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Redistribution, homing and organ-invasion of neoplastic stem cells in myeloid neoplasms

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Abstract

The development of a myeloid neoplasm is a step-wise process that originates from leukemic stem cells (LSC) and includes pre-leukemic stages, overt leukemia and a drug-resistant terminal phase. Organ-invasion may occur in any stage, but is usually associated with advanced disease and a poor prognosis. Sometimes, extra-medullary organ invasion shows a metastasis-like or even sarcoma-like destructive growth of neoplastic cells in local tissue sites. Examples are myeloid sarcoma, mast cell sarcoma and localized blast phase of chronic myeloid leukemia. So far, little is known about mechanisms underlying re-distribution and extramedullary dissemination of LSC in myeloid neoplasms. In this article, we discuss mechanisms through which LSC can mobilize out of the bone marrow niche, can transmigrate from the blood stream into extramedullary organs, can invade local tissue sites and can potentially create or support the formation of local stem cell niches. In addition, we discuss strategies to interfere with LSC expansion and organ invasion by targeted drug therapies.

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Authors' contributions

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Declaration of Competing Interest

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Keywords

Neoplastic stem cells; Stem cell niche; Extramedullary myelopoiesis; Leukemia; Stem cell invasion; Myeloid sarcoma; Mast cell sarcoma

1 Introduction

A widely accepted theory is that cancer evolution is a step-wise process that is associated with molecular diversification and clonal selection of neoplastic stem cells (NSC) [1–7]. Blood cancer evolution may begin early during lifetime (sometimes even before birth) or later in adulthood, and includes premalignant, indolent phases and aggressive (malignant) stages. One good example is the development of chronic myeloid neoplasms where multiple disease-phases have been described [7–11].

Chronic (indolent) myeloid neoplasms include, among others, myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), chronic myeloid leukemia (CML) and mast cell neoplasms (mastocytosis) [12]. In all these neoplasms, the disease can be divided into premalignant, chronic phases and a terminal aggressive phase that resembles a secondary acute myeloid leukemia (sAML) [7–11]. However, even before an indolent myeloid neoplasm develops, a clonal prephase may be identified by chance or by screening apparently healthy individuals [12–17]. Such pre-phases are defined by a normal or near normal blood count and by one or more somatic mutations which *per se* (as isolated lesion/s) exhibit(s) low oncogenic potential [12–19]. Whereas in some of these individuals, a myeloid neoplasm develops in the follow up, others will never develop an overt malignancy. Therefore, this condition has been described as clonal hematopoiesis of indeterminate potential (CHIP) [14–17]. As the incidence of CHIP mutations increases with age, the condition has also been termed age-related clonal hematopoiesis (ARCH) [13,18].

We and others have recently proposed a model of cancer/leukemia evolution where the earliest stages of carcinogenesis are defined by expression of somatic mutations in small-sized clones containing pre-leukemic NSC (pre-L-NSC) [7,15,16,20–23]. Over time, one or multiple sub-clones expand and replace normal hematopoiesis in the bone marrow (BM) and/or other organs, depending on additional somatic lesions [7,15,16,20–23]. As long as the neoplastic (stem) cells retain full differentiation- and maturation potential and can be controlled by the niche and the immune system, all neoplastic (sub)clones will remain indolent and may even mimic normal organ function after having replaced healthy cells [7,15,16,20–23]. However, as soon as the dominant clone(s) and their NSC escape(s) most control mechanisms, the disease can further expand and can progress to an overt malignancy. At that time, the pre-L-NSC convert into leukemic stem cells (LSC) [7,15,16,20–23]. The end-stage of such malignancy (sAML) is usually resistant to most or all therapeutic interventions.

In most patients with chronic myeloid neoplasms, the disease is largely restricted to lymphohematopoietic organs, including BM and spleen, and less frequently lymph nodes or other organs (Table 1). An exception is systemic mastocytosis (SM), where the skin is also involved quite frequently (Table 1) [24–26]. In addition, the gastrointestinal tract and liver

may be affected in SM [24–26]. In advanced SM, the disease may also progress into a leukemia or present as a sarcoma-like expansion in local organ sites. Even a primary mast cell sarcoma has been described and is included in the WHO classification of mastocytosis [26].

In patients with sAML following MDS, MPN, MDS/MPN or SM, the disease process may still be limited to the BM, blood and spleen. However, in many cases of sAML, multiple internal organs are involved. In all these patients, leukemic (stem) cells are considered to mobilize out of the BM niches and to redistribute into the circulation, and then, after having entered the peripheral (extramedullary) tissues by transendothelial migration, invade into these target organs.

In myeloid neoplasms, clinically relevant organ-invasion of neoplastic stem cells may occur at any stage, but is usually associated with advanced disease (often sAML) and a poor prognosis. Sometimes, organ invasion presents with a metastasis-like or even sarcoma-like, destructive growth of neoplastic cells in local tissue sites. Examples are myeloid sarcoma, localized blast phase of CML, and as mentioned above, mast cell sarcoma [26–31]. So far, little is known about mechanisms underlying re-distribution and extramedullary dissemination of pre-L-NSC and leukemic NSC (LSC) in myeloid neoplasms.

In this article, we discuss mechanisms through which LSC can mobilize out of the BM niche, can transmigrate from the blood stream into tissues, and support the creation of local extramedullary stem cell niches.

2 Phenotype of LSC and molecules regulating LSC homing to BM niches

So far, little is known about the phenotype of long-term disease-propagating NSC/LSC in myeloid neoplasms. In most myeloid malignancies, these cells are considered to reside within a CD34⁺ sub-fraction of the malignant clone, based on their *in vivo* long-term disease-propagating capacity [32–42]. In chronic myeloid neoplasms where neoplastic cells retain a substantial differentiation and maturation potential, like in CML, low risk MDS, or advanced SM, the disease-initiating and -propagating cells are detected preferentially in a CD34⁺/CD38[−] subpopulation, thereby resembling the basic phenotype of normal hematopoietic stem cells [41–44]. However, in high risk MDS, AML, and in the blast phase of CML, the disease-initiating and propagating stem cells may also reside in a CD34⁺/CD38⁺ cell compartment [41,45,46].

The phenotype of CD34⁺/CD38[−] cells has been analyzed extensively in AML and CML. Whereas in CML, LSC display a homogeneous phenotype, the LSC-phenotype in AML is variable and includes different patterns of abnormally expressed antigens. In almost all patients with CML, CD34⁺/CD38[−] LSC aberrantly express CD25, CD26 and IL-1RAP (Table 2) [47–52]. In addition, these cells usually express CD56 in an aberrant manner (Table 2). Moreover, CML LSC express substantially higher levels of cell surface CD33, CD93 and CD123 compared to normal BM stem cells (Table 2) [53,54].

In AML, LSC reside in both, the CD34⁺/CD38[−] and CD34⁺/CD38⁺ compartment of the clone. Compared to normal CD34⁺/CD38[−] stem cells, CD34⁺/CD38[−] AML LSC express

high levels of CD33, CD47 and CD123 (Table 2) [53,55,56]. In addition, AML LSC often display CD44 and CD371 (CLL-1) in an aberrant manner (Table 2) [57–59]. In a smaller fraction of patients, CD34⁺/CD38[−] AML cells exhibit CD25, CD96 and IL-1RAP. Most of these antigens are also expressed on CD34⁺/CD38⁺ stem/progenitor cells in these patients. Overall, the phenotype of AML LSC is heterogeneous with variable combinations of surface antigens, thereby contrasting CML. With the exception of a subset of patients with *FLT3*-mutated leukemia, CD34⁺/CD38[−] AML LSC lack CD26 (Table 2). Independent of the type of AML and the phase of CML, CD34⁺/CD38[−] and CD34⁺/CD38⁺ LSC display CD44 (Fig. 1).

A number of cell surface antigens may be responsible for specific interactions between NSC/LSC and the tissue-specific microenvironment (niches). Many of these surface antigens, such as CD44 (Hermes receptor), CD117 (KIT) or CD184 (CXCR4), are responsible for homing to BM niches (and niches in other organs) and are detectable on NSC/LSC (Figs. 1 and 2) [53,60–65]. These molecules are also expressed on normal BM stem cells and serve as receptors for ligands expressed in niche cells. For example, the KIT ligand stem cell factor (SCF) is expressed by fibroblasts and vascular endothelial cells and the CXCR4 ligand stroma cell-derived factor-1 (SDF-1) is displayed by osteoblasts, endothelial cells and other stromal cells (Fig. 2). An important aspect is that these molecules are often produced by niche-cells in membrane bound and/or soluble form (Fig. 2). In soluble form, these molecules act as strong chemo-attractants for stem cells expressing CXCR4, whereas in membrane-bound form these molecules mediate binding/homing of stem- and progenitor cells to BM niches (Fig. 2). Both interactions, KIT-SCF and CXCR4-SDF-1, are considered to play an essential role in directed migration, homing, and (re)distribution of normal stem cells as well as NSC/LSC in AML, CML and other myeloid neoplasms (Fig. 2) [65–68].

The Hermes receptor CD44, also termed homing cell adhesion molecule (HCAM), is a multi-functional invasion receptor that mediates homing of leukocytes and stem cells in hematopoietic organs [69,70]. CD44 can act as a ligand and as a receptor. When decorated by sialyl Lewis x carbohydrate-moieties, CD44 serves as a major E-selectin ligand and mediates endothelial transmigration [71]. Through this interaction CD44 is considered to play an essential role in endothelial transmigration and invasion of NSC/LSC in myeloid neoplasms, including CML, AML and mast cell neoplasms (SM) [72–76]. In addition, CD44 acts as a receptor for several matrix-based ligands, including hyaluronic acid, osteopontin, collagens and matrix metalloproteinases and regulates (stem) cell invasion and (stem) cell distribution in diverse vascularized organs [69,70].

In AML and CML, LSC also express a number of additional cell surface adhesion receptors, including, among others, integrins (such as LFA-1 = CD11a/CD18 or VLA-4 = CD29/CD49d) and selectin ligands such as CD44 or P-selectin glycoprotein ligand-1 (PSGL-1 = CD162), both of which are involved in endothelial transmigration and distribution of LSC [74,76–81]. An overview of adhesion antigens displayed by stem cells is provided in Table 3, and examples of expression of CD18, CD44 and CD162 on AML LSC and CML LSC are shown in Fig. 1. It is generally assumed that pre-L-NSC and LSC employ these antigens to interact with endothelial cells, other niche-related cells and various matrix molecules in the

tissues. In addition, pre-L-NSC and LSC are considered to interact through these antigens with endothelial cells before they transigrate into tissues (Fig. 3). However, before these cells enter the circulation, they have to be mobilized out of their niches in the BM.

3 Mechanisms underlying transition of LSC from the BM into blood: stem cell mobilization

A number of different cellular interactions and molecular mechanisms contribute to the mobilization of normal hematopoietic stem cells (HSC) from the BM into the peripheral blood [82–86]. Cell types involved in this process are neutrophils, basophils, monocytes (macrophages), fibroblasts, endosteal cells and endothelial cells [82–88]. Most of these cells produce and release enzymes that can cleave adhesion molecules, cytokines or chemokines relevant to stem cell adhesion and homing in BM niches. For example, upon exposure to granulocyte colony-stimulating factor (G-CSF), neutrophils become activated, which leads to the release of neutrophil elastase (NE) and cathepsin G (CG) from their granules. These enzymes, in turn, cleave vascular cell adhesion molecule 1 (VCAM-1) and disrupt the interaction between SDF-1 and CXCR4 on BM stem cells [82–88]. Other relevant enzymes are dipeptidyl-peptidase IV (DPPIV=CD26) and matrix metalloproteinase 9 (MMP9) [82–91]. Both enzymes can degrade various cytokines and also disrupt SDF-1-CXCR4 interactions [82–91]. MMP9 is also involved in IL-8-induced mobilization of stem cells. DPPIV (CD26) is expressed on G-CSF-mobilized stem cells as well as on endothelial cells and basophils. Most importantly, this enzyme degrades SDF-1 into inactive fragments [83–91]. As a result, SDF-1-CXCR4-mediated cell-cell interactions relevant to stem cell migration and homing are weakened or disappear, which leads to stem cell mobilization out of the niche.

In general, the mechanisms underlying mobilization of NSC/LSC out of the niche and their redistribution into the peripheral blood may be similar or identical to those contributing to mobilization of normal BM HSC [92]. However, whereas the mobilization of normal stem cells depends on cytokine activation and granulocyte-derived enzymes, NSC/LSC mobilization is (in addition) often intrinsically triggered and may in part be independent of 'cytokine-exposure' [92–96]. In fact, in myeloid neoplasms, a number of (additional) mechanisms may contribute to the mobilization of pre-L-NSC and LSC in the leukemic BM niche. First, these cells often express low amounts of or lack one or more of the critical cell surface adhesion/homing molecules [64,68,93–96]. In several instances, the oncogenic machineries may directly lead to decreased expression of these cell surface antigens. Likewise, BCR-ABL1, RUNX1-RUNX1T1 and PML1-RARA have been implicated in the decreased expression of surface homing receptors on leukemic (stem) cells [96–98].

Loss of adhesion molecules may also lead to a decrease in cell-cell aggregation and may thus facilitate mobilization of neoplastic cells when these cells are packed in dense infiltrates, as often seen in AML, SM, MDS or MPN. For example, during transformation from non-advanced SM to MCL, LSC may lose several surface adhesion receptors, including LFA-2 (CD2) [99]. This is of particular importance as normal mast cells and neoplastic mast cells in indolent systemic mastocytosis (ISM) display both CD2 and the

counter-receptor CD58 which is considered most relevant for the formation of mast cell clusters and aggregates in ISM [100]. Therefore, it has been hypothesized that the leukemic spread of neoplastic mast cells into the peripheral blood (normal mast cells do not circulate in the blood) in mast cell leukemia (MCL) is in part mediated by the loss of CD2 during malignant progression.

As mentioned before, the mechanisms of mobilization of neoplastic (stem) cells out of the niche may sometimes be similar to those applying in normal hematopoiesis. Especially in neoplasms where pre-L-NSC or LSC are still capable of producing mature (enzyme storing) cells, these mechanisms may play a role in their mobilization. For example, in CML, the excess of protease-producing cells (neutrophilic granulocytes, basophils) may well lead to a decreased expression of adhesion and homing molecules on pre-L-NSC and LSC and thus to their mobilization out of the BM niches.

Another mechanism is loss or enzymatic degradation of niche-targeting cytokines (such as SDF-1 or SCF) involved in the chemotactic migration and binding of NSC/LSC in BM niches. In certain types of leukemia, this loss is triggered by certain enzymes produced by leukemic cells. For example, CML LSC and CML basophils display the SDF-1-degrading enzyme DPPIV (CD26) in excess over normal cells [48,101]. An intriguing fact is that CML LSC themselves express CD26 in an aberrant and disease-specific manner (Tables 2 and 3, Figs. 1 and 2). It is also worth noting that basophilia is a pathognomonic feature of CML. In addition, endothelial cells increase in number and express CD26 in CML. Since SDF-1 is a major substrate of DPPIV the hypothesis has been raised that the massive extramedullary spread of CML LSC results in part from CD26-induced degradation of SDF-1 in the BM niches [48,101]. It has also been described that the AML-related stem cell niche can promote mobilization of leukemic (and normal residual) BM stem cells through anti-angiogenic and osteoblast-targeting cytokines produced by AML cells, with consecutive loss of niche cells, and/or through nitric oxide (NO) and NO-induced dis-integration of endothelial layers [102,103].

Another important aspect is that mobilization is also associated with blood-directed migration through the BM matrix and trans-endothelial migration. These processes are regulated by various mediators and cytokines, including vascular endothelial growth factor (VEGF) also known as vascular permeability factor (VPF), metalloproteinases, other proteases and histamine [82–88,104]. Such molecules are often produced by leukemic cells. Especially in CML and MPN, but also in certain AML variants and MCL, neoplastic cells produce large amounts of VEGF (immature myeloid cells, megakaryocytes, basophils, mast cells), hepatocyte growth factor (HGF) (basophils) and/or histamine (basophils, mast cells) [104–108].

A summary of mechanisms responsible for mobilization of leukemic stem and progenitor cells out of the BM niche is provided in Table 4.

4 Mechanisms underlying trans-endothelial migration of LSC into extramedullary tissues

Once released into the blood, normal BM stem cells can circulate and can leave the blood stream to enter various tissues *via* homing-related mechanisms upon demand or/and in the steady state [109–113]. Whereas the numbers of circulating stem cells transmigrating into extra-hematopoietic organs in the steady are low, a substantial transmigration of stem cells and more mature lympho-hematopoietic cells is seen under certain pathologic conditions (with leukocyte demand) such as local inflammation, auto-immune disorders, infectious diseases or hypersensitivity reactions [114–116]. Under such conditions the endothelial cell phenotype changes into a pro-inflammatory state that includes expression of various cyto-adhesive molecules (inter-cellular CAMs, selectins, others), cytokines and other pro-inflammatory molecules which contributes to trans-endothelial trafficking of stem cells and other leukocytes [114,115]. In inflamed tissues other local cells may also be activated and provide additional mediators (histamine, prostaglandins, chemokines, cytokines) which facilitates transmigration of BM-derived stem cells and other leukocytes, mostly by transforming the endothelial wall into a ‘trans-migratable’ layer.

A number of different adhesion molecules and surface receptors are involved in the homing of normal leukocytes and stem cells to various tissue sites. These molecules include, among others, selectins, selectin-ligands, integrins and integrin-binding cyto-adhesive receptors displayed by endothelial cells [109–116]. The adhesion of leukocytes to endothelial cells is a complex and tightly regulated process, called the leukocyte adhesion cascade [117]. The initial process in this cascade is the capturing of cells in the blood stream by E- and P-selectins expressed on the surface of endothelial cells. These selectins bind to sialyl Lewis^{x,a} glycotopes on the surface of leukocytes, which results in rolling and tethering of the leukocytes on the endothelial surface. Subsequent firm binding of leukocytes is then mediated by leukocyte integrins and cell adhesion molecules of the immunoglobulin superfamily displayed by endothelial cells. Since stem cells also express selectin ligands and integrins (Table 3), the adhesion cascade is also considered to mediate NSC/LSC transmigration into tissues. Once transmigrated, normal stem cells undergo differentiation under the influence of local cytokines, and these cytokines may also induce chemotaxis in transmigrated cells. For example, soluble SCF is a potent growth factor for stem cells and mast cell progenitors (mast cell differentiation is considered a major pathway in the steady state) and can also act as a chemoattractant for KIT + stem cells and mast cells [117,118]. Once the KIT + mast cells ‘arrive’ at local sites where tissue-fixed cells express membrane-bound SCF these cells home in local microenvironments through KIT-SCF interaction [117,118].

In most leukemia models, especially when the disease progresses, LSC are also capable of transmigrating into tissues in various extra-medullary organs. In most instances, lympho-hematopoietic organs are affected (Table 1). However, depending on the neoplasm and the expression of surface adhesion and homing molecules, NSC/LSC may enter any type of vascularized organ. A number of different adhesion receptors, including the classical integrins, various selectin ligands, and the invasion molecule CD44, have been detected on

LSC in myeloid leukemias (Fig. 2, Table 3). Therefore, the hypothesis has been raised that the transmigration process of NSC/LSC into tissues is the same or very similar to that applying to normal stem cells. Indeed, it has been described that myeloid leukemia cells utilize these classical adhesion receptors for binding to endothelium and for their transmigration into tissues in mouse xenotransplantation models [119–124]. For example, E- and P-selectins were found to be essential for the repopulation of CML cells and chronic eosinophilic leukemia cell lines in a SCID mouse xenograft model [123]. In most of these disease models, the Hermes receptor CD44 appears to be an additional critical molecule that mediates both, the transmigration and invasion of leukemic (stem) cells [69–71]. During transmigration, the critical role of CD44 is to bind to E-selectin [71].

5 Invasion of LSC and formation of LSC-Related stem cell niches

After having entered local tissue sites, pre-L-NSC and LSC have the capacity to migrate and home in extramedullary organs. As mentioned before, these organs usually are classical hematopoietic organs, such as the spleen or lymph nodes. A key receptor involved in invasion and homing of stem cells in local niches is CD44, also known as Hermes receptor (Figs. 2 and 3) [69,70]. CD44 is expressed on CD34⁺ pre-L-NSC and LSC in virtually all myeloid neoplasms, including AML, CML and advanced SM (Fig. 1, Table 3) [53,70,72–76]. In addition, CD44 is expressed on all mature cells produced by these neoplasms, including leukemic blast cells, neoplastic eosinophils, neoplastic mast cells and neoplastic monocytes [75–78]. Therefore, CD44 is considered to be involved not only in stem cell invasion but also in the distribution of more mature clonal (leukemic) cells in extramedullary organs. However, tissue invasion and homing is a complex process that depends on several different molecules and cell-cell interactions. In this process, local migration needs to be induced and facilitated which requires a complex interplay between chemotactic molecules (chemokines, cytokines), surface receptors and mediators that induce an ‘invasion-milieu’. These mediators include proteases, histamine and VEGF (Figs. 2 and 3). As mentioned before, the most important chemotactic factors for normal and neoplastic myeloid stem- and progenitors cells are SDF-1 and SCF (Fig. 2).

An important aspect is that neoplastic (stem) cells, once transmigrated, can contribute to niche formation in extramedullary organs. In fact, homing of pre-L-NSC and LSC in peripheral tissues and organs is often followed by stem cell expansion and the formation of local pools of self-renewing and disease-propagating cells (Fig. 3). Moreover, it has been postulated that leukemic cells may be able to create their own stem cell niches and can thereby trigger self-renewal and promote drug resistance of LSC (Fig. 3) [125–132]. This process may be triggered by many different factors, including niche-creating and pro-inflammatory cytokines produced by invading leukemic cells and their LSC [125–132]. Niche-promoting cytokines include VEGF, HGF, fibroblast growth factors (FGF) and other fibrogenic and/or angiogenic cytokines. These cytokines are considered to recruit local stromal cells and endothelial cells (Fig. 3). In myeloid neoplasms neoplastic cells indeed express multiple fibrogenic and angiogenic cytokines [104–108]. Especially myeloblasts, megakaryocytes, macrophages, eosinophils, mast cells and basophils are a rich source of these cytokines. As mentioned before, HGF is preferentially produced and released by basophils [104,108]. However, the leukemic niche may be triggered by additional (pro-

inflammatory and remodeling) cytokines and signaling pathways [125–132]. It has also been postulated that pre-L-NSC and LSC may even be capable of producing niche cells themselves. Especially in the chronic myeloid neoplasms (MPN and CML) trans-differentiation of LSC into stromal cells or endothelial cells has been postulated [133–136]. As a consequence, endothelial cells in these neoplasms may sometimes be clonal cells and can rapidly expand to support the formation of LSC niches and thus the evolution and self-renewal of pre-L-NSC and LSC [133–136].

6 Pharmacological concepts to block mobilization, transmigration or extramedullary niche formation in myeloid neoplasm

Based on their obvious impact in disease evolution and drug resistance, NSC/LSC-niche interactions have been recognized as an emerging therapeutic target in myeloid neoplasms and a number of related treatment concepts have been proposed over the past two decades. A quite old concept is to directly attack niche cells, including (microvascular) endothelial cells and other stroma cells [137–142]. These agents were often developed as specific blockers of VEGF receptors (mostly KDR) or other angiogenic target receptors [136–143]. Thalidomide and lenalidomide were also considered to exert anti-neoplastic effects in part through their inhibitory effects on angiogenesis [144,145]. However, with the advent of imatinib and other driver-specific small molecules, anti-angiogenic (anti-niche cell) effects were no longer a top focus in applied leukemia research. However, several tyrosine kinase inhibitors (TKI) used for the treatment of myeloid neoplasms, such as sorafenib, nilotinib or ponatinib, turned out to be rather strong inhibitors of growth and function of vascular endothelial cells [144–147]. Such additional off-target effects are easily overlooked but may help eradicate clonal cells because further niche-cell expansion is blocked, a hypothesis that is currently tested in preclinical trials. However, targeting the vascular niche and thus endothelial cell-related physiologic functions of the cardiovascular system also bears the risk of cardiovascular side effects [146,148–150].

Another strategy is to disrupt the protective effect of niche cells on NSC/LSC [143,151–157]. However, little is known about the mechanisms underlying niche-induced drug resistance and how this form of resistance can be overcome [151–155]. Recent data suggest that osteoblast-mediated resistance is mediated by the BET-MYC axis and that co-application of BET-inhibitors together with BCR-ABL1 TKI can overcome niche-induced resistance in CML LSC [156].

Another approach is to block the expansion of LSC in the niche and/or the LSC mobilization out of the niche, with the hope that niche-fixed LSC are less capable of proliferating/expanding in the leukemic BM. These concepts involve protease inhibitors and drugs suppressing inflammation. One specific example is CML where DPPIV (CD26) is a major driver of LSC mobilization [48]. Since gliptins are specific inhibitors of DPPIV activity, their use has been discussed in CML. The hope is that gliptin-induced blockage of DPPIV prevents SDF-1 degradation and thus the release of CML LSC from the niche [48]. Whether this concept can work *in vivo* is currently unknown. In single case reports, treatment with a gliptin was indeed found to lead to a major decrease in BCR-ABL1 mRNA levels in patients

with TKI-resistant CML [48]. However, gliptin-treatment of NSG mice did not consistently augment the imatinib- or nilotinib-induced suppression of engraftment of CML (stem) cells in a xenotransplantation model [157].

A completely different concept is to mobilize LSC out of the niche in myeloid neoplasms to sensitize these cells against chemotherapy [153,155,158]. This concept has initially been introduced in AML (where it is not clear whether LSC are always niche-fixed cells) with the hope to kill more LSC by targeted drugs or chemotherapy [153,155,158,159]. Indeed the plerixafor-induced mobilization of AML LSC may sensitize these cells against certain anti-leukemic drugs [158,159]. More recently, the clinical efficacy of this approach has been tested in clinical trials [160–163]. However, although the combination approaches were well tolerated and mobilization of AML cells and their response to targeted drugs or cytotoxic drugs could be documented, no substantial clinical benefit or improved survival was observed. In CML, the intrinsic mobilization of LSC is a pathognomonic feature of the disease. Nevertheless, experiments with plerixafor have been conducted in preclinical studies [164,165]. However, although mobilizing and drug-sensitizing effects of plerixafor on CML cells were reported, drug combinations consisting of plerixafor and BCR-ABL1-targeting drugs failed to eradicate the disease in mouse models [164,165]. An interesting aspect is that most BCR-ABL1-targeting drugs are also directed against KIT and that the SCF-KIT pathway must be inhibited to enable apoptosis induced by these drugs in CML cells [166]. In addition, several of the novel BCR-ABL1 TKI (but not imatinib) can suppress angiogenesis and thus the expansion of the vascular niche [146,147]. Whether these off-target effects indeed contribute to the superior clinical efficacy of the second and third generation BCR-ABL1 TKI remains unclear.

Another strategy is to block the re-distribution and invasion of LSC by blocking surface adhesion molecules. In fact, it has been described that drugs directed against integrins, CD44 or other homing molecules suppress leukemic distribution and spread in AML and other disease models [72,73,167–169]. However, only a few of these drugs have been developed far enough to reach clinical application. For example, the safety and efficacy of RG7356, an anti-CD44 humanized antibody was tested in a Phase I study in patients with refractory or relapsed AML [170]. One of 44 patients achieved a complete response with incomplete platelet recovery, one a partial response, and one a stable disease with hematologic improvement [170].

There have also been attempts to block other adhesion receptors, such as VLA-4 or E-selectin in AML and CML models with the aim to suppress the leukemic spread *in vivo* [123,169,171]. Other studies have explored whether leukemic cells can be sensitized against chemotherapy by blocking critical adhesion receptors. Whether this approach can be translated into application is currently tested in clinical trials. However, currently, it remains unknown whether such combination therapies can increase efficacy of standard treatment in myeloid neoplasms.

A summary of pharmacological approaches and drugs targeting niche cells and/or niche-NSC/LSC interactions is provided in Table 5.

7 Concluding remarks and future perspectives

Mobilization, redistribution, and extramedullary organ invasion by neoplastic stem cells is often seen in myeloid neoplasms, including the classical chronic MPN, CML, CMML, AML and mast cell neoplasms. The clinical features associated with invasion and local expansion depend on the type and stage of disease and the stem cells involved. Sometimes, organ invasion with a sarcoma-like spread of disease is seen. In other cases, the dissemination follows an indolent course like in indolent SM where skin lesions are even indicative of a less aggressive disease. The mechanisms of redistribution and organ invasion of NSC/LSC may be similar or the same as that found in normal hematopoietic (stem) cells and often involve classical adhesion receptors and homing molecules, including selectins, integrins, and selectin ligands like CD44. This concept has been confirmed in most neoplasms and offers the opportunity to interfere with organ invasion by application of specific drugs targeting certain adhesion and/or homing molecules, niche cells, or the local expansion of LSC. Whether these drugs can help counteract (or prevent) the extramedullary spread of LSC in myeloid neoplasms remains to be determined in clinical trials.

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Fig. 1. Expression of cell surface adhesion receptors on CD34⁺/CD38⁻ stem cells in normal bone marrow and patients with myeloid neoplasms.

The expression of cell surface antigens on aspirated CD34⁺/CD38⁻ bone marrow (BM) cells was determined by fluorochrome-conjugated monoclonal antibodies and multi-color flow cytometry. BM was obtained from control donors without a hematologic neoplasms (NBM), patients with myelodysplastic syndromes (MDS), patients with acute myeloid leukemia (AML), and patients with chronic myeloid leukemia (CML) at the time of diagnosis (routine BM investigations). All patients gave written informed consent before BM aspiration was performed. The study was approved by the ethics committee of the Medical University of Vienna. Reactivity of the test antibodies (NBM: grey histograms; MDS/AML/CML: blue histograms) was assessed on a FACSCantoII (BD Biosciences). Antibody-reactivity was controlled by isotype-matched control-antibodies (open black histograms). Analysis of flow cytometry data was performed using FlowJo 8.8.7 software (Flowjo).

Fig. 2. Schematic overview of major molecular cell-cell interactions relevant to stem cell homing and mobilization in the leukemic bone marrow and blood in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).

Leukemic stem cells (LSC) in AML (upper cell) and CML (lower cell) display a number of different functionally relevant cell surface adhesion molecules. Both types of cells exhibit the stem cell factor (SCF) receptor KIT (CD117), the chemokine receptor CXCR4 (CD184), LFA-1 (CD11a/CD18), and VLA-4 (CD29/CD49d). Stroma cell-derived factor-1 (SDF-1), the ligand of CXCR4, and the KIT ligand SCF are expressed in bone marrow (BM) niche cells and vascular endothelial cells in membrane-bound form and/or soluble form. As soluble molecule, these cytokines induce migration of LSC whereas in membrane-bound form, these cytokines mediate adhesion and homing of LSC in the niche(s). However, CML LSC also express dipeptidyl-peptidase IV (DPPIV = CD26), a surface enzyme that degrades SDF-1 into inactive fragments. Based on this notion, CML LSC are considered to be intrinsically mobilized cells that produce persistent extramedullary hematopoiesis. Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; VLA-4, very late antigen-4; VCAM-1, vascular cell adhesion molecule-1; LFA-1, leukocyte function-associated antigen-1; ICAM-1, intercellular adhesion molecule-1.

Fig. 3. Extramedullary march of neoplastic stem cells in myeloid neoplasms.

In most myeloid neoplasms, neoplastic stem cells (NSC), in the context of a leukemia also called leukemic stem cells (LSC), initially reside in the bone marrow (left part). Due to their self-renewal capacity and their ability to generate more mature neoplastic cells (not shown) these cells can propagate the malignancy for unlimited time periods. NSC/LSC reside in so called stem cell niches of the bone marrow where stromal cells produce soluble and membrane bound forms of cytokine ligands, like stem cell factor (SCF) or stroma cell-derived factor-1 (SDF-1) through which these cells interact with and attract NSC/LSC. A number of different mechanisms may lead to the mobilization of NSC/LSC out of the niche. These mechanisms involve proteases and cytokines produced by niche cells, neoplastic cells and other cells in the bone marrow. Once mobilized NSC/LSC transmigrate from the bone marrow into the blood, and after blood-based dissemination, these cells enter the peripheral, extramedullary organs after transmigration local endothelial cell barriers. Transmigration is dependent on various adhesion molecules (integrins, selectins), and is supported by various proteases, cytokines and histamine. After transmigration into extramedullary organs, NSC/LSC invade local tissue sites. Invasion is facilitated by several surface molecules such as CD44 and is promoted by chemotactic factors (chemokines), proteases and cytokines. After invasion, NSC/LSC finally create their stem cell niches through the generation and release of cytokines and subsequent, cytokine-induced, recruitment and activation of tissue-resident local stromal cells forming the niche.

Table 1
Recurrent extramedullary organ-involvement in myeloid neoplasms.

Myeloid Neoplasm	Recurrently involved organs and estimated frequency ^a						
	Spleen	Liver	Lymph Nodes	Skin	CNS	Heart	GI-Tract
ISM/SSM ^b	+/-	-/+	-/+	++	-	-	-/+
ASM/MCL ^b	+	+	+/-	+/-	-	-	+/-
CEL ^c	+/-	-	-/+	-/+	-	+/-	-/+
MDS LR	-	-	-	-	-	-	-
MDS HR	-/+	-/+	-	-	-	-	-
CMML	++	+/-	-	-	-	-	-
CML CP	++	+/-	-/+	-	-	-	-
CML AP/BP	++	+	+/-	-	-/+	-/+	-
AML	+/-	+/-	-/+	-/+	-/+	-	-

Abbreviations: CNS, central nervous system; GI-Tract, gastrointestinal tract; ISM, indolent SM; SSM, smoldering SM; ASM, aggressive SM; MCL, mast cell leukemia; CEL, chronic eosinophilic leukemia; MDS, myelodysplastic syndrome; LR, low risk; HR, high risk; CMML, chronic myelomonocytic leukemia; CML, chronic myeloid leukemia; CP, chronic phase; AP, accelerated phase; BP, blast phase; AML, acute myeloid leukemia; WHO, World Health Organization.

^aFrequencies were estimated based on data obtained from the recent available literature and data obtained in the authors' labs.

^bSystemic mastocytosis (SM) can essentially be divided into non-advanced SM (ISM + SSM) and advanced SM (ASM + MCL).

^cOnly patients with a rearranged *PDGFRA* or *PDGFRB* and clinical features resembling CEL were examined. In these patients the primary WHO-based diagnosis is myeloid/lymphoid neoplasm with eosinophilia with rearranged *PDGFRA*, *PDGFRB* or *FGFR1*, or with *PCM1-JAK2*. Definition of score: ++, seen in a majority of patients; + seen in 10–50% of all cases; +/-, seen in less than 10% of patients; -/+, occasionally seen (< 1%).

Table 2
Cell surface molecules expressed on neoplastic/leukemic stem cells in myeloid neoplasms
and comparison to normal hematopoietic stem cells^a.

Surface molecule	Expression on CD34 ⁺ /CD38 ⁻ stem cells in ^b					
	NBM	MDS	CMML	CML	AML	MCL
CD25 (IL2RA)	–	–/+	–	+	+/-	–
CD26 (DPPIV)	–	–	–	+	–/+ ^c	–/+
CD33 (Siglec-3)	+/-	+/-	+	+	+	+
CD44 (Hermes)	+	+	+	+	+	+
CD45 (LCA)	+	+	+	+	+	+
CD47 (IAP)	+	+	+	+	+	+
CD56 (NCAM)	–	n.k.	n.k.	+	–	n.k.
CD93 (C1QR1)	–/+	n.k.	n.k.	+	+/-	n.k.
CD96 (Tactile)	–	n.k.	n.k.	–	+/-	n.k.
CD117 (KIT)	+	+	+	+	+	+
CD123 (IL-3RA)	+/-	+	+	+	+	+
CD371 (CLL-1)	–	n.k.	n.k.	–	+/-	–
IL-1RAP	–	n.k.	–/+	+	–/+	–/+

Abbreviations: NBM, normal bone marrow-derived CD34⁺/CD38⁻ hematopoietic stem cells; IL2RA, interleukin-2 receptor alpha chain; DPPIV, dipeptidyl peptidase IV; LCA, leukocyte common antigen; IAP, integrin associated protein; NCAM, neural cell adhesion molecule; CLL-1, C-type lectin-like molecule-1; IL1-RAP, interleukin-1 receptor accessory protein; n.k., not known.

^aResults refer to multi-color flow cytometry data obtained from the available literature and/or from studies performed in the authors' labs.

^bScore: +, strongly expressed on most or all cells in most donors; +/- weak expression on most cells or subsets of cells (10–50%) clearly express the marker in most donors; -/+, weak expression on a minor subset of cells or subsets of cells express the antigen in a small subset of donors; –, not expressed on stem cells in most donors.

^cIn AML, CD34⁺/CD38⁻ LSC usually lack CD26. However, in a subset of patients with *FLT3* ITD-mutated AML, CD34⁺/CD38⁻ LSC display CD26.

Table 3
Expression of adhesion- and homing molecules on CD34⁺/CD38⁻ neoplastic/leukemic stem cells in myelodysplastic syndromes, acute myeloid leukemia and chronic myeloid leukemia; and comparison to normal bone marrow stem cells.

Surface adhesion molecule	CD	Expression on CD34 ⁺ /CD38 ⁻ stem cells in ^a			
		NBM	MDS	CML	AML
Integrin α-L	CD11a	+	+	+	+
Integrin β-2	CD18	+/-	+/-	+	+
DPPIV	CD26	-	-	+	-/+
Integrin β-1	CD29	-/+	+/-	+/-	+/-
Integrin α-IIb	CD41	-/+	-/+	-/+	-/+
Hermes	CD44	+	+	+	+
Integrin α-4	CD49d	+	+/-	+	+
Integrin α-6	CD49f	+	+	+	+
Integrin α-V	CD51	-	-	-	-
NCAM	CD56	-	n.k.	+	-
LFA-3	CD58	n.k.	n.k.	+	+
Integrin β-3	CD61	-	-	-	-
L-Selectin	CD62L	+	n.k.	+/-	+/-
KIT	CD117	+	+	+	+
PSGL-1	CD162	+	+	+	+
CXCR4	CD184	+	+/-	+	+

**Score: +, strongly expressed on most or all cells in most donors; +/- weak expression on most cells or subsets of cells (10–50%) clearly express the marker in most donors; -/+, weak expression on a minor subset of cells or subsets of cells express the antigen in a small subset of donors; -, not expressed on stem cells in most donors.

Abbreviations: NBM, normal bone marrow; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; DPPIV, dipeptidyl-peptidase IV; NCAM, neural cell adhesion molecule; LFA-3, lymphocyte function-associated antigen 3; PSGL-1, P-selectin glycoprotein ligand-1; CXCR4, C-X-C chemokine receptor type 4; n.k., not known.

^aResults refer to multi-color flow cytometry data obtained from the available literature and/or from studies performed in the authors' labs.

Table 4
Potential mechanisms underlying the mobilization of neoplastic/leukemia stem cells out of the BM niche.

Mechanism	Involved cell(s)	Typical example
Oncoprotein-induced loss of stem cell homing receptors	Leukemic cells	RUNX1/ETO-induced downregulation of PSGL-1
Loss of cell-cell aggregation receptors counteracting the leukemic spread of cells	Leukemic cells	Loss of CD2 during development from non-advanced SM to MCL ^a
Protease-induced degradation of adhesion/homing receptors	Leukemic cells, neutrophil granulocytes ^b	Neutrophil elastase and cathepsin G-induced VCAM-1 degradation
Protease-induced degradation of LSC-attracting cytokines	Leukemic stem cells, basophil granulocytes ^b , endothelial cells	DPPIV-induced degradation of SDF-1 in CML
Preparing endothelial cells for transmigration prior to evasion from the BM	Leukemic cells, basophils ^b , mast cells ^b , endothelial cells (NO)	VEGF, NO-, and histamine-induced endothelial cell-leakiness
Blockage and loss of niche cells by anti-angiogenic cytokines and/or other cytotoxic mediators	Leukemic cells, macrophages, mast cells, niche cells	TNF- α -induced destruction of niche cells in the BM of patients with AML

Abbreviations: NSC, neoplastic stem cells; LSC, leukemic stem cells; BM, bone marrow; PSGL-1, P-selectin glycoprotein ligand-1; SM, systemic mastocytosis; MCL, mast cell leukemia; VCAM-1, vascular cell adhesion molecule-1; DPPIV, dipeptidyl-peptidase IV; SDF-1; stroma cell-derived factor-1; CML, chronic myeloid leukemia; VEGF, vascular endothelial growth factor; NO, nitric oxide; TNF- α , tumor necrosis factor-alpha.

^aIn non-advanced SM, mast cells typically form densely packed aggregates in the BM; however, when the disease progresses to mast cell leukemia (MCL), a more diffuse spread is seen and mast cells also leave the BM to enter the peripheral blood (normal mast cells and mast cells in non-advanced SM do not circulate in the peripheral blood).

^bIn chronic myeloid neoplasms, the residual differentiation and maturation capacity of the clone (their LSC) creates mature myeloid cells, including neutrophil and basophil granulocytes. Therefore, in these malignancies, the leukemic (granulocytic) cells themselves produce these mediators and cytokines.

Table 5
Potential pharmacologic concepts to disrupt the mobilization or transmigration of
leukemic stem cells (LSC), or LSC-dependent niche-formation in myeloid neoplasms.

Concept	Types of targeted drugs - examples
Blocking the growth and expansion of niche cells:	Niche cell-targeting drugs
a. Endothelial cells (vascular niche):	Anti-angiogenic drugs: mTOR inhibitors; VEGFR2/KDR-targeting drugs, like sorafenib, or ponatinib; lenalidomide
b. Osteoblasts (endosteal/osteoblast niche):	BET-targeting drugs: BRD4 inhibitors disrupt niche-induced LSC resistance
Blocking the mobilization of NSC/LSC out of the niche	Drugs blocking the activity of the SDF-1 degrader DPPIV: gliptins, CD26 antibodies
Mobilizing NSC/LSC out of the niche(s)	CXCR4-targeting drugs: plerixafor
Inhibiting adhesion, migration and/or invasion of circulating NSC/LSC	Integrin-targeting drugs: VLA4 blocker; CD44-targeting drugs

LSC, leukemic stem cells; mTOR, mechanistic target of rapamycin; VEGFR2, vascular endothelial cell growth factor receptor-2; NSC, neoplastic stem cells (includes also pre-leukemic neoplastic stem cells in pre-leukemia models); SDF-1, stroma cell-derived factor-1; DPPIV, dipeptidyl-peptidase IV; VLA4, very late antigen 4.