Special Issue: Mesenchymal stem cells

Mini Review

Mesenchymal stem cells as an essential hematopoietic stem cell niche component

Keiyo Takubo

Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, Keio University School of Medicine, Tokyo, Japan

Hematopoietic stem cells (HSCs) are capable of self-renewal and multi-lineage differentiation, which are characteristics that enable them to maintain the stem cell population and produce large numbers of blood cells. Quiescent mammalian adult HSCs localize within the bone marrow niche, primarily in the endosteal region of the trabecular bone. These cells scarcely participate in the cell cycle, but are instead maintained by assorted niche cells within the bone marrow, including mesenchymal stem cells (MSCs). This review summarizes the crucial role(s) that MSCs play as niche cells for the support of HSCs.

Rec.12/3/2012, Acc.12/16/2012, pp13-18

* Correspondence should be addressed to:
Keiyo Takubo, M.D., Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: +81-3-5363-3475, Fax: +81-3-5363-3474, E-mail: keiyot@gmail.com

Key words hematopoietic stem cell, hypoxia, mesenchymal stem cell, niche

Introduction

Adult stem cells sustain tissue homeostasis by generating differentiated progeny throughout the lifetime of an organism, a process essential for the proper maintenance of the stem cell system[1, 2]. If stem cells are improperly regulated, various processes, including development, aging, and control of disease progression, are adversely affected[1, 2].

Stem cells are maintained by dynamic microenvironments, or "niches", which control stem cell fate. The stem cell niche comprises niche cells and niche factors, which are derived from the niche cells[3] (e.g., cytokines and extracellular matrix molecules). Niche factors retain stem cells within the niche, maintain stem cell homeostasis, and trigger the generation of stem cell progeny.

Hematopoietic stem cells (HSCs) give rise to many different hematopoietic cells, including immune cells of the lymphoid lineage (e.g., T cells and B cells) and non-immune cells of the myeloid lineage (e.g., macrophages, granulocytes, erythrocytes, and platelets)[4, 5]. Adult mammalian HSCs are mainly sustained within the bone marrow niche[6] by mesenchymal stem cells (MSCs). This mini-review will focus on the role of MSCs as a critical niche cell for the support of HSCs in the bone marrow.

The Hematopoietic Stem Cell Niche

HSCs are capable of self-renewal and multi-lineage differentiation, characteristics that enable proper maintenance of the HSC population and the generation of a large num-

ber of myeloid and lymphoid lineage cells. Quiescent mammalian adult HSCs are localized within the bone marrow niche[7], primarily in the endosteal region of the trabecular bone. Bone marrow niche cells in the endosteum regulate the retention of HSCs within the niche. They also regulate the balance between HSC quiescence, self-renewal/proliferation, and differentiation by providing support in the form of cytokines, chemokines, and other extracellular molecules and by expressing adhesion molecules. Moreover, niche cell-derived factors can induce the homing and lodgment of HSCs to the bone marrow after stem cell transplantation. Therefore, manipulation of niche signals is a promising strategy to ensure the proper engraftment and/or efficient mobilization of HSCs.

HSC niche cells comprise non-hematopoietic and hematopoietic cells. For example, quiescent HSCs are directly attached to, and retained within, the bone marrow niche by N-cadherin+ osteoblasts and osteolineage progenitor cells[8, 9]. These cells transduce non-canonical Wnt signaling in HSCs, which is mediated by Flamingo and Frizzled 8. This induces the long-term maintenance of HSC quiescence by suppressing the calcium ion/nuclear factor of activated T cells (NFAT)/interferon (IFN)-γ pathway and by antagonizing canonical Wnt signaling[10].

The trabecular region of the bone marrow is well vascularized. Accordingly, HSCs are located adjacent to endothelial cells in the bone marrow[11, 12]. Bone marrow endothelial cells are required to support hematopoiesis[13-15], and act to protect the HSC compartment from premature exhaustion during stress hematopoiesis and hematological stress. For example, endothelial cells and perivascular reticular cells, in addition to osteolineage cells, retain HSCs within the bone marrow by secreting chemokine (C-X-C motif) ligand 12 (CXCL12) and KitL[16, 17]. Osteoblastic/osteolineage cells also produce CXCL12[18], indicating that the maintenance of HSCs by niche cells is regulated by multiple cell types that use common niche factor(s).

In addition to the canonical niche components (i.e., niche cells and proteins), non-canonical components of the HSC bone marrow niche include calcium ions and molecular oxygen. The concentration of calcium ions in the endosteal bone marrow region is higher than that in other bone marrow regions. HSCs recognize increased extracellular calcium ion levels in the niche via the calcium-sensing receptor (CaR) during lodgment in the adult bone marrow stem cell microenvironment. The genetic loss of CaR prevents HSCs from homing to the niche[19].

In contrast to the high concentration of calcium ions in the endosteal zone, the partial pressure of oxygen is low, resulting in hypoxia. This induces the activation of hypoxia inducible factor (HIF)-1α, a master regulator of systemic and cellular hypoxia responses, in the endosteal niche. Under normoxic conditions, HIF-1α is hydroxylated at its oxygen-dependent degradation domain (ODD) by oxygen-dependent prolyl hydroxylases (Phds). Hydroxylated HIF-1α is recognized by an E3 ubiquitin ligase, von Hippel-Lindau protein (VHL), resulting in degradation via the ubiquitin-proteasome pathway. Under hypoxic conditions, Phds lose their enzymatic activity, and HIF-1α is maintained in its dehydroxylated form, resulting in protein stabilization. Stabilized HIF-1α then binds to Arnt (aryl hydrocarbon receptor nuclear translocator), and the heterodimer translocates to the nucleus where it binds to the hypoxia-responsive element in the promoter region of various hypoxia-responsive genes[20].

HSCs exist in the bone marrow under hypoxic conditions; this stabilizes HIF-1α within the niche. The genetic loss of HIF-1α results in the loss of HSC quiescence, resulting in increased sensitivity to various hematological stresses

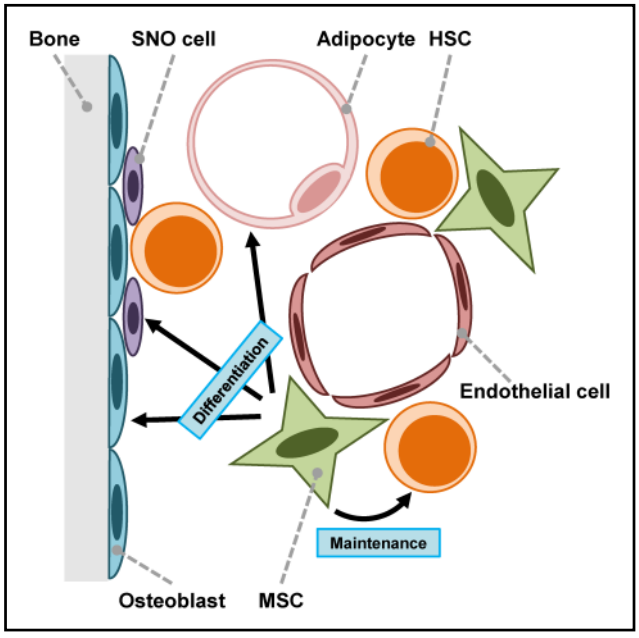

Fig.1 Hematopoietic stem cells in the bone marrow niche
Hematopoietic stem cells (HSCs) are maintained by multiple niche cellular components, including osteoblasts, small N-cadherin+ osteoblastic cells (SNO cells), endothelial cells, adipocytes, and mesenchymal stem cells (MSCs). MSCs also indirectly affect HSCs by differentiating into mesenchymal-lineage niche cells.

(such as those caused by transplantation, exposure to chemotherapeutic agents, and aging). Therefore, the hypoxic conditions within the bone marrow enable HSCs to resist stress through HIF-1α [21]. Thus, these cellular and non-cellular niche components coordinately regulate the homeostasis of HSCs in the bone marrow (Fig.1).

Mesenchymal Progenitors as A New Niche Component

MSCs play an important role as a niche cell for HSCs. MSCs are able to grow *in vitro* and give rise to multiple mesenchymal lineages, such as osteoblasts, adipocytes, and chondrocytes. In addition to the putative developmental roles played by MSCs, they are involved in homeostasis and in several pathological conditions.

Méndez-Ferrer et al. reported that MSCs in the bone marrow could be identified by their expression of nestin[22]. Nestin+ MSCs contain all the bone marrow colony-forming-unit fibroblastic activity (CFU-F), can be expanded in a serial transplantation system, and retain the capacity to differentiate into different mesenchymal cell lineages. They are located in close proximity to HSCs, and the genetic depletion of nestin+ cells results in the rapid loss of HSCs from the bone marrow. Therefore, MSCs are a crucial niche cell that supports HSCs. Nestin+ cells produce various hematopoiesis-related factors, including CXCL12, KitL, Angpt1, IL-7, Vcm1, and Spp1, suggesting that these niche factors are critical for supporting HSCs[22].

Another group[16] reported that genetic ablation of CXCL12-abundant reticular (CAR) cells, which have both adipogenic and osteogenic capacity, did not affect osteoblasts or endothelial cells, but decreased the production of KitL and CXCL12 in the bone marrow. This resulted in a marked reduction in the number of cycling progenitors and in the size of the HSC pool. Thus, the niche comprising adipo-osteogenic progenitors is required for the proliferation of both progenitors and HSCs through the production of niche factors including KitL and CXCL12[16].

An attempt to prospectively isolate MSCs from the bone marrow of adult mice identified a subset of MSCs ($PDGFR\alpha^{+}Sca\text{-}1^{+}CD45^{-}TER119^{-}$)[23], which showed high expression of Angpt1; however, CXCL12 expression was lower than that in the $PDGFR\alpha^{+}Sca\text{-}1^{-}CD45^{-}TER119^{-}$ fraction. Therefore, MSCs in the bone marrow niche comprise a mixture of different subsets. Nakamura et al. subdivided bone marrow endosteal cells into immature mesenchymal cell-enriched $ALCAM^{-}Sca\text{-}1^{+}$ cells, osteoblast-enriched $ALCAM^{+}Sca\text{-}1^{-}$ cells, and $ALCAM^{-}Sca\text{-}1^{-}$ cells[24]. They found that all three fractions supported the long-term repopulating (LTR) activity of HSCs in an *in vitro* culture. Notably, the $ALCAM^{+}Sca\text{-}1^{-}$ cells significantly increased the LTR activity of HSCs by up-regulating the expression of homing- and cell adhesion-related genes in HSCs. Transcriptome analysis revealed that the $ALCAM^{-}Sca\text{-}1^{+}$ fraction showed high expression of cytokine-related genes, whereas the $ALCAM^{+}Sca\text{-}1^{-}$ fraction expressed multiple cellular adhesion molecules. These observations suggest that the bone marrow niche in adults comprises multiple subsets of MSCs and their progeny, all of which regulate HSCs.

Type XVII collagen is highly expressed by hair follicle stem cells (HFSCs) and is required to maintain both HFSCs and melanocyte stem cells; the latter do not express Col17a1, but directly adhere to the HFSCs within the niche[25]. Thus, stem cells within the niche may be maintained by other stem cells, which act as niche cells; this might be a common regulatory mechanism in tissues that contain multiple types of stem cell.

Modulation of The Mesenchymal Niche by MSCs

MSCs not only act as a niche cell for HSCs, they also regulate the microenvironment by differentiating into mesenchymal-lineage cells, including osteoblasts and adipocytes. The frequency of HSCs and their progenitors in adipocyte-rich vertebrae is low compared with that in adipocyte-free vertebrae[26]. Thus, adipocytes negatively regulate the bone marrow niche.

Under normoxic conditions, MSCs lose their stem cell potential, whereas hypoxia promotes their self-renewal capacity and maintains the undifferentiated state[27]. Hypoxia also reversibly suppresses osteoblastic and adipocytic differentiation[27-29]. Osteoblastic differentiation is dependent on the HIF pathway, whereas adipocytic differentiation is not; however, the latter is dependent on the activation of the unfolded protein response pathway. In addition, increases in the colony formation capacity of MSCs are also HIF-independent[27]. Thus, the increased self-renewal capacity of MSCs under hypoxic conditions is regulated both in a HIF-dependent and -independent manner. These findings suggest that HIF plays a limited, albeit pivotal, role in maintaining MSCs in the BM niche.

Clinical Implications

Recently, much research has focused on the immunomodulatory properties of MSCs. MSCs are thought to play a role in T cell suppression[30, 31]; therefore, they may be a potent tool for treating immunological disorders that are resistant to conventional forms of therapy, such as acute graft-versus-host disease (GvHD), which disrupts the bone marrow niche[32]. Thus, controlling GvHD is crucial for hematopoiesis. Although MSCs play a clinically significant role in several human diseases[30, 31], data regarding the biological mechanisms underlying MSC-mediated immunosuppression are inconsistent.

Recently, a new model of MSC-mediated immunosuppression was proposed[33]. Proinflammatory cytokines (IFN-γ, plus one of the three following cytokines: TNF-α, IL-1α, or IL-1β) derived from activated T cells target MSCs and induce the release of chemokines. The MSC-derived chemokines recruit CXCR3+ immune cells, including T cells, to MSCs. In addition, MSCs produce nitric oxide (NO), which suppresses T cell proliferation by inhibiting Stat5 phosphorylation and causing cell-cycle arrest[33]. However, because MSCs also modulate other immune cells, including B cells, NK cells, regulatory T cells, and dendritic cells, further studies are required to fully clarify the mechanism(s) underlying MSC-mediated immunosuppression[31].

MSCs themselves are thought to be involved in pathological BM fibrosis. Transgenic mice with osteoblasts that express the constitutively-active form of parathyroid hormone (PTH)/PTH-related peptide receptors show BM fibrosis[34, 35]. MSCs express receptors for PTH, proliferating and redistributing within the adult bone marrow upon PTH treatment. This partly explains the occurrence of pathological BM fibrosis in individuals with severe hyperparathyroidism.

In addition, a study using a chimeric BM model of primary myelofibrosis induced by thrombopoietin shows that SPARC, a matricellular protein derived from MSCs, contributes to the development of BM stromal fibrosis. Interestingly, SPARC deficiency within the radio-resistant stromal compartment impairs myelofibrosis but increases the reactive myeloproliferative response to thrombopoietin[36]. Thus, MSC-derived SPARC regulates the hematopoietic BM response in individuals with myeloproliferative disorders.

Conclusion

Recent research has clarified the critical role(s) of MSCs as a niche cell that supports the homeostasis of HSCs. A strategy that combines genetic dissection and real-time imaging of the bone marrow stem cell niche would likely facilitate a more complete understanding of this multi-faceted interplay between HSCs and MSCs, as well as the development of novel MSC-based approaches to treating immunological disorders.

Funding source

K.T. was supported in part by a MEXT Grant-in-Aid for Young Scientists (A), a MEXT Grant-in-Aid for Scientific Research on Innovative Areas and the Project for Realization of Regenerative Medicine from MEXT.

Conflict(s) of interest

None declared.

References

1) Fuchs E: The tortoise and the hair: slow-cycling cells in the stem cell race. Cell. 2009; 137: 811-819.
2) Li L, Clevers H: Coexistence of quiescent and active adult stem cells in mammals. Science. 2010; 327: 542-545.
3) Fuchs E, Tumbar T, Guasch G: Socializing with the neighbors: stem cells and their niche. Cell. 2004; 116: 769-778.
4) Mikkola HK, Orkin SH: The journey of developing hematopoietic stem cells. Development. 2006; 133: 3733-3744.
5) Seita J, Weissman IL: Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdiscip Rev Syst Biol Med. 2010; 2: 640-653.
6) Mercier FE, Ragu C, Scadden DT: The bone marrow at the crossroads of blood and immunity. Nat Rev Immunol. 2012; 12: 49-60.
7) Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR, Trumpp A: Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008; 135: 1118-1129.
8) Xie Y, Yin T, Wiegraebe W, He XC, Miller D, Stark D, Perko K, Alexander R, Schwartz J, Grindley JC, Park J, Haug JS, Wunderlich JP, Li H, Zhang S, Johnson T,

Feldman RA, Li L: Detection of functional haematopoietic stem cell niche using real-time imaging. Nature. 2009; 457: 97-101.

9) Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L: Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003; 425: 836-841.

10) Sugimura R, He XC, Venkatraman A, Arai F, Box A, Semerad C, Haug JS, Peng L, Zhong XB, Suda T, Li L: Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. Cell. 2012; 150: 351-365.

11) Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ: SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell. 2005; 121: 1109-1121.

12) Sugiyama T, Kohara H, Noda M, Nagasawa T: Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006; 25: 977-988.

13) Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, Kopp HG, Shido K, Petit I, Yanger K, James D, Witte L, Zhu Z, Wu Y, Pytowski B, Rosenwaks Z, Mittal V, Sato TN, Rafii S: Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. Cell Stem Cell. 2009; 4: 263-274.

14) Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M, Witte L, May C, Shawber C, Kimura Y, Kitajewski J, Rosenwaks Z, Bernstein ID, Rafii S: Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell Stem Cell. 2010; 6: 251-264.

15) Kobayashi H, Butler JM, O'Donnell R, Kobayashi M, Ding BS, Bonner B, Chiu VK, Nolan DJ, Shido K, Benjamin L, Rafii S: Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. Nat Cell Biol. 2010; 12: 1046-1056.

16) Omatsu Y, Sugiyama T, Kohara H, Kondoh G, Fujii N, Kohno K, Nagasawa T: The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity. 2010; 33: 387-399.

17) Ding L, Saunders TL, Enikolopov G, Morrison SJ: Endothelial and perivascular cells maintain haematopoietic stem cells. Nature. 2012; 481: 457-462.

18) Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS: Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006; 124: 407-421.

19) Adams GB, Chabner KT, Alley IR, Olson DP, Szczepiorkowski ZM, Poznansky MC, Kos CH, Pollak MR, Brown EM, Scadden DT: Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature. 2006; 439: 599-603.

20) Semenza GL: Oxygen homeostasis. Wiley Interdiscip Rev Syst Biol Med. 2010; 2: 336-361.

21) Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, Shima H, Johnson RS, Hirao A, Suematsu M, Suda T: Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell Stem Cell. 2010; 7: 391-402.

22) Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS: Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010; 466: 829-834.

23) Morikawa S, Mabuchi Y, Kubota Y, Nagai Y, Niibe K, Hiratsu E, Suzuki S, Miyauchi-Hara C, Nagoshi N, Sunabori T, Shimmura S, Miyawaki A, Nakagawa T, Suda T, Okano H, Matsuzaki Y: Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. J Exp Med. 2009; 206: 2483-2496.

24) Nakamura Y, Arai F, Iwasaki H, Hosokawa K, Kobayashi I, Gomei Y, Matsumoto Y, Yoshihara H, Suda T: Isolation and characterization of endosteal niche cell populations that regulate hematopoietic stem cells. Blood. 2010; 116: 1422-1432.

25) Tanimura S, Tadokoro Y, Inomata K, Binh NT, Nishie W, Yamazaki S, Nakauchi H, Tanaka Y, McMillan JR, Sawamura D, Yancey K, Shimizu H, Nishimura EK: Hair follicle stem cells provide a functional niche for melanocyte stem cells. Cell Stem Cell. 2011; 8: 177-187.

26) Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ: Bone marrow adipocytes as negative regulators of the haematopoietic microenvironment.

Nature. 2009; 460: 259-263.
27) Tamama K, Kawasaki H, Kerpedjieva SS, Guan J, Ganju RK, Sen CK: Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition. J Cell Biochem. 2011; 112: 804-817.
28) Potier E, Ferreira E, Andriamanalijaona R, Pujol JP, Oudina K, Logeart-Avramoglou D, Petite H: Hypoxia affects mesenchymal stromal cell osteogenic differentiation and angiogenic factor expression. Bone. 2007; 40: 1078-1087.
29) Valorani MG, Germani A, Otto WR, Harper L, Biddle A, Khoo CP, Lin WR, Hawa MI, Tropel P, Patrizi MP, Pozzilli P, Alison MR: Hypoxia increases Sca-1/CD44 co-expression in murine mesenchymal stem cells and enhances their adipogenic differentiation potential. Cell Tissue Res. 2010; 341: 111-120.
30) Keating A: Mesenchymal stromal cells. Curr Opin Hematol. 2006; 13: 419-425.
31) Nauta AJ, Fibbe WE: Immunomodulatory properties of mesenchymal stromal cells. Blood. 2007; 110: 3499-3506.
32) Shono Y, Ueha S, Wang Y, Abe J, Kurachi M, Matsuno Y, Sugiyama T, Nagasawa T, Imamura M, Matsushima K: Bone marrow graft-versus-host disease: early destruction of hematopoietic niche after MHC-mismatched hematopoietic stem cell transplantation. Blood. 2010; 115: 5401-5411.
33) Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y: Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008; 2: 141-150.
34) Ohishi M, Chiusaroli R, Ominsky M, Asuncion F, Thomas C, Khatri R, Kostenuik P, Schipani E: Osteoprotegerin abrogated cortical porosity and bone marrow fibrosis in a mouse model of constitutive activation of the PTH/PTHrP receptor. Am J Pathol. 2009; 174: 2160-2171.
35) Ohishi M, Schipani E: PTH and stem cells. J Endocrinol Invest. 2011; 34: 552-556.
36) Tripodo C, Sangaletti S, Guarnotta C, Piccaluga PP, Cacciatore M, Giuliano M, Franco G, Chiodoni C, Sciandra M, Miotti S, Calvaruso G, Care A, Florena AM, Scotlandi K, Orazi A, Pileri SA, Colombo MP: Stromal SPARC contributes to the detrimental fibrotic changes associated with myeloproliferation whereas its deficiency favors myeloid cell expansion. Blood. 2012; 120: 3541-3554.