Thrombopoietin, a Direct Stimulator of Viability and Multilineage Growth of Primitive Bone Marrow Progenitor Cells

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Abstract. Thrombopoietin (TPO), the ligand for c-*mpl*, has recently been demonstrated to be the primary regulator of megakaryocytopoiesis and platelet production. In addition, several studies have demonstrated that c-*mpl* is expressed on hematopoietic cell populations highly enriched in primitive progenitor cells. Here we summarize and discuss recent studies from our laboratory, as well as others, demonstrating that TPO has effects on primitive hematopoietic progenitor cells. When acting alone, TPO stimulates little or no growth, but promotes viability and suppresses apoptosis of murine multipotent (Lin$^-$Sca-1$^+$) bone marrow progenitor cells in vitro. In addition, TPO directly and potently synergizes with other early acting cytokines (kit ligand, flt3 ligand and interleukin 3) to promote multilineage growth of the same progenitor cell population. Although it remains to be established whether TPO also acts on the long-term reconstituting pluripotent stem cells, these studies combined with progenitor cell studies in c-*mpl*-deficient mice, suggest that TPO, in addition to its key role in platelet production, might also have an important impact on early hematopoiesis. *Stem Cells 1996;14(suppl 1):173-180*

Cytokine Regulation of Hematopoiesis

Hematopoiesis is a lifelong process involving the production of mature blood cells of all lineages from a pool of pluripotent long-term reconstituting stem cells (LTRC) capable of producing hematopoietic progenitor cells (HPC) with different proliferative as well as differentiation potentials [1-3]. The daily turnover of approximately 10^{12} cells in a normal adult human must be tightly regulated and involves, in part, a complex interaction between membrane-bound, as well as soluble stimulatory and inhibitory cytokines, and their corresponding receptors [1-3]. In steady state, the most primitive hematopoietic stem cells are thought to be quiescent, and triggering their growth appears to require combined stimulation by multiple cytokines, whereas more committed progenitors frequently can be induced to proliferate by single cytokines [2, 3].

Cytokines demonstrated to have activities on HPC can be classified based on distinct patterns of functional activities (Table 1) [2, 3]. The first class of "colony-stimulating factors" (CSFs) is characterized by the unique ability of its members to efficiently stimulate the in vitro clonogenic growth of more committed progenitor cells when acting individually, and includes G-CSF, GM-CSF, M-CSF or CSF-1, interleukin 3 (IL-3), erythropoietin (EPO), IL-5 and IL-7. In addition, many of the CSFs can interact with cytokines of the other classes to synergistically enhance the growth of primitive HPC. The second class of "stem cell regulators" acts predominantly, but not exclusively, on primitive HPC, and includes the ligands for c-*kit* and flt3 (KL and FL, respectively). These two tyrosine kinase receptor ligands have the unique ability to potently trigger the in vitro proliferation of primitive HPC with multilineage potential when acting in synergy with most of the cytokines in class I or class III [2-4]. In addition, it has become increasingly clear that although KL and FL to a large degree act in an overlapping manner, they also each have distinct biological activities, and also synergize with each other to enhance the growth of primitive HPC [4, 5]. The third class of "synergistic cytokines" which

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Table 1. Classification of cytokines based on their ability to affect the growth of HSC

Class		Members
I.	CSFs	G-CSF, CSF-1, GM-CSF, IL-3, IL-5, IL-7, EPO
II.	SCF	SCF, FL
III.	Pure synergistic cytokines	IL-1, IL-6, IL-9, IL-10, IL-11, IL-12, LIF
IV.	Inhibitors/bidirectional regulators	TGF-β, TNF, MIP-1α, IFN-γ, IL-4

This classification of cytokines is solely based on the functional ability of different cytokines to stimulate the in vitro growth of HPC. The listing of class III and IV cytokines is not meant to be complete, but rather represents examples of important cytokines within each of these classes.

includes multiple IL and leukemia inhibitory factor (LIF), has little or no ability to stimulate the in vitro growth of HPC when acting individually, but can enhance growth of primitive progenitor cells in response to members of the "CSFs" or "stem cell regulators." Synergistic interactions between cytokines are particularly evident with regard to their ability to trigger the growth of primitive HPC. Such synergy occurs predominantly through interactions between cytokines of different classes, although synergy between cytokines of the same class also takes place [2, 3].

In addition to the above-mentioned three classes of cytokines, which mostly have a direct stimulatory effect on the growth of HPC in vitro, a number of cytokines have been demonstrated to have growth inhibitory effects on HPC although most of them under certain conditions might rather directly enhance progenitor cell growth, particularly in combination with some of the CSFs [6]. These "bidirectional growth regulators" (class IV cytokines; Table 1) include transforming growth factor-β (TGF-β), tumor necrosis factor (TNF), interferon-γ (IFN-γ), IL-4 and macrophage inflammatory protein-1α (MIP-1α).

Although decades of research had implicated the presence of thrombopoietin (TPO), a humoral regulator of platelet production, it was first in 1994 that several groups reported on the cloning and initial characterization of TPO [7-13]. Through in vitro and in vivo studies, as well as studies in c-*mpl*-deficient mice, it rapidly became clear that TPO was the primary regulator of megakaryocytopoiesis and platelet production [7, 9-14]. Specifically, TPO has been demonstrated to potently stimulate the growth of HPC committed to the megakaryocyte (MK) lineage [13, 15-17]. Although it initially appeared that TPO might act in a lineage-specific manner, several groups demonstrated expression of c-*mpl* on murine, as well as human populations of highly enriched primitive HPC, potentially implicating a role of TPO in early hematopoiesis [18-20].

TPO Directly Promotes the In Vitro Viability of Primitive Murine Bone Marrow Progenitor Cells and Suppresses Apoptosis of Lin$^-$Sca-1$^+$ Bone Marrow Cells in the Absence of Other Cytokines

Although constituting only 0.1%-0.2% of the nucleated cells, Lin$^-$Sca-1$^+$ and Lin$^-$Sca-1$^+$ c-*kit*$^+$ cells contain all the LTRC of the bone marrow [1, 21, 22]. In addition, this population is depleted in lineage-committed progenitors and represents predominantly multipotent progenitor cells important for short-term as well as long-term reconstitution [23, 24]. It seems that these primitive progenitor cells can grow under defined in vitro conditions, and that their optimal growth can only be achieved in response to the combined action of multiple cytokines [23, 25-27]. In fact, only KL and IL-3 can as single cytokines act to promote growth of a low fraction of these primitive progenitor cells [23, 25]. We have recently utilized this population of primitive progenitor cells to investigate the potential role of TPO in early hematopoiesis [28, 29]. When Lin$^-$Sca-1$^+$ bone marrow cells were cultured in fetal calf serum (FCS)-containing medium at the single cell level to avoid indirect effects of potentially contaminating accessory cells, no clones were formed in response to TPO alone, whereas a low number of clones were formed when cells were cultured in a serum-depleted medium. In addition, almost all of these clones contained fewer than 50 cells and would thus not be scored according to commonly used criteria for colony formation in vitro. Despite little or no ability to stimulate growth

of Lin$^-$Sca-1$^+$ progenitor cells, TPO when acting alone maintained the viability of a large fraction of the progenitors through prolonged culture. As previously demonstrated [23, 30], most if not all Lin$^-$Sca-1$^+$ progenitor cells lost viability following 40 h incubation in medium alone, whereas more than 35% of the clonogenic progenitor cells survived following 40 h incubation in TPO alone. Most progenitors surviving in response to TPO displayed a very high proliferative potential when stimulated with a combination of cytokines (TPO + KL + G-CSF + IL-3), underscoring their primitive nature. Although 38% of the Lin$^-$Sca-1$^+$ progenitor cells produced megakaryocytes in response to the same cytokine combination, almost all of these progenitor cells produced other myeloid cell lineages (granulocytes and macrophages) as well, proving that TPO promotes the survival of high proliferative multipotent progenitor cells. In other experiments we found that a fraction of the Lin$^-$Sca-1$^+$ progenitor cells surviving in response to TPO also had a B-lymphoid potential, as determined by their ability to produce B220$^+$ cells in response to KL + FL + IL-7, a cytokine combination recently demonstrated to selectively promote B cell commitment and development from uncommitted Lin$^-$Sca-1$^+$ progenitor cells [31].

As would be expected from previous studies demonstrating that all clonogenic, as well as LTRC are c-*kit*$^+$ [22, 32], we also found that all Lin$^-$Sca-1$^+$ progenitors surviving in response to TPO are c-*kit*$^+$.

Although induction of optimal growth of primitive progenitor cells requires the combined action of multiple early-acting cytokines, it has been demonstrated that some early-acting cytokines (in particular KL) when acting individually are capable of increasing viability of Lin$^-$Sca-1$^+$ progenitor cells in vitro [30, 33-36]. Thus, we directly compared the ability of TPO to enhance viability of KL + IL-3 + G-CSF + TPO-responsive progenitors in serum-depleted medium to the viability-enhancing effects of KL, G-CSF and IL-1 [28]. Following 40 h incubation, a slightly higher number of Lin$^-$Sca-1$^+$ progenitors remained viable in TPO than in G-CSF or IL-1, whereas KL was significantly better than TPO. However, following more prolonged incubation (72 h), no significant difference was observed between the viability-promoting effects of KL and TPO.

Since TPO and its receptor show considerable homology to EPO and the EPO receptor, respectively, we also investigated whether EPO might promote viability of Lin$^-$Sca-1$^+$ progenitor cells. However, unlike TPO, EPO had no effect on the viability of Lin$^-$Sca-1$^+$ progenitors. The reason for this is most likely that whereas c-*mpl* appears to be expressed on primitive/multipotent progenitors [18-20], the EPO receptor might not be. In fact, previous studies have suggested that even the most primitive stages of erythroid progenitor cells might not yet express the EPO receptor [37, 38].

Based on these and other studies [5, 30, 33-35, 39-43], we can conclude that TPO along with some other early-acting cytokines can promote viability of primitive HPC in the presence of little or no proliferation (Table 2). At least on Lin$^-$Sca-1$^+$ bone marrow progenitor cells, TPO has a potent ability to promote viability of in vitro clonogenic and multipotent progenitor cells. This ability appears to be somewhat less than that of KL and IL-3, comparable to that of FL, and more pronounced than G-CSF or any of the purely synergistic cytokines (i.e., IL-1, IL-6, IL-11).

Due to the heterogeneity (with regard to short- and LTRC) of the Lin$^-$Sca-1$^+$ progenitor cell population or any other enriched stem cell population [1], it is not yet possible to state whether TPO has a viability-promoting effect on the LTRC. So far, KL is in fact the only cytokine demonstrated to promote the viability of LTRC [33, 43], although it has also been demonstrated that KL is not essential for such viability [44]. However, since the flt3$^+$ fraction of murine stem cells contains LTRC [45], it appears likely that FL, like stem cell factor (SCF), might act on the LTRC. Similar studies with c-*mpl*$^+$ Lin$^-$Sca-1$^+$ stem cells could reveal whether c-*mpl* is in fact expressed on LTRC.

Also, due to the heterogeneity, viability studies of primitive progenitor cells have almost exclusively been done in an indirect manner, by delayed addition studies, implicating that the progenitor cells are preincubated in medium alone or the putative viability factor [28, 30, 33, 34, 39-43]. Following this preincubation period, cultures are pulsed with a potent proliferative stimulus capable of recruiting most, if not all, in vitro clonogenic progenitor cells. When compared to cultures stimulated with this potent proliferative stimulus from initiation of culture, a reduction in number of clonogenic progenitor cells is thought to reflect progenitor cells which

Table 2. Cytokines with viability-promoting effects on Lin⁻Sca-1⁺ bone marrow progenitor cells

Cytokine	% Surviving in vitro clonogenic Lin⁻Sca-1⁺ progenitor cells[a]	Viability-promoting effect on LTRC[b]
SCF	40%-75% [28, 30, 33-35]	+(33)
FL	35%-50% [35, 57]	nd
IL-3	65% [34]	–(33)
TPO	35% [28]	nd
G-CSF	10%-30% [28, 30, 34]	–(33)
IL-1	10%-25% [28, 30, 34]	–(33)
IL-6	10%-20% [30, 34]	–(33)
IL-11	10%-15% [30, 34]	nd
EPO	none [28]	nd

List is exclusively based on studies investigating effects of cytokines on viability of Lin⁻Sca-1⁺ progenitor cells. Studies on other primitive murine or human progenitor cell populations have reached similar conclusions [39-43, 46].
[a]Numbers show the range (from different studies) of the percentage of Lin⁻Sca-1⁺ progenitor cells surviving in response to each cytokine, as measured by delayed addition studies, at which time less than 2%-5% of the progenitors could be recovered from cells cultured in medium alone.
[b]This column shows cytokines demonstrated to have (+) or not to have (–) a viability-promoting effect on LTRC as measured in a long-term reconstituting assay (33). nd = not determined. All numbers in brackets represent references from which data have been accumulated.

have lost viability due to lack of appropriate viability signals. Although measuring viability in an indirect manner, this appears to represent a better assay than the direct assays available (such as propidium iodine [PI] uptake, trypan blue exclusion, and different apoptosis assays). The reason for this is that these specific viability assays do not allow the specific identification of the progenitors surviving, and thus a reduction (or increase) in viability in these assays does not necessarily reflect effects on the primitive progenitor cells in the candidate stem cell populations. However, this does not mean that such direct viability assays are not important supplements to the indirect viability assays. On the contrary, there is usually a good correlation between the indirect and direct viability assays [5, 30, 46]. This was also the case for TPO which enhanced the number of viable Lin⁻Sca-1⁺ bone marrow cells in culture, as determined by PI exclusion as well as an established apoptosis assay [47]. Thus, TPO might promote viability of Lin⁻Sca-1⁺ progenitor cells through its ability to suppress apoptosis.

TPO Promotes the In Vitro Growth of Multipotent Candidate Murine Bone Marrow Stem Cells in Synergy with other Early-Acting Cytokines

In addition to establishing that TPO, when acting alone, has a selective viability-promoting effect on Lin⁻Sca-1⁺ bone marrow progenitor cells [28], we have also examined whether TPO might interact with other cytokines to enhance the growth of this primitive progenitor cell population [29]. In particular, TPO synergized with KL and FL (individually as well as when combined) and IL-3 to enhance the number as well as the size of clones formed by Lin⁻Sca-1⁺ progenitor cells. This effect was observed in serum-depleted, as well as FCS-supplemented cultures, and appeared to be directly mediated on the primitive progenitor cells since the effect was observed when cells were plated individually. The synergistic effect of TPO was observed regardless of whether cells were stimulated with a serum-free conditioned medium containing murine TPO or with purified recombinant human TPO, and was neutralized with soluble murine c-*mpl* (*Ramsfjell* and *Jacobsen*, unpublished observation).

The clones formed in response to TPO in combination with other cytokines contained erythroid as well as multiple myeloid cell lineages, demonstrating that TPO acts on multipotent progenitor cells. Whereas KL enhanced the production of high ploidy MK from Lin⁻Sca-1⁺ progenitor cells when combined with TPO, the increased cell production observed in response to the combined action of FL + TPO was not accompanied by any increase in MK. TPO displayed little or no synergy when combined with G-CSF, GM-CSF, EPO,

IL-11 or IL-12, and unlike TPO, EPO showed no ability to synergize with KL, FL or IL-3, again suggesting that c-*mpl* is expressed on more primitive progenitor cells than the EPO receptor.

TPO Acts on Primitive HPC with Multilineage Potential: Just Another Cytokine with Redundant Activities on Primitive HPC?

Our findings fall in line with other recent studies [48, 49], suggesting that TPO, through its receptor c-*mpl*, can affect the in vitro growth and viability of primitive HPC with multilineage potential. Using $Lin^-Sca\text{-}1^+$ c-kit^+ bone marrow cells isolated from 5-fluorouracil-treated mice, *Ku et al.* recently demonstrated that TPO could enhance multilineage growth, particularly in combination with KL or IL-3 [48]. Similarly, *Sitnicka et al.* used a murine bone marrow stem cell population characterized by low Hoescht 33342 and low Rhodamine 123 fluorescence, and found that TPO in combination with SCF, IL-3 or both, enhanced the cloning frequency and proliferative capacity of these progenitors in vitro [49]. It is noteworthy that the cytokines with which TPO appears to most potently interact to enhance growth of primitive progenitor cells (KL, IL-3 and FL) are also the cytokines (together with TPO itself) which individually have the best viability-promoting effect on the same progenitor cells (Table 2).

Collectively, these studies confirm what already had been implicated through studies of c-*mpl* expression on murine and human candidate stem cell populations [18-20], meaning that TPO is not a lineage-specific cytokine, and can in fact maintain the viability and promote the clonal growth of very primitive multipotent murine bone marrow progenitor cells. Yet other studies have demonstrated that TPO can directly enhance EPO-stimulated growth of murine and human erythroid progenitor cells [50, 51], demonstrating that TPO can affect multiple stages of hematopoiesis (Fig. 1). A recent study on human

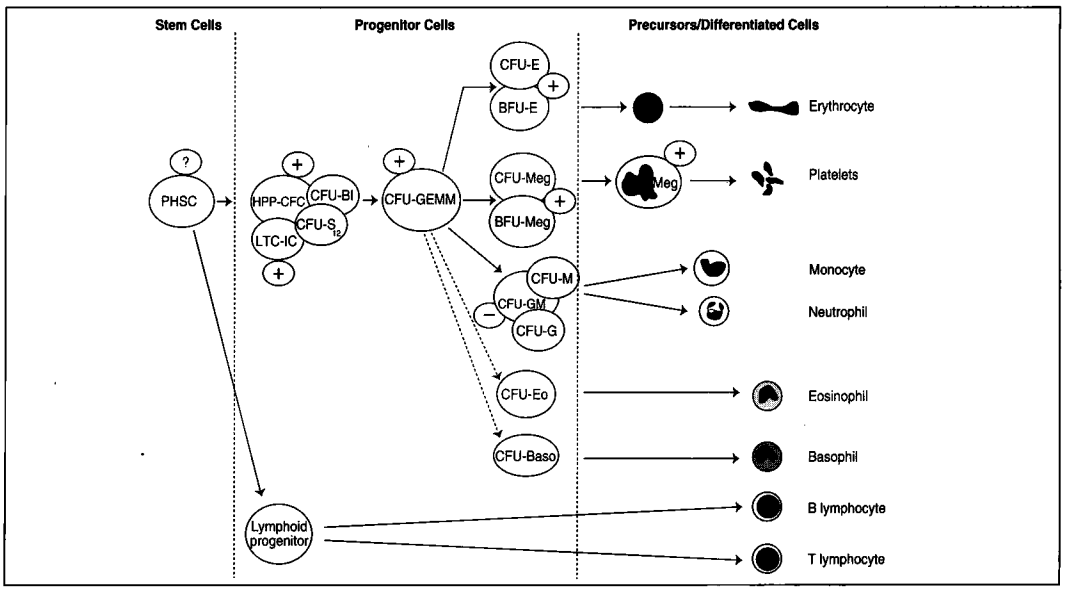

Figure 1. Thrombopoietin acts at multiple levels in the hematopoietic hierarchy. *Figure shows different stages of hematopoietic stem and progenitor cells, as well as precursors and fully differentiated cells upon which TPO has been demonstrated to have (+) or not to have (–) effects. The "?" at the long-term reconstituting pluripotent hematopoietic stem cell indicates that TPO is likely, but not yet proven, to have activity on this cell population. On progenitor cells not labeled with a + or –, it is not yet established whether TPO has any activity.*
Abbreviations not defined elsewhere: CFU-Bl = colony-forming unit-blast; HPP-CFC = high proliferative potential colony-forming cell; CFU-GEMM = CFU-granulocyte-erythroid-macrophage-megakaryocyte; BFU-E = burst-forming unit-erythroid; CFU-E = CFU-erythroid; BFU-Meg = BFU-megakaryocyte; CFU-GM = CFU-granulocyte-macrophage; CFU-Eo = CFU-eosinophil; CFU-Baso = CFU-basophil.

$CD34^+CD38^-$ bone marrow cells demonstrated that TPO maintained or even slightly expanded long-term culture-initiating cells [52], suggesting that TPO also acts on very primitive human HPC. However, it remains (as discussed above) to be established whether it also can affect the LTRC.

A number of cytokines can affect the growth of primitive HPC (Table 1), but the potential clinical and, in particular, physiological significance of such findings remains questionable in many cases [2, 3]. The early studies in c-*mpl*-deficient and later TPO-deficient mice clearly established that TPO is a critical regulator of megakaryocytopoiesis and platelet production, but gave no indications (through studies of peripheral blood) that TPO might be involved in steady-state regulation of other blood cell lineages [14, 53]. However, in more recent studies of progenitor cell levels in c-*mpl*-deficient mice, the total number of progenitor cells was found to be reduced to less than 50% of that observed in wild-type littermate controls [54]. This reduction involved all types of investigated progenitors, including GM, erythroid, MK and multipotent progenitor cells. In addition to providing important support for TPO acting on very primitive HPC, this study clearly implicated a role for c-*mpl* and its ligand in the production of primitive HPC, as well as progenitor cells committed to different cell lineages. In addition, data have emerged suggesting that the activity of TPO on early progenitor cells might have clinical significance as well in that TPO, in addition to promoting platelet recovery, also accelerates recovery of red cells and neutrophils [55, 56]. Thus, in addition to being the principal regulator of megakaryocytopoiesis and platelet production, TPO might also prove to be a key regulator in early hematopoiesis.

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References

1 Spangrude GJ, Smith L, Uchida N et al. Mouse hematopoietic stem cells. Blood 1991;78:1395-1402.

2 Metcalf D. Hematopoietic regulators: redundancy or subtlety. Blood 1993;82:3515-3523.

3 Ogawa M. Differentiation and proliferation of hematopoietic stem cells. Blood 1993;81:2844-2853.

4 Lyman SD. Biology of flt3 ligand and receptor. Int J Hematol 1995;62:63-73.

5 Jacobsen SEW, Okkenhaug C, Myklebust J et al. The FLT3 ligand potently and directly stimulates the growth and expansion of primitive murine bone marrow progenitor cells in vitro: synergistic interactions with interleukin (IL) 11, IL-12, and other hematopoietic growth factors. J Exp Med 1995;181:1357-1363.

6 Moore MAS. Clinical implications of positive and negative hematopoietic stem cell regulators. Blood 1991;78:1-19.

7 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;91:11104-11108.

8 Sohma Y, Akahori H, Seki N et al. Molecular cloning and chromosomal localization of the human thrombopoietin gene. FEBS Lett 1994;353:57-61.

9 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;77:1117-1124.

10 Lok S, Kaushansky K, Holly RD et al. Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. Nature 1994;369:565-568.

11 de Sauvage FJ, Hass PE, Spencer SD et al. Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature 1994;369:533-538.

12 Wendling F, Maraskovsky E, Debili N et al. c-Mpl ligand is a humoral regulator of megakaryocytopoiesis. Nature 1994;369:571-574.

13 Kaushansky K. Thrombopoietin: the primary regulator of platelet production. Blood 1995;86:419-431.

14 Gurney AL, Carver-Moore K, de Sauvage FJ et al. Thrombocytopenia in c-*mpl*-deficient mice. Science 1994;265:1445-1447.

15 Kaushansky K, Lok S, Holly RD et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. Nature 1994;369:568-571.

16 Broudy VC, Lin NL, Kaushansky K. Thrombopoietin (c-*mpl* ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy in vitro. Blood 1995;85:1719-1726.

17 Debili N, Wendling F, Katz A et al. The Mpl-ligand or thrombopoietin or megakaryocyte

growth and differentiative factor has both direct proliferative and differentiative activities on human megakaryocyte progenitors. Blood 1995;86:2516-2525.

18 Methia N, Louache F, Vainchenker W et al. Oligodeoxynucleotides antisense to the proto-oncogene c-*mpl* specifically inhibit in vitro megakaryocytopoiesis. Blood 1993;82:1395-1401.

19 Zeigler FC, de Sauvage F, Widmer HR et al. In vitro megakaryocytopoietic and thrombopoietic activity of c-*mpl* ligand (TPO) on purified murine hematopoietic stem cells. Blood 1994;84:4045-4052.

20 Berardi AC, Wang A, Levine JD et al. Functional isolation and characterization of human hematopoietic stem cells. Science 1995;267:104-108.

21 Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988;241:58-62.

22 Li CL, Johnson GR. Murine hematopoietic stem and progenitor cells: I. Enrichment and biologic characterization. Blood 1995;85:1472-1479.

23 Heimfeld S, Hudak S, Weissman IL et al. The in vitro response of phenotypically defined mouse stem cells and myeloerythroid progenitors to single or multiple growth factors. Proc Natl Acad Sci USA 1991;88:9902-9906.

24 Uchida N, Fleming WH, Alpern EJ et al. Heterogeneity of hematopoietic stem cells. Curr Opin Immunol 1993;5:177-184.

25 Li CL, Johnson GR. Rhodamine123 reveals heterogeneity within murine Lin^-, $Sca\text{-}1^+$ hemopoietic stem cells. J Exp Med 1992;175:1443-1447.

26 Randall TD, Lund FE, Howard MC et al. Expression of murine CD38 defines a population of long-term reconstituting hematopoietic stem cells. Blood 1996;87:4057-4067.

27 Trevisan M, Iscove NN. Phenotypic analysis of murine long-term hematopoietic reconstituting cells quantitated competitively in vivo and comparison with more advanced colony-forming progeny. J Exp Med 1995;181:93-103.

28 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 (in press).

29 Ramsfjell V, Borge OJ, Veiby OP et al. Thrombopoietin, but not erythropoietin, directly stimulates multilineage growth of primitive murine bone marrow progenitor cells in synergy with early acting cytokines: distinct interactions with the ligands for c-*kit* and FLT3. Blood 1996 (in press).

30 Jacobsen FW, Stokke T, Jacobsen SE. Transforming growth factor-β potently inhibits the viability-promoting activity of stem cell factor and other cytokines and induces apoptosis of primitive murine hematopoietic progenitor cells. Blood 1995;86:2957-2966.

31 Veiby OP, Lyman SD, Jacobsen SEW. Combined signaling through IL-7 receptor and flt3 but not c-*kit* potently and selectively promotes B cell commitment and differentiation from uncommitted murine bone marrow progenitor cells. Blood 1996;88:1256-1265.

32 Ikuta K, Weissman IL. Evidence that hematopoietic stem cells express mouse c-*kit* but do not depend on steel factor for their generation. Proc Natl Acad Sci USA 1992;89:1502-1506.

33 Li CL, Johnson GR. Stem cell factor enhances the survival but not the self-renewal of murine hematopoietic long-term repopulating cells. Blood 1994;84:408-414.

34 Katayama N, Clark SC, Ogawa M. Growth factor requirement for survival in cell-cycle dormancy of primitive murine lymphohematopoietic progenitors. Blood 1993;81:610-616.

35 Rasko JE, Metcalf D, Rossner MT et al. The flt3/flk-2 ligand: receptor distribution and action on murine haematopoietic cell survival and proliferation. Leukemia 1995;9:2058-2066.

36 Jacobsen FW, Veiby OP, Stokke T et al. TNF-α bidirectionally modulates the viability of primitive murine hematopoietic progenitor cells in vitro. J Immunol 1996;157:1193-1199.

37 Sawada K, Krantz SB, Kans JS et al. Purification of human erythroid colony-forming units and demonstration of specific binding of erythropoietin. J Clin Invest 1987;80:357-366.

38 Youssoufian H, Longmore G, Neumann D et al. Structure, function, and activation of the erythropoietin receptor. Blood 1993;81:2223-2236.

39 Bodine D, Crosier P, Clark SC. Effects of hematopoietic growth factors on the survival of primitive stem cells in liquid suspension culture. Blood 1991;78:914-920.

40 Itoh Y, Ikebuchi K, Hirashima K. Interleukin-3 and granulocyte colony-stimulating factor as survival factors in murine hemopoietic stem cells in vitro. Int J Hematol 1992;55:139-145.

41 Leary AG, Zeng HQ, Clark SC et al. Growth factor requirements for survival in G_0 and entry into the cell cycle of primitive human hemopoietic progenitors. Proc Natl Acad Sci USA 1992;89:4013-4017.

42 Sasaki H, Hirabayashi Y, Inoue T et al. Promotion of survival and proliferation by

interleukin 3, kit-ligand and erythropoietin on early and late appearing spleen colony forming units in culture. STEM CELLS 1993;11:555-561.

43 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;86:1757-1764.

44 Wineman JP, Nishikawa S, Muller-Sieburg CE. Maintenance of high levels of pluripotent hematopoietic stem cells in vitro: effect of stromal cells and c-*kit*. Blood 1993;81:365-372.

45 Zeigler FC, Bennett BD, Jordan CT et al. Cellular and molecular characterization of the role of the flk-2/flt-3 receptor tyrosine kinase in hematopoietic stem cells. Blood 1994;84:2422-2430.

46 Brandt JE, Bhalla K, Hoffman R. Effects of interleukin-3 and c-*kit* ligand on the survival of various classes of human hematopoietic progenitor cells. Blood 1994;83:1507-1514.

47 Gorczyca W, Gong J, Darzynkiewicz Z. Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. Cancer Res 1993;53:1945-1951.

48 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 of mice. Blood 1996;87:4544-4551.

49 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.

50 Kaushansky K, Broudy VC, Grossmann A et al. Thrombopoietin expands erythroid progenitors, increases red cell production, and enhances erythroid recovery after myelosuppressive therapy. J Clin Invest 1995;96:1683-1687.

51 Kobayashi M, Laver JH, Kato T et al. Recombinant human thrombopoietin (Mpl ligand) enhances proliferation of erythroid progenitors. Blood 1995;86:2494-2499.

52 Petzer AL, Zandstra PW, Piret JM et al. Differential cytokine effects on primitive ($CD34^+CD38^-$) human hematopoietic cells: novel responses to Flt3-ligand and thrombopoietin. J Exp Med 1996;183:2551-2558.

53 de Sauvage FJ, Carver-Moore K, Luoh S et al. Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. J Exp Med 1996;183:651-656.

54 Warren SA, Roberts AW, Nicola NA et al. Deficiencies in progentior cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietin receptor c-Mpl. Blood 1996;87:2162-2170.

55 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;86:4486-4492.

56 Farese AM, Hunt P, Granb LB et al. Combined administration of recombinant human megakaryocyte growth and development factor and granulocyte colony-stimulating factor enhances multilineage hematopoietic reconstitution in nonhuman primates after radiation-induced marrow aplasias. J Clin Invest 1996;97:2145-2151.

57 Veiby OP, Jacobsen FW, Cui L et al. The Flt3 ligand promotes survival of primitive hematopoietic progenitor cells with myeloid as well as B-lymphoid potenetial: suppression of apoptosis and counteraction by TNF-α and TGF-β. J Immunol 1996 (in press).