1. Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders characterized by abnormal proliferation and expansion of one or more myeloid lineages [1, 2]. The WHO classification of MPN comprises four classic MPN and additional nonclassic MPN. The group of the common, classic MPN includes chronic myeloid leukemia (CML) defined by the Philadelphia chromosome (Ph) and the three Ph-negative entities’ polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The group of nonclassic MPN includes systemic mastocytosis (SM), chronic neutrophilic leukemia (CNL), and chronic eosinophilic leukemia (CEL) [1, 3].

Aberrant tyrosine kinase (TK) signaling is a common hallmark in MPN and has been shown to represent a key driver of the disease. The BCR-ABL1 fusion gene, which results in a constitutive activation of ABL1 kinase activity, characterizes CML [4–6]. In a majority of patients with PV, ET, and PMF, the activating V617F mutation in the receptor-associated TK JAK2 is detected [7–10]. In addition, mutations in exon 12 of JAK2 and mutations in the thrombopoietin receptor (MPL W515K/L) have been described in these entities [11, 12]. More recently, somatic mutations in CALR were found in JAK2- and MPL-negative patients with ET or PMF [13, 14]. The activating point mutation D816V in the KIT receptor TK is a diagnostic criterion for SM and is found in more than 80% of all patients with SM [15].
A constitutively activated *FIP1L1-PDGFRα* fusion TK has been identified in patients with CEL with or without an accompanying hypereosinophilic syndrome (HES) [16, 17]. More recently, *CSF3R* mutations have been described as a recurrent defect in patients with CNL [18].

Common pathogenic mechanisms are observed despite the variety of different oncogenic mutations underlying specific MPN types. Aberrant expression of inflammatory cytokines has been associated with patients’ symptoms and alterations of the bone marrow (BM) microenvironment as well as progression of the disease. Several different studies have suggested an important role for the BM microenvironment in the pathogenesis of hematologic malignancies including MPN. In fact, alterations in the BM microenvironment such as increased microvessel density (angiogenesis), fibrosis, and thickening of bone trabeculae are typical pathological findings in MPN and may contribute to disease phenotypes and disease progression. This review focuses on the cytokine regulation of microenvironmental cells with special emphasis on common as well as distinct pathogenetic mechanisms in various MPN. In particular, expression and functional relevance of interleukin-6 (IL-6), IL-8, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF)-b, hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) in the myeloproliferative neoplasms chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocytosis (SM) is shown. The numbers indicate selected references for elevated expression of the cytokine in the given myeloproliferative neoplasm.

### Table 1: Increased expression of cytokines in myeloproliferative neoplasms

| Disease | CML | PV, ET, PMF | SM |
|---------|-----|-------------|----|
| Oncogene | BCR-ABL1 | JAK2 V617F, CALR, MPL | KIT D816V |
| FGF | [31, 36, 37] | [36–46] | [47, 48] |
| HGF | [30, 31, 33] | [38, 39, 49, 50] | [54–56] |
| IL-6 | [51, 52] | [38, 49, 51, 53] | [54–56] |
| IL-8 | [57] | [49, 53, 58] | |
| OSM | [59] | [60, 61] | |
| PDGF | [35] | [22, 46, 62–65] | [48] |
| TGF-β | [66, 67] | [40, 42, 46, 68–72] | [74] |
| TNF-α | [31, 52] | [38, 49, 73] | [74] |
| VEGF | [26, 27, 29, 31, 33, 36, 75–77] | [21, 36, 38, 45, 46, 49, 58, 75, 76, 78–87] | [54, 88–91] |

The mammalian target of rapamycin (mTOR) contributes to BCR-ABL1-dependent expression of VEGF [27]. Targeting of mTOR by rapamycin in CML cells inhibited not only VEGF expression but also the *in vitro* growth of leukemic cells [28]. CD34+ BM cells derived from CML patients secreted up to 10 times more VEGF, FGF-b, HGF, and IL-8 compared to normal donors’ BM CD34+ cells. Furthermore, BM mononuclear cells isolated from CML patients induced vascularization of matrigel implants in mice [29]. A number of additional studies described expression of HGF in CML cells [30–33]. In particular, elevated HGF levels in BM and peripheral blood and a correlation of HGF expression with microvessel density in the BM were found. Zhelyazkova and colleagues reported evaluated plasma HGF, cellular HGF, and expression of the HGF receptor c-MET in CML patients. The plasma HGF level correlated with markers reflecting the tumor burden as well as with the phase of CML and overall survival in these patients. In contrast, no prognostic relevance for VEGF levels in chronic phase CML was observed in this study [33]. Also, contrary to VEGF, BCR-ABL1 did not induce synthesis of HGF *in vitro* and targeting of BCR-ABL1 with imatinib showed no effect on HGF expression [34]. Although various cell types may express and release HGF, immunostaining of BM sections revealed that basophils are a primary source of HGF in CML [32]. Expression of PDGF was reported to be associated with BM fibrosis in accelerated and blast phase CML [35].

IL-2 and IL-6 serum levels in patients with CML were found to be significantly elevated compared to controls.

### 2. Cytokine Expression in Classical MPN

#### 2.1. Chronic Myeloid Leukemia

CML is characterized by the reciprocal chromosome translocation t(9;22) and the resulting *BCR-ABL* fusion gene [5, 6]. The BCR-ABL oncoprotein exhibits constitutive TK activity and triggers key signaling pathways, including the RAS-RAF-MEK-ERK pathway, the phosphoinositide 3-kinase-AKT pathway, and STAT5 [19, 20]. Cytokines and other effector molecules downstream of these aberrant signaling cascades have been implicated in the pathogenesis of CML [21].

Aguayo et al. investigated BM vascularity and cytokine levels in CML and other hematologic neoplasms [22]. CML patients reportedly have increased BM vessel density and elevated serum levels of VEGF, HGF, FGF-b, and TNF-α compared to controls [23, 24]. Furthermore, high VEGF levels were found to correlate with a shorter survival of patients in chronic phase CML [25]. Immunohistochemical staining of BM sections showed that VEGF is expressed primarily in myeloid progenitor cells, megakaryocytes, and mature granulomonocytic cells in chronic phase CML as well as in myeloid differentiated blast cells in the blast phase of CML [26]. The BCR-ABL1 oncoprotein was found to upregulate expression of VEGF in CML cells, and analysis of signaling pathways downstream of BCR-ABL1 revealed that...
Moreover, IL-6 levels in CML patients were found to correlate with BM angiogenesis and reportedly increase during disease progression [51, 52, 92]. The BCR-ABL1 targeting TK inhibitor (TKI) imatinib was found to downregulate IL-6 and IL-8 release in primary CML cells in vitro [93]. Hantschel et al. identified IL-8 as one of the strongest downregulated genes in CML upon treatment with the TKI dasatinib [57]. Expression of BCR-ABL1 resulted in a substantial upregulation of IL-8 which was inhibited by dasatinib or nilotinib [57]. TNF-\(\alpha\) has recently been implicated in stem cell biology of MPN [94, 95]. A study investigated IL-1, IL-6, and TNF-\(\alpha\) serum levels in CML patients and described no significant difference for TNF-\(\alpha\) compared to controls [52]. However, CML stem/progenitor cells were found to produce TNF-\(\alpha\) in a kinase-independent fashion, and at higher levels relative to their normal CD34+ counterparts. In addition, TNF-\(\alpha\) concentrations were found to be elevated in BM supernatants derived from BCR-ABL1 transgenic mice compared to wild type mice [95].

### 2.2. Polycythemia Vera, Essential Thrombocythemia, and Primary Myelofibrosis

Elevated levels of inflammatory cytokines have been reported in all entities of classical MPN [38, 49, 51, 53, 58, 96–99]. In particular in PMF, patients suffer from severe constitutional symptoms, and increasing evidence shows that several of these symptoms are mediated by proinflammatory cytokines. Tefferi et al. investigated the prognostic significance of cytokines in PMF by determining serum levels of a comprehensive cytokine panel. In this study, IL-1\(\beta\), IL-1RA, IL-2R, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, TNF-\(\alpha\), granulocyte colony-stimulating factor (G-CSF), interferon \(\alpha\) (IFN-\(\alpha\)), macrophage inflammatory protein 1\(\alpha\) (MIP-1\(\alpha\)), MIP-1\(\beta\), HGF, IFN-\(\gamma\)-inducible protein 10 (IP-10), monokine induced by IFN-\(\gamma\) (MIG), monocyte chemotactic protein 1 (MCP-1), and VEGF levels were found to be elevated in PMF patients. In addition, the authors identified IL-8, IL-2R, IL-12, and IL-15 levels as independent prognostic factors for survival of patients with PMF [49]. These findings are in line with other studies showing elevated cytokine level in PMF, ET, and PV [38, 51, 53, 58, 96–98]. However, the methods applied in these studies differed and the panels of elevated cytokines within different studies showed some inconsistencies between these studies as reviewed by Hasselbalch [99]. Thus, better standardization is apparently needed to directly compare cytokine production in different MPN cohorts. Nevertheless, increasing evidence indicates that the disease burden of MPN is not only mediated by the primary neoplastic clone but also mediated by a secondary inflammation with an aberrant cytokine production and changes of the BM microenvironment. The concept of cytokines contributing to tissue fibrosis, angiogenesis, and osteosclerosis/osteopenia in MPN has been well established. In particular, FGF-b, IL-8, VEGF, HGF, PDGF, TGF-\(\beta\), TNF-\(\alpha\), and OSM have been implicated in BM microenvironment alterations in patients with MPN [21, 22, 39–41, 68, 69]. Evidence for expression of these cytokines in PV, ET, and PMF is discussed in the next paragraphs.

FGF-b was found to be elevated in the serum of MPN patients. While Musolinio et al. reported increased FGF-b levels in PV, ET, and PMF [36], Vaidya et al. found FGF-b—together with IL-1\(\beta\), IL-1RA, IL-2R, EGF, IL-10, FGF-b, IL-12, IFN-\(\alpha\), and RANTES—to be particularly elevated in PMF when compared to PV patients [38]. Moreover, high levels of IL-6 and FGF-b were observed in a coculture model of \(\text{JAK2 V617F}\) positive hematopoietic cells and stroma cells [34]. Emadi et al. studied IL-8 production in PMF. IL-8 serum levels were significantly increased in patients with PMF; and IL-8 expression was observed in various hematopoietic cell types, including granulocytes, monocytes, megakaryocytic cells, CD34+ progenitor cells, and platelets [100]. Increased serum levels of IL-8 have also been described in patients with PV and ET [38, 58, 101], and IL-8 was found to enhance formation of erythroid colonies in vitro [102]. Within a PMF patient cohort, IL-8 serum level was an independent prognostic factor for survival [49].

A number of studies have described elevated VEGF serum levels in MPN [36, 49, 78]. Immunohistochemical studies performed on BM sections of ET, PV, and PMF patients revealed an increased expression of VEGF and its receptor in all MPN groups compared to controls [79, 80]. Megakaryocytes, macrophages, and immature myeloid precursors showed positive immunostaining while erythroid (precursor) cells stained negative for VEGF [80]. Boissinot et al. detected elevated levels of HGF in the serum and BM plasma obtained from PV patients compared to secondary erythrocytosis patients that were employed as controls. Furthermore, BM stem cells and clonal erythroblasts were identified as the major sources of HGF in patients with PV [50]. Further studies analyzing cytokine panels in plasma of MPN patients confirmed elevated HGF levels in PMF, PV, and ET [38, 49].

Wickenhauser et al. described production of TGF-\(\beta\) and PDGF in normal human megakaryocytes [103]. Subsequent studies found higher levels of TGF-\(\beta\) in megakaryocytes in the BM of patients with myelofibrosis compared to controls. In contrast, no increase in TGF-\(\beta\) was found in BM cells of patients with ET [70]. TNF-\(\alpha\) was found to be elevated in a subset of patients with PMF. Tefferi et al. studied 127 PMF patients and observed significantly higher levels of TNF-\(\alpha\) compared to controls. However, a substantial number of patients showed no detectable TNF-\(\alpha\) in peripheral blood and no association with clinical parameters and disease progression was observed [49]. Another study identified TNF-\(\alpha\) as one of two cytokines that were differentially expressed when stratifying ET and PV patients according to their JAK2 V617F mutation status [58]. In line with this finding, a murine BM transplant model for \(\text{JAK2 V617F}\) showed a marked increase of TNF-\(\alpha\) serum levels. This increased TNF-\(\alpha\) level was found to be accompanied by a decrease in erythropoietin and G-CSF, which the authors discussed as a possible suppressive effect of TNF-\(\alpha\) on normal hematopoiesis [73]. We studied \(\text{JAK2 V617F}\)-mediated gene expression and identified IL-6 and the IL-6 family members OSM and leukemia inhibitory factor (LIF) to be directly upregulated by V617F-mutated \(\text{JAK2}\). Furthermore, oncogene-dependent upregulation of IL-8 and VEGF was observed [59]. Immunohistochemistry
staining of BM section from patients with PMF, ET, and PV showed that megakaryocytes, endothelial cells, and myeloid progenitors stain positive for OSM, whereas erythroid cells were OSM negative. This pattern correlates with expression of phosphorylated STAT5, which was identified as the major signaling pathway of oncogene-dependent OSM expression [59].

3. Cytokine Expression in Nonclassical MPN: Systemic Mastocytosis

SM is MPN characterized by an abnormal accumulation of mast cells in the BM and other organs [104]. In a substantial subset of patients, SM is accompanied by increased release of various mediators from mast cells and consecutive clinical symptoms [105–107]. The majority of SM patients harbor the somatic KIT point mutation D816V. KIT is a receptor TK, and activation of KIT signaling through its ligand stem cell factor (SCF) mediates cell proliferation and survival in immature progenitor cells and mast cell differentiation, as well as mast cells migration, activation, and adhesion [108]. KIT D816V shows constitutively active TK signaling and induces the recruitment of several downstream signaling pathways, including PI3-kinase/AKT [109], mTOR [110], and STAT5 [109, 111].

Brockow et al. measured levels of growth factors in plasma and skin blister fluid of patients with SM [54]. IL-3 and IL-4 were not detectable, and SCF as well as VEGF levels showed no significant difference between patient samples and controls. In contrast, IL-6 was significantly increased in plasma of SM patients and correlated with serum tryptase levels [54]. Subsequent studies confirmed increased IL-6 plasma levels in SM cohorts and suggested a correlation with the severity of symptoms and the presence of osteoporosis [55]. Moreover, IL-6 levels were found to correlate with disease category, severity of BM pathology, organomegaly, and extent of skin involvement. Thus, the authors suggested that IL-6 was a useful surrogate marker of severity of disease [56].

Moreover, Rabenhorst et al. investigated cytokines potentially involved in the development of osteopenia or osteoporosis in SM. Again, elevated levels of the proinflammatory cytokine IL-6 were found in patients with SM. High levels of IL-6 were accompanied by increased levels of the osteoclast-regulating factors receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin. The authors argue that cytokines produced by mast cells might shift the balance of bone turnover towards increased bone resorption and decreased bone formation [112]. IL-31 has been implicated in the induction of chronic skin inflammation and was found to be increased in patients with SM and to correlate with disease severity [113]. Gene expression studies of purified BM mast cells in SM detected high expression of CCL-23 in indolent and aggressive SM, whereas IL-1β, IL-13, or OSM were particularly upregulated in aggressive SM [60].

A number of studies used mast cell lines, in particular the KIT D816V mutated human mast cell line HMC-1, to investigate cytokine expression in SM. Selvan and colleagues described expression of MCP-1, MIP-1α, MIP-1β, RANTES, and IL-8 in HMC-1 cells [114]. Subsequent studies showed expression of TNF-α [74], IL-1β [74], and OSM [61] in HMC-1 cells. FGF-b was found to be expressed in a number of murine mast cell lines and to be regulated by SCF, TGF-β, and TNF-α [47]. Immunohistochemical staining of BM sections derived from patients with SM showed expression of FGF-b and in some cases weak expression of TGF-β [48]. Furthermore, mast cell infiltrates expressed VEGF as determined by immunohistochemistry of BM sections [88]. Although no significant elevation of VEGF levels was found in plasma of SM patients [54], it is likely that VEGF is locally increased in the BM microenvironment and contributes to increased angiogenesis in SM. Comparative oncology studies in dogs showed expression of VEGF in neoplastic mast cells [89, 90]. Moreover, a correlation of VEGF plasma levels with tumor grade and microvascular density was observed in canine mastocytoma [91].

We studied the effect of KIT D816V on cytokine expression in various in vitro models. The cytokine profile induced by KIT D816V showed a marked overlap when compared to the profile induced by JAK2 V617F and FIP1L1-PDGFRα. A number of cytokines, including OSM, were found to be regulated by all three oncogenes [59, 61, 115]. These studies suggest that the mutant TK in MPN activate common signaling pathways resulting in overlapping effects on cytokine production. Moreover, these and other data indicate that targeting of TK signaling or relevant downstream signaling molecules will reduce the aberrant inflammatory cytokine production not only in PMF, ET, and PV but also in other MPN. A comprehensive analysis of cytokine serum levels in a large cohort of SM patients would be useful to compare the expression of inflammatory cytokines in SM with the pattern observed in other MPN.

4. Cytokine Regulation of Microenvironmental Cells

4.1. Fibrosis in MPN. Fibrosis is considered to be a reactive process that is often associated with tissue remodeling and tissue repair. Tissue fibrosis may occur in various organs and involves fibroblasts and other connective tissue cells [116]. Concerning development and characteristics of MPN, fibrosis is one of the major pathological findings [117]. The process of fibrosis involves not only local fibroblasts and infiltrating leukocytes resulting in persistence of inflammation in the tissue, but also the proliferation of cells with a myofibroblast phenotype. The pathological mechanisms underlying the development of fibrosis in MPN patients are still not fully understood. Involved cells produce different growth factors, proteolytic enzymes, angiogenic factors, and fibrogenic cytokines, which results in enhancement of connective tissue elements’ deposition. This leads to progressively remodeling and finally destruction of physiological tissue architecture [116].

PMF and CML have the highest potential of inducing myelofibrosis. In general, all MPN can develop BM fibrosis, although the likelihood for this varies considerably between the subtypes. The fibrotic potential of MPN with predominant thrombocytosis such as ET can be differentiated from PMF on the basis of morphology. In PMF, the stromal
reaction that accompanies clonal hematopoietic stem cell proliferation is characterized by a consistent myelofibrosis associated with osteosclerosis and neoangiogenesis. Thus, fibrosis is a disease-defining hallmark of PMF at diagnosis [118]. In addition, a higher fibrosis grade in patients with PMF correlated with worse prognosis [119]. In patients with PV or ET, reticulin fibrosis at the time of diagnosis is associated with an increased risk of transformation to post-PV or post-ET myelofibrosis [119]. In CML, BM fibrosis occurs in up to 40% of patients at diagnosis and is associated with a poor prognosis [120]. Recently, BM fibrosis in CML was proposed as an independent predictor of responses to TKI therapy [127]. In the BM of SM patients, mast cell infiltration is often accompanied by fibrosis. In addition, mast cell infiltration with consecutive fibrosis may also occur in the liver, spleen, and lymph nodes [48, 117]. Mast cells produce fibrogenic cytokines including TGF-β and FGF-b. Immunohistochemical studies show a close correlation between the mast cell expression of FGF-b and the reticulin fibrosis of mastocytosis lesions [48].

Concerning PMF development, the megakaryocytic lineage seems to play an essential role in promoting myelofibrosis [68]. Megakaryocytic cells were found to produce a variety of growth factors and cytokines leading to proliferation of fibroblast and the development of fibrosis. PDGF is one of the first growth factors that has been implicated in the role of megakaryocytes in development of BM fibrosis [122]. Several studies described increased levels of PDGF in patients with PMF [62, 63], and immunohistochemical staining showed that megakaryocytes and erythroid precursors were highly positive for PDGF [64]. Patients with ET showed increased plasma levels of PDGF; in particular the subgroup of patients with reticulin fibrosis had higher PDGF plasma levels. In contrast, no alteration of intraplatelet PDGF levels was observed in this study [65]. PDGF not only enhances the replication, survival, and migration of myofibroblasts but also modulates the production and secretion of pro- and anti-inflammatory mediators in the pathogenesis of fibrotic diseases [123].

Further studies revealed that the expression and production of TGF-β were increased in patients suffering from MPN. Several groups have evaluated TGF-β expression in PMF, PV, and ET. These groups reported on quantitative alterations of TGF-β and its receptors in megakaryocytic, platelet, and CD34+ progenitor cells and concluded that TGF-β was involved in myelofibrosis and myeloproliferation [39, 40, 42, 69–72, 124, 125]. TGF-β is a growth factor displaying potent fibrogenic properties and is furthermore associated with not only BM fibrosis, but also clonal hematopoietic expansion and angiogenesis. Moreover, TGF-β has been described to negatively regulate progenitor cell growth [126, 127]. In addition, TGF-β reportedly promotes the deposition of extracellular matrix in different tissues [128, 129]. In PMF, the pathogenic relevance of TGF-β is based on the ability to induce production of types I, III, and IV collagen, fibronectin, tenascin, and proteoglycans. Furthermore, TGF-β blocks matrix degradation by reducing collagenase-like protease synthesis, while enhancing protease inhibitor expression [116]. Importantly, TGF-β downstream signaling, through SMAD2/3 phosphorylation, has been shown to be active in megakaryocytes extending proplatelets, indicating an autocrine stimulation in megakaryocyte development [125]. TGF-β induced PI3-kinase/AKT/NIκB signaling in hemangioblasts, and activation of this pathway enhanced the production of matrix metalloproteinase-9 [66, 67].

Apart from TGF-β and PDGF, FGF-b is considered to be a cytokine with potent fibrogenic characteristics. Several groups analyzed expression of FGF-b in different MPN. The levels of circulating FGF-b were significantly higher in the serum of MPN patients when compared to healthy controls, the highest levels being measured in patients with marked BM fibrosis [37, 39–41, 43, 44, 124]. FGF-b was found to promote fibroblast proliferation in cortical kidney [130]. Furthermore FGF-b promotes cardiac hypertrophy and fibrosis by activating MAPK signaling [131]. Further studies are required to identify the importance of FGF-b in development and progression of MPN. Dalley et al. determined concentration of FGF-b and calmodulin in urine. They showed a significantly elevated calmodulin excretion in PMF patients when compared to PV, ET, and CML. Using a neutralizing antibody to calmodulin influenced the in vitro proliferation of normal human fibroblasts. Extracellular calmodulin should also be considered a potential mitogen involved in the stroma cell reaction in patients with PMF [43].

A special situation is FIP1L-PDGFRα+ CEL. In these patients, fibrosis is usually detected in the endomyocardium, which is not the case in other MPN types. One hypothesis is that eosinophils, once entering cardiac tissues, can promote local fibrosis. Eosinophil-related tissue fibrosis has been attributed to infiltration of the tissues with eosinophils and deposition of eosinophil granule proteins [132]. Furthermore, eosinophils were shown to produce the cytokines IL-α, IL-2, IL-3, IL-4, IL-5, IL-6, GM-CSF, TGF-α, TGF-β, TNF-α, MIP-1α, RANTES, eotaxin, and OSM [133]. Many of the eosinophil-derived cytokines have the potential to stimulate fibroblast proliferation and contribute to local inflammation as well as recruitment of other leukocytes [115, 132]. However, further in vitro and in vivo models are mandatory to deeply understand the role of cytokines for organ specific fibrosis in CEL.

4.2. Angiogenesis in MPN. Angiogenesis, the formation of new vessels from preexisting vessels, plays an important role in development and progression of different tumor types, and targeting of angiogenesis has been successfully translated into clinical practice in various solid tumor models [134]. The process of angiogenesis in hematological malignancies is comparable to the process observed in solid tumors. Endothelial cells from preexisting vessels are activated in the BM by an angiogenic stimulus (e.g., VEGF) and proliferate, migrate, and form new vessels. Initiation of leukemia-induced angiogenesis involves secretion of angiogenic cytokines by leukemic cells and their interaction with the BM stroma [135]. Apart from solid tumors, the importance of angiogenesis becomes increasingly evident in various MPN and other hematologic malignancies. Angiogenesis in the BM of MPN patients was described to correlate
with disease burden, progression, and prognosis [36, 79]. Among the classical BCR-ABL1 negative MPN, increased BM microvessel density (MVD) has been observed in a number of studies in all MPN entities but is most abundant in PMF [45, 75, 80–83, 136]. Not only does PMF show the highest MVD among the classical MPN, but MVD was also described as an independent adverse prognostic factor in PMF [137]. Significantly higher MVD was also found in the BM of patients with post-PV or post-ET myelofibrosis compared to PV or ET [138]. The association of JAK2 V617F mutation status with MVD showed no significant difference between JAK2 wild type and mutant MPN patients in two out of three studies [79, 81, 138]. In contrast to the observation that the increase in BM vascularity seems to be generally independent of the JAK2 V617F status, MVD correlated with JAK2 V617F mutant allele burden within the JAK2 V617F+ subgroup [79]. In chronic phase CML, the BM is hypercellular, with a prominent myeloid compartment and left shift in the granulomonocytic cell compartment [23]. Along with myeloid hyperplasia, augmented BM angiogenesis is a typical finding [24, 75]. In particular, the BM of patients with CML shows a significant increase in MVD, functionally associated with elevated levels of angiogenic cytokines [23]. Furthermore, the BCR-ABL1 targeting TKI imatinib was found to reduce the MVD in CML [137]. Alterations of the BM microenvironment are frequently noticed not only in classical MPN but also in SM. These alterations include angiogenesis, thickened bone trabeculae, and sometimes massive BM fibrosis [48, 88, 117, 139]. Our group studied MVD and expression of VEGF in SM. The median BM MVD was found to be significantly higher in SM compared to cutaneous mastocytosis or controls. Furthermore, MVD correlated with the grade of mast cell infiltration in the BM [88].

The process of angiogenesis is tightly controlled by a variety ofangiogenic and antiangiogenic cytokines. Leukemic cells upregulate several angiogenic factors leading to increased BM vascularity. VEGF is the most important proangiogenic cytokine that is involved in tumor angiogenesis. VEGF is able to bind to three receptors: VEGF receptor-1 (VEGFR-1; fms-like tyrosine kinase-1, Flt-1), VEGFR-2 (human kinase domain region, KDR/murine fetal liver kinase-1, Flk-1), and VEGFR-3 (Flt-4). VEGFR-2 was found to be both necessary and sufficient to mediate effects of VEGF on endothelial cells, like induction of vascular permeability and angiogenesis [135]. In addition, VEGFR-1 is expressed on hematopoietic stem cells and frequently on leukemic cells [140], whereas megakaryocytes express VEGFR-2 [135], and VEGFR-3 is mainly involved in the regulation of lymphangiogenesis. VEGFR not only promotes BM neovascularization but was also found to signal through VEGFRs expressed on the surface of neoplastic hematopoietic cells [141]. Thus, secreted VEGF has been considered to contribute to disease progression by an autocrine or paracrine mechanism [135]. Numerous studies reported increased levels of VEGF in the blood as well as expression in the BM of patients with PV, ET, and PMF [21, 36, 46, 49, 75, 76, 80, 83–87]. Increased expression of VEGF was also found in CML [23, 25, 26, 77] and in BM section of SM patients [88].

HGF and FGF-b are other cytokines with potent angio-

genic potential. Endothelial cells express the HGF receptor c-MET and the role of HGF in angiogenesis is well established [142]. HGF enhances vascular matrix degradation and endothelial cell invasion and migration, as well as proliferation of vascular endothelial cells. Furthermore, HGF induces capillary tube formation in a matrigel assay and promotes angiogenesis in vivo. HGF acts synergistically with VEGF on endothelial growth but has also been shown to induce angiogenesis independent of VEGF [142]. Elevated levels of HGF have been described in patients with PV, ET, and PMF [38, 49, 50], as well as in CML [30–33]. FGF-b regulates proliferation and function of various mesenchymal cells. It induces growth of fibroblasts and endothelial cells in vitro [143] and stimulates angiogenesis and fibrosis in vivo [144]. Elevated levels of FGF-b have been described in PV and ET, but particular high levels were found in patients with PMF [36, 38]. In addition, CML patients showed also increased expression of FGF-b [24, 29].

IL-6 is a proinflammatory cytokine that has been implicated in the pathogenesis of various MPN. Among many other functions, IL-6 has been reported to stimulate angiogenesis in the tumor microenvironment and to enhance proliferation and migration of endothelial cells [145–147]. A recent study reported defective pericyte coverage of vessels after IL-6 stimulation compared to VEGF-stimulated vessels [148]. We identified the IL-6 family member OSM as an oncprotein-dependent cytokine in neoplastic cells of JAK2 V617F, KIT D816V, and FIP1L1-PDGFRα positive MPN [59, 61, 115]. OSM has been described to act as a growth factor for various mesenchymal cells, including fibroblasts, osteoblasts, and endothelial cells and to induce angiogenesis in vitro and in vivo [149–152]. Thus, OSM has been implicated in tissue remodeling, inflammation, and tissue fibrosis [151, 153–155]. Similarly, IL-8 is a multifunctional proinflammatory cytokine which is highly expressed in various MPN. It has been implicated in tumor growth and angiogenesis. In particular, IL-8 was shown to promote endothelial cell proliferation, capillary tube organization, and matrix metalloproteinase expression in endothelial cells [156]. In summary, a number of inflammatory cytokines, abundantly expressed in various MPN, have the potential to trigger angiogenesis in the BM and other organ systems. This pathogenetic process has therefore been proposed as a potential target in CML and other MPN and is best studied for targeted drugs against VEGF/VEGFR and HGF/c-MET.

4.3. Bone Marrow Niche Interactions. Apart from direct effects on endothelial cells and fibroblast, neoplastic cell-derived inflammatory cytokines are also involved in auto-
crine and paracrine loops between neoplastic (stem) cells and mesenchymal (stem) cells (Figure 1). Hematopoietic stem cells (HSC) rely on their interactions with the BM niche to maintain their quiescent state and to protect their integrity and functions but also to undergo asymmetrical cell division and differentiation in order to regulate and support blood cell production on demand. Similarly, disease-initiating leukemic stem cells (LSC) interact with the BM niche. However, the BM niche in hematopoietic malignancies is commonly altered
Inflammatory cytokines induce alterations of the bone marrow microenvironment and mediate autocrine and paracrine stimulation of neoplastic cells in myeloproliferative neoplasms. Neoplastic hematopoietic cells in chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and systemic mastocytosis (SM) secrete various cytokines including fibroblast growth factor (FGF), hepatocyte growth factor (HGF), interleukin-6 (IL-6), IL-8, oncostatin M (OSM), platelet derived growth factor (PDGF), transforming growth factor-$\beta$ (TGF-$\beta$), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and vascular endothelial growth factor (VEGF). These cytokines bind to their receptors on the surface of fibroblast, endothelial cells, and other cells of the bone marrow stroma and induce fibrosis and angiogenesis. In turn, cytokine production in stromal cells (e.g., stroma derived factor-1, SDF-1, or stem cell factor, SCF) has been implicated in proliferation, migration, and clonal selection of hematopoietic cells as well as in resistance to therapy.

and the leukemia-induced remodeling of the niche may directly contribute to the aberrant function of LSC [157]. A number of these complex interactions have been described as potentially interesting targets in MPN, of which some are exemplified in this section.

Arranz et al. recently described the effect of nestin-positive mesenchymal stromal cells and sympathetic nerve fibers on the regulation of hematopoietic stem cells in JAK2 V617F positive MPN. Sympathetic nerve fibers, supporting Schwann cells, and nestin-positive mesenchymal stromal cells were found to be reduced in the BM of MPN patients and murine MPN models, a process that may be triggered by IL-$\beta$ produced by mutated MPN cells. Depletion of nestin-positive cells or their production of stroma derived factor-1 (SDF-1, CXCL12) accelerated MPN progression. This elegant study demonstrates how inflammatory cytokines produced by neoplastic cells alter or even damage the niche-forming mesenchymal stromal cells in MPN. Furthermore, neuroprotective or sympathomimetic drugs were described as potential therapeutic agents to target this interaction [158].

Expanded myeloid CML cells were found to produce the proinflammatory cytokine IL-6 in inducible BCR-ABL1 transgenic mouse model recapitulation features of human chronic phase CML. IL-6 served as a positive feedback loop to sustain CML development in this model and reprogrammed both normal and leukemic multipotent progenitor cells towards myeloid development at the expense of lymphoid differentiation. Interestingly, knockout of IL-6 signaling was observed to delay CML development. These results suggest that blocking of IL-6 or targeting the IL-6 signal transduction pathway could represent a valuable target in CML. Moreover, the authors suggested that such self-reinforcing loop—invoking IL-6, or other secreted proinflammatory factors—might be relevant in a broad spectrum of MPN [92]. Traer and colleagues studied the effect of the BM microenvironment on imatinib resistance in CML. FGF released from stromal cells was found to promote growth of CML cells through the FGF receptor and the MAP-kinase pathway. In line with the in vitro data, CML patients resistant to imatinib without kinase domain mutations showed increased expression of FGF in the BM. Resistance could be overcome with ponatinib, a multikinase inhibitor that targets the FGF receptor in addition to BCR-ABL1 [159]. Another study focused on the effect of stromal cells on the resistance to JAK2 inhibitor treatment in JAK2 V617F+ disease. Cytokines were found to contribute to this protective effects of stromal cells, and neutralizing antibodies against IL-6, FGF, or CXCL-10 restored the apoptosis induced by JAK2 inhibition [34].

We found that OSM secreted by neoplastic cells did not only stimulate growth of fibroblasts, osteoblasts and microvascular endothelial cells but also induced the production of the angiogenic and profibrogenic cytokines HGF and VEGF in human fibroblasts [59]. In addition, marked production of SDF-1 was induced by OSM in these cells. Thus,
specific tumor cell-stroma cell interactions may potentiate the cytokine storm observed in MPN, that is, by inducing the production and release of cytokines that modulate growth of stromal cells as well as their activation, with consecutive expression of additional cytokines and cytokine receptors [58]. Schwaller et al. showed that retroviral overexpression of OSM in BM cells was sufficient to induce a lethal MPN with marked BM fibrosis and polyclonal expansion of myeloid cells [160].

Fleischman and colleagues studied the effect of the proinflammatory cytokine TNF-α in MPN. JAK2 V617F induced TNF-α expression in cell lines and primary MPN cells. TNF-α in turn was found to reduce colony formation in normal hematopoietic cells while JAK2 V617F+ progenitor cells were resistant to TNF-α. Thus, oncogenic JAK2 generates a TNF-α rich environment which facilitates clonal expansion of mutant cells in MPN [94]. Similarly, CML stem and progenitor cells were found to produce higher levels of TNF-α than their normal CD34+ counterparts. TNF-α promoted survival of CML stem cells in an autocrine manner by the nuclear factor κB/p65 pathway and expression of IL-3. Importantly, TNF-α inhibition induced apoptosis of CML cells and acted synergistically with nilotinib [95]. Together, these findings suggest TNF-α as a new putative therapeutic target in MPN.

5. Targeting the Cytokine Storm and the Microenvironment in MPN: A Novel Concept

5.1. JAK Inhibitors and Cytokine Production in MPN. Increased cytokine production was described as a hallmark of classical MPN that contributes to symptom burden of the patients and was referred to as cytokine storm. Targeting of this increased overall cytokine production has been successfully implicated in PMF, PV, and ET. In particular, the identification of the JAK2 V617F mutation led to the development of various JAK2 inhibitors. Ruxolitinib is the first JAK2 inhibitor approved for treatment of PMF. It targets wild type and mutant JAK2 as well as JAK1 and was found to induce marked and durable reductions in splenomegaly and symptoms in patients with PMF [161]. Despite having only limited effects on the JAK2 V617F allele burden, significant improvements of fatigue, pain, night sweats, and pruritus were observed after ruxolitinib treatment. In addition, a reduction of cytokine serum levels—including IL-6, IL-8, TNF-α, VEGF, and FGF-b—was found. Changes in cytokine level correlated with reduction in spleen size and coincided with symptom improvement [161]. Thus, it is tempting to speculate that the cytokine storm observed in PMF significantly contributes to the symptom burden in PMF. Interestingly, these changes were not related to the patients’ JAK2 mutational status [161]. This is in line with the observation of similar activation patterns of downstream signaling pathways in JAK2 mutant and wild type cases. The majority of JAK2 wild type patients harbor CALR mutations. Initial observations suggest that mutant CALR also activates JAK-STAT signaling [13]. Therefore, targeting of JAK1/JAK2 is effective to reduce proinflammatory cytokines in PMF irrespective of the JAK2/MPL/CALR mutation status [162].

Autocrine GM-CSF stimulation was identified as mechanism of imatinib resistance in CML leading to BCR-ABL1-independent activation of JAK/STAT signaling. Wang et al. used the JAK2 inhibitor AG490 to target GM-CSF induced activation of JAK/STAT signaling and could thus overcome resistance to imatinib and nilotinib in vitro [163]. Furthermore, activated JAK2/STAT5 signaling has been described as a potential target in LSC in CML [164, 165]. BCR-ABL1 was shown to activate JAK2 and subsequently STAT5 [166]. In addition, BCR-ABL1 was also found to activate STAT5 directly and independently of JAK2, and high levels of STAT5 activation contributed to imatinib resistance [167]. Gallipoli et al. showed that the JAK1/2 inhibitor ruxolitinib synergized with nilotinib in inhibiting the proliferation of CD34+ cells in patients with CML [164]. These findings provide a rationale for the application of JAK2 inhibitors to eradicate residual disease in CML. Clinical trials combining these drugs are now warranted to test this concept in patients.

5.2. Targeting of the VEGF/VEGFR Axis. Targeting of VEGF and/or the VEGF receptors (VEGFRs) is a widely used concept of antiangiogenesis in oncology. Neutralizing antibodies and soluble receptors are used to inhibit the interaction between VEGF and its receptors (Figure 2(b)). In addition, small molecule inhibitors targeting the kinase activity of VEGFR are applied [168]. Targeting of VEGFR with kinase inhibitors resulted in a reduction in stromal fibroblasts, macrophages, and endothelial cells in in vitro cultures of human BM whereas hematopoietic colony formation was not impaired [169].

Bevacizumab is a humanized monoclonal antibody against VEGF approved for antiangiogenic treatment in solid tumors. Mesa and colleagues performed a phase II study enrolling 13 patients with myelofibrosis. None of the patients treated with bevacizumab had an objective response, but significant toxicity was observed. Therefore, this study was terminated early [170]. VEGF promotes angiogenesis mainly through VEGFR-1 and VEGFR-2. Small molecule inhibitors targeting VEGFR and other kinases, for example, sorafenib and sunitinib, have been approved for treatment of patients with renal cell and hepatocellular carcinoma [168]. Sunitinib was tested in a small cohort of patients with PMF. Only one out of 14 patients showed clinical improvement, whereas a high rate of adverse events was observed [171]. Vatalanib is a VEGFR kinase inhibitor with greater potency against VEGFR-2 than against VEGFR-1 or VEGFR-3. In addition, inhibitory effects on PDGF receptor and KIT are observed. A phase I study in PMF showed modest activity with clinical improvement in 20% of the patients examined [172].

mTOR was identified to mediate BCR-ABL1-dependent VEGF expression in CML [27]. Targeting of mTOR by rapamycin in CML cells inhibited not only VEGF expression but also the in vitro growth of leukemic cells [28]. A clinical pilot study to evaluate the antileukemic and antiangiogenic effects of rapamycin in patients with imatinib-resistant CML showed transient antileukemic effects in a subset of cases [173]. In summary, despite promising data in preclinical
models, direct targeting of VEGF resulted only in modest clinical effects on patients with MPN so far.

5.3. Targeting of the HGF/c-MET Axis. Aberrant activation of HGF and/or its receptor c-MET has been described in solid tumors as well as in acute myeloid leukemia (AML), myeloma, and MPN. Production of HGF was found to be independent of BCR-ABL1 in CML and independent of JAK2 V617F in other MPN [32]. Thus, blocking of the HGF/c-MET function was suggested as an independent therapeutic target which could synergize with TKI treatment in MPN [174].

c-MET neutralizing antibodies bind to the extracellular domain of the receptor and prevent binding of HGF to c-MET (Figure 2(c)) [160]. These antibodies have shown promising effects on solid tumors [174]. In vitro studies have shown that c-MET neutralizing antibodies can effectively suppress the growth of JAK2 V617F-mutated cells, including PV erythroblasts and the HEL cell line, which expresses HGF at high levels [50]. In addition, small molecule inhibitors targeting c-MET and the c-MET-related RON receptor have been developed. The c-MET inhibitors SU-11274 and PHA-665752 decreased the survival of AML cells in a dose dependent manner [174]. SU-11274 was found to inhibit colony formation, to reduce viability, and to induce differentiation in A9M, U937, and OCI-AML cells [175]. Moreover, the c-MET inhibitors were found to block the response to HGF in a myeloma model [176]. Our group tested the effects of SU-11274 and PF-02341066 (crizotinib) on BCR-ABL1 positive cells and found that both drugs induce a significant growth reduction in KU812 cells and K562 cells [34]. Furthermore, c-MET inhibitors were found to reduce proliferation of primary
CML cells *in vitro* [32]. Boissinot and colleagues tested the efficacy of combining c-MET and JAK inhibitors on the proliferation of the JAK2 V617F positive HEL and UKE-1 cell lines. Only a weak inhibition was observed when molecules were tested separately, whereas the combination of the c-MET inhibitor PF-2341066 and the JAK inhibitor ruxolitinib inhibited growth of UKE-1 cells [174]. In summary, preclinical models show promising results for HGF/c-MET inhibition in MPN. The clinical efficacy of this targeting approach remains to be tested in clinical trials.

5.4. **Targeting of the SDF-1/CXCR4 Axis.** Increasing evidence suggests an important role of the BM microenvironment in the regulation of proliferation and survival of normal and leukemic hematopoietic stem cells. Thus, targeting of the specific BM niches and stem cell-niche interactions has been suggested as a promising therapeutic strategy [177]. SDF-1 (CXCL-12) is a chemokine produced by mesenchymal cells of the BM stroma (e.g., endothelial cells and osteoblasts) with particularly high expression in perivascular, niche-forming mesenchymal stromal cells [178]. Hematopoietic stem and progenitor cells express the SDF-1 receptor CXCR4 and migrate specifically towards SDF-1. Plerixafor (AMD3100) inhibits the SDF-1/CXCR4 interaction and is clinically used to mobilize hematopoietic stem and progenitor cells in stem cell transplant donors [179]. The SDF-1/CXCR4 axis is one potential target in the interplay of leukemic stem cells (LSC) and the BM microenvironment (Figure 2(d)).

CXCR4 is highly expressed on the surface of malignant cell in chronic lymphocytic leukemia (CLL), and SDF-1 was found to promote chemotaxis of CLL cells and their interaction with stromal cells, which was shown to induce resistance of CLL cells to cytotoxic agents, and was furthermore suggested to mediate persistence of minimal residual disease in the BM during therapy. In line with this concept, CXCR4 antagonists were successfully used to block interactions between CLL and stromal cells and to mobilize CLL cells from their protective microenvironments, becoming thus accessible to conventional drugs [180]. Similar targeting concepts were applied in preclinical models for AML and acute lymphoblastic leukemia (ALL). CXCR4 antagonist inhibited the proliferation of AML cells and reduced protection against chemotherapy by stromal cells *in vitro* and *in vivo* [181–183]. Leukemic cells in T-ALL were found to be in direct, stable contact with SDF-1-producing BM stroma. Furthermore, genetic targeting of CXCR4 in murine T-ALL led to rapid, sustained disease remission and CXCR4 antagonism suppressed human T-ALL in primary xenograft models [184].

Partly ambivalent results have been published for the role of SDF-1/CXCR4 in MPN, and although increased levels of SDF-1 have been reported, this may not necessarily result in a sustained activation of CXCR4 signaling in neoplastic cells [185, 186]. On the one hand, mobilization of CD34+ cells in patients with PMF has been attributed to reduced CXCR4 expression and hypermethylation of the CXCR4 promoter [187, 188]. Moreover, although elevated levels of immunoreactive forms of SDF-1 were found in the BM and peripheral blood of patients with PMF and PV, detailed studies using mass spectrometry have shown that SDF-1 was mainly truncated and thus expressed in an inactive form in these patients. The authors of this study concluded that reduced levels of intact SDF-1 due to proteolytic degradation would contribute to the mobilization of hematopoietic stem cells in PMF [186]. In line with these data, CD34+ cells in CML showed an impaired chemotactic response to SDF-1 although no decrease in CXCR4 expression was observed [189, 190]. Our group identified the cell surface enzyme dipeptidylpeptidase-IV (CD26) as a marker of CML LSC. CD26 was shown to disrupt the SDF-1/CXCR4 axis by cleaving SDF-1, and targeting of CD26 by gliptins suppressed the expansion of BCR-ABL1+ cells. CD26 expression may explain the mobilization of LSC and the observed extramedullary spread of hematopoietic stem and progenitor cells in CML, and inhibition of CD26 may revert abnormal LSC function [191].

On the other hand, the SDF-1/CXCR4 axis between stroma and leukemic cells contributes to resistance to TKI treatment in CML. Imatinib was found to enhance migration of CML cells towards stromal cell layers, which may in turn promote nonpharmacological resistance to imatinib [192, 193]. Mechanistically, this finding was linked to CXCR4 redistribution into the lipid raft fraction, in which CXCR4 colocalized with active LYN after TKI treatment [193]. The CXCR4 inhibitor plerixafor diminished migration of BCR-ABL1 positive cells and reduced adhesion of these cells to extracellular-matrix components and to BM stromal cells *in vitro*. Moreover, plerixafor was also found to decrease the drug resistance of CML cells induced by coculture with BM stromal cells *in vitro*. Importantly, plerixafor was shown to mobilize leukemic cells *in vivo* and to act synergistically with nilotinib to reduce the leukemia burden in a mouse model. The authors of this study argue that the combination of CXCR4 inhibition with TKI treatment in CML might be a useful approach to override drug resistance and to achieve deeper responses in CML [194]. In contrast, another study tested the effects of plerixafor in combination with either imatinib or dasatinib in a murine CML BM transplant model. In this study, no beneficial effect of plerixafor over TKI monotherapy was observed. Moreover, an increase in CNS infiltration after plerixafor treatment was described [195]. The discrepancy of these date can partly be explained by difference in the CML mouse model (e.g., irradiation possibly contributing to CNS infiltration) and in the TKI administration. Weisberg et al. applied plerixafor after marked reduction of disease burden with nilotinib as a model of minimal residual disease and argued that the absence of significant disease burden was relevant for the beneficial effects of the combination therapy [194].

Thus, the SDF-1/CXCR4 axis is a promising but still controversial target in CML and other types of MPN. The effect of CXCR4 inhibitors in PV, ET, PMF, and SM remains to be addressed in further preclinical models.

6. **Concluding Remarks and Future Perspectives**

The complex interplay between neoplastic cells and microenvironmental cells in MPN has gained increasing interest in
recent years. The resulting research revealed new important insights into the pathogenesis of MPN. One important aspect is that oncogenic signaling promotes cytokine production in MPN cells and alters their interaction with the BM stroma. A number of pathogenetic mechanisms are found to be conserved between various MPN, and lessons learned from one disease can be exploited for the other MPN types. Thus, it will be important to compare systematically the various common as well as rare MPN-variants in terms of basic and clinical science.

More recently, the pathologically altered interactions between neoplastic cells and their microenvironment have been investigated with the aim of defining new potential targets of therapy and to develop novel therapeutic approaches. First, the increased angiogenesis and BM fibrosis may serve as novel targets of therapy in MPN. Indeed, several TKI used to treat MPN may also suppress angiogenesis and/or fibrosis through inhibition of vascular target kinases. Thus, the VEGF/VEGFR, HGF/c-MET, and SDF-1/CXCR4 axis are potential targets in MPN, and a number of other molecular targets are under investigation. Many open questions still have to be addressed in preclinical model, and so far only few of the many exciting approaches were successfully translated to the clinic. Best evidence for targeting of the inflammatory cytokine storm is derived from the clinical efficacy of JAK inhibitors in MPN, which show marked benefits in patients with the cytokine storm is derived from the clinical efficacy of JAK inhibitors in MPN, which show marked benefits in patients. First, the increased angiogenesis and BM fibrosis may serve as novel targets of therapy in MPN. Indeed, several TKI used to treat MPN may also suppress angiogenesis and/or fibrosis through inhibition of vascular target kinases. Thus, the VEGF/VEGFR, HGF/c-MET, and SDF-1/CXCR4 axis are potential targets in MPN, and a number of other molecular targets are under investigation. Many open questions still have to be addressed in preclinical model, and so far only few of the many exciting approaches were successfully translated to the clinic. Best evidence for targeting of the inflammatory cytokine storm is derived from the clinical efficacy of JAK inhibitors in MPN, which show marked benefits in patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] A. Tefferi and J. W. Vardiman, “Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms,” Leukemia, vol. 22, no. 1, pp. 14–22, 2008.
[2] R. L. Levine and D. G. Gilliland, “Myeloproliferative disorders,” Blood, vol. 112, no. 6, pp. 2190–2198, 2008.
[3] A. Tefferi and W. Vainchenker, “Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies,” Journal of Clinical Oncology, vol. 29, no. 5, pp. 573–582, 2011.
[4] P. C. Nowell and D. A. Hungerford, “A minute chromosome in human granulocytic leukemia,” Science, vol. 132, pp. 1497–1499, 1960.
[5] J. D. Rowley, “A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining,” Nature, vol. 243, no. 5405, pp. 290–293, 1973.
[6] A. de Klein, A. G. van Kessel, G. Grosveld et al., “A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia,” Nature, vol. 300, no. 5894, pp. 765–767, 1982.
[7] J. D. Rowley, “A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining,” Nature, vol. 243, no. 5405, pp. 290–293, 1973.
[8] A. de Klein, A. G. van Kessel, G. Grosveld et al., “A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia,” Nature, vol. 300, no. 5894, pp. 765–767, 1982.
[21] F. Di Raimondo, M. P. Azzaro, G. A. Palumbo et al., “Elevated vascular endothelial growth factor (VEGF) serum levels in idiopathic myelofibrosis,” *Leukemia*, vol. 15, no. 6, pp. 976–980, 2001.

[22] O. Kato, A. Kimura, T. Itoh, and A. Kuramoto, “Platelet derived growth factor messenger RNA is increased in bone marrow megakaryocytes in patients with myeloproliferative disorders,” *American Journal of Hematology*, vol. 35, no. 3, pp. 145–150, 1990.

[23] C. Sillaber, M. Mayerhofer, K. J. Aichberger, M.-T. Krauth, and P. Valent, “Expression of angiogenic factors in chronic myeloid leukemia: role of the bcr/abl oncogene, biochemical mechanisms, and potential clinical implications,” *European Journal of Clinical Investigation*, vol. 34, supplement 2, pp. 2–11, 2004.

[24] A. Aguayo, H. Kantarjian, T. Manshouri et al., “Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes,” *Blood*, vol. 96, no. 6, pp. 2240–2245, 2000.

[25] S. Verstovsek, H. Kantarjian, T. Manshouri et al., “Prognostic significance of cellular vascular endothelial growth factor expression in chronic phase chronic myeloid leukemia,” *Blood*, vol. 99, no. 6, pp. 2265–2267, 2002.

[26] M.-T. Krauth, I. Simonitsch, K. J. Aichberger et al., “Immunohistochemical detection of VEGF in the bone marrow of patients with chronic myeloid leukemia and correlation with the phase of disease,” *American Journal of Clinical Pathology*, vol. 121, no. 4, pp. 473–481, 2004.

[27] M. Mayerhofer, P. Valent, W. R. Sperr, J. D. Griffin, and C. Sillaber, “BCR/ABL induces expression of vascular endothelial growth factor and its transcriptional activator, hypoxia inducible factor-alpha, through a pathway involving phosphoinositide 3-kinase and the mammalian target of rapamycin,” *Blood*, vol. 100, no. 10, pp. 3767–3775, 2002.

[28] M. Mayerhofer, K. J. Aichberger, S. Florian et al., “Identification of mTOR as a novel bifunctional target in chronic myeloid leukemia: dissection of growth-inhibitory and VEGF-suppressive effects of rapamycin in leukemic cells,” *The FASEB Journal*, vol. 19, no. 8, pp. 960–962, 2005.

[29] A. Janowska-Wieczorek, M. Majka, L. Marquez-Curtis, J. A. Wertheim, A. R. Turner, and M. Z. Ratajczak, “Bcr-abl-positive cells secrete angiogenic factors including matrix metalloproteinases and stimulate angiogenesis in vivo in Matrigel implants,” *Leukemia*, vol. 16, no. 6, pp. 1160–1166, 2002.

[30] M. Hino, M. Inaba, H. Goto et al., “Hepatocyte growth factor levels in bone marrow plasma of patients with leukaemia and its gene expression in leukaemic blast cells,” *British Journal of Cancer*, vol. 73, no. 1, pp. 119–123, 1996.

[31] J. G. Kim, S. K. Sohn, D. H. Kim et al., “Clinical implications of angiogenic factors in patients with acute or chronic leukemia: Hepatocyte growth factor levels have prognostic impact, especially in patients with acute myeloid leukemia,” *Leukemia & Lymphoma*, vol. 46, no. 6, pp. 885–891, 2005.

[32] S. Cerny-Reiterer, V. Ghanim, G. Hoermann et al., “Identification of basophils as a major source of hepatocyte growth factor in chronic myeloid leukemia: a novel mechanism of BCR-ABL-independent disease progression,” *Necropolis*, vol. 14, no. 7, pp. 572–584, 2012.

[33] A. G. Zhelyazkova, A. B. Tonchev, P. Kolova, L. Ivanova, and L. Gercheva, “Prognostic significance of hepatocyte growth factor and microvessel bone marrow density in patients with chronic myeloid leukemia,” *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 68, no. 6, pp. 492–500, 2008.

[34] T. Manshouri, Z. Estrov, A. Quintás-Cardama et al., “Bone marrow stroma-secreted cytokines protect JAK2V617F-mutated cells from the effects of a JAK2 inhibitor,” *Cancer Research*, vol. 71, no. 11, pp. 3831–3840, 2011.

[35] A. Kimura, Y. Nakata, H. Hyodo, A. Kuramoto, and Y. Satow, “Platelet-derived growth factor expression in accelerated and blastic phase of chronic myelogenous leukemia with myelofibrosis,” *British Journal of Haematology*, vol. 86, no. 2, pp. 303–307, 1994.

[36] C. Musolino, L. Calabro, G. Bellomo et al., “Soluble angiogenic factors: implications for chronic myeloproliferative disorders,” *The American Journal of Hematology*, vol. 69, no. 3, pp. 159–163, 2002.

[37] N. Sayin, H. Cinar, A. Usen et al., “Plasma basic fibroblast growth factor and bone marrow fibrosis in clonal myeloproliferative disorders,” *Clinical and Laboratory Haematology*, vol. 26, no. 4, pp. 265–268, 2004.

[38] R. Vaidya, N. Gangat, T. Jimma et al., “Plasma cytokines in polycythaemia vera: phenotypic correlates, prognostic relevance, and comparison with myelofibrosis,” *The American Journal of Hematology*, vol. 87, no. 11, pp. 1003–1005, 2012.

[39] M.-C. Le Bousse-Kerdiles, S. Chevillard, A. Charpentier et al., “Differential expression of transforming growth factor-beta, basic fibroblast growth factor, and their receptors in CD34+ hematopoietic progenitor cells from patients with myelofibrosis and myeloid metaplasia,” *Blood*, vol. 88, no. 12, pp. 4534–4546, 1996.

[40] M. C. Le Bousse-Kerdiles and M. C. Martyrè, “Involvement of the fibrogenic cytokines, TGF-β and bFGF, in the pathogenesis of idiopathic myelofibrosis,” *Pathologie Biologie*, vol. 49, no. 2, pp. 153–157, 2001.

[41] M.-C. Martyrè, M.-C. Le Bousse-Kerdiles, N. Romquin et al., “Elevated levels of basic fibroblast growth factor in megakaryocytes and platelets from patients with idiopathic myelofibrosis,” *British Journal of Haematology*, vol. 97, no. 2, pp. 441–448, 1997.

[42] S.-Y. Yoon, C.-Y. Li, R. V. Lloyd, and A. Tefferi, “Bone marrow histochemical studies of fibrogenic cytokines and their receptors in myelodysplastic syndrome with myelofibrosis and related disorders,” *International Journal of Hematology*, vol. 72, no. 3, pp. 337–342, 2000.

[43] A. Dalley, J. M. Smith, J. T. Reilly, and S. MacNeil, “Investigation of calmodulin and basic fibroblast growth factor (bFGF) in idiopathic myelofibrosis: evidence for a role of extracellular calmodulin in fibroblast proliferation,” *British Journal of Haematology*, vol. 93, no. 4, pp. 856–862, 1996.

[44] A. Wehmeier and T. Sudhoff, “Elevated plasma levels of basic fibroblast growth factor in patients with essential thrombocythaemia and polycythaemia vera,” *British Journal of Haematology*, vol. 98, no. 4, pp. 1050–1051, 1997.

[45] H. Ni, G. Barosi, and R. Hoffman, “Quantitative evaluation of angiogenic factors: implications for chronic myeloproliferative disorders,” *Neoplasia*, vol. 87, no. 11, pp. 1003–1005, 2012.

[46] J. M. Chou, C.-Y. Li, and A. Tefferi, “Bone marrow immunohistochemical studies of angiogenic cytokines and their receptors in myelofibrosis with myeloid metaplasia,” *Leukemia Research*, vol. 27, no. 6, pp. 499–504, 2003.

[47] Z. Qu, X. Huang, P. Ahmadi et al., “Synthesis of basic fibroblast growth factor by murine mast cells. Regulation by transforming growth factor beta, tumor necrosis factor alpha, and stem cell...
factor,” *International Archives of Allergy and Immunology*, vol. 115, no. 1, pp. 47–54, 1998.

[48] C.-Y. Li and J.-Y. Baek, “Mastocytosis and fibrosis: role of cytokines,” *International Archives of Allergy and Immunology*, vol. 127, no. 2, pp. 123–126, 2002.

[49] A. Tefferi, R. Vaidya, D. Caramazza, C. Finke, T. Lasbo, and A. Pardanani, “Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study,” *Journal of Clinical Oncology*, vol. 29, no. 10, pp. 1356–1363, 2011.

[50] M. Boissinot, C. Cleyrat, M. Vilaine, Y. Jacques, I. Corre, and S. Hermouet, “Anti-inflammatory cytokines hepatocyte growth factor and interleukin-11 are over-expressed in Polycthemia vera and contribute to the growth of clonal erythroblasts independently of JAK2V617F,” *Oncogene*, vol. 30, no. 8, pp. 990–1001, 2011.

[51] K. E. Panteli, E. C. Hatzimichael, P. K. Bouranta et al., “Serum interleukin (IL)-1, IL-2, sIL-2Ra, IL-6 and thrombopoietin levels in patients with chronic myeloproliferative diseases,” *British Journal of Haematology*, vol. 130, no. 5, pp. 709–715, 2005.

[52] M. Anand, S. K. Chodda, P. M. Parikh, and J. S. Nadkarni, “Abnormal levels of proinflammatory cytokines in serum and monocyte cultures from patients with chronic myeloid leukemia in different stages, and their role in prognosis,” *Hematological Oncology*, vol. 16, no. 4, pp. 143–154, 1998.

[53] H.-C. Hsu, W.-H. Tsai, M.-L. Jiang et al., “Circulating levels of thrombopoietic and inflammatory cytokines in patients with clonal and reactive thrombocytoysis,” *The Journal of Laboratory and Clinical Medicine*, vol. 134, no. 4, pp. 392–397, 1999.

[54] K. Brockow, C. Akin, M. Huber, L. M. Scott, L. R. Schwartz, and D. D. Metcalfe, “Levels of mast-cell growth factors in plasma and in suction skin blister fluid in adults with mastocytosis: correlation with dermal mast-cell numbers and mast-cell tryptase,” *The Journal of Allergy and Clinical Immunology*, vol. 109, no. 1, pp. 82–88, 2002.

[55] T. C. Theoharides, W. Boucher, and K. Spear, “Serum interleukin-6 reflects disease severity and osteoporosis in mastocytosis patients,” *International Archives of Allergy and Immunology*, vol. 128, no. 4, pp. 344–350, 2002.

[56] K. Brockow, C. Akin, M. Huber, and D. D. Metcalfe, “IL-6 levels predict disease variant and extent of organ involvement in patients with mastocytosis,” *Clinical Immunology*, vol. 115, no. 2, pp. 216–223, 2005.

[57] O. Hantschel, A. Gstoettenbauer, J. Colinge et al., “The chemokine interleukin-8 and the surface activation protein CD69 are markers for Bcr-Abl activity in chronic myeloid leukemia,” *Molecular Oncology*, vol. 2, no. 3, pp. 272–281, 2008.

[58] E. Pourcelot, C. Trocmé, J. Mondet, S. Bailly, B. Toussaint, and P. Mossuz, “Cytokine profiles in polycthemia vera and essential thrombocythemia patients: clinical implications,” *Experimental Hematology*, vol. 42, no. 5, pp. 360–368, 2014.

[59] G. Hoermann, S. Cerny-Reiterer, A. Perné et al., “Identification of oncostatin M as a STAT5-dependent mediator of bone marrow remodeling in KIT D816V-positive systemic mastocytosis,” *The American Journal of Pathology*, vol. 178, no. 5, pp. 2344–2356, 2011.

[60] G. M. Gersuk, R. Carmel, and P. K. Pattengale, “Platelet-derived growth factor concentrations in platelet-poof plasma and urine from patients with myeloproliferative disorders,” *Blood*, vol. 74, no. 7, pp. 2330–2334, 1989.

[61] O. Bock, G. Loch, G. Büsche, R. von Wasielewski, J. Schlué, and H. Kreipe, “Aberrant expression of platelet-derived growth factor (PDGF) and PDGF receptor-α is associated with advanced bone marrow fibrosis in idiopathic myelofibrosis,” *Haematologica*, vol. 90, no. 1, pp. 133–134, 2005.

[62] S.-Y. Yoon, A. Tefferi, and C.-Y. Li, “Cellular distribution of platelet-derived growth factor, transforming growth factor-β, basic fibroblast growth factor, and their receptors in normal bone marrow,” *Acta Haematologica*, vol. 104, no. 4, pp. 151–157, 2000.

[63] P. R. Lev, R. F. Marta, P. Vassallu, and F. C. Molinas, “Variation of PDGF, TGFβ, and bFGF levels in essential thrombocythemia patients treated with anagrelide,” *American Journal of Hematology*, vol. 70, no. 2, pp. 85–91, 2002.

[64] K. Miyazono, “Tumour promoting functions of TGF-β in CML-initiating cells,” *Journal of Biochemistry*, vol. 152, no. 5, pp. 383–385, 2012.

[65] X. Zhu, L. Wang, B. Zhang, J. Li, X. Dou, and R. C. Zhao, “TGF-beta-induced P13K/Akt/NF-kappaB/MMP9 signalling pathway is activated in Philadelphia chromosome-positive chronic myeloid leukaemia haemangioblasts,” *Journal of Biochemistry*, vol. 149, no. 4, pp. 405–414, 2011.

[66] M. C. Martyre, N. Romquin, M. C. Le Bousse-Kerdiles et al., “Transforming growth factor-beta and megakaryocytes in the pathogenesis of idiopathic myelofibrosis,” *British Journal of Haematology*, vol. 88, no. 1, pp. 9–16, 1994.

[67] T. Terui, Y. Niitsu, K. Mahara et al., “The production of transforming growth factor-beta in acute megakaryoblastic leukemia and its possible implications in myelofibrosis,” *Blood*, vol. 75, no. 7, pp. 1540–1548, 1990.

[68] C. C. Ponce, F. C. M. de Lourdes, S. S. M. Ihara, and M. R. R. Silva, “The relationship of the active and latent forms of TGF-beta with marrow fibrosis in essential thrombocythemia and primary myelofibrosis,” *Medical Oncology*, vol. 29, no. 4, pp. 2337–2344, 2012.

[69] T. Akiyama, T. Matsunaga, T. Terui et al., “Involvement of transforming growth factor-β and thrombopoietin in the pathogenesis of myelodysplastic syndrome with myelofibrosis,” *Leukemia*, vol. 19, no. 9, pp. 1558–1566, 2005.

[70] R. Campanelli, V. Rosti, L. Villani et al., “Evaluation of the bioactive and total transforming growth factor β1 levels in primary myelofibrosis,” *Cytokine*, vol. 53, no. 1, pp. 100–106, 2011.

[71] T. G. P. Bumm, C. Elsea, A. S. Corbin et al., “Characterization of murine JAK2V617F-positive myeloproliferative disease,” *Cancer Research*, vol. 66, no. 23, pp. 11156–11165, 2006.

[72] C. Sillaber, D. Bevec, J. H. Butterfield et al., “Tumor necrosis factor α and interleukin-1β mRNA expression in HMC-1 cells: differential regulation of gene product expression by recombinant interleukin-4,” *Experimental Hematology*, vol. 21, no. 9, pp. 1271–1275, 1993.

[73] L. G. Lundberg, R. Lerner, P. Sundelin, R. Rogers, J. Folkman, and J. Palmblad, “Bone marrow in polycythemia vera, chronic
myelocytic leukemia, and myelofibrosis has an increased vascu-
arity,” The American Journal of Pathology, vol. 157, no. 1, pp. 15–19, 2000.

[76] K. Panteli, M. Bai, E. Hatzimichael, N. Zagorianakou, N. J. Agnantis, and K. Bourantas, “Serum levels, and bone mar-
row immunohistochemical expression of, vascular endothelial
growth factor in patients with chronic myeloproliferative dis-
ees;” Hematology, vol. 12, no. 6, pp. 481–486, 2007.

[77] P. Liu, J. Li, Z. C. Han et al., “Evaluated plasma levels of
vascular endothelial growth factor is associated with marked
splenomegaly in chronic myeloid leukemia,” Leukemia & Lym-
phoma, vol. 46, no. 12, pp. 1761–1764, 2005.

[78] A. Alonci, A. Allegra, G. Bellomo et al., “Evaluation of circu-
ulating endothelial cells, VEGF and VEGFR2 serum levels in
patients with chronic myeloproliferative diseases,” Hematol-
ogical Oncology, vol. 26, no. 4, pp. 235–239, 2008.

[79] M. Medinger, R. Skoda, A. Gratwohl et al., “Angiogenesis
and vascular endothelial growth factor-receptor expression in
myeloproliferative neoplasms: correlation with clinical param-
eters and JAK2-V617F mutational status,” British Journal of
Haematology, vol. 146, no. 2, pp. 150–157, 2009.

[80] L. Boiocchi, C. Vener, F. Saviet al., “Increased expression of vas-
cular endothelial growth factor receptor 1 correlates with VEGF
and microvessel density in Philadelphia chromosome-negative
myeloproliferative neoplasms,” Journal of Clinical Pathology,
vol. 64, no. 3, pp. 226–231, 2011.

[81] U. Gianelli, C. Vener, P. R. Raviele et al., “VEGF expression cor-
relates with microvessel density in Philadelphia chromosome-
negative chronic myeloproliferative disorders,” American Jour-
nal of Clinical Pathology, vol. 128, no. 6, pp. 966–973, 2007.

[82] T. Wróbel, G. Mazur, P. Surowiak, D. Wołowiec, M. Jeleń, and
K. Kuliczkowski, “Increased expression of vascular endothe-
lial growth factor (VEGF) in bone marrow of patients with
myeloproliferative disorders (MPD),” Pathology and Oncology
Research, vol. 9, no. 3, pp. 170–173, 2003.

[83] M. Steurer, H. Zoller, F. Augustin et al., “Increased angiogenesis in
chronic idiopathic myelofibrosis: vascular endothelial growth
factor as a prominent angiogenic factor,” Human Pathology, vol.
38, no. 7, pp. 1057–1064, 2007.

[84] R. R. Cacciola, E. Di Francesco, R. Giustolisi, and E. Cacciola,
“Evaluated serum vascular endothelial growth factor levels in
patients with polycythemia vera and thrombotic complicati-
on,” Haematologica, vol. 87, no. 7, pp. 774–775, 2002.

[85] P. Murphy, N. Ahmed, and H. T. Hassan, “Increased serum
levels of vascular endothelial growth factor correlate with splenomegaly in polycythemia vera,” Leukemia Research, vol.
26, no. 11, pp. 1007–1010, 2002.

[86] R. R. Cacciola, E. Di Francesco, C. Ferlito, G. G. Guaraccia,
R. Giustolisi, and E. Cacciola, “Vascular endothelial growth
factor and thrombopoietin in patients with essential throm-
bocytemia and polycythemia vera and thrombotic complica-
tions,” Acta Haematologica, vol. 110, no. 4, pp. 202–203, 2003.

[87] S. Theodoridou, T. Vyzantiadis, S. Vakalopoulou et al., “Evaluated
levels of serum vascular endothelial growth factor in patients
with polycythemia vera,” Acta Haematologica, vol. 110, no. 1,
pp. 16–19, 2003.

[88] F. Wimazal, J.-H. Jordan, W. R. Sperr et al., “Increased angiogen-
esis in the bone marrow of patients with systemic mastocytosis,”
The American Journal of Pathology, vol. 160, no. 5, pp. 1639–1645,
2002.

[89] L. Rebuzzi, M. Willmann, K. Sonneck et al., “Detection of vascular endothelial growth factor (VEGF) and VEGF recep-
tors Flt-1 and KDR in canine mastocytoma cells,” Veterinary
Immunology and Immunopathology, vol. 115, no. 3–4, pp. 320–333,
2007.

[90] O. Mederle, N. Mederle, E. V. Bocan, R. Ceauşu, and M.
Raica, “VEGF expression in dog mastocytoma,” Revista Medico-
Chirurgical a Societatii de Medici si Naturalişti din Iasi, vol. 114,
no. 1, pp. 185–188, 2010.

[91] R. Patruno, N. Arpaia, C. D. Gadaleta et al., “VEGF con-
centration from plasma-activated platelets rich correlates
with microvascular density and grading in canine mast cell
tumour spontaneous model,” Journal of Cellular and Molecular
Medicine, vol. 13, no. 3, pp. 555–561, 2009.

[92] D. Reynaud, E. Pitras, K. Barry-Holson et al., “IL-6 controls
leukemic multipotent progenitor cell fate and contributes to
chronic myelogenous leukemia development,” Cancer Cell, vol.
20, no. 5, pp. 661–673, 2011.

[93] R. Ciarcia, M. T. Vitiello, M. Galdiero et al., “Imatinib treatment
inhibit IL-6, IL-8, NF-KB and AP-1 production and modulate intracellular calcium in CML patients,” Journal of Cellular
Physiology, vol. 227, no. 6, pp. 2798–2803, 2012.

[94] A. G. Fleischman, K. I. Aichberger, S. B. Luty et al., “TNFα
facilitates clonal expansion of JAK2V617F positive cells in
myeloproliferative neoplasms,” Blood, vol. 118, no. 24, pp. 6392–
6398, 2011.

[95] P. Gallipoli, F. Pellicano, H. Morrison et al., “Autocrine TNF-α
production supports CML stem and progenitor cell survival and
enhances their proliferation,” Blood, vol. 122, no. 19, pp. 3335–
3339, 2013.

[96] V. Skov, T. S. Larsen, M. Thomassen et al., “ Molecular profiling
of peripheral blood cells from patients with polycythemia vera
and related neoplasms: identification of deregulated genes of
significance for inflammation and immune surveillance,”
Leukemia Research, vol. 36, no. 11, pp. 1387–1392, 2012.

[97] V. Skov, M. Thomassen, C. H. Riley et al., “Gene expression pro-
file with principal component analysis depicts the biological
continuum from essential thrombocythemia over polycythemia
vera to myelofibrosis,” Experimental Hematology, vol. 40, no. 9,
pp. 771.e19–780.e19, 2012.

[98] C.-L. Ho, T. L. Lasho, J. H. Butterfield, and A. Tefferi, “Global
cytokine analysis in myeloproliferative disorders,” Leukemia
Research, vol. 31, no. 10, pp. 1389–1392, 2007.

[99] H. C. Hasselbalch, “The role of cytokines in the initiation
and progression of myelofibrosis,” Cytokine & Growth Factor
Reviews, vol. 24, no. 2, pp. 133–145, 2013.

[100] S. Emadi, D. Clay, C. Desterke et al., “IL-8 and its CXCR1 and
CXCR2 receptors participate in the control of megakaryocytic
proliferation, differentiation, and ploidy in myeloid metaplasia
with myelofibrosis,” Blood, vol. 105, no. 2, pp. 464–473, 2005.

[101] S. Hermouet, A. Godard, D. Pineau et al., “Abnormal produc-
tion of interleukin (IL)-11 and IL-8 in polycythemia vera,”
Cytokine, vol. 20, no. 4, pp. 178–183, 2002.

[102] I. Corre-Buscail, D. Pineau, M. Boissinot, and S. Hermouet,
“Erythropoietin-independent erythroid colony formation by
bone marrow progenitors exposed to interleukin-11 and interleukin-8,” Experimental Hematology, vol. 33, no. 11, pp.
1299–1308, 2005.

[103] C. Wickenhauser, A. Hillenhor, K. Junghem et al., “Detection
and quantification of transforming growth factor beta (TGFTGF-beta) and platelet-derived growth factor (PDGF)
release by normal human megakaryocytes,” *Leukemia*, vol. 9, no. 2, pp. 310–315, 1995.

[104] M. Arock and P. Valent, “Pathogenesis, classification and treatment of mastocytosis: state of the art in 2010 and future perspectives,” *Expert Review of Hematology*, vol. 3, no. 4, pp. 497–516, 2010.

[105] L. Escribano, C. Akin, M. Castells, and L. B. Schwartz, “Current options in the treatment of mast cell mediator-related symptoms in mastocytosis,” *Inflammation and Allergy — Drug Targets*, vol. 5, no. 1, pp. 61–77, 2006.

[106] P. Valent, C. Akin, L. Escribano et al., “Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria,” *European Journal of Clinical Investigation*, vol. 37, no. 6, pp. 435–453, 2007.

[107] D. D. Metcalfe, “Mast cells and mastocytosis,” *Blood*, vol. 112, no. 4, pp. 946–956, 2008.

[108] Y. Okayama and T. Kawakami, “Development, migration, and survival of mast cells,” *Immunologic Research*, vol. 34, no. 2, pp. 97–115, 2006.

[109] N. Harir, C. Boudot, K. Friedbichler et al., “Oncogenic Kit controls neoplastic mast cell growth through a Stat5/P13-kinase signaling cascade,” *Blood*, vol. 112, no. 6, pp. 2463–2473, 2008.

[110] M. Gabillot-Carré, Y. Lepelletier, M. Humbert et al., “Rapamycin inhibits growth and survival of D816V-mutated c-kit mast cells,” *Blood*, vol. 108, no. 3, pp. 1065–1072, 2006.

[111] C. Baumgartner, S. Cerny-Reiterer, K. Sonneck et al., “Expression of activated STAT5 in neoplastic mast cells in systemic mastocytosis: subcellular distribution and role of the transforming oncoprotein KIT D816V,” *The American Journal of Pathology*, vol. 175, no. 6, pp. 2416–2429, 2009.

[112] A. Rabenhorst, B. Christopeit, S. Leja et al., “Serum levels of bone cytokines are increased in indolent systemic mastocytosis associated with osteopenia or osteoporosis,” *The Journal of Allergy and Clinical Immunology*, vol. 132, no. 5, pp. 1234.e7–1237.e7, 2013.

[113] K. Hartmann, N. Wagner, A. Rabenhorst et al., “Serum IL-31 levels are increased in a subset of patients with mastocytosis and correlate with disease severity in adult patients,” *The Journal of Allergy and Clinical Immunology*, vol. 132, no. 1, pp. 232.e4–235.e4, 2013.

[114] R. S. Selvan, J. H. Butterfield, and M. S. Kranget al., “Expression of multiple chemokine genes by a human mast cell leukemia,” *The Journal of Biological Chemistry*, vol. 269, no. 19, pp. 13893–13898, 1994.

[115] G. Hoermann, S. Cerny-Reiterer, I. Sadovnik et al., “Oncostatin M is a FIP1L1/PDGFRα-dependent mediator of cytokine production in chronic eosinophilic leukemia,” *Allergy*, vol. 68, no. 6, pp. 713–723, 2013.

[116] M.-C. Le Bousse-Kerdiles, M.-C. Martyre, and M. Samson, “Cellular and molecular mechanisms underlying bone marrow and liver fibrosis: a review,” *European Cytokine Network*, vol. 19, no. 2, pp. 69–80, 2008.

[117] H.-P. Horny and P. Valent, “Histopathological and immunohistochemical aspects of mastocytosis,” *International Archives of Allergy and Immunology*, vol. 127, no. 2, pp. 115–117, 2002.

[118] J. Thiele and H. M. Kvasnicka, “Myelofibrosis in chronic myeloproliferative disorders—dynamics and clinical impact,” *Histochemistry and Histopathology*, vol. 21, no. 12, pp. 1367–1378, 2006.

[119] O. Pozdynyakova, R. P. Hasserjian, S. Verstovsek, and A. Orazi, “Impact of bone marrow pathology on the clinical management of Philadelphia chromosome-negative myeloproliferative neoplasms,” *Clinical Lymphoma, Myeloma & Leukemia*, vol. 15, no. 5, pp. 253–261, 2015.

[120] R. Dekmezian, H. M. Kantarjian, M. J. Keating, M. Talpaz, K. B. McCredie, and E. J. Freireich, “The relevance of reticulin stain-measured fibrosis at diagnosis in chronic myelogenous leukemia,” *Cancer*, vol. 59, no. 10, pp. 1739–1743, 1987.

[121] E. Eliacik, A. Isik, C. Aydin et al., “Bone marrow fibrosis may be an effective independent predictor of the ‘TKI drug response level’ in chronic myeloid leukemia,” *Hematology*, vol. 20, no. 7, pp. 392–396, 2015.

[122] H. Castro-Malaspina and S. C. Jhanwar, “Properties of myelofibrosis-derived fibroblasts,” *Progress in Clinical and Biological Research*, vol. 154, pp. 307–322, 1984.

[123] J. C. Bonner, “Regulation of PDGF and its receptors in fibrotic diseases,” *Cytokine and Growth Factor Reviews*, vol. 15, no. 4, pp. 255–273, 2004.

[124] P. R. Lev, J. P. Salim, L. I. Kornblith et al., “PDGF-A, PDGF-B, TGFβeta, and bFGF mRNA levels in patients with essential thrombocytopenia treated with anagrelide,” *American Journal of Hematology*, vol. 78, no. 2, pp. 155–157, 2005.

[125] S. Badalucco, C. A. Di Buduo, R. Campanelli et al., “Involvement of TGFβ in autocrine regulation of proplatelet formation in healthy subjects and patients with primary myelofibrosis,” *Haematologica*, vol. 98, no. 4, pp. 514–517, 2013.

[126] J. Hatzfeld, M.-L. Li, E. L. Brown et al., “Release of early human hematopoietic progenitors from quiescence by anti-sense transforming growth factor beta or Rb oligonucleotides,” *The Journal of Experimental Medicine*, vol. 174, no. 4, pp. 925–929, 1991.

[127] N. O. Fortunel, A. Hatzfeld, and J. A. Hatzfeld, “Transforming growth factor-β: pleiotropic role in the regulation of hematopoiesis,” *Blood*, vol. 96, no. 6, pp. 2022–2036, 2000.

[128] C. S. Curran and P. J. Keely, “Breast tumor and stromal cell responses to TGF-β and hypoxia in matrix deposition,” *Matrix Biology*, vol. 32, no. 2, pp. 95–105, 2013.

[129] S. Gupta, M. R. Clarkson, J. Duggan, and H. R. Brady, “Connective tissue growth factor: potential role in glomerulosclerosis and tubulointerstitial fibrosis,” *Kidney International*, vol. 58, no. 4, pp. 1389–1399, 2000.

[130] F. Strutz, M. Zeisberg, B. Hemmerlein et al., “Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation,” *Kidney International*, vol. 57, no. 4, pp. 1521–1538, 2000.

[131] N. Itoh and H. Ohta, “Pathophysiological roles of FGF signaling in the heart,” *Frontiers in Physiology*, vol. 4, article 247, 2013.

[132] P. Valent, G. J. Gleich, A. Reiter et al., “Pathogenesis and classification of eosinophil disorders: a review of recent developments in the field,” *Expert Review of Hematology*, vol. 5, no. 2, pp. 157–176, 2012.

[133] G. M. Walsh, “Advances in the immunobiology of eosinophils and their role in disease,” *Critical Reviews in Clinical Laboratory Sciences*, vol. 36, no. 5, pp. 453–496, 1999.

[134] J. Folkman, “Angiogenesis in cancer, vascular, rheumatoid and other disease,” *Nature Medicine*, vol. 1, no. 1, pp. 27–31, 1995.

[135] M. Medinger and J. Passweg, “Role of tumour angiogenesis in haematological malignancies,” *Swiss Medical Weekly*, vol. 144, Article ID w4050, 2014.

[136] F. Di Raimondo, G. A. Palumbo, S. Molica, and R. Giustolisi, “Angiogenesis in chronic myeloproliferative diseases,” *Acta Haematologica*, vol. 106, no. 4, pp. 177–183, 2001.
inhibitors on long-term cultures from normal human bone marrow,” *Growth Factors*, vol. 19, no. 1, pp. 1–17, 2001.

[170] R. A. Mesa, R. T. Silver, S. Verstovsek et al., “Single agent bevacizumab for myelofibrosis: results of the myeloproliferative disorders research consortium trial,” *Haematologica*, vol. 98, no. 9, pp. 1421–1423, 2013.

[171] E. Apostolidou, H. Kantarjian, D. Thomas, I. Burger, G. Borthakur, and S. Verstovsek, “Phase II study of sunitinib in patients with primary or post-polycythemia vera/essential thrombocytopenia myelofibrosis,” *Clinical Lymphoma, Myeloma & Leukemia*, vol. 10, no. 4, pp. 281–284, 2010.

[172] F. J. Giles, A. F. List, M. Carroll et al., “PTK787/ZK 222584, a small molecule tyrosine kinase receptor inhibitor of vascular endothelial growth factor (VEGF), has modest activity in myelofibrosis with myeloid metaphasia,” *Leukemia Research*, vol. 31, no. 7, pp. 891–897, 2007.

[173] C. Sillaber, M. Mayerhofer, A. Böhm et al., “Evaluation of antileukemic effects of rapamycin in patients with imatinib-resistant chronic myeloid leukaemia,” *European Journal of Clinical Investigation*, vol. 38, no. 1, pp. 43–52, 2008.

[174] M. Boissinot, M. Vilaine, and S. Hermouet, “The Hepatocyte Growth Factor (HGF)/met axis: a neglected target in the treatment of chronic myeloproliferative neoplasms?” *Cancers*, vol. 6, no. 3, pp. 1631–1669, 2014.

[175] N. M. Mulgrew, L. M. J. Kettle, J. M. Ramsey et al., “c-Met inhibition in a HOXA9/Meis1 model of CN-AML,” *Developmental Dynamics*, vol. 243, no. 1, pp. 172–181, 2014.

[176] H. Hov, R. U. Holt, T. B. Ro et al., “A selective c-met inhibitor blocks an autocrine hepatocyte growth factor growth loop in ANBL-6 cells and prevents migration and adhesion of myeloma cells,” *Clinical Cancer Research*, vol. 10, no. 19, pp. 6686–6694, 2004.

[177] D. T. Scadden, “Nice neighborhood: emerging concepts of the stem cell niche,” *Cell*, vol. 157, no. 1, pp. 41–50, 2014.

[178] S. Méndez-Ferrer, T. V. Michurina, F. Ferraro et al., “Mesenchymal and haematopoietic stem cells form a unique bone marrow niche,” *Nature*, vol. 466, no. 7308, pp. 829–834, 2010.

[179] M. P. Rettig, G. Ansstas, and J. F. Dipersio, “Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4,” *Leukemia*, vol. 26, no. 1, pp. 34–53, 2012.

[180] T.-T. Han, L. Fan, J.-Y. Li, and W. Xu, “Role of chemokines and their receptors in chronic lymphocytic leukemia: function in microenvironment and targeted therapy,” *Cancer Biology and Therapy*, vol. 15, no. 1, pp. 3–9, 2014.

[181] B. Cho, Z. Zeng, H. Mu et al., “Antileukemia activity of the novel peptidic CXCR4 antagonist LY2510924 as monotherapy and in combination with chemotherapy,” *Blood*, vol. 126, no. 2, pp. 222–232, 2015.

[182] L. Zaitseva, M. Y. Murray, M. S. Shafat et al., “Ibrutinib inhibits SDF1/CXCR4 mediated migration in AML,” *Oncotarget*, vol. 5, no. 20, pp. 9930–9938, 2014.

[183] Z. Zeng, Y. X. Shi, I. J. Samudio et al., “Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML,” *Blood*, vol. 113, no. 24, pp. 6215–6224, 2009.

[184] L. A. Pitt, A. N. Tikhonova, H. Hu et al., “CXCL12-producing vascular endothelial niches control acute T cell leukemia maintenance,” *Cancer Cell*, vol. 27, no. 6, pp. 755–768, 2015.

[185] I. Onishi, Y. Nakagawa, T. Murayama et al., “Expression of multidrug resistance 1 gene in association with CXCL12 in chronic myelogenous leukaemia,” *Pathology*, vol. 46, no. 7, pp. 623–629, 2014.

[186] S. Y. Cho, M. Xu, J. Roboz, M. Lu, J. Mascarenhas, and R. Hoffman, “The effect of CXCL12 processing on CD34+ cell migration in myeloproliferative neoplasms,” *Cancer Research*, vol. 70, no. 8, pp. 3402–3410, 2010.

[187] C. Bogani, V. Ponzi, P. Guglielmelli et al., “Hypermethylation of CXCR4 promoter in CD34+ cells from patients with primary myelofibrosis,” *Stem Cells*, vol. 26, no. 8, pp. 1920–1930, 2008.

[188] V. Rosti, M. Massa, A. M. Vannucchi et al., “The expression of CXCR4 is down-regulated on the CD34+ cells of patients with myelofibrosis with myeloid metaphasia,” *Blood Cells, Molecules & Diseases*, vol. 38, no. 3, pp. 280–286, 2007.

[189] J. Dürig, C. Rosenthal, A. Elmaagaci et al., “Biological effects of stroma-derived factor-1 on normal and CML CD34+ haemopoietic cells,” *Leukemia*, vol. 14, no. 9, pp. 1652–1660, 2000.

[190] A. Peled, I. Hardan, L. Trakhtenbrot et al., “Immature leukemic CD34+CXCR4+ cells from CML patients have lower integrin-dependent migration and adhesion in response to the chemokine SDF-1,” *Stem Cells*, vol. 20, no. 3, pp. 259–266, 2002.

[191] H. Herrmann, I. Sadovnik, S. Cerny-Reiterer et al., “Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia,” *Blood*, vol. 123, no. 25, pp. 3951–3962, 2014.

[192] L. Jin, Y. Tabe, S. Konoplev et al., “CXCR4 up-regulation by imatinib induces chronic myelogenous leukemia (CML) cell migration to bone marrow stroma and promotes survival of quiescent CML cells,” *Molecular Cancer Therapeutics*, vol. 7, no. 1, pp. 48–58, 2008.

[193] Y. Tabe, L. Jin, K. Iwabuchi et al., “Role of stromal microenvironment in nonpharmacological resistance of CML to imatinib through Lyn/CXCR4 interactions in lipid rafts,” *Leukemia*, vol. 26, no. 5, pp. 883–892, 2012.

[194] E. Weisberg, A. K. Azab, P. W. Manley et al., “Inhibition of CXCR4 in CML cells disrupts their interaction with the bone marrow microenvironment and sensitizes them to nilotinib,” *Leukemia*, vol. 26, no. 5, pp. 985–990, 2012.

[195] A. Agarwal, A. G. Fleischman, C. L. Petersen et al., “Effects of plerixafor in combination with BCR-ABL kinase inhibition in a murine model of CML,” *Blood*, vol. 120, no. 13, pp. 2658–2668, 2012.