

## The bone marrow stroma in hematological neoplasms—a guilty bystander

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**Abstract** | In the setting of hematological neoplasms, changes in the bone marrow (BM) stroma might arise from pressure exerted by the neoplastic clone in shaping a supportive microenvironment, or from chronic perturbation of the BM homeostasis. Under such conditions, alterations in the composition of the BM stroma can be profound, and could emerge as relevant prognostic factors. In this Review, we delineate the multifaceted contribution of the BM stroma to the pathobiology of several hematological neoplasms, and discuss the impact of stromal modifications on the natural course of these diseases. Specifically, we highlight the involvement of BM stromal components in lymphoid and myeloid malignancies, and present the most relevant processes responsible for remodeling the BM stroma. The role of bystander BM stromal elements in the setting of hematological neoplasms is discussed, strengthening the rationale for treatment strategies that target the BM stroma.

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### Introduction

The interaction between hematopoietic stem cells (HSCs) and bone marrow (BM) mesenchymal cells occurs within functional niches and is responsible for the maintenance of BM homeostasis. Two distinct niches with specialized microenvironments can be identified in the BM that are able to support localization, survival, and self-renewal of stem cells—the osteoblastic (or endosteal) niche and the vascular niche.<sup>1</sup> The osteoblastic niche, in close proximity to bone trabeculae and mainly composed of endosteal fibrocytes and osteoblasts, is associated with HSCs showing a maturation gradient towards the intertrabecular areas of the BM parenchyma.<sup>2</sup> The vascular niche displays a broad distribution along the BM parenchyma and is composed of endothelial cells and other vascular components, such as smooth muscle cells and adventitial reticular cells.<sup>3</sup> The microenvironment of each niche is interconnected, forming a complex network, but the two niches differentially influence HSCs. The osteoblastic niche favors HSC homing during development through chemotactic signals that originate from the interaction between CXCL-12 (SDF-1) and its receptor CXCR-4 on HSCs. This process is recapitulated by transplanted HSCs and several neoplastic populations that display BM tropism.<sup>4</sup> The osteoblastic niche also provides a nest for HSCs, in which signals from endosteal fibroblasts and osteoblasts maintain their quiescence while enabling self-renewal. These signals include cell–cell and cell–matrix adhesive interactions mediated by  $\beta$ 1 integrins ( $\alpha$ 4,  $\alpha$ 7, and  $\alpha$ 9) and their ligands VCAM-1, type I collagen, tenascin-C, fibronectin, laminin, and osteopontin.<sup>5</sup> In addition to cell adhesion pathways, the

Notch pathway is involved in both BM homeostasis and in the pathogenesis of lymphoid and myeloid malignancies.<sup>6</sup> In particular, engagement of Notch on HSCs prevents HSC differentiation, and contributes to their quiescence in the osteoblastic niche.<sup>5,7</sup> In addition to cellular components, soluble factors such as growth factors, cytokines, and matrix components also contribute to the composition of the BM osteoblastic and vascular niches to differently regulate HSC proliferation, mobilization, and differentiation dynamics (Table 1).

The vascular niche delivers maturation and activation signals to differentiating cells and controls the intravasation and extravasation of BM and foreign elements.<sup>2</sup> Blood vessels are evenly distributed throughout the BM parenchyma and considerable vascularity is also observed in the BM osteoblastic niche (Figure 1), where the unique composition of the extracellular matrix (ECM) and soluble mediator milieu renders the osteoblastic niche functionally different from that of the vascular niche. Cells that are characteristic of the vascular niche include endothelial cells, BM mesenchymal stem cells (BM-MSCs) that express the markers Nestin and CD146, and stromal cells expressing CD10, which all display different activation and adhesion phenotypes that influence their preference for selective hematopoietic elements.<sup>8,9</sup> Lineage fate can be determined by stromal cues. Multipotent, transformed CD34<sup>+</sup> human cord blood cells transplanted into immunodeficient mice either lacking  $\beta$ 2-microglobulin or expressing myeloid growth factors resulted in leukemias of lymphoid or myeloid lineages, respectively.<sup>10</sup>

The adipocyte component of the BM cannot be easily assigned to either of the two specialized niches. BM adipocyte content shows an inverse correlation with the

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### Competing interests

The authors declare no competing interests.

hematopoietic cellularity in physiological (age-related changes) and pathological (BM failure) conditions. Adipocytes have an active role in regulating hematopoietic homeostasis through a direct suppressive influence on HSCs by reducing G-CSF and GM-CSF, and the release of adiponectin and tumor necrosis factor (TNF).<sup>11</sup>

The BM stroma has a critical role in the maintenance, renewal, and differentiation of HSCs. In lymphoid malignancies, the crosstalk between the BM stroma and neoplastic cells eventually contributes to the establishment of a permissive microenvironment for clone survival and progression. In myeloid malignancies, the stromal involvement is frequently characterized by profound changes, including angiogenesis, fibrosis, and/or new bone formation (osteosclerosis), which may retain clinical and prognostic significance as predictors of BM failure.<sup>12–15</sup> We highlight the role of the BM stroma in the development and course of lymphoid, plasma cell, and myeloid malignancies. The direct interactions of neoplastic elements with the BM stroma, its clone-related modifications, and its emerging immunomodulatory role eventually favoring the generation and progression of clones are discussed.

### The BM stroma in lymphoid neoplasms

BM infiltration is a common feature of lymphoproliferative disorders, characterized by specific patterns depending on the disease entity. The relationship between BM infiltration and other histological parameters (including tissue architecture, stromal organization, and fibrosis) possibly reflects specific interactions with microenvironmental factors of the BM.<sup>16–18</sup> In most low-grade lymphomas, BM infiltration is associated with blood vessel proliferation and marked differences in the distribution of lymphoma and stromal cells.<sup>19</sup> Aggregates in follicular lymphoma, lymphoplasmacytic lymphoma, and mantle-cell lymphoma seem to maintain contact, at least initially, with the BM stromal architecture, whereas aggregates in chronic lymphocytic leukemia (CLL) and marginal zone lymphomas predominantly displace the pre-existing stroma.<sup>19</sup> Several factors, including adhesion molecules, contribute to the variation in BM-infiltration dynamics among lymphoid neoplasms. Indeed, leukemias and lymphomas that express BM-homing markers, such as CD44 (hyaluronan receptor) and/or CD22, but lack adhesive ligands required for the stable adhesion to the sinusoidal endothelium (E-selectin and P-selectin ligands), display a specific intrasinusoidal infiltration (Figure 1c).<sup>20</sup>

Lymphoma cells that spread to the BM interstitium or form nodules are associated with the expression of a broader spectrum of adhesion markers, including P-selectin glycoprotein ligand-1 (CD162), and the integrins  $\alpha_1\beta_2$  and  $\alpha_4\beta_1$  (ligands of VCAM-1 and ICAM-1, respectively).<sup>20</sup> B-cell and T-cell leukemias and lymphomas characterized by an intrasinusoidal infiltration pattern in the BM, such as splenic marginal zone lymphomas, hairy-cell leukemia, and hepatosplenic T-cell lymphoma, frequently involve the spleen and the liver, where they display a prominent intravascular distribution.<sup>21</sup> Such characteristic behavior might imply a neoplastic cell preference for the BM vascular niche, which shares several

### Key points

- BM stromal changes in lymphoid malignancies are engendered by neoplastic cells to support their localization, proliferation and survival, and to suppress effective antitumor immune responses
- Lymphoid neoplastic clones are able to manipulate the BM environment either directly, or through the co-optation of accessory cells such as macrophages and mast cells
- Treatment strategies interfering with the axes involved in crosstalk between neoplastic cells and BM stroma may prove effective in lymphoid malignancies, when combined with therapies that target the neoplastic clones
- Stromal alterations associated with myeloid malignancies, such as BM fibrosis, could be profound and negatively influence the clinical course of the disease and response to therapy
- Drugs that could potentially control the proliferation of BM stromal components, such as tyrosine kinase inhibitors, proteasome inhibitors, and immunomodulatory agents are promising for the treatment of myeloid malignancies

features with the spleen and liver sinusoidal compartment, characterized by fenestrated sinusoids abundantly exposing hyaluronan and prominent subendothelial reticular cells expressing CXCL12. The tight interaction with the vascular niche through the CXCL12/CXCR4 axis may represent a drug resistance mechanism, and anti-CXCR4 therapeutic strategies have been proposed.<sup>22</sup>

Other lymphomas that infiltrate the BM, display a specific paratrabeular pattern in the osteoblastic niche (Figure 1d).<sup>16,17</sup> Non-Hodgkin lymphomas derived from germinal centers are often associated with large infiltrates, BM fibrosis, and megakaryocytic hyperplasia.<sup>17</sup> The prototypic example is follicular lymphoma, commonly presenting with paratrabeular aggregates containing small T lymphocytes, probably recruited through CCL17/CCL22, and possibly sustaining the neoplastic clone.<sup>23</sup> A similar BM histopathology also extends to other B-cell malignancies (for example, T-cell/histiocyte-rich diffuse large B-cell lymphoma) and T-cell lymphomas of follicular derivation (angioimmunoblastic T-cell lymphoma).<sup>24–26</sup> The extracellular meshwork of lymphoid follicles is associated with the stromal follicular dendritic cell and microvascular network, and shares several components with that of the BM osteoblastic niche, including integrin- $\beta_1$ , type I collagen, and matricellular protein expression.<sup>27,28</sup> These elements might provide a common adhesive background to neoplastic cells by favoring their location in the BM osteoblastic compartment, and signaling through the Notch stromal ligands Delta-like-1 and Jagged-1.<sup>29</sup>

Thus, the BM stroma may condition the degree and pattern of lymphoid infiltration and contribute to neoplastic cell survival. Notably, besides selectively homing to specific favorable microenvironments, neoplastic lymphocytes can actively promote BM stroma modifications to produce an *ad hoc* neoplastic niche.<sup>30</sup> These modifications mainly include blood vessel formation, and changes in the adhesion profile of the stromal meshwork (Figure 2).

In xenograft models of B-cell and T-cell acute lymphoblastic leukemia (ALL), leukemic cells affect the BM vascular niche by promoting angiogenesis through the release of proangiogenic molecules, including VEGF, bFGF, angiopoietins, metalloproteinases, interleukin (IL)-6 and IL-8,

**Table 1** | Soluble factors involved in BM niche maintenance

Reference	Factor	Function
<b>Growth factors</b>		
Sugiyama <i>et al.</i> (2006) <sup>4</sup>	CXCL-12	HSC homing to the BM niche
Miura <i>et al.</i> (2006) <sup>125</sup>	PDGF	Promotes the formation of BM niches Directs the fate of MSC
Gotoh <i>et al.</i> (2009) <sup>126</sup>	bFGF	Maintains the self-renewal and efficient proliferative capabilities of stem cells BM-MSD differentiation
Blank <i>et al.</i> (2008) <sup>5</sup>	VEGF	Organization of the vascular niche Improves survival of MSC
<b>Cytokines</b>		
Karlsson <i>et al.</i> (2009) <sup>127</sup>	TGF- $\beta$	It is a potent inhibitor of HSC proliferation <i>in vitro</i> , its function <i>in vivo</i> is context-dependent Dispensable effect <i>in vivo</i> related to dependence upon redundant processes and interactions with other growth factors BM-MSD differentiation
Fibbe <i>et al.</i> (1991) <sup>128</sup>	IL-1	Maintenance and self-renewal of uncommitted progenitors Acts synergistically with colony-stimulating factors in the proliferation of primitive HSCs
Kopf <i>et al.</i> (1995) <sup>129</sup>	IL-6	Increases proliferation and maintains primitive hematopoietic progenitor cells Stimulates megakaryocytopoiesis and the formation of monocytic colonies from hematopoietic progenitors IL-6-deficient mice have reduced numbers of early progenitors in the bone marrow and blood
Pearl-Yafe <i>et al.</i> (2010) <sup>130</sup>	TNF	It acts as negative regulator of differentiated clone expansion Supports murine hematopoietic progenitor function in the early stages of engraftment
<b>Other</b>		
Naveiras <i>et al.</i> (2009) <sup>11</sup>	Adiponectin	Increases HSCs proliferation while retaining the cells in a functionally immature state
Haylock <i>et al.</i> (2006) <sup>131</sup>	Osteopontin	Synthesized by osteoblasts has critical roles in HSC regulation Attraction and retention of HSC in the endosteal niche Negative regulator of HSC proliferation
Emerson <i>et al.</i> (2007) <sup>132</sup>	Thrombopoietin	Survival and maintenance of HSC survival at the osteoblast surface
Abbreviations: bFGF, basic fibroblast growth factor; BM, bone marrow; CXCL-12, C-X-C motif chemokine 12; HSC, hematopoietic stem cell; IL, interleukin; MSC, mesenchymal stem cell; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.		

and eventually establishing a niche that promotes ALL survival via IL-7 and IL-3.<sup>30</sup> The leukemic niche is characterized by low HSC support capability owing to down-regulated CXCL12 stromal expression, yet it outcompetes native stromal niches for HSC engraftment owing to production of stem-cell factor by neoplastic cells.<sup>31</sup> In addition to ALL, increased BM angiogenesis is associated with unfavorable prognosis in several other lymphoid malignancies, including CLL.<sup>32,33</sup> In specific settings, such as in angioimmunoblastic T-cell lymphoma, overexpression of VEGF may not only sustain the increase in microvessel density, but also promote tumor cell proliferation and survival by autocrine and paracrine loops.<sup>34</sup> Expression of CD38 and CD49d adhesion molecules by the neoplastic clone in CLL has been associated with a more-aggressive disease course.<sup>35</sup> CD38 engagement by CD31 favors the synthesis and release of CCL3 and CCL4 chemokines by neoplastic cells, which induce the recruitment of CD68<sup>+</sup> macrophages at sites of lymphoid infiltration.<sup>35</sup> Recruited macrophages influence the activation of BM stromal cells, inducing expression of VCAM-1 and release of proinflammatory cytokines such as TNF (Figure 2).<sup>35</sup> A strong prosurvival feedback signal is delivered to CLL cells by the concomitant CD38/CD31 and CD49d/VCAM-1 pathway. Similarly, other lymphoid neoplasms may benefit from the recruitment of cellular partners for the

induction of stromal modifications at sites of infiltration. This process may occur in lymphoplasmacytic lymphoma and in angioimmunoblastic T-cell lymphoma, in which lymphomatous infiltrates may be enriched by mast cells attracted by neoplastic cell-derived CCL5 and CXCL13.<sup>36</sup> Lymphoma-infiltrating mast cells may foster neoplastic growth and survival through the CD40L/CD40 or OX40L/OX40 axes, and also contribute to the orchestration and maintenance of a supportive stromal microenvironment through the synthesis of mediators, including TNF, IL-6, matrix metalloproteinase-9, and interferon- $\gamma$ .<sup>36</sup>

Nodular BM infiltrates from mature B-cell lymphoproliferative disorders may foster a meshwork of mesenchymal cells that express markers of follicular dendritic cells, such as CD21, conferring to neoplastic aggregates the appearance of 'true follicles' (Figure 1e). Although the occurrence of a follicular organization in BM lymphoid infiltrates has never been related with specific biological features of the neoplastic clone, interplay between such follicular dendritic cells and neoplastic cells can be predicted in terms of cell adhesion (for example, mediated by ICAM-1 expression) and antigenic stimulation (Figure 1f). Interestingly, signals stemming from surface antigen receptors and signals derived from the actin network may support activation and survival of lymphoid cancer cells through a common pathway.<sup>22,37</sup>



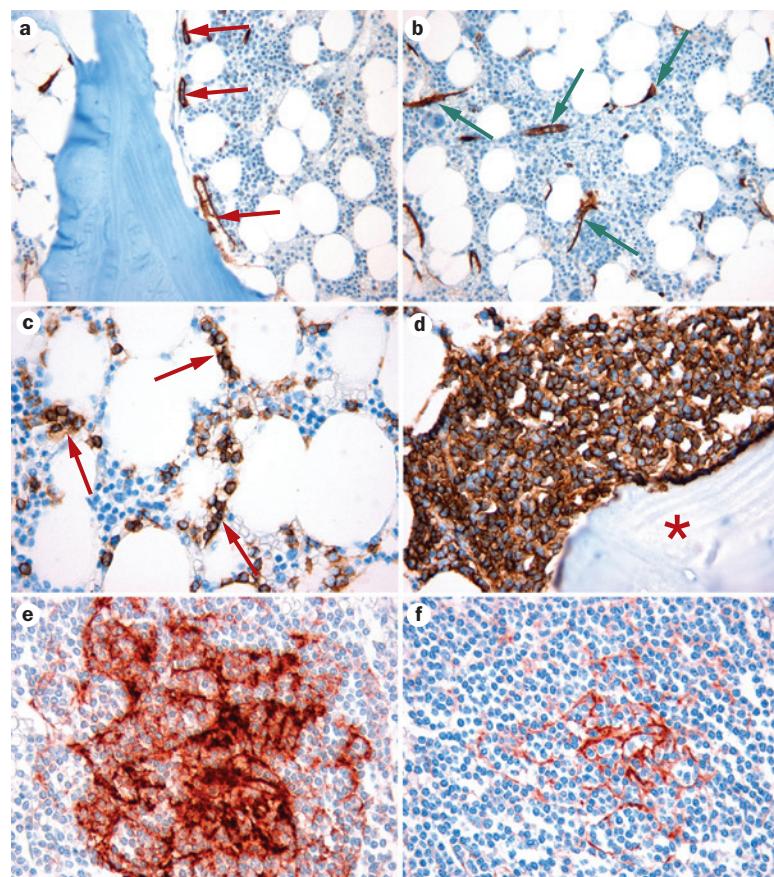
BM stromal changes associated with lymphoid neoplasms might also comprise reticulin fibrosis and fibroblastic proliferation. The former is often slight to moderate and associated with specific lymphoproliferative neoplasms, such as hairy-cell leukemia or peripheral T-cell lymphomas, whereas the latter is mainly observed in BM infiltrates from classic Hodgkin lymphoma.<sup>38</sup> Importantly, the degree of fibrosis may be helpful in differentiating non-Hodgkin lymphoma and benign lymphoid hyperplasia characterized by central localization and absence of considerable amounts of reticulin.<sup>39</sup>

Available evidence indicates that neoplastic lymphocytes can shape the neighboring BM microenvironment by directly affecting stromal composition or co-opting other immune effectors that are active in tissue remodeling (Figure 2). Notably, such changes in the BM microenvironment eventually favor neoplastic cell survival and influence the clinical course of the disease.

### The BM stroma in multiple myeloma

Although the BM is the primary site of multiple myeloma, it is not the site of origin. Emerging data indicate that the malignant plasma cell arises from a postgerminal center B cell that has undergone somatic hypermutation, lacks intracлонаl variation, and expresses CD19 and CD27, consistent with a central memory B-cell phenotype.<sup>40</sup> Interestingly, several groups have identified critical cell populations for multiple myeloma BM engraftment, ranging from CD38<sup>high</sup>/CD19<sup>+</sup>/CD138<sup>-</sup> to CD19<sup>+</sup>/CD27<sup>+</sup>/CD138<sup>-</sup>.<sup>41,42,43,44</sup> This latter population has self-renewal potential, which supports the hypothesis of a memory B cell as the cell responsible for sustaining the malignant phenotype in plasma cell dyscrasias. The presence of this population in the peripheral circulation raises the question of what mechanisms regulate trafficking and homing to the BM in plasma cell dyscrasias and how neoplastic-cell survival is maintained within the BM microenvironment.

Many factors are involved in the crosstalk between multiple myeloma cells and the BM stroma, including soluble factors, such as chemokines, cytokines, growth factors, cell-surface receptors, ECM proteins, and cellular constituents of the BM stromal niches. BM stromal cells contribute to IL-6 synthesis, which in turn stimulates multiple myeloma-cell proliferation and confers chemoresistance through activation of pathways, such as PI3K/Akt, MAPK, and Janus-kinase/STAT3, and synergizes with adhesive  $\beta_1$ -integrin signals to increase survival of multiple myeloma cells.<sup>45,46</sup> Interestingly, IL-6 production by BM stromal cells requires multiple myeloma-cell binding and subsequent activation through nuclear factor kappa B signaling.<sup>47</sup> IGF-1 can overcome the effects of IL-6-blockade in multiple myeloma through binding to its receptor IGF-1R.<sup>48</sup> IGF-1R expression has been associated with more-aggressive disease and is found to a greater extent in the poor prognosis t(4;14) multiple myeloma.<sup>48</sup> High circulating levels of IGF-1 are associated with a dismal prognosis.<sup>49</sup> Through binding of IGFBP-3, IGF1 is targeted to multiple myeloma cells that express high levels of the cell surface adhesion molecule syndecan-1 (CD138). CD138 mediates IGF-1 uncoupling from IGFBP-3 and enables

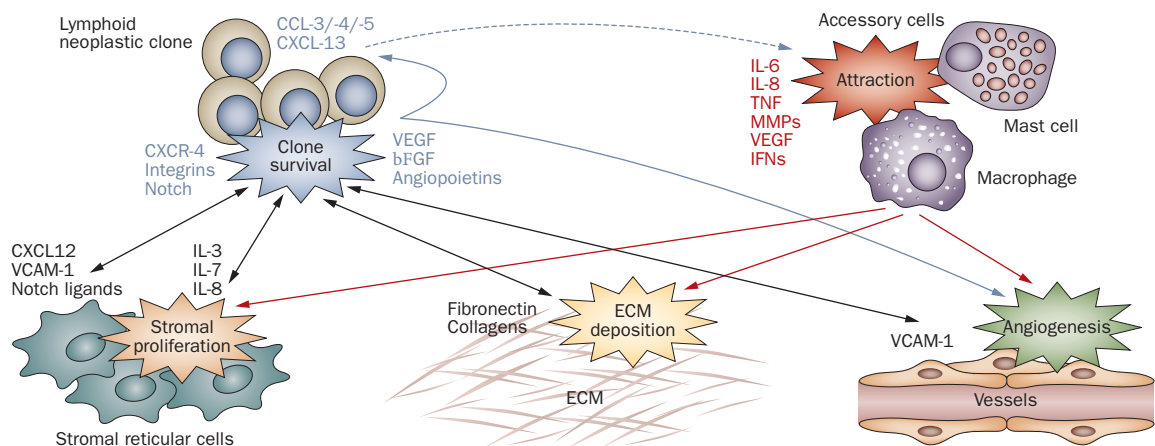


**Figure 1** | BM stroma and lymphoid infiltration. **a** | CD34 immunostaining highlights the presence of blood vessels in the osteoblastic compartment of the BM lining the bone trabeculae (red arrows). **b** | A high density of finely branching vessels (green arrows) is observed in the intertrabecular space of the BM, which corresponds to the vascular niche. **c** | CD20 immunostaining reveals the presence of neoplastic B cells (red arrows) lodged inside dilated bone marrow sinusoids in a case of splenic marginal zone lymphoma with prominent intrasinusoidal infiltration. **d** | Aggregates of CD20<sup>+</sup> neoplastic B cells can be observed adjacent to the endosteal lining of the bone trabeculae (asterisk) in a case of follicular lymphoma with prominent BM paratrabecular infiltration. **e** | The follicular dendritic cell meshwork of a BM nodular infiltrate is highlighted by CD23 immunostaining in a case of splenic marginal zone lymphoma. **f** | In the same nodule, follicular dendritic cells provide an adhesive background to neoplastic B cells due to the expression of intercellular adhesion molecule 1. Abbreviation: BM, bone marrow.

IGF-1 interaction with its receptor on multiple myeloma cells, in turn promoting cell survival and proliferation.<sup>48</sup> Furthermore, soluble CD138 also binds IGF-1, leading to further increased local delivery of IGF-1 to myeloma cells, representing a link between adhesion and soluble mediator-induced cell survival.<sup>48</sup>

### Role of RANKL

Additional soluble factors have been implicated in the osteoclast activation leading to lytic bone disease. RANKL produced by stromal cells and osteoblasts binds to RANK on osteoclasts resulting in osteoclast differentiation.<sup>50</sup> Osteoprotegerin is a stromal-derived RANKL inhibitor that regulates the amount of functional RANKL. Myeloma induces upregulation of RANKL and inhibition of osteoprotegerin, thus altering the ratio of these proteins and



**Figure 2** | Schematic representation of the interactions between lymphoid neoplastic cells and the BM stromal microenvironment. The lymphoid neoplastic clone can directly interact with BM stromal elements through several axes (gray arrows), including adhesion molecules and their cellular and extracellular ligands, growth factors, cytokines, and chemokines. The combined effects of these interactions sustain neoplastic clone growth and survival. The neoplastic clone can also induce modifications in the BM stromal environment either directly by influencing angiogenesis (blue arrow), or indirectly by recruiting other immune effectors such as macrophages and mast cells at sites of infiltration (dashed blue arrow). Through the synthesis of a whole plethora of mediators, these recruited cells induce changes in the BM stromal composition by promoting angiogenesis, ECM deposition, and stromal cell proliferation (red arrows). Abbreviations: bFGF, basic fibroblast growth factor; CCL, CC-motif chemokine ligand; CXCL, CXC-motif chemokine ligand; CXCR, CXC-motif chemokine receptor; ECM, extracellular matrix; IFN, interferon; IL, interleukin; MMP, metalloproteinase; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

enhancing osteoclast activation.<sup>51</sup> Macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ) is produced by myeloma cells, stimulates multiple myeloma cell proliferation and survival, macrophage recruitment, and osteoclast formation. Moreover, it increases interactions between myeloma and stromal cells through upregulation of RANKL. Interestingly, MIP-1 $\alpha$  levels in the BM correlated with disease stage and activity.<sup>52</sup> Another link between multiple myeloma and BM stroma is Dickkopf-1, a Wnt antagonist that increases BM-MSC survival, inhibits osteoblast differentiation, and increases osteoclast-mediated bone resorption, which eventually alters the osteoblastic BM stromal niche.<sup>53</sup> The complex network of soluble factors produced by the myeloma-associated microenvironment is critical for promoting and maintaining multiple myeloma survival within the BM niche.

### Role of the immune system

The clinical manifestations of multiple myeloma result from the complex interactions between tumor cells, BM stromal elements, and the immune system. Effective T-cell recognition of the embryonal antigen SOX2 in the pre-malignant condition known as monoclonal gammopathy of unknown significance (MGUS), inhibits the clonogenic growth of MGUS cells. Cellular immunity to SOX2 is lost as disease progresses to more active forms, indicating a role in immune surveillance; however, the factors responsible for this loss of immunity remain unknown.<sup>54</sup>

In multiple myeloma, a Th17-oriented immunological microenvironment could also have a relevant role in maintaining myeloma cell survival. Resident BM dendritic cells can induce a Th17 phenotype in multiple myeloma.<sup>55</sup> The Th17 phenotype in the BM tightly correlates with the development of osteoclast-induced bone disease,

independently of tumor burden. In one study, activation of BM-infiltrating lymphocytes to a Th1 phenotype dramatically reduced osteoclast differentiation.<sup>56</sup> Moreover, an interaction between IL-17 and IL-17R with the BM microenvironment and myeloma cells augments myeloma cell growth.<sup>57</sup> Taken together, these findings suggest an intricate immune network of multiple myeloma cells, T cells, and stromal cells that all contribute to multiple myeloma cell survival and osteolytic bone disease. Notably, immunomodulatory agents, such as thalidomide, lenalidomide, and the proteasome inhibitor bortezomib for the treatment of multiple myeloma,<sup>58</sup> interfere with the malignant immunological and stromal BM microenvironment restoring an effective immune response against the neoplastic clone. These drugs also limit the proinflammatory pressure of multiple myeloma-associated BM accessory cells, such as dendritic cells and macrophages,<sup>59</sup> and contribute to the normalization of angiogenesis,<sup>60</sup> thus rendering the BM milieu less supportive of multiple myeloma growth and survival.

### The BM stroma in myeloid neoplasms

Myeloid neoplasms are a heterogeneous group of clonal HSC disorders. Although alterations in the BM stroma in these neoplasms are considered secondary to cytokine effects, the BM stroma is not just an 'innocent bystander' but has an active role in the pathogenesis and disease progression, affecting both clinical course and response to therapy. The major morphologically recognizable BM stromal alterations include fibrosis (Box 1), angiogenesis, and osteosclerosis. Such stromal changes have been described in acute myeloid leukemia (AML), myelodysplastic syndromes, and myeloproliferative neoplasms.



### Acute myeloid leukemia with fibrosis

Two AML subtypes are consistently associated with stromal fibrosis: acute panmyelosis with myelofibrosis (APMF), and acute megakaryoblastic leukemia. APMF accounts for <1% of AML cases and its diagnostic criteria have been clearly outlined.<sup>61,62</sup> Acute megakaryoblastic leukemia accounts for 3–5% of AML and its diagnosis requires >20% blasts, with >50% showing megakaryocytic differentiation.<sup>62</sup> Both diseases are characterized by dysplastic dwarf megakaryocytes with hypolobulated or non-lobulated nuclei, and a marked degree of reticulin fibrosis; the latter is most likely owing to abnormal cytokine release from megakaryocytes. The BM stroma composition is altered in AML as a result of cytokine and growth factor production from the neoplastic clone, which increases leukemia growth and progression through the interaction with the modified BM stroma.<sup>63,64</sup> It is not known whether the altered BM microenvironment occurring in AML actively promotes leukemia development or is a secondary change. New therapeutic approaches targeting the BM environment in AML, such as inhibitors of CXCL12/CXCR4 axis, integrin inhibitors, and antiangiogenic agents are being evaluated.<sup>65–67</sup>

### Myelodysplastic syndromes with fibrosis

Myelodysplastic syndromes with fibrosis refer to cases with fibrosis grade  $\geq 2$  based on the European Consensus Grading System (Box 1).<sup>68</sup> Most patients with this condition have multilineage dysplasia, high transfusion requirement, and aggressive disease course.<sup>15,69</sup> In patients stratified according to the International Prognostic Scoring System (IPSS) and WHO classification-based Prognostic Scoring System (WPSS),<sup>70,71</sup> the presence of fibrosis places the patient in a more-advanced risk group, representing an independent prognostic factor that could be useful in clinical decision making.<sup>15</sup> Patients with grade 2 or grade 3 fibrosis might be considered for aggressive treatment earlier than the prognostic score would indicate.<sup>15</sup>

### Myeloproliferative neoplasms

The group of myeloproliferative neoplasms (MPN) is broadly divided into Philadelphia-chromosome-positive/BCR-ABL-positive chronic myelogenous leukemia (CML) and Philadelphia-chromosome-negative/BCR-ABL-negative MPN (Ph-MPN). In CML, the presence of marked fibrosis has been associated with a worse outcome than cases without fibrosis, which remains true even in the current imatinib era.<sup>72</sup> Ph-MPN includes essential thrombocythemia, polycythemia vera, and primary myelofibrosis (PMF).<sup>73</sup> Common stromal alterations in these diseases are BM fibrosis, osteosclerosis, and increased angiogenesis.<sup>74</sup> Patients with essential thrombocythemia usually display no or only minimal fibrosis; the presence of fibrosis in such patients should prompt exclusion of early polycythemia vera or PMF as an alternative diagnosis.<sup>74</sup> This diagnosis has a considerable prognostic implication since most cases of essential thrombocythemia do not progress to myelofibrosis and have a favorable outcome.<sup>75</sup>

#### Box 1 | Assessment of bone marrow fibrosis

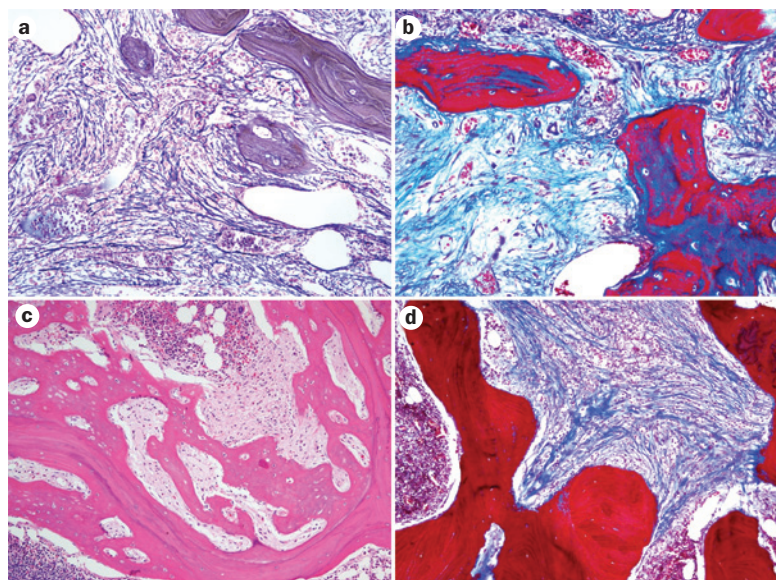
In routine clinical practice, evaluation of bone marrow fibrosis includes assessing the quantity and pattern of reticulin fiber deposition as demonstrated by a reticulin stain, such as Gomori methenamine silver stain. Reticulin stains all types of fibers, including mature collagen (type I collagen). The presence of type I collagen, as seen in cases of severe myelofibrosis, can be most specifically demonstrated by Masson's trichrome stain. The evaluation of fibrosis in the clinical setting is performed using Gomori-stained bone marrow biopsies in which the amount of fibrosis is assessed semi-quantitatively by using, for example, the European Consensus Grading System.<sup>47</sup> This method, originally proposed for assessing fibrosis in primary myelofibrosis, distinguishes four degrees of fibrosis (MF-0 to MF-3) with MF-0 indicating normal fiber content:

- MF-0: Scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow
- MF-1: Loose network of reticulin with many intersections, especially in perivascular areas
- MF-2: Diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis
- MF-3: Diffuse and dense increase in reticulin with extensive intersections of coarse collagen bundles, often associated with substantial osteosclerosis

Patients with PMF and polycythemia vera can develop overt marrow fibrosis as the disease progresses from a cellular phase to a 'terminal' myelofibrotic/osteosclerotic phase associated with leukoerythroblastosis and extramedullary hematopoiesis, where the latter is usually manifested as splenomegaly. In the fibrotic stage of PMF, the marrow cellularity shows patchy hematopoiesis alternated with hypocellular fibrotic areas often surrounded by osteosclerotic bone (Figure 3a,b). Atypical megakaryocytes forming tight clusters can be found even in the hypocellular areas, and dilated marrow sinuses, often containing intravascular hematopoiesis, are other typical findings.

Disease progression occurs within the first 5 years of observation in about 30% of patients with PMF diagnosed with early-stage disease.<sup>74</sup> The degree of BM fibrosis is an important prognostic factor in PMF as patients with grade 3 fibrosis have a significantly shorter overall survival than patients with grade 0, 1, or 2 fibrosis ( $P=0.001$  and  $P=0.003$ , respectively).<sup>14</sup> In patients with advanced PMF, the stromal changes are the most prominent feature of the pathology, and are related to the development of extramedullary hematopoiesis, whose clinical manifestations account for significant morbidity.<sup>74</sup>

The myelofibrosis and osteosclerosis seen in MPN have a complex pathogenesis. The release of profibrogenic and proangiogenic cytokines, such as PDGF, FGF, TGF- $\beta$ , and VEGF, from neoplastic myeloid cells, activate BM stromal cells, which induces excess ECM deposition and triggers angiogenesis.<sup>76–78</sup> Stromal changes relating to MPN indicate a proliferation of adventitial reticular cells, which can be assessed with the use of antibodies for low-affinity nerve growth factor, and the enrichment of the MSC component, which can be identified by CD146 immunostaining.<sup>79,80</sup> Increased production of osteoprotegerin and synthesis of bone morphogenetic protein-1 might also contribute to the unbalanced osteoblastic activity, which results in the osteosclerosis frequently seen in patients with APMF.<sup>76,77</sup>



**Figure 3** | Bone marrow stromal changes in myeloid malignancies. Bone marrow biopsy section stained for **a** | reticulin (Gomori stain) and **b** | trichrome. Both stains highlight the presence of severe myelofibrosis with abundant collagen deposition (MF-3 grade according to the European Consensus Grading System). **c** | A bone marrow biopsy section stained with hematoxylin and eosin showing compact aggregates of abnormal mast cells surrounded by dense fibrosis, typical of systemic mastocytosis. **d** | A corresponding trichrome-stained section confirms the presence of abundant collagen fibrosis.

Alterations of the CXCL12/CXCR4 axis have been extensively investigated in myeloid neoplasms. In PMF, HSCs display decreased CXCR4 expression and the BM milieu is deprived of functional CXCL12 because of increased CXCL12 uptake by neoplastic megakaryocytes and truncated inactive CXCL12 owing to action of matrix metalloproteinases.<sup>81,82</sup> The lack of CXCL12 in conjunction with decreased CXCR4 might prevent HSCs from localizing to the osteoblastic niche, thus facilitating their exit from the BM into the peripheral circulation, and homing to extramedullary sites. Since therapies targeted at HSC alone have limited efficacy in advanced MPN, attempts at correcting stromal abnormalities might represent a useful approach. In patients with PMF, a total regression of the pre-existing fibrosis is usually seen within 6–12 months of successful stem-cell transplantation,<sup>83</sup> although osteosclerosis was unchanged during this timeframe.<sup>83</sup>

### Systemic mastocytosis

Systemic mastocytosis is another myeloid neoplasm with characteristic stromal alterations. Systemic mastocytosis BM lesions are characterized by abundant reticulin and collagen fibrosis, reduced vascularization, and a paucity of cells that express low-affinity nerve growth factor receptor and/or show myofibroblastic differentiation (Figure 3c,d).<sup>79</sup> The stromal changes in systemic mastocytosis are different, therefore, from those observed in PMF. The paucity of microvessels in systemic mastocytosis fibrotic aggregates raise doubts about the effectiveness of antiangiogenic treatments in this disease.<sup>84</sup> Therapies for systemic mastocytosis include observation or cytoreductive therapy including IFN alpha, 2-chlorodeoxyadenosine, and imatinib mesylate.<sup>85</sup>

### Angiogenesis in myeloid neoplasms

Increased angiogenesis has been found in the BM of patients with AML, myelodysplastic syndromes, and MPN.<sup>12,79,86–91</sup> Results obtained from patients with myelodysplastic syndromes indicate a correlation between increased angiogenesis and progression to AML and CML transformation.<sup>90,91</sup> In patients with CML treated with imatinib, a decrease in microvessel density attributed to reduction in BCR-ABL-mediated secretion of VEGF, has been associated with reversal of fibrosis and cytogenetic and molecular response.<sup>92</sup> In Ph-MPN, the extent of angiogenesis was much greater in PMF than in polycythemia vera or essential thrombocythemia.<sup>12</sup> In PMF, microvascular proliferation is thought to be associated with increased passage of progenitor or precursor cells from the BM, their circulation in the peripheral blood, and subsequent development of extramedullary hematopoiesis.<sup>12</sup> Use of therapeutic agents with anti-angiogenic activity in PMF is being evaluated in several clinical trials.<sup>93–96</sup> Ongoing studies support the hypothesis that angiogenesis has an important role in the pathogenesis of myeloid neoplasms, and the promise of a possible role for antiangiogenic therapies in this setting.<sup>97</sup>

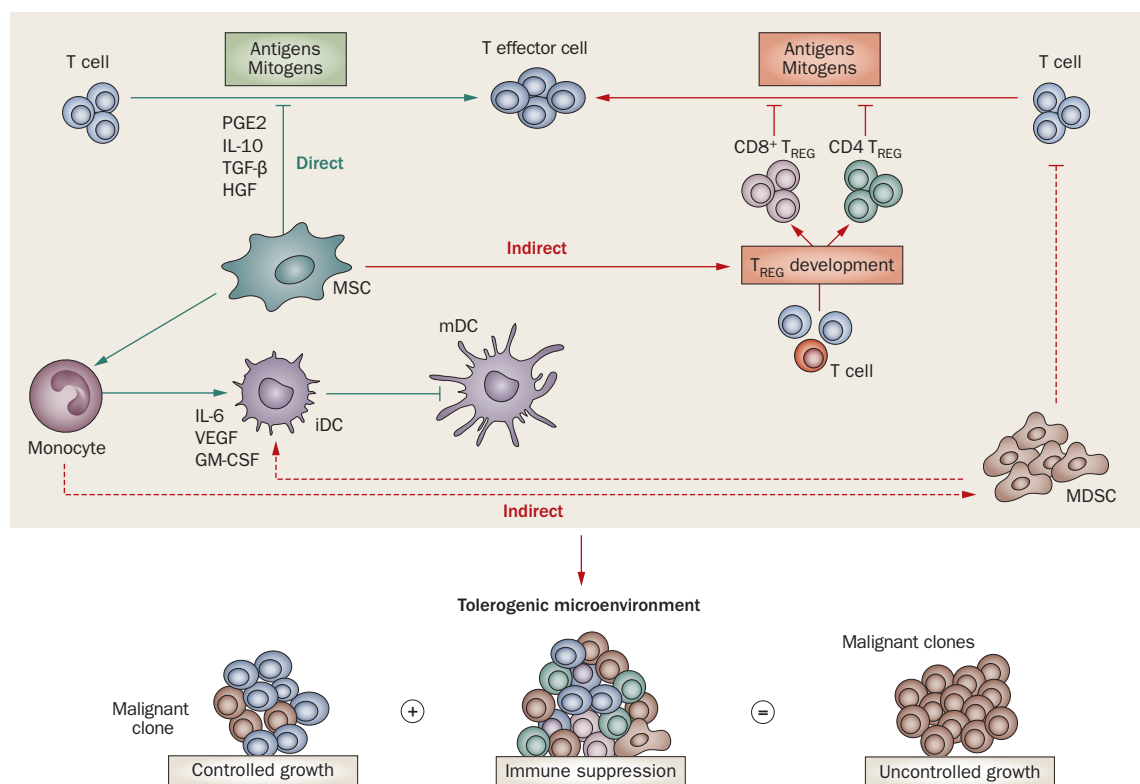
### Immunomodulation by BM stromal cells

BM stromal cells have a distinct immunological role from that of stromal cells of secondary lymphoid organs, which organize the memory response, or initiate the adaptive immune response, respectively.<sup>98</sup> Specialized BM stromal cells provide a survival niche for plasma cells and helper T lymphocytes. Similar mechanisms can operate to support survival of lymphoid malignant clones, whereas opposing mechanisms could enable hematological malignancies to escape from the control of the immune system.

Immune escape could occur in malignancies whose immunogenicity is confirmed by the identification of specific tumor-associated antigens. Chromosomal translocation can generate new antigens potentially perceived as non-self by the immune system. An example of this is the BCR-ABL fusion protein in CML, which is recognized by T lymphocytes.<sup>99,100</sup> Also, the proteins elastase and proteinase 3 induce cytotoxic T-cell responses against CML.<sup>101</sup> In both AML and CML, the Wilms tumor gene product, *WT1*, is a target of cytotoxic T cells and a potential candidate for immunotherapy.<sup>102</sup> These examples support the existence of immune suppression, which enables escape from immune control.

In the BM environment, BM-MSCs are capable of immune regulation in several settings, including transplantation, tolerance and, indeed, tumor evasion.<sup>103</sup> BM-MSCs exert their immunosuppressive functions directly on B cells, T cells, and dendritic cells via the release of specific soluble mediators, and indirectly through regulatory cells such as T regulatory ( $T_{REG}$ ) cells and, probably, myeloid-derived suppressor cells (Figure 4).

BM-MSCs can inhibit the proliferation of T cells in response to alloantigens, mitogens, CD3 and CD28 as well as the proliferation and immunoglobulin secretion of reactive B cells to CD40 or IL-4.<sup>104–106</sup> The most relevant mediators of such effects include TGF- $\beta$ , hepatocyte



**Figure 4** | Possible suppressive mechanisms of BM stromal cells. Through a direct suppressive mechanism, MSCs produce soluble factors, such as PGE<sub>2</sub>, IL-10, TGF-β, HGF, that inhibit responding T cells or block DC maturation from BM progenitors (IL-6) into a tolerogenic phenotype. Through an indirect mechanism, MSCs induce regulatory T cells that can either suppress effectors or block APC function. Factors released by MSCs are also expected to induce MDSC, the other suppressive population that together with regulatory T cells increase in patients with hematological malignancies. Immune suppression by MSC might have a complex role in hematological neoplasms by directly contribute in controlling the proliferation of the malignant clone by limiting proinflammatory signals and then exerting a prevalent suppressive function on antitumor immunity, thus contributing to the uncontrolled growth of the malignant clone. Abbreviations: APC, antigen presenting cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; iDC, immature dendritic cell; IL, interleukin; mDC, mature dendritic cell; MDSC, myeloid-derived suppressor cell; MSC, mesenchymal stem cell; PBMC, peripheral blood mononuclear cell; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TGF-β, transforming growth factor-β; T<sub>REG</sub>, T regulatory cells; VEGF, vascular endothelial growth factor.

growth factor, IL-10, IL-6 and prostaglandin E<sub>2</sub>.<sup>106</sup> BM-MSCs also secrete galectin-1 and semaphorin-3A, which inhibit T-cell proliferation via neuropilin-1.<sup>107,108</sup> IL-6 derived from BM-MSCs, in particular, seems to inhibit the differentiation of CD34<sup>+</sup> and monocyte precursors into mature dendritic cells, maintaining them in an immature, tolerogenic state.<sup>109</sup> Dendritic-cell differentiation from HSCs requires the activation of the Notch pathway and Wnt, whose ligands are produced by nearby stromal cells, a regulatory model that further implicates BM-MSCs in the immune response.<sup>110</sup>

BM-MSCs induce a T<sub>REG</sub> cell fate from CD4 or CD8 T cells upon their interaction.<sup>111</sup> Once T<sub>REG</sub> cells are activated, they suppress nearby lymphocyte and natural killer cells and as such can also offer escape from immune control to tumors. Tumor progression is associated with the expansion of the T<sub>REG</sub> cell compartment, partially explaining the tumor-associated immune suppression; whether T<sub>REG</sub> cells have a role in the initial phase of transformation and immune surveillance is less clear.<sup>112</sup> Regardless of their thymic origin or peripheral conversion, T<sub>REG</sub> cells are endowed with plasticity and sensing

different microenvironments can deflect toward the prevalent cytokine milieu including the inflammatory Th17.<sup>113</sup> Even in the context of lymphoid neoplasms, such as angioimmunoblastic T-cell lymphoma, T<sub>REG</sub> cells can be skewed towards Th17 cells, which in turn maintain the proinflammatory conditions and promote remodeling of the infiltrated tissue.<sup>36</sup> In hematological malignancies, increased numbers of T<sub>REG</sub> cells have been reported in patients with MGUS, multiple myeloma, and AML.<sup>114,115</sup> AML cells can induce T-cell tolerance by directly converting CD4<sup>+</sup>CD25<sup>-</sup> T cells into T<sub>REG</sub> cells through indoleamine 2,3-dioxygenase,<sup>116</sup> a mechanism that, originally described immunosuppressive in placenta, might characterize BM-MSCs.<sup>117,118</sup>

The other immunosuppressive population potentially induced by BM-MSCs in the BM environment is that of myeloid-derived suppressor cells. In the presence of solid tumors, the BM responds and produces abundant immature myeloid cells unable to complete their differentiation program and collectively named myeloid-derived suppressor cells, which provide potent immune suppression.<sup>119</sup> Factors inducing the expansion of myeloid-derived



suppressor cells are prostaglandins, IL-6, GM-CSF, and indoleamine 2,3-dioxygenase produced by BM-MSCs, which indicates a potential complementary mechanism through which BM-MSCs might induce a tolerogenic BM microenvironment.<sup>119</sup> Myeloid-derived suppressor cells can inhibit immune cell responses through different mechanisms: T-cell activation is suppressed by the production of arginase and reactive oxygen species, the nitration of the T-cell receptor, cysteine deprivation, and the induction of T<sub>REG</sub> cells. Innate immunity is impaired by the down-regulation of IL-12 from macrophages, the increase in IL-10 from myeloid-derived suppressor cells, and the suppression of natural killer cell cytotoxicity. Antigen presentation is limited by the expansion of myeloid-derived suppressor cells at the expense of dendritic cells.<sup>120</sup>

The outcome of the immunosuppressive functions of BM-MSCs *in vivo* has been investigated in animal models and human pathological settings. In rats and mice, administration of BM-MSCs ameliorated autoimmune myasthenia gravis or enabled successful immune escape of cancer cells in an allogeneic background, respectively.<sup>121,122</sup> Moreover, BM-MSCs have been used in patients with severe graft-versus-host disease following solid organ and HSC transplantation, where they proved to be safe and showed substantial clinical efficacy in association with conventional immunosuppressive therapy.<sup>123</sup> The immunomodulatory properties of BM-MSCs discussed above may also impact on the establishment and progression of hematologic neoplasms. T<sub>REG</sub> cells, which might be induced directly by BM-MSCs from effector cells, or recruited in the BM via the CXCL12/CXCR4 axis, may directly control the proliferation of the malignant clone and help maintain an homeostatic condition in the BM. They might also impair the establishment of an effective antitumor immune response, thus favoring uncontrolled growth of the clones.<sup>115,124</sup> In myeloid malignancies, the detrimental contribution of BM-MSC-induced and/or

BM-MSC-recruited T<sub>REG</sub> cells might be enriched by the direct participation of T<sub>REG</sub> cells to myeloid cancer-related stromal changes via the release of TGF- $\beta$ , which directly contributes to remodeling of the BM stroma.

## Conclusions

In hematological malignancies, the BM stroma is not an innocent bystander, but rather an accomplice of tumor development by favoring neoplastic cell growth and survival in the BM microenvironment, co-evolving alongside tumor progression, and forming part of the tumor microenvironment at extramedullary sites. Thus, treatment strategies that interfere with the major axes involved in crosstalk between neoplastic cells and BM stromal cells, such as monoclonal antibodies that target cell-adhesion or cytokine pathways, may prove effective in lymphoid malignancies, when combined with therapies that target the neoplastic clones. Efforts should also focus on limiting the stromal alterations that eventually determine BM failure in several types of myeloid neoplasms. The adoption of drugs that control the proliferation of BM stromal and vascular cells, such as tyrosine kinase inhibitors, proteasome inhibitors, and immunomodulatory agents could represent a promising perspective.

## Review criteria

Data for this Review was compiled by searching the PubMed database for articles published before 1 September 2010. Only articles published in English were considered. The search terms included "bone marrow" and "stroma" in association with "lymphoma", "leukemia", "lymphoproliferative", "myeloproliferative", "stem cell", "niche", "fibrosis", and "angiogenesis". Where possible, primary sources have been quoted. References were chosen on the basis of the best experimental, clinical or laboratory evidence, especially if the results had been corroborated by published work from other centers.

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## Author contributions

C. Tripodo, S. Sangaletti, P. P. Piccaluga, S. Prakash, G. Franco, and I. Borrello researched data to include in the article. C. Tripodo, S. Sangaletti, I. Borrello, A. Orazi, M. P. Colombo, and S. A. Pileri contributed to discussion to of content for the article. All the authors contributed to the writing of the manuscript. C. Tripodo, S. Sangaletti, A. Orazi, M. P. Colombo, and S. A. Pileri reviewed/edited the manuscript before submission.