregulated by other cytokines (e.g., IL-1, IL-6, IL-8, and tumor necrosis factor

[TNF]-α) as part of a generalized pro-inflammatory response. The primary form of the protein is secreted, but like SCF and Flt3L, an immobilized form that associates with the extracellular matrix can be generated by alternative splicing [76]. LIF is not normally detected in the serum, due to its sequestration by circulating soluble LIF receptors. This serves to restrict LIFs action to local sites of production and helps explain how such a pleiotropic factor can elicit unique responses in different organs without simultaneous effects on other tissues.

As a single agent, LIF does not stimulate progenitors of any hematopoietic lineage. In combination with other cytokines, LIF also has no effects on in vitro colony-forming cells, with the exception of augmenting proliferation of murine megakaryocytic progenitors stimulated with IL-3 [77] and late-stage myeloid progenitors (CFU-GM, CFU-M) stimulated with IL-3, GM-CSF, M-CSF, and SCF [78]. However, addition of LIF to stromal cell-based cultures significantly improves the maintenance of HSCs with competitive long-term in vivo repopulating ability [79]. LIF appears to act indirectly because alone it does not support the proliferation of enriched HSCs in stroma-free cultures. Rather, LIF induces the expression of multiple HGFs (IL-1, IL-2, and IL-6; G-CSF and GM-CSF; transforming growth factors; SCF; and LIF itself), of which IL-6 and SCF have been shown to be able to substitute for LIF in stimulating stem cell proliferation [79].

The first study of LIFs actions in vivo employed mice that were chronically exposed to high levels by transplanting them with a LIF-producing hematopoietic cell line. These animals died relatively rapidly of a syndrome characterized by diverse hematopoietic and non-hematopoietic abnormalities, again highlighting the pleiotropic nature of this molecule [80, 81]. In subsequent studies in which purified recombinant protein was injected into mice to more carefully control the exposure profile, LIF stimulated a rise in megakaryocyte and platelet numbers and a tenfold increase in megakaryocytic progenitor cells in the spleen that peaked after 7–10 days [82]. Notably, the half-life of injected LIF was extremely short (<1 h), most likely due to clearance of the molecule by binding to circulating LIF receptors as described above. LIF was studied intensively in mice and non-human primates to identify a potential therapeutic use in HSC transplantation or amelioration of chemotherapy-induced thrombocytopenia (CIT). It is worth remembering that at this time TPO had only recently been cloned and studies to evaluate its clinical utility had not yet been completed so the therapeutic value of other thrombopoietic agents was of great interest. In non-myelosuppressed mice, as little as 0.2 μg/day rHuLIF increased platelet counts, but at tenfold higher doses this was associated with remarkable body weight loss. In carboplatin-treated mice, 4 μg/day rHuLIF beginning 24 h after chemotherapy stimulated the recovery of platelet counts beginning on day 5 [83]. Recombinant HuLIF also stimulated the expansion of hematopoietic progenitor cells in vivo and resulted in significantly faster recovery of circulating platelets and leukocytes when BM from LIF-treated mice was transplanted into lethally irradiated syngeneic hosts [84]. In rhesus monkeys, rHuLIF dose-dependently increased platelet counts with peak levels of ~twofold above normal observed on day 11 after treatment [85]. This effect was similar to that elicited by rHuIL-6, which was also

being evaluated as a thrombopoietic drug at that time, but considerably less than that obtained with rHuTPO. LIF did not induce changes in leukocyte counts or the number of progenitor cells in the peripheral blood.

Recombinant HuLIF (emfilermin) was studied in a phase I clinical trial to promote platelet recovery in patients with advanced cancer [86]. As observed previously in animals, the half-life of rHuLIF in humans was very short (1–5 h). Two patients who were injected with 4 μg/kg rHuLIF three times daily for 7 days, but who had not received chemotherapy or any other HGFs, exhibited >tenfold increases in CFU-GM and BFU-E and a three or eightfold increase in CFU-Mk numbers in the peripheral blood. Circulating platelet levels in these patients increased 1.5-fold and 2-fold. The hematological actions of emfilermin were more apparent in the chemotherapy setting where it was administered for 7 days beginning on the day before or for 14 days beginning on the day after chemotherapy. In such patients, platelets recovered to baseline levels earlier and the neutrophil nadir was less severe at doses of ≥4 μg/kg/day of rHuLIF (Fig. 2.5) [86]. These modest hematological effects were accompanied by dose-limiting toxicities of hypotension and rigors. Clinical development of emfilermin has been halted so any potential future therapeutic applications of rHuLIF await the outcome of ongoing research.

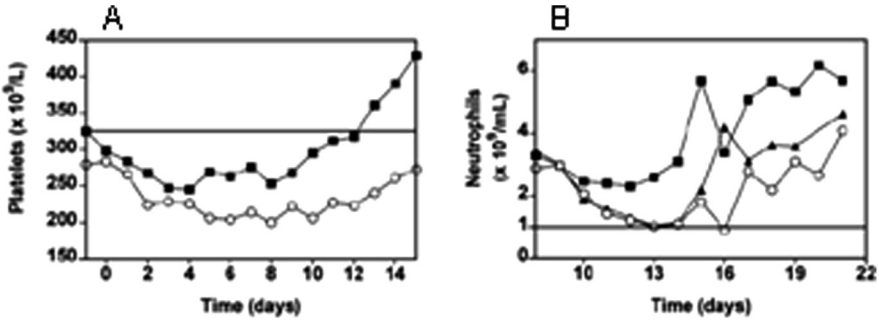


Fig. 2.5 Exposure to IL-3 *in vivo* abrogates the re-transplantation potential of human hematopoietic stem cells. Panel A: Human cord blood $CD34^+$ cells (10^5/mouse) were transplanted into immunodeficient *NOD/SCID* mice together with 5×10^6 rat fibroblasts that were stably transfected with either a control vector (Rat-1) or a vector containing the human IL-3 gene (Rat-IL-3). Injection of the latter cells resulted in the presence of 3-5 ng/ml HuIL-3 in the mouse peripheral blood. Six to 9 weeks later the proportion of human cells (%$HLA\text{-}I^+$) of hematopoietic origin (%$CD45^+$) in the BM, and their contribution to T (CD2) and B (CD19) lymphoid, myeloid (CD14, CD33), and stem/progenitor (CD34, CD38) cell compartments was determined by flow cytometry. The data show that co-injection of IL-3-producing fibroblasts increased the level of human cell chimerism in primary recipients. Panel B: Six weeks after primary transplantation, BM cells were re-transplanted into secondary *NOD/SCID* mice (107/mouse) and the level of human cell engraftment observed 6-9 weeks later was analyzed as above. Despite the superior level of human cell engraftment observed in primary mice containing Rat-IL-3 cells, this was not maintained in secondary animals, indicating that HSCs were depleted during their prior exposure to IL-3 *in vivo*. Reproduced with permission from Nitsche *et al.* Stem Cells. 2003;21:236–244

Interleukin-3 (IL-3, Multi-CSF)

Murine interleukin IL-3 has the distinction of being the first hematopoietic cytokine that was cloned (in 1984) [87], followed shortly thereafter by the cloning of human IL-3 [88]. The 140 amino acid sequence of human and murine IL-3 is only 29% identical and they exhibit no species cross-reactivity in their actions. As with most HGFs, native IL-3 is glycosylated resulting in a molecular weight of the human factor of 23–30 kDa, but the carbohydrate component is not necessary for biological activity. One of many names originally assigned to this cytokine was "multi-CSF" because it exhibits an exceptionally broad range of proliferative effects on myeloid, erythroid, and megakaryocytic progenitors, eosinophils, mast cells, and multipotential stem cells. Given this polyfunctionality, it was surprising that *IL-3* knock-out mice exhibit no abnormalities in steady-state hematopoiesis [89] but this provides another example of the redundancy which characterizes many of the hematopoietic regulators. IL-3 is produced by activated T cells and various stromal cell populations such as monocytes/macrophages and mast cells in vitro. It is the primary component of "conditioned medium" that was used by early experimental hematologists to stimulate the proliferation of myeloid colony-forming cells before purified recombinant HGFs were widely available. Because IL-3 has been available for study so long, the literature describing its effects on hematopoietic cells is vast. Focusing here on its early actions on HSCs simplifies the survey, but reveals striking contradictions in the data as to whether IL-3 amplifies or depletes long-term hematopoietic repopulating ability [90]. Differences in the results of in vitro studies performed by different laboratories depend largely on the presence of serum in the culture system, which is generally (but not universally) deleterious to IL-3-stimulated HSC self-renewal. In serum-free cultures of highly enriched human and murine HSCs, the addition of IL-3 to optimized HGF cocktails can increase the yield of total cells and hematopoietic progenitors by >tenfold and long-term repopulating HSCs by ~threefold [91]. However, this effect in vitro is completely eliminated by the addition of serum. Definitive proof of the exhausting effect of IL-3 on long-term repopulating HSCs in vivo was provided by Nitsche et al. [92] who transplanted human $CD34^+$ cord blood cells into immunodeficient mice with or without a rat fibroblast cell line engineered to express human IL-3. Mice co-transplanted with IL-3-expressing fibroblasts were reconstituted to a higher level by human cells than mice injected with $CD34^+$ CB cells alone. However, when BM from these primary recipients was re-transplanted into secondary mice, human chimerism was virtually completely lost (Fig. 2.6), indicating that repopulating HSCs were depleted during their residence in the primary hosts where they were exposed to high levels of IL-3. This effect on long-term repopulating HSCs contrasts with that on more mature progenitor cells such as spleen colony-forming units (CFU-S), which expand >25-fold in animals perfused with IL-3 [93]. Expansion of the progenitor compartment is accompanied by moderate increases in the number of circulating neutrophils, eosinophils, monocytes, erythrocyte precursors, and particularly mast cells. This prompted investigation of the use of IL-3 to enhance hematological recovery in humans.

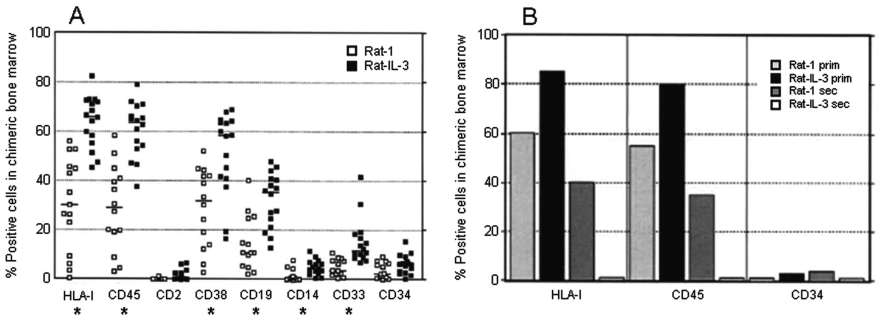


Fig. 2.6 Exposure to IL-3 in vivo abrogates the re-transplantation potential of human hematopoietic stem cells. **Panel a**: Human cord blood $CD34^+$ cells (10^5/mouse) were transplanted into immunodeficient *NOD/SCID* mice together with 5×10^6 rat fibroblasts that were stably transfected with either a control vector (Rat-1) or a vector containing the human *IL-3* gene (Rat-IL-3). Injection of the latter cells resulted in the presence of 3–5 ng/mL HuIL-3 in the mouse peripheral blood. Six to nine weeks later the proportion of human cells (%$HLA\text{-}I^+$) of hematopoietic origin (%$CD45^+$) in the BM, and their contribution to T (CD2) and B (CD19) lymphoid, myeloid (CD14, CD33), and stem/progenitor (CD34, CD38) cell compartments was determined by flow cytometry. The data show that co-injection of IL-3-producing fibroblasts increased the level of human cell chimerism in primary recipients. **Panel b**: Six weeks after primary transplantation, BM cells were re-transplanted into secondary *NOD/SCID* mice (10^7/mouse) and the level of human cell engraftment observed 6–9 weeks later was analyzed as above. Despite the superior level of human cell engraftment observed in primary mice containing Rat-IL-3 cells, this was not maintained in secondary animals, indicating that HSCs were depleted during their prior exposure to IL-3 in vivo. Reproduced with permission from Ref. [92]

A broad clinical development program was conducted throughout the 1990s to assess the therapeutic potential of human IL-3. Initial results of some phase I/II trials were promising, indicating that subcutaneous administration of 5–10 μg/kg rHuIL-3 daily for 5–10 days in patients with various types of cancer either alone or as an adjunct to stem cell transplantation reduced chemotherapy delays in dose-intensive treatment regimens and induced faster regeneration of neutrophils and platelets [94]. However, these early data were not supported by larger phase III studies of IL-3 alone or in combination with other cytokines (e.g. G-CSF, GM-CSF) to enhance hematopoiesis following cancer therapy or in patients with myelodysplastic syndrome (MDS). This experience precipitated a period of particularly innovative research to produce novel synthetic cytokines that exhibited greater biological activity than native IL-3 but with fewer inflammatory side effects [94]. Thus were created Synthokine (SC-55494 or daniplestim), a potent IL-3 receptor agonist [95], and an array of hybrid factors wherein the *IL-3* gene was fused to genes encoding GM-CSF (PIXY321 or Pixykine) [96], G-CSF (Myelopoietin [MPO] or Leridistim) [97], TPO (Promegapoietin-1 or PMP) [98], or insulin-like growth factor (compound 406) [99]. These too were evaluated in vitro for their ability to increase the expansion of myeloid and megakaryocytic progenitor cells, in vivo in non-human primates and humans for their ability to mobilize hematopoietic stem/progenitor cells for transplantation, and as supportive care agents to promote

hematological reconstitution following chemotherapy or radiation-induced myelosuppression. Many proved moderately effective but were ultimately abandoned in favor of r-metHuG-CSF (filgrastim, NEUPOGEN®), rHuGM-CSF (sargramostim, LEUKINE®), and rHuEPO (epoetin alfa, EPOGEN®), which exhibited more desirable pharmacodynamic properties as discussed elsewhere in this volume and which have now been used in millions of patients for the treatment of neutropenia and anemia.

Summary

From the first studies demonstrating that certain HGFs exhibit early actions that reach to the apex of the hematopoietic hierarchy, and exemplified by the naming of c-kit ligand as "stem cell factor," scientists and clinicians have had high hopes for the therapeutic value of early-acting hematopoietins. It is therefore notable that 25 years after the discovery of the first cytokine with demonstrated actions on HSCs, none have proven to be revolutionizing medicines despite some utility as potentiators of the action of later-acting lineage-specific growth factors. This lack of clinical and commercial success underscores the reality that patients with cancer, MDS, and congenital or treatment-related cytopenias do not in fact succumb to their diseases because they harbor inadequate numbers of HSCs, even though the genetic lesion that led to disease may have in some cases originated in this cell compartment. Rather, patients die because of functional or quantitative deficiencies in the end products of stem cell proliferation and differentiation, namely mature erythrocytes, granulocytes, and platelets. Thus it is the HGFs that drive these later stages of hematopoiesis that have proven more clinically useful. Defective stem cell self-renewal is not in itself a terminal condition though molecules that enhance HSC replication without simultaneous differentiation may eventually find utility in niche therapies such as ex vivo expansion. Even for these applications, various intracellular signaling proteins and stem cell transcription factors (e.g., Wnt 3A, HOXB4) that have now been expressed in stable form and used as soluble factors have proven more effective than conventional early-acting HGFs, at least in an experimental setting. This is certainly not to imply that the early-acting HGFs are uninteresting or not worthy of additional study. LIF is critical for the maintenance of embryonic stem cell lines in vitro and will continue to play an important albeit supportive role in the burgeoning field of stem cell therapy and tissue engineering. In the nascent discipline of immunotherapy, Flt3L remains untested in humans under optimal conditions in which it is employed together with dendritic cell maturation factors. Finally, novel mpl ligands such as Nplate® (romiplostim) and Promacta® (eltrombopag), a non-peptidyl molecule, have also only recently been approved and these agents may, like TPO, activate key transcriptional and developmental pathways in early stem cells. Thus in many ways the potential utility of the early-acting factors is just beginning to be explored as the field of stem cell biology reaches its zenith – the next decade may yield interesting surprises.

References

1. Szilvassy SJ. The biology of hematopoietic stem cells. Arch Med Res. 2003;34:446–60.
2. Russell ES. Hereditary anemias of the mouse: a review for geneticists. Adv Genet. 1979;20:357–459.
3. Chabot B, Stephenson DA, Chapman VM, et al. The proto-oncogene *c-kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. Nature. 1988;335: 88–9.
4. Geissler EN, Ryan MA, Houseman DE. The dominant-white spotting (*W*) locus of the mouse encodes the *c-kit* proto-oncogene. Cell. 1988;55:185–92.
5. Huang E, Nocka K, Beier DR, et al. The hematopoietic growth factor KL is encoded by the *Sl* locus and is the ligand of the *c-kit* receptor, the gene product of the *W*locus. Cell. 1990;63:225–33.
6. Martin FH, Suggs SV, Langley KE, et al. Primary structure and functional expression of rat and human stem cell factor cDNAs. Cell. 1990;63:203–11.
7. Williams DE, Eisenman J, Baird A, et al. Identification of a ligand for the *c-kit* proto-oncogene. Cell. 1990;63:167–74.
8. Anderson DM, Lyman SD, Baird A, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell. 1990;63: 235–43.
9. Brannan CI, Lyman SD, Williams DE, et al. Steel–Dickie mutation encodes a c-kit ligand lacking transmembrane and cytoplasmic domains. Proc Natal Acad Sci USA. 1991;88: 4671–4.
10. Toksoz D, Zsebo KM, Smith KA, et al. Support of human hematopoiesis in long-term bone marrow cultures by murine stromal cells selectively expressing the membrane-bound and secreted forms of the human homolog of the steel gene product, stem cell factor. Proc Natal Acad Sci USA. 1992;89:7350–4.
11. Zsebo KM, Wypych J, McNiece IK, et al. Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver-conditioned medium. Cell. 1990;63:195–201.
12. Li CL, Johnson GR. Stem cell factor enhances the survival but not the self-renewal of murine hematopoietic long-term repopulating cells. Blood. 1994;84:408–14.
13. Keller JR, Ortiz M, Ruscetti FW. Steel factor (c-kit ligand) promotes the survival of hematopoietic stem/progenitor cells in the absence of cell division. Blood. 1995;86: 1757–64.
14. McNiece IK, Langley KE, Zsebo KM. Recombinant human stem cell factor synergizes with GM-CSF, G-CSF, IL-3 and Epo to stimulate human progenitor cells of the myeloid and the erythroid lineages. Blood. 1991;19:226–31.
15. Kobayashi M, Laver JH, Kato T, et al. Thrombopoietin supports proliferation of human primitive hematopoietic cells in synergy with steel factor and/or interleukin-3. Blood. 1996;88:429–36.
16. Bernstein ID, Andrews RG, Zsebo KM. Recombinant human stem cell factor enhances the formation of colonies by $CD34^+$ and $CD34^+Lin^-$ cells, and the generation of colony-forming cell progeny from $CD34^+Lin^-$ cells cultured with interleukin-3, granulocyte colony-stimulating factor, or granulocyte–macrophage colony-stimulating factor. Exp Hematol. 1991;77: 2316–21.
17. Zsebo KM, Williams DA, Geissler EN, et al. Stem cell factor is encoded at the *Sl* locus of the mouse and is the ligand for the *c-kit* tyrosine kinase receptor. Cell. 1990;63:213–24.
18. Molineux G, Migdalska A, Szmitkowski M, et al. The effects on hematopoiesis of recombinant stem cell factor (ligand for *c-kit*) administered in vivo to mice either alone or in combination with granulocyte colony-stimulating factor. Blood. 1991;78:961–6.
19. Yan X-Q, Briddell R, Hartley C, et al. Mobilization of long-term hematopoietic reconstituting cells in mice by the combination of stem cell factor plus granulocyte colony-stimulating factor. Blood. 1994;84:795–9.

20. de Revel T, Appelbaum FR, Storb R, et al. Effects of granulocyte colony-stimulating factor and stem cell factor, alone and in combination, on the mobilization of peripheral blood cells that engraft lethally irradiated dogs. Blood. 1994;83:3795–9.
21. Andrews RG, Briddell RA, Knitter GH, et al. Rapid engraftment by peripheral blood progenitor cells mobilized by recombinant human stem cell factor and recombinant human granulocyte colony-stimulating factor in nonhuman primates. Blood. 1995;85:15–20.
22. Nocka K, Buck J, Levi E, et al. Candidate ligand for the c-kit transmembrane kinase receptor: KL, a fibroblast derived growth factor stimulates mast cells and erythroid progenitors. EMBO J. 1990;9:3287–94.
23. Lacerna L Jr, Sheridan WP, Basser R, et al. Stem cell factor. In: Ho AD, Haas R, Champlin RE, editors. Hematopoietic stem cell transplantation. New York, NY: Marcel Dekker Inc; 2000. pp. 31–46.
24. Shpall EJ, Wheeler CA, Turner SA, et al. A randomized phase 3 study of peripheral blood progenitor cell mobilization with stem cell factor and filgrastim in high-risk breast cancer patients. Blood. 1999;93:2491–501.
25. Brandt J, Briddell RA, Srour EF, et al. Role of *c-kit* ligand in the expansion of human hematopoietic progenitor cells. Blood. 1992;79:634–41.
26. Holyoake TL, Freshney MG, McNair L, et al. Ex vivo expansion with stem cell factor and interleukin-11 augments both short-term recovery post-transplant and the ability to serially transplant marrow. Blood. 1996;87:4589–95.
27. Möhle R, Kanz L. Hematopoietic growth factors for hematopoietic stem cell mobilization and expansion. Semin Hematol. 2007;44:193–202.
28. McNiece IM, Jones R, Bearman SI, et al. Ex vivo expanded peripheral blood progenitor cells provide rapid neutrophils recovery after high-dose chemotherapy in patients with breast cancer. Blood. 2000;96:3001–7.
29. Prince HM, Simmons PJ, Whitty G, et al. Improved haematopoietic recovery following transplantation with ex vivo-expanded mobilized blood cells. Br J Haematol. 2004;126: 536–45.
30. Brugger W, Heimfeld S, Berenson RJ, et al. Reconstitution of hematopoiesis after high-dose chemotherapy by autologous progenitor cells generated ex vivo. N Engl J Med. 1995;333:283–7.
31. Rosnet O, Marchetto S, deLapeyriere O, et al. Murine *flt3*, a gene encoding a novel tyrosine kinase receptor of the PDGFR/CSF1R family. Oncogene. 1991;6:1641–50.
32. Lyman SD, James L, Vanden Bos T, et al. Molecular cloning of a ligand for the flt3/flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell. 1993;75:1157–67.
33. Hannum C, Culpepper J, Campbell D, et al. Ligand for flt3/flk2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant RNAs. Nature. 1994;368:643–8.
34. Lyman SD, James L, Johnson L, et al. Cloning of the human homologue of the murine flt3 ligand: a growth factor for early hematopoietic progenitor cells. Blood. 1994;83:2795–801.
35. Christensen JL, Weissman IL. Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. Proc Natl Acad Sci USA. 2001;98: 14541–6.
36. Sitnicka E, Buza-Vidas N, Larsson S, et al. Human $CD34^+$ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells. Blood. 2003;102:881–6.
37. Kikushige Y, Yoshimoto G, Miyamoto T, et al. Human flt3 is expressed at the hematopoietic stem cell and the granulocyte/macrophage progenitor stages to maintain cell survival. J Immunol. 2008;180:7358–67.
38. Rasko JEJ, Metcalf D, Rossner MT, et al. The flt3/flk-2 ligand: receptor distribution and action on murine haemopoietic cell survival and proliferation. Leukemia. 1995;9:2058–66.

39. Jacobsen SEW, Okkenhaug C, Myklebust J, et al. The FLT3L potently and directly stimulates the growth and expansion of primitive murine bone marrow progenitor cells in vitro: synergistic actions with interleukin (IL) 11, IL-12, and other hematopoietic growth factors. J Exp Med. 1995;181:1357–63.
40. Broxmeyer HE, Lu L, Cooper S, et al. Flt3 ligand stimulates/costimulates the growth of myeloid stem/progenitor cells. Exp Hematol. 1995;23:1121–9.
41. Brasel K, McKenna HJ, Morrissey PJ, et al. Hematologic effects of flt3 ligand in vivo in mice. Blood. 1996;88:2004–12.
42. Molineux G, McCrea C, Yan XQ, et al. Flt-3 ligand synergizes with granulocyte colony-stimulating factor to increase neutrophils numbers and to mobilize peripheral blood stem cells with long-term repopulating potential. Blood. 1997;89:3998–4004.
43. Lynch DH, Andreasen A, Maraskovsky E, et al. Flt3 ligand induces tumor regression and antitumor immune responses in vivo. Nat Med. 1997;3:625–31.
44. Esche C, Subbotin VM, Maliszewski C, et al. FLT3 ligand administration inhibits tumor growth in murine melanoma and lymphoma. Cancer Res. 1998;58:380–3.
45. Reber AJ, Ashour AE, Robinson SN, et al. Flt3 ligand bioactivity and pharmacology in neoplasia. Curr Drug Targets – Immune Endocr Metabol Disord. 2004;4:149–57.
46. Borges L, Miller RE, Jones J, et al. Synergistic action of fms-like tyrosine kinase 3 ligand and CD40 ligand in the induction of dendritic cells and generation of antitumor immunity in vivo. J Immunol. 1999;163:1289–97.
47. Fong L, Hou Y, Rivas A, et al. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci USA. 2001;98:8809–14.
48. Cheever MA. Twelve immunotherapy drugs that could cure cancers. Immunol Rev. 2008;222:357–68.
49. Sitnicka E, Bryder D, Theilgaard-Mönch K, et al. Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. Immunity. 2002;17:463–72.
50. Keleman E, Cserhati I, Tanos B. Demonstration of some properties of human thrombopoietin in thrombocythaemic sera. Acta Haematologica. 1958;20:350–5.
51. Kato T, Ogami K, Shimada Y, et al. Purification and characterization of thrombopoietin. J Biochem. 1995;118:229–36.
52. Bartley TD, Bogenberger J, Hunt P, et al. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. Cell. 1994;77:1117–24.
53. de Sauvage FJ, Hass PE, Spencer SD, et al. Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature. 1994;369:533–8.
54. Lok S, Kaushansky K, Holly RD, et al. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. Nature. 1994;369:565–8.
55. Kuter DJ, Beeler DL, Rosenberg RD. The purification of megapoietin: a physiological regulator of megakaryocyte growth and platelet production. Proc Natl Acad Sci USA. 1994;91:11104–8.
56. Linden HM, Kaushansky K. The glycan domain of thrombopoietin enhances its secretion. Biochemistry. 2000;39:3044–51.
57. Alexander WS, Roberts AW, Nicola NA, et al. Deficiencies in progenitor cells of multiple hematopoietic lineages and effective megakaryocytopoiesis in mice lacking the thrombopoietin receptor c-mpl. Blood. 1996;87:2162–70.
58. Carver-Moore K, Broxmeyer HE, Luoh S-M, et al. Low levels of erythroid and myeloid progenitors in thrombopoietin- and c-mpl-deficient mice. Blood. 1996;88:803–8.
59. Kaushansky K, Lin N, Grossmann A, et al. Thrombopoietin expands erythroid, granulocyte–macrophage, and megakaryocytic progenitor cells in normal and myelosuppressed mice. Exp Hematol. 1996;24:265–9.
60. Hokom MM, Lacey D, Kinstler OB, et al. Pegylated megakaryocyte growth and development factor abrogates the lethal thrombocytopenia associated with carboplatin and irradiation in mice. Blood. 1995;86:4486–92.

61. Sitnicka E, Lin N, Priestley GV, et al. The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells. Blood. 1996;87:4998–5005.
62. Ku H, Yonemura Y, Kaushansky K, et al. Thrombopoietin, the ligand for the mpl receptor, synergizes with steel factor and other early acting cytokines in supporting proliferation of primitive hematopoietic progenitors in mice. Blood. 1996;87:4544–51.
63. Borge OJ, Ramsfjell V, Veiby OP, et al. Thrombopoietin, but not erythropoietin promotes viability and inhibits apoptosis of multipotent murine hematopoietic progenitor cells in vitro. Blood. 1996;88:2859–70.
64. Matsunaga T, Kato T, Miyazaki H, et al. Thrombopoietin promotes the survival of murine hematopoietic long-term reconstituting cells: comparison with the effects of Flt3/Flk-2 ligand and interleukin-6. Blood. 1998;92:452–61.
65. Solar GP, Kerr WG, Zeigler FC, et al. Role of c-mpl in early hematopoiesis. Blood. 1998;92: 4–10.
66. Kirito K, Fox N, Kaushansky K. Thrombopoietin stimulates *Hoxb4* expression: an explanation for the favorable effects of TPO on hematopoietic stem cells. Blood. 2003;102: 3172–8.
67. Kirito K, Fox N, Kaushansky K. Thrombopoietin induces HOXA9 nuclear transport in immature hematopoietic cells: potential mechanism by which the hormone favorably affects hematopoietic stem cells. Mol Cell Biol. 2004;24:6751–62.
68. Gerber HP, Malik AK, Solar GP, et al. VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature. 2002;417:954–8.
69. Danet GH, Pan Y, Luongo JL, et al. Expansion of human SCID-repopulating cells under hypoxic conditions. J Clin Invest. 2003;112:126–35.
70. Kirito K, Fox N, Komatsu N, et al. Thrombopoietin enhances expression of vascular endothelial growth factor (VEGF) in primitive hematopoietic cells through induction of HIF-1a. Blood. 2005;105:4258–63.
71. Metcalf D. The unsolved enigmas of leukemia inhibitory factor. Stem Cells. 2003;21:5–14.
72. Escary J-L, Perreau J, Duménil D, et al. Leukaemia inhibitory factor is necessary for maintenance of haematopoietic stem cells and thymocyte stimulation. Nature. 1993;363:361–4.
73. McKinstry WJ, Li C-L, Rasko JEJ, et al. Cytokine receptor expression on hematopoietic stem and progenitor cells. Blood. 1997;89:65–71.
74. Gough NM, Gearing DP, King JA, et al. Molecular cloning and expression of the human homologue of the murine gene encoding myeloid leukemia-inhibitory factor. Proc Natal Acad Sci USA. 1988;85:2623–7.
75. Chodorowska G, Glowacka A, Tomczyk M. Leukemia inhibitory factor (LIF) and its biological activity. Ann Univ Mariae Curie Sklodowska Med. 2004;59:189–93.
76. Rathjen PD, Toth S, Willis A, et al. Differentiation inhibiting activity is produced in matrix-associated and diffusible forms that are generated by alternate promoter usage. Cell. 1990;62:1105–14.
77. Metcalf D, Hilton D, Nicola NA. Leukemia inhibitory factor can potentiate murine megakaryocyte production in vitro. Blood. 1991;77:2150–3.
78. Keller JR, Gooya JM, Ruscetti FW. Direct synergistic effects of leukemia inhibitory factor on hematopoietic progenitor cell growth: comparison with other hematopoietins that use the gp130 receptor subunit. Blood. 1996;88:863–9.
79. Szilvassy SJ, Weller KP, Lin W, et al. Leukemia inhibitory factor upregulates cytokine expression by a murine stromal cell line enabling the maintenance of highly enriched competitive repopulating stem cells. Blood. 1996;87:4618–28.
80. Metcalf D, Gearing DP. Fatal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. Proc Natl Acad Sci USA. 1989;86:5948–52.
81. Metcalf D, Gearing DP. A myelosclerotic syndrome in mice engrafted with cells producing high levels of leukemia inhibitory factor (LIF). Leukemia. 1989;3:847–52.
82. Metcalf D, Nicola NA, Gearing DP. Effects of injected leukemia inhibitory factor on hematopoietic and other tissues in mice. Blood. 1990;76:50–6.

83. Akiyama Y, Kikuchi Y, Matsuzaki J, et al. Protective effect of recombinant human leukemia inhibitory factor against thrombocytopenia in carboplatin-treated mice. Jpn J Cancer Res. 1997;88:584–9.
84. Pruijt JFM, Lindley IJD, Heemskerk DPM, et al. Leukemia inhibitory factor induces in vivo expansion of bone marrow progenitor cells that accelerate hematopoietic reconstitution but do not enhance radioprotection in lethally irradiated mice. Stem Cells. 1997;15: 50–55.
85. Mayer P, Geissler K, Ward M, et al. Recombinant human leukemia inhibitory factor induces acute phase proteins and raises the blood platelet counts in nonhuman primates. Blood. 1993;81:3226–33.
86. Gunawardana DH, Basser RL, Davis ID, et al. A phase I study of recombinant human leukemia inhibitory factor in patients with advanced cancer. Clin Cancer Res. 2003;9: 2056–65.
87. Fung MC, Hapel AJ, Ymer S, et al. Molecular cloning of cDNA for murine interleukin-3. Nature. 1984;307:233–7.
88. Yang YC, Ciarletta AB, Temple PA, et al. Human interleukin 3 multi-colony-stimulating factor identification by expression cloning of a novel hematopoietic growth factor related to murine interleukin 3. Cell. 1986;47:3–10.
89. Mach N, Lantz CS, Galli SJ, et al. Involvement of interleukin-3 in delayed-type hypersensitivity. Blood. 1998;91:778–83.
90. Ivanovic Z. Interleukin-3 and ex vivo maintenance of hematopoietic stem cells: facts and controversies. Eur Cytokine Netw. 2004;15:6–13.
91. Bryder D, Jacobsen SEW. Interleukin-3 supports expansion of long-term multilineage repopulating activity after multiple stem cell divisions in vitro. Blood. 2000;96:1748–55.
92. Nitsche A, Junghahn I, Thulke S, et al. Interleukin-3 promotes proliferation and differentiation of human hematopoietic stem cells but reduces their repopulation potential in NOD/SCID mice. Stem Cells. 2003;21:236–44.
93. Kindler V, Thorens B, Vassalli P. In vivo effect of murine recombinant interleukin 3 on early hematopoietic progenitors. Eur J Immunol. 1987;17:1511–14.
94. Mangi MH, Newland AC. Interleukin-3 in hematology and oncology: current state of knowledge and future directions. Cytokines Cell Mol Ther. 1999;5:87–95.
95. Ahmed N, Khokher MA, Hassan HT. Synthetic cytokines containing interleukin-3 exert potent megakaryocytic activity. Haematologica. 2000;30:167–76.
96. Vadhan-Raj S. PIXY321 (GM-CSF/IL-3 fusion protein): biology and early clinical development. Stem Cells. 1994;12:253–61.
97. Nabholtz J-M, Cantin J, Chang J, et al. Phase III trial comparing granulocyte colony-stimulating factor to leridistim in breast cancer patients treated with docetaxel/doxorubicin/cyclophosphamide: results of the BCIRG 004 trial. Clin Breast Cancer. 2002;3:268–75.
98. Farese AM, Smith WG, Giri JG, et al. Promegapoietin-1a, an engineered chimeric IL-3 and mpl-L receptor agonist, stimulates hematopoietic recovery in conventional and abbreviated schedules following radiation-induced myelosuppression in nonhuman primates. Stem Cells. 2001;19:329–38.
99. Difalco MR, Dufresne L, Congote LF. Efficacy of an insulin-like growth factor-interleukin-3 fusion protein in reversing the hematopoietic toxicity associated with azidothymidine in mice. J Pharmacol Exp Ther. 1998;284:449–54.

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Hematopoietic Growth Factors in Oncology
Lyman, G.; Dale, D.C. (Eds.)
2011, XV, 433 p., Hardcover
ISBN: 978-1-4419-7072-5