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Review

The role of microenvironment and immunity in drug response in leukemia☆

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ABSTRACT

Leukemia is a cancer of the white blood cells, with over 54,000 new cases per year diagnosed worldwide and a 5-year survival rate below 60%. This highlights a need for research into the mechanisms behind its etiology and causes of therapy failure. The bone marrow microenvironment, in which adult stem cells are maintained in healthy individuals, has been implicated as a source of chemoresistance and disease relapse. Here the various ways that the microenvironment can contribute to the resistance and persistence of leukemia are discussed. The targeting of the microenvironment by leukemia cells to create an environment more suitable for cancer progression is described. The role of soluble factors, drug transporters, microvesicles, as well as the importance of direct cell–cell contact, in addition to the effects of inflammation and immune surveillance in microenvironment-mediated drug resistance are discussed. An overview of the clinical potential of translating research findings to patients is also provided. Understanding of and further research into the role of the bone marrow microenvironment in leukemia progression and relapse are crucial towards developing more effective treatments and reduction in patient morbidity. This article is part of a Special Issue entitled: Tumor Microenvironment Regulation of Cancer Cell Survival, Metastasis, Inflammation, and Immune Surveillance edited by Peter Ruvolo and Gregg L. Semenza.

1. Introduction

Recent advances in understanding the role of the tumor microenvironment (TME) in cancer and the central role of the stromal cells have uncovered new potential therapeutic opportunities to target cancer development and progression. Of particular interest is the effect of the microenvironment on hematological malignancies, usually referring to several levels of crosstalk between leukemia cells and the bone marrow microenvironment. This process is mediated in several different ways, for example by soluble factors and cell–cell contact and is suggested to affect chemotherapeutic response, potentially contributing to leukemia relapse. Different pathways play a role in the interaction between leukemia and the microenvironment depending on the type of leukemia. For example B cell receptor (BCR) associated kinases are important for chronic lymphocytic leukemia (CLL) expansion and maintenance. Adhesion molecules, cytokines and other signaling molecules all play a role in the movement and homing of leukemia, and have been identified as potential targets for drug development, and are at different stages of clinical trials [1]. In chronic myeloid leukemia (CML) therapeutic resistance can be induced by altered signaling between microenvironment and leukemia cells that involves aberrant mRNA splicing of GSK3 beta and BCL-2 family [2]. The microenvironment has also been shown to be important for the acute myeloid leukemia (AML), with leukemic cells interfering with the function of hematopoietic niche [3]. However, the molecular details and clinical utility of these and other findings relevant to understanding the role of the bone marrow microenvironment in leukemia progression and treatment are yet to be fully elucidated.

This review will describe how the crosstalk between the bone marrow niche and leukemia affects drug response. In particular the focus will be on the role of soluble factors and cell–cell interactions in this crosstalk. The relevance of inflammation and immune surveillance in leukemia drug response and niche function will also be discussed. Lastly, a summary of clinical applications to date of manipulating microenvironment for leukemia therapy will be discussed.

1.1. Hematopoiesis and the bone marrow microenvironment

Cells in the blood consist of a variety of types, each differentiated to carry out a specialized function. For example there are erythrocytes (red blood cells, involved in oxygen transport), platelets (involved in blood clotting) and leukocytes (white blood cells, involved in the immune

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response). Each of these cell types share a common progenitor cell, known as a hematopoietic stem cell (HSC) [4]. The process of an HSC differentiating into a more specialized cell is known as hematopoiesis. Due to the fact that the body produces a large number of different blood cells every day [5], the process of hematopoiesis and the maintenance of HSCs are tightly regulated. HSCs are maintained in quiescent states through specialized microenvironment known as niches. HSCs specifically are known to be maintained within the bone marrow. In order for cells to be affected by the bone marrow microenvironment they must first be localized to that area. Homing describes the phenomenon of the migration of HSCs through the blood to different organs and to the niches of the bone marrow [6]. It has been shown that expression of signaling molecules and integrins is crucial to the homing of leukemic cells to the bone marrow and other locations [7].

The word microenvironment is used to describe certain parts of the body where surrounding cells and tissues can affect the growth of specific cells by altering the pH, oxygen levels and other factors. The link between microenvironment and cancer was described by Stephen Paget who developed the seed (tumor cells) and soil (microenvironment) hypothesis, building on earlier idea by Ernst Fuchs, stating that some tumor cells grow preferentially in certain organ microenvironment [8]. Microenvironment plays an important role in both health and disease. For example, the healthy body contains microenvironment which supports the maintenance of stem cells, often referred to as stem cell niches. However, when dysfunctional, these niches can ultimately play an important role in disease progression, especially in cancer [9].

The bone marrow was classically described to have two niches: the endosteal (osteoblastic) niche and the vascular (sinusoidal) niche (Fig. 1) [9]. Early observations showed that primitive hematopoietic cells tended to localize near the endosteal margins, thus promoting the theory that bone played a role in the regulation of hematopoiesis. This work was later expanded upon, where it was discovered that osteoblasts could support hematopoietic cells in culture [10]. The endosteal niche comprises the inner surface of the bone cavity and is lined by cells such as osteoblasts and osteoclasts, involved in osteogenesis.

The vascular niche of the bone marrow comprises a variety of structures. Sinusoids are small blood vessels with thin walls that serve as an interface for communication between the marrow cavity and circulating blood, allowing cells to traffic in and out of the bone marrow [11]. The interface between bone cells, HSCs and endothelial cells are crucial for hematopoiesis and bone formation. The vascular niche plays three important roles in bone marrow: supplying oxygen, nutrients and growth factors to HSCs to promote proliferation and differentiation; encouragement of homing and mobilization through chemokines and sinusoidal endothelium and lastly the vascular niche is also important for cells outside of the bone marrow, such as in the spleen which may replace the niche in case of bone marrow suppression [12].

Understanding the maintenance of stem cells in the absence of disease is crucial, as these niches and the cells themselves may be altered through the course of illness. Evidence has shown that during cancer, there exists a class of cells known as cancer stem cells (CSCs), a subtype of a tumor that has stem-like properties, are capable of self-renewal but have deregulated pathways [13]. Cancer stem cells have been identified in a variety of cancers with leukemic stem cells (LSCs) being identified in different types of leukemia [14]. For a comprehensive review of CSCs, see Ajani et al. (2015) [13].

As described above, stem cells are maintained in specific niches that allow them to remain in a quiescent state. During cancer, however, these niches can be used by CSCs, and can also be affected by them to create an environment more favorable to cancer [15]. It has been shown in leukemia that LSCs can overtake the microenvironment within the bone marrow resulting in its altered function [14]. Therefore, successful treatment of leukemia means not only treating the leukemia cells which circulate through the blood, but also eliminating LSCs. The paragraphs below discuss the role of the bone marrow niches in maintenance of HSCs and LSCs.

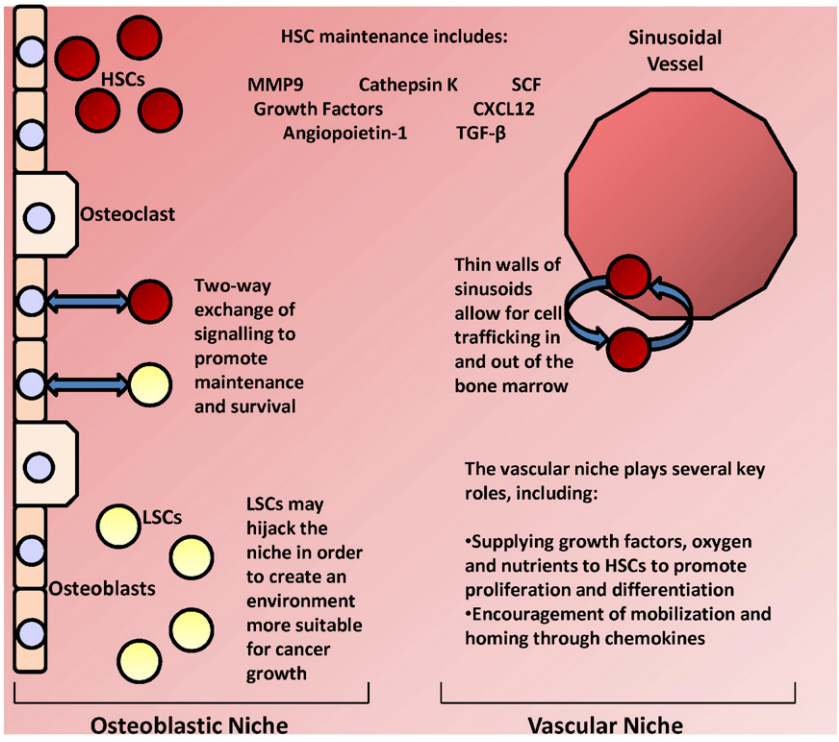

Fig. 1. The niches of the bone marrow. The bone marrow is classically described to contain two niches, the osteoblastic (endosteal, left) and vascular (sinusoidal, right) niches. Both are involved in the maintenance of HSCs (red circles) and LSCs (yellow circles) that may alter the niche to their favor. Sinusoids (red dodecagon) in the vascular niche allow for trafficking in and out of the bone marrow and play a role in the vascular niche's role in supporting and mobilizing HSCs. A variety of factors have been suggested to regulate HSCs, such as MMP9 and CXCL12.

It was originally thought that the endosteal niche exerted its effect potentially through cell contact (via adhesion molecules) of HSCs with osteoblasts. The osteoblastic niche was thus thought to contribute positively and negatively to HSC-state. Positive effects include osteoblasts signaling through Jagged-1, reaching the corresponding receptor NOTCH on the HSC cell, ultimately expanding the HSC pool. However, osteoblasts also express osteopontin, which reduces HSC levels by the inhibition of proliferation and promotion of apoptosis. Chemokine (C-X-C motif) ligand 12 (CXCL12), a chemotactic molecule produced by osteoblasts, was thought to be important for the recruitment of HSCs to the bone marrow [16]. Recent evidence has suggested that the endosteum can secrete factors that help maintain HSCs into surrounding niches [11] and exerts its effect on HSCs, as has been reviewed in-depth [10,14]. It was also shown, through in vivo imaging, that few HSCs are in contact with osteoblasts [10]. Factors important in the maintenance of HSCs by the endosteal niche include the proteases matrix metalloproteinase 9 (MMP9), cathepsin K and growth factors [11]. Other factors include angiopoietin-1, stem cell factor (SCF), thrombopoietin and CXCL12, all of which are involved in HSC maintenance [9,16].

It has also been shown that interruption of the signals that maintain HSCs can improve chemotherapy. For example, TGF-β plays a role in the quiescence of HSCs; however, blockage of TGF-β improved sensitivity to chemotherapy [14]. LSCs have been implicated in drug resistance and disease relapse and retaining minimal residual disease (small number of leukemic cells that remain after chemotherapy) [17]. Understanding the mechanisms behind drug resistance that allow LSCs to survive is therefore crucial to improve therapy.

There are two classifications for chemoresistance in LSCs: intrinsic factors (i.e., signaling pathways within the LSCs themselves) and extrinsic factors (i.e., components generated by the bone marrow/other microenvironment) [18]. One of the more important regulators of chemoresistance is the cell cycle status of the cell; many chemotherapeutic drugs target cycling cells. However, like HSCs, LSCs often reside in a quiescent state, likely reducing the efficacy of chemotherapy drugs that target cycling cells [18]. Use of drugs that target LSCs regardless of cycling status is a feasible option, though it has also been shown that inducing the cell cycle in LSCs, and following this with traditional chemotherapy despite potential risks could be effective [19].

One of the main intrinsic pathways deregulated in LSCs is NF-κB pathway that has been shown to be constitutively activated in LSCs, indicating a role for it in survival; furthermore many of the drugs which are shown to be effective against LSCs are known NF-κB inhibitors [18]. Other pathways and mediators have been implicated in LSC/HSC survival, such as Akt phosphatidylinositol-3 kinase (PI3K) [20], NOTCH signaling [18], and the Wnt/β-catenin pathway [21]. It is possible to target these pathways, such as the case of inhibiting the activation of Akt by phosphatidylinositol 3 kinase (PI3K) inhibitors. Sinclair and colleagues [21] provide a detailed overview of the various survival pathways in CML stem cells, in addition to discussing methods to target the pathways.

Extrinsic factors have been described as "environment-mediated drug resistance" [18], arising from the protective effect of the microenvironment. Niche stromal cells affect malignant hematological cells by preventing apoptosis through the positive regulation of anti-apoptotic proteins. It has been reported that stromal cells protected co-cultured leukemia cells from both drug-induced (cytarabine/ara-C) apoptosis and apoptosis following serum starvation, correlating with the patient's response to chemotherapy in vivo. Further to this, this protection was shown to be through soluble factors as opposed to a mechanism requiring cell–cell contact. It was shown that BCL-2 was significantly upregulated in leukemia cells co-cultured with stromal cells, when compared to cells that had not undergone co-culture, highlighting a way that this protection could have occurred [22].

Drug resistance can develop in the continuous presence of chemotherapeutic drugs, leading to genetic and epigenetic changes in the cells that confer resistance (Table 1) [23–51]. One of the main causes of drug resistance is altered function of efflux transporters that under certain conditions in cancer transport drugs out of the cell. The following section will discuss in more depth the role of drug transporters in leukemia resistance.

1.2. Efflux pumps as a means of chemoresistance

One of the primary ways in which cancer and stem cells may employ protection is through the expression of adenosine triphosphate-binding cassette (ABC) efflux transporters, which are known to be multifunctional [52]. Some cancer cells are resistant to chemotherapy and their continued proliferation may ultimately lead to development to multidrug resistance (MDR) [53]. The aforementioned ABC transporters have been implicated in MDR, as they are known to shield CSCs from the chemotherapeutic effect by active excretion of a variety of chemotherapy drugs. This links the ABC transporters to MDR, which is an important process to understand as it represents a major issue in chemotherapy treatment [54]. MDR in general may arise not only through efflux by ABC transporters, but also through phenomena such as reduction in drug uptake and imbalances in cell growth, survival and death signaling [55].

There are over 40 different human ABC genes, and each of them has been linked to disease when their function is defective and deregulated [54,56]. ABCB1, also known as MDR1/P-glycoprotein 1 has been implicated in MDR in cancer through efflux of xenobiotics. ABCC1/MRP1 has also been implicated in MDR cancer. ABCB1 is composed of six putative transmembrane domains and a nucleotide binding fold, which is the site at which ATP is processed to provide energy for the efflux/transport [54]. ABCB1 has been linked to the efflux of many chemotherapeutic drugs such as anthracyclines, and its expression is also known to be increased in drug-resistant cancer [57]. This highlights ABCB1 as a potential target for drug development, however, strategy to overcome MDR is challenging due to the widespread expression of ABCB1 in normal cells [55].

It was previously thought that random genetic mutations could explain the subset of cancer cells that are drug resistant [58]. However, recent findings have suggested that the tumor microenvironment can facilitate an MDR phenotype through a variety of mechanisms that depend on extracellular matrix signaling to cancer cells and cell adhesion (reviewed in [58]). Resistance to treatment can be attributed to the induction of efflux pump proteins by the microenvironmental factors. Several members of the family of the ABC transporters such as the breast cancer resistance protein (BCRP) have been shown to be upregulated in tumor cells under low oxygen conditions. High levels of multidrug resistance-associated protein-1 (MRP1) and ABCB1 correlate with drug resistance in leukemia. ABCB1 expression is increased in hypoxic cells, and this increase is mediated by the hypoxia-inducible factor-1 (HIF-1) [59].

A link between the microenvironment and MDR in leukemia was recently demonstrated [60], where it was shown that incubation with bone marrow-derived stromal cells altered the expression of ABC transporters MDR1, MRP1, MRP2, MRP3 and BCRP in a myeloid leukemia cell lines and this process was dependent on insulin-like growth factor1. Our own unpublished data suggest that bone marrow derived conditioned media increases expression of ABC transporter genes ABCC4, ABCC5, ABCC10, ABCA5 and TAP2 in ALL cell lines. Gene expression of ABC transporters is controlled through complex networks of signals that include numerous transcription factors and epigenetic mechanisms [59,61]. These factors link extracellular signals that include inflammatory response (for example IL-6) and growth factors (such as IGF1), to gene expression of ABC transporter family and thus may mediate microenvironment effects of drug resistance. This highlights a possibility that the microenvironment can promote an MDR phenotype. Obtaining a full awareness and understanding of how the microenvironment may protect cancer cells is crucial, as there are many ways by which leukemia may be affected. The following sections will discuss more in-depth the mechanisms by which the microenvironment exerts its effect.

Table 1
Drugs used to treat leukemia and mechanisms of resistance.

Type of leukemia	Drug	Mechanisms of resistance	References
ALL	Asparaginase	De novo expression of Asn and changes in the expression levels of genes that regulate apoptosis	[24]
	Clofarabine	Downregulation of nucleoside transporters, and upregulation of anti-apoptotic proteins such as Bcl-2	[25]
	Daunorubicin	Change of MDR gene expression levels	[26]
	Doxorubicin	Upregulation in MDR genes such as MDR1 and MRP1	[27]
	Methotrexate	Impaired drug uptake, increased drug efflux, impaired intracellular polyglutamation, changes in target enzyme activity and increased intracellular folate pools	[28]
	Vincristine	Increased microtubule stability, mutations in β-tubulin and increased levels of polymerized tubulin Increased levels of expression of ABC transporters	[29]
	Corticosteroids (dexamethasone or prednisone)	Low levels of GR protein and reduced activity Bcl-2 overexpression	[30–34]
AML	Cytarabine	Reduced drug influx by the hENT1 transporter. Reduced phosphorylation of cytarabine by deoxycytidine kinase. Increased degradation by 5′-nucleotidase and/or cytidine deaminase. Increased levels of DNA polymerase alpha. Reduced levels of topoisomerase I/II	[35]
	Idarubicin	P-glycoprotein-mediated multiple drug resistance	[36]
	Mitoxantrone	Increased Mcl-1 expression and downregulation of topoisomerase IIβ	[37]
APL	Daunorubicin	Change of MDR gene expression levels	[26]
	Idarubicin	P-glycoprotein-mediated multiple drug resistance.	[36]
	Arsenic trioxide and all-*trans* retinoic acid	Increased expression of multidrug resistance-associated protein (MRP1) Increased intracellular content of glutathione Mutations in the arsenic-binding domain of PML-RARA	[38,39]
CLL	Bendamustine	Increased activity of DNA repair enzymes Increased expression of sulfhydryl proteins, e.g., glutathione and glutathione-related enzymes	[40,41]
	Chlorambucil	Mutations in p53, increased DNA-PK activity, and increased formation of Rad51 foci	[42]
	Cyclophosphamide	Cyclophosphamide-resistant cell lines exhibit decreased initial levels of DNA interstrand cross-links following cyclophosphamide treatment (likely due to elevated aldehyde dehydrogenase). These cross-links are removed more rapidly compared to cyclophosphamide-sensitive cell lines. Glutathione has also been implicated in resistance (increased DNA repair).	[43–45]
	Fludarabine	It has been demonstrated that Ku, which is involved in DNA repair, can bind telomerase and that this may be a mechanism of resistance to fludarabine.	[46]
	Alemtuzumab	Decreased complement dependent cytotoxicity	[47,48]
	Rituximab	Mesenchymal stromal cells can downregulate CD20, which may be required for Rituximab effect	[49]
	Corticosteroids (prednisone)	See previous entry for ALL	[30–34]
CML	Cyclophosphamide	See previous entry for CLL	[43–45]
	Cytarabine	See previous entry for AML	[35]
	Tyrosine kinase inhibitors (such as dasatinib, imatinib, or nilotinib)	Mutations in the BCR-ABL oncogene, increased expression of efflux proteins and deregulated apoptotic pathways	[50]
	Plerixafor	Increased expression of adhesion molecules and CXCR4	[51]

Mechanisms of resistance may reflect findings from another type of leukemia.

1.3. Microenvironment/leukemia cells crosstalk: soluble factors and microvesicles affecting leukemia development and drug resistance

Several studies have demonstrated that bone marrow stromal cells could mediate chemoresistance and protect leukemic cells from the cytotoxic effects of therapy. Asparaginase is routinely used in the therapy of childhood acute lymphoblastic leukemia (ALL) treatment due to the fact that ALL cells often depend on exogenous asparagine, as their own capacity for its production is generally low [62]. Despite this, however, not all patients respond to this therapy, which indicates further sources of asparagine production and potential interference with asparaginase treatment. Mesenchymal stem cells (MSCs), which are multipotent stromal cells that are thought to reside in hematopoietic stem cell (HSC) niches, are known to upregulate asparagine synthesis and protect sensitive leukemia cells from death. It has also been shown that a subset of leukemic blast cells themselves express lysosomal cysteine proteases that degrade the drug asparaginase, leading to resistance [63]. While it is unclear why only some leukemic cells upregulate the expression of these proteases and others do not, the most likely explanation lies in the host–tumor interaction [64].

Bone marrow stromal cells have been implicated in the resistance of a variety of drugs. Asparaginase is one mentioned above, and in addition to this MSCs have been shown to protect leukemic cells from the cytotoxic effects of cytarabine [22]. Another class of drugs commonly used to treat leukemia are steroids and they regulate several cellular pathways to increase apoptosis of white blood cells [32,65]. Effects of microenvironment on steroid response has been investigated in several experimental systems [66], including the xenograft models where the mechanisms of steroid resistance in leukemic cell lines are different from those seen in xenografts [67]. Thus, mechanisms of therapeutic failure need further investigation of molecular details at the basis of host–tumor interactions.

The tumor microenvironment and primary cancer cells exchange signals in a two-way process that can lead to evasion of immune response, change in TME function, uncontrolled proliferation of cancer cells, metastasis to form secondary tumors and altered drug response [68]. The mechanistic details of this process are not entirely known but involve soluble factors such as secreted signaling molecules and microvesicles. One of the main categories of soluble factors are cytokines, signaling molecules that are able to induce chemotaxis of target cells. CXCL12 that is produced by osteoblasts in bone marrow serves as a ligand for the G-protein coupled receptor CXCR4 and was suggested to be one of attractants for hematopoietic stem cells [69,70]. Leukemia stem cells are also attracted by CXCL12; however, they alter the niche and secrete another cytokine (SCF, also known as the KIT ligand) which regulates hematopoiesis and can contribute to the disruption of normal HSC function. SCF therefore represents an interesting therapeutic target, as its inhibition could potentially maintain normal HSC function. This has been demonstrated in a mouse model of leukemia where SCF function was inhibited by neutralizing SCF antibodies [71,72]. Another cytokine, proposed to have important function and links immune response, inflammation and microenvironment is interleukin 6 (IL6). IL6 is cytokine with pro and anti-inflammatory effects that controls numerous cellular functions. IL6 expression is increased in ALL patients bone marrow, correlates with severity of disease and it facilitates proliferation of multiple myeloma (MM) cells [73,74]. IL6 also mediates

resistance to chemotherapy in epithelial cancers and hematological stroma-mediated drug resistance [75]. In vitro experiments indicated that blocking IL6 production by stroma restores dexamethasone sensitivity of myeloma cells that have become resistant due to stroma influence [76]. Inhibition of IL6 actions with monoclonal antibody has shown some improvements in clinical trials and mouse models, although further development of antibody properties is needed to improve its therapeutic efficacy [77,78].

In addition to cytokines, several growth factors have been reported to play a role in leukemia–microenvironment crosstalk. Numerous growth factors have been hypothesized to contribute to drug resistance to anticancer kinase inhibitors [79] (Table 2). TGF-β has been documented to display an inhibitory effect on hematopoiesis. However, leukemia cells are often resistant to this inhibition despite increased production of TGF-β by bone marrow stromal cells from leukemia patients [80–83]. Although TGF-β has been named the molecular Jekyll and Hyde of cancer, recent evidence indicate that TGF-β neutralizing antibody in combination with cytarabine (that interferes with DNA synthesis) and CXCR4 antagonist plerixafor increased survival in leukemia mouse model [84]. Despite the complex picture regarding its actions these finding suggest the possibility of using TGF-β as a drug target for therapy of leukemia.

Other growth factors including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) have also been reported as important in affecting leukemia development and bone marrow-mediated resistance to chemotherapy [80]. Increased levels of bFGF have been observed in CLL patients and correlate with the resistance to fludarabine [85]. Further deregulation of the FGF pathway has been reported in patients with multiple myeloma [80]. Anti-apoptotic effects of VEGF on ALL and CLL cells have been documented and involve alteration of BCL-2 family members and signaling through MAPK pathways. Another family of growth factors (tumor necrosis factor—TNF family) has been reported to play a role in microenvironment–leukemia crosstalk. TNFα is important in hematopoiesis, it has context-dependent effects on cellular proliferation and it plays important role in bone marrow function. Deregulation of its signaling can lead to BM failure and to leukemia stem cell survival [14]. Investigation of the B-cell receptor (BCR) signaling in CLL indicated that the use of irreversible BTK inhibitor PCI-32765 (Bruton tyrosine kinase (BTK) that is essential to BCR function) inhibits CLL cell proliferation and TNF-α-mediated prosurvival signals [86]. Further support for the role of growth factors was provided by studies that identified CC-5013 that is a derivative of thalidomide as therapy for MM that inhibited bone marrow communication with myeloma cells that included inhibition of TNF-α, VEGF and interleukin-6 leading to inhibition of tumor growth, and patient survival [87]. In addition, the interplay between angiogenic factors and cells in microenvironment in MM have been reported and described in detail [88]. Therefore modulating pathways controlled by growth factors holds promise for development of new therapies of leukemia.

It has also been shown that cell supernatants from CLL cells ("conditioned medium") stimulates the expression of platelet-derived growth factor receptors (PDGFRs) on mesenchymal stromal cells [89]. Examination of the conditioned medium showed that platelet-derived growth factor (PDGF) was present, having been secreted by the leukemia cells. The authors also showed that high-risk leukemia patients had elevated levels of PDGF. The authors speculated that because PDGF stimulates mesenchymal stromal cell function, this activity would enhance the ability of the mesenchymal cells to protect CLL cells from drug cytotoxicity [89]. This highlights how the cells may mutually affect each other to nurture a drug-resistant phenotype.

The microenvironment can also contribute to leukemic cell survival through microvesicles such as exosomes. Exosomes are small vesicles secreted by all cells and found in numerous species from bacteria to humans suggesting they are conserved through evolution [90,91]. Exosomes are a part of cell–cell communication and can carry various cargo including proteins, lipids and nucleic acids [92,93]. They are present in fluids in the body and have specialized functions such as cell signaling and elimination of cellular waste and are hypothesized to deliver these signals to specific target cells [94]. TME and tumor cells interact via exosomes which can also package immunosuppressive molecules and micro RNAs (miRs) that promote metastasis and can cause activation, proliferation or death of target cells [95]. Plasma from patients with AML and CLL contains higher levels of exosomes than normal plasma [96], indicating a role for exosomes in cancer development. Exosomes released by AML cells contain leukemia-specific coding and non coding RNA that can modulate target cell function [97]. The concept that tumor cells can modify bone marrow microenvironment has been shown in the case of malignant melanoma suggesting alteration of the bone marrow progenitor cells towards a cancerous state through exosome-dependent delivery of oncogenes [68]. Other reports have indicated a similar role of exosomes in hematological malignancies. Exosomes from B-CLL have been shown to alter AKT signaling and hypoxia response, affecting cyclin D and c-MYC function in bone marrow stromal cells [98]. It has been shown in CML that the leukemia cells can produce exosomes which communicate to bone marrow stromal cells, leading to increased IL8 production. This represents a positive feedback loop, as IL8 ultimately promotes the survival of leukemia cells [99]. Bone marrow mesenchymal stromal cells also release exosomes and they can be different between normal and malignant bone marrow in solid tumors [94,100]. However, less is known about the role of bone marrow derived exosomes in this crosstalk in hematological malignancies. Roccaro and colleagues reported that stromal cells release exosomes containing micro RNAs, oncogenic proteins, cytokines and adhesion factors, that are then transferred to multiple myeloma cells [101]. Exosomes derived from the normal BM have inhibitory, whereas exosomes derived from patients with MM had stimulatory effect on MM tumor growth.

The chemoprotective effect of the bone marrow has been shown on ALL cells as well [102]. Liu and colleagues simulated the bone marrow microenvironment in vitro by generating bone marrow stromal conditioned media which was shown to exert a chemoprotective effect. Tests indicated that this chemoprotective effect was retained in the proteinase K, RNase and heat resistant fraction of the conditioned medium. Furthermore, as confirmed by confocal microscopy, the effect was most likely due to ALL uptake of stromal-derived exosomes enriched in micro RNAs. This resulted in aberrant PI3K/AKT and oxidative phosphorylation in ALL [102]. Our unpublished data indicated that exposure of ALL cells to conditioned media obtained from the bone marrow cells causes alteration in ALL cell transcriptome in numerous genes of unknown cellular function (Qattan et al., unpublished observations) highlighting the need for further studies and potential for therapeutic use of abovementioned findings.

1.4. Microenvironment–leukemia cells crosstalk: cell–cell interaction

The importance of cell–cell contact between leukemia cells and cells of the bone marrow has been investigated previously, with several interesting findings and approaches. Although accumulation of knowledge regarding mechanisms of cell–cell communication induced drug resistance has been slow due to technical challenges, it is possible to model cell–cell contact in vitro through co-culture of leukemia cells with bone marrow stromal cells. What is important to acknowledge is that as well as the niche affecting cancer cells, the cancer cells affect the niche [103].

Obtaining a full understanding of the interactions between the niche and leukemia is complicated by the fact that there are several different types of leukemia, and each may interact with the niche differently. However, despite this, it has been shown that there are some common signaling pathways to several types of leukemia and lymphoma; for instance, it has been shown that contact with leukemia cells can induce protein kinase C (PKC)-βII expression in bone marrow stromal cells,

for CLL and ALL, as well as mantle cell lymphoma [104]. This increased expression of PKC-βII leads to the activation of NF-κB in tumor stromal cells, and this pathway promotes the survival of malignant B cells in vivo [104]. Indeed, due to the fact that the microenvironment nurtures cancer survival, it has been argued that the microenvironment itself should be a target for treatment, to bypass the drug resistance that can occur in cancer cells [104].

Pro-survival effects of the microenvironment may occur through the inhibition of apoptosis, both spontaneous and drug-induced. It has been shown that mesenchymal marrow stromal cells are able to protect CLL cells from spontaneous apoptosis and apoptosis induced by the chemotherapeutic drugs dexamethasone and cyclophosphamide [105]. Furthermore, it was shown that direct cell–cell contact was necessary for this protective effect, at least in this instance, as Kurtova et al. (2009) [105] separated the leukemia cells from the mesenchymal cells and found virtually no protective effect.

Several molecules have been shown to be crucial for homing and retention of HSCs and LSCs/leukemic cells to the bone marrow. As previously stated, CD44 and CD49d (also known as integrin alpha4) were shown to be crucial in the homing of leukemic cells to the bone marrow [7,106]. CD49d binds other molecules, forming active complexes. High expression levels of CD49d was identified as an adverse risk factor in childhood ALL [106], thereby marking it as a potential therapeutic target. It has been shown that CD49d enhanced chemoresistance to doxorubicin in ALL cells [106,107] and that adhesion of ALL cells to the bone marrow microenvironment is mediated by CD49d. Several mechanisms for how it may exert its chemoprotective effect have been described. NF-κB, as previously stated, is involved in pro-survival signaling, and has been shown to be activated by CD49d signaling. Anti-integrin treatments are currently a widely explored area, with over 260 anti-integrin drugs currently in clinical evaluation [106]. However, only a few of these have been approved for use clinically. Shishido et al.(2014) [106] provide an in-depth discussion of drugs currently available, citing examples such as antibodies that target integrin combinations, as well as discussing potential alternative treatments.

Ultimately, an increased understanding of the mechanisms behind how cell–cell contact encourages a drug-resistant phenotype will have important implications for future therapies. The microenvironment has been shown to affect drug response through both soluble factors and direct cell–cell contact. If this is more clearly understood, it is possible that future therapies could be developed that either target the niche itself to reduce its effect, or target its effector molecules such that they are no longer able to affect cancerous cells. It has been shown that some drugs have the ability to reduce cell–cell contact between the cells of the niche and cancer cells as discussed in the clinical potential section below.

The microenvironment, however, is not the only way in which tumors may utilize cell–cell contact to survive. As well as contact with the niche, cell–cell contact is important for tumor survival in a variety of other ways. Natural killer cells are known to have reduced activity in CLL, and cell contact with the leukemia cells has been thought to be a potential mechanism behind this [103]. Again, similar to the above, patients in early stages of disease had higher counts of natural killer cells, possibly implying a potential protective effect of natural killer cells regarding cancer progression [103]. There has been much research on the role of the inflammation and the immune system and cancer progression, some of which will be discussed further on in this review.

1.5. Inflammation and its role in tumor microenvironment

Inflammation is a physiological process that occurs as a response to infection and injuries, producing inflammatory mediators such as cytokines and chemokines [108]. The link between chronic inflammation and cancer was proposed in 1863 by Rudolf Virchow after infiltration of leukocytes in the malignant tissue was observed [109]. In the inflammatory response macrophages are the most abundant immune cells in the tumor microenvironment. They are key regulators of the link between inflammation and cancer and have both positive and negative effects on tumor progression [109–112]. There are different factors that mediate survival of the macrophages in the tumor which include the chemokine CCL2 and molecules such as VEGF, PDGF, TGF-β and macrophage colony-stimulating factor (M-CSF) [110]. Numerous studies have revealed that chronic inflammation is increased risk factor to certain types of cancer, and that the inflamed tumor microenvironment can promote tumor progression, modulating the expression of growth factors, increasing angiogenesis and suppressing immune response [109].

Inflammation is considered an important factor of cancer development through different molecular mechanisms. One transcription factor crucial for the inflammatory response is the NF-κB, which regulates the expression of a wide range of inflammatory molecules, including cytokines and adhesion factors and has also been demonstrated to have a negative regulatory effect on apoptosis in cancer [113,114]. NF-κB also contributes to cancer development by stimulating cell proliferation by positive regulation of growth factor genes, the proto-oncogene c-Myc, and the cell cycle regulator cyclin D1 [115].

The second group of mediators of inflammation are the cytokines, including interleukins (ILs), TNF-α, growth factors, and colony-stimulating factors, all of which play a regulatory role in the growth, differentiation, and activation of immune cells [116]. Cytokine signaling contributes to the progression of tumors in two ways: stimulating cell growth or inhibition of apoptosis of cells affected by inflammation. Moreover, TNF-α and chemokines are believed to be the linking molecules between inflammation and cancer, TNF-α is produced mainly by activated macrophages and plays an important role in inflammation through tissue damage, recovery, remodeling and communication with microenvironment [117]. TNF-α stimulates inflammatory cytokines and increases the expression of growth and adhesion factors. TNF-α is crucial for NF-κB activation and can induce DNA damage, inhibit DNA repair and acts as a growth factor for cancer cells. Furthermore, TNF-α plays a role in promoting angiogenesis and cancer cells proliferation, and in linking inflammation to cancer through regulation of chemokines [118].

Cytokine family of factors includes chemokines, that are important for the control of leukocytes migration to the inflammatory site. Moreover, chemokines have also been suggested to play a role in facilitation of cancer growth, invasion and metastasis and in regulating angiogenesis. Chemokines may direct tumor cells to their metastatic target tissue in a similar manner as they control leukocytes [119].

A role of inflammation in hematological malignancies is complex given that cells that mediate immune response are themselves cancerous. Several examples include the effect of chronic inflammation in development of lymphoid cancer including lymphomas associated with infections in the gastric, ocular and cutaneous systems. Viral infections also play a role in development of certain types of lymphoma [120,121]. Increase in the levels of TNF-α and its receptors have been observed in lymphoma and leukemia patients and are prognostic markers associated with the poor outcome [111,122].

ALL is the most common cancer in children with unknown causes and increasing incidence [123]. Potential risk factors include environmental exposure to chemicals (such as cigarette smoke), pollution, pesticides and infectious agents. The age and frequency at which infections occur appear to be important and inverse correlation exists between exposure to infectious agents and development of ALL (as the hygiene hypothesis would predict) [124,125]. There also may be a genetic link with low IL-10 expression at birth in children who later develop ALL. Further to this, a history of exposure to infectious agents, autoimmune reactions and chronic inflammation have been described as a factor in development of CLL [126]. signaling pathways involved were activation of TNF family members, cytokine IL-4, VEGF and chemokine SDF-1. The microenvironment was also shown to have influence [127–129]. Microenvironment inflammation can affect multiple myeloma through similar mechanisms and increased IL6 production has been associated with

shorter survival and drug resistance. Molecular mechanisms include increased IL6 cytokine production as well as numerous other factors that affect myeloma and stroma cells (reviewed in [130]). In clinical studies monoclonal antibodies against IL6 have been shown to demonstrate biological effect in six myeloma patients [78]. Drugs that inhibit IL6 production (thalidomide and its derivative lenalidomide), in combination with either dexamethasone or proteasome inhibitors have shown significant positive activity in myeloma patients [131].

Inflammation in general can lead to an increase of inflammatory mediators in the blood and inhibition of the effects of anticancer drugs through actions of cytokines and other molecules. Examples of this include IL6 and acute phase proteins inactivating anticancer drugs in experimental mouse systems [131,132]. Also, CYP450 proteins that are involved in drug metabolism in the liver have shown altered activity in inflammatory states, thus indirectly affecting drug response [133]. Although some members of this family are expressed in tissues other than liver including breast cancer [134] and white blood cells in response to various stimuli, their function is not well established [135]. Finally, cancer therapy can increase inflammation leading to adverse effects in myeloma and other types of leukemia [130]. In a study of 27 survivors of childhood ALL and 20 controls, authors have reported deregulation of immune response and chronic inflammation and proposed this to be the cause of late cardiovascular morbidity [136].

1.6. Immune system and microenvironment

Immune system has been exploited for therapy of numerous diseases and includes use of vaccines and monoclonal antibodies. In cancer, few successful examples based on traditional methods include vaccine against the HPV virus [137] and monoclonal antibodies for cancer treatment [138]. There are 12 antibodies approved for use in therapy of solid tumors with the best results obtained with the antibodies against VEGF and ErbB family. Rituximab and ofatumumab monoclonal antibodies that target CD20 and alemtuzumab that targets CD52 have been approved for therapy of CLL and alter apoptotic pathways. Another approach in treatment of hematological malignancies was to use antibodies in delivery of anticancer agents [138]. Recent approaches have focussed on investigation of immune surveillance of cancer development and potential exploitation of this knowledge to modify immune system in order to eradicate cancer progression [119,139].

1.7. Evasion of the immunosurveillance in leukemia

The immune surveillance theory proposes that the immune system recognizes precursor cancer cells and attempts to eradicate them. Several mechanisms by which the tumor evades immune surveillance have been described in the literature which could be divided in two general categories. The first refers to the interaction between the tumor and the immune system mediating changes of the immune cells that render them incapable to identify tumor cells as foreign to the host, and the second to the inability of the immune system to detect the tumor for reasons related either to the ability of the tumor to hide from the immune system or its capacity to disguise as benign host tissue [119,139]. Here selected pathways of tumor evasion of immune surveillance which lead to resistance to anti-cancer therapy are described in the context of the bone marrow microenvironment and elucidate potential targets to reverse resistance to therapy focusing on hematological malignancies.

1.8. Leukemia immune microenvironment

Immune response against leukemic cells is mainly elicited by cytotoxic T lymphocytes (CTLs) which recognize leukemia specific antigens such as BCR/ABL expressed in CML and ALL and PML/RAR in AML [reviewed in [14]]. CTLs eliminate efficiently virally infected or tumor cells through binding to an antigen that is presented to them by the infected or tumor cell in complex with MHC class I molecules. Binding between the T-cell receptor on CTLs and the MHC class I-antigen complex on the infected or tumor cell takes place through the so-called 'three-signal activation model', which results in CTL activation that are then competent to kill target cells. The process of the specific interaction between the CTL and antigens perceived by the immune system as foreign to the host requires interaction between the CD8 + molecule on the surface of the T cell and regions on the MHC class I molecule that are not bound to the antigen, as well as the presence of other co-stimulatory signals and release of cytokines by the infected or cancer cells that fully activate CTL specific response. T regulatory cells (Treg) play important role in distinguishing foreign from self-antigen and therefore in the induction of the CTL killing activity. Leukemic cells differentiate and develop in the immunosuppressive Treg-rich bone marrow microenvironment and are thereby protected from regular immune responses [140].

Apart from the inhibitory role of the Treg cells, CTLs might be unable to elicit response due to the fact that leukemia associated antigens are not immunogenic because of diminished MHC class I expression on leukemic cells which results in deficiencies in antigen presentation and thus malignant cells escaping immunosurveillance [141,142].

Inhibition of CTLs activation can also occur in the presence of negative regulatory receptors such as the members of the CD28 family cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death-1 (PD-1) which bind to the B7 ligands B7.1 (CD80) and B7.2 (CD86) found on activated antigen presenting cells thereby producing a co-inhibitory signal decreasing CTLs activity [143]. The T cell immunoglobulin domain and mucin domain 3 (TIM-3) is another negative regulatory receptor that was originally found on Th1 cells inducing tolerance through interaction with its ligand, galectin-9 [144], but later was detected on other T cells as well, including CD8 + T cells [145]. TIM-3 is expressed in "exhausted" T cells which exhibit functional unresponsiveness to infections [146] and its blockade using specific antibodies has shown enhanced anti-tumor T cell responses implying that TIM-3 inactivation may re-activate T cell responses in cancer patients [147].

L-tryptophan (L-TRP) is an essential amino acid obtained from food mainly involved in protein synthesis and is a precursor for several compounds including melatonin, serotonin, kynurenine and nicotinamide [148]. The heme-containing enzyme indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan producing as major metabolite kynurenine [149],which in turn inhibits T-cell proliferation and induces T-cell death [150]. It has been shown that IDO is expressed in professional antigen presenting cells (APCs), dendritic cells particularly, induced by cytokines, including IFN-γ [151]. Transcriptional activation of IDO gene expression and enzymatic activity following association of CTLA-4 with CD80/CD86 on the surface of DCs has also been reported [152,153] indicating that IDO possesses powerful immunomodulatory activity. Indeed APCs expressing IDO activate Tregs and induce T cell differentiation of Tregs thereby intensifying immunosuppression in the tumor microenvironment [154,155]. IDO expression has been observed in a substantial number of AML patients implying a significant role of this enzyme in the AML pathophysiology and response to drug treatment [156].

2. Clinical potential

There have been several studies on microenvironment that show interesting translational potential. Although many studies described previously in this review focused on the effect of the microenvironment and bone marrow cells on hematological malignancy survival, it has been shown that it is possible to target bone marrow stromal cells to reduce this protective effect. In a study of AML, treatment of bone marrow stromal cells with cytarabine was shown to decrease their protective effect on AML cell death in a dose-dependent manner [157]. This

highlights the potential for targeting BMSCs as well as leukemic cells in order to improve chemotherapy efficacy.

As well as in AML, this concept of targeting the stroma has been shown for other types of leukemia, including CLL. It has been suggested that microenvironment offers protection from chemotherapy, often by aberrant expression of BCL-2 proteins which allows resistance to BCL-2 inhibitors [158]. However, treatment of co-cultured CLL and stromal cells with gossypol was shown to overcome the stroma-induced resistance to a BCL-2 inhibitor. More importantly, this study was performed ex vivo, using CLL cells derived from patients [158],thus providing immediate clinical relevance and potential.

Another interesting target is angiogenesis. Although obviously important for the metastasis and spread of solid tumors, its role in hematological malignancies was originally less clear [159]. However it has since been established that angiogenesis does play a role in the development of leukemia, suggesting that potential use of anti-angiogenic therapy may improve treatment response in hematological malignancies [160]. Medinger and Passweg (2014) [160] provide a comprehensive review on the role of angiogenesis in hematological malignancies as well as discussing treatment options. Many anti-angiogenic agents are already in use in the clinic for other forms of cancer, implying that their safe use is already well-established. This therefore represents an interesting clinical opportunity as the drugs may be used directly to treat hematological malignancies without the pre-clinical and clinical screening required for a novel drug.

Interestingly, it has been shown that co-culture of multiple myeloma cells with BMSCs leads to a significant increase in the secretion of both VEGF and interleukin-6 [161]. This shows that the bone marrow microenvironment itself can drive angiogenesis, and also affects the immune response. This has interesting therapeutic implications. It has also been shown that bortezomib can target multiple myeloma and decrease their adherence to BMSCs, inhibiting their growth and also reducing NF-κB-dependent induction of interleukin-6 secretion [162].

Another approach that exploits the immune microenvironment for therapeutic purposes includes targeting the immune system for drug development. One method to overcome evasion of immunosurveillance elicited by the tumor and enhance anti-tumor response is to deplete Tregs. Several approaches have been used to this direction mainly involving the use of anti-CD25 monoclonal antibodies (daclizumab used in T-cell leukemia patients) [163] to target CD4+ CD25+ FoxP3+ Tregs but the disadvantage of this is that anti-CD25 antibodies can also target CD25+ T cells [164]. Productive immune response requires the presence of co-stimulatory and the absence of co-inhibitory signals such as the CTLA-4 and PD-1. The main role of these immunosuppressive molecules is to protect against self-directed immune responses by inducing activation of Tregs and hence tolerance, but their overexpression in cancer cells facilitates evasion of immunosurveillance of tumors. Therefore targeting co-inhibitory signals with specific antibodies to block their function would theoretically increase the possibility that tumors become recognizable by the immune system and offers the potential for the generation of novel efficient immunotherapies [165]. The anti-CTLA-4 antibodies ipilimumab and tremelimumab which block the interaction between CTLA-4 and its ligands CD80 and CD86 have been tested in a variety of tumors but although they initially showed promising results in cancer patients [166], serious adverse effects have restricted their use to limited types of tumors (mainly melanoma) and studies continue to investigate their efficacy when they are used in combination with peptide vaccines [167].

Similarly to CTLA-4 the immunosuppressive function of PD-1 and the expression of its ligands PD-L1 and PDL-2 on tumor cells [168] has provided the theoretical basis for the development of immunotherapeutic approaches against the tolerogenic function of the PD-1 pathway targeting the PD-1/PD-L1 and PD-1/PD-L2 interaction [169]. Several antibodies blocking the PD-1/PD-L1 and PD-1/PD-L2 pathways are in different phases of clinical trials studying their effectiveness in different types of cancer including hematological malignancies [170]. The optimism for the use of these antibodies originates from the fact that PD-1 is expressed more abundantly than CTLA-4 and therefore it could offer higher immunogenicity [171]. Some examples of these antibodies are the MDX-1106 (anti-PD1 mAb, Bristol Myers Squibb) [172], CT-011 (anti-PD1 mAb, CureTech/Teva) [173] and MK-3475 (antiPD-1 mAb, Merck) [174]. Apart from the antibodies blocking PD-1 function immunotherapeutic approaches targeting the PD-1 ligands have been developed given that PD-L1 and PD-L2 might exert functions independent from their role as PD-1 ligands. Examples of antibodies against PD-1 ligands are the MPDL3280A/RG7446 (anti-PD-L1, Genentech) [175], and the MDX-1105 (anti PD-L1 mAb Bristol Myers Squibb) [176].

Combination of immune checkpoint CTLA4, PD-1, PD-L1, and PD-L2 blockade with inhibition of the exhausted T cells' biomarker TIM-3 [177] partially restore T cell function and induce antitumor immunity [178]. The advantage of targeting TIM-3 is that this immunomodulatory receptor is expressed in specific types of T cells and mainly in T cells localized within tumors [179] and hence its inhibition is less likely to lead to autoimmune responses as compared to CTLA-4 and PD-1 blockade [178].

Alternative methods to modulate T cell immunity include the inhibition of the IDO enzymatic activity which has been associated with induction of immunotolerance, increase of the Tregs number [180] and poor prognosis [181]. Despite the advantages of the use of IDO inhibitors in cancer immunotherapy (reviewed in [182]), side effects such as the development of autoimmune encephalomyelitis (EAE) [183] mean that further research is required before they are introduced in clinical practice.

The above examples have highlighted several potential mechanisms for research of the microenvironment to be brought into the clinic. Eventually, the ideal is that a more suitable preclinical model could be developed that more accurately represents what occurs in vivo. One potential model is of tumor-derived xenografts, which seems to be a technique that faithfully mimics what occurs in patients [184], allowing for more accurate preclinical data to be gathered. This may ultimately lead to clinical trials and successful therapy.

3. Conclusions

Accumulated evidence has highlighted an essential role for the bone marrow microenvironment in the development, survival, and persistence of various types of leukemia. The microenvironment affects various leukemia cell functions and through a variety of different mechanisms leads to a chemoresistant phenotype (Fig. 2). However, the multifaceted effects of the microenvironment on drug resistance complicate treatment. A variety of approaches are currently being researched to target the microenvironment and reduce its effect on cancer cells. Many chemotherapeutic drugs target cycling cells, despite the fact that LSCs are maintained in a quiescent state (leading to relapse as they are not eliminated). The use of drugs that target cancer cells regardless of cycling status has been shown to be potentially effective. Additional reported strategy was to first induce the cell cycle in LSCs and then use normal chemotherapy that targets cycling cells. Targeting the chemotactic molecules that drive LSCs towards the niche as well as targeting the various self-renewal pathways that LSCs use to survive are also options. This review has discussed several mechanisms behind microenvironment-mediated drug resistance including the role of inflammation and immune surveillance, as well as potential approaches to mitigate this. Translational research, bringing basic findings from bench to bedside, is crucial to improving patient outlook, as is development of preclinical models that accurately simulates what occurs in patients such as tumor-derived xenografts. The immune system has the potential to eliminate cancer cells and if it can be educated to recognize them, it would provide a powerful approach to improve survival of cancer patients. With a fuller understanding of the microenvironment and further research into abrogating its chemoprotective effect, it is hoped

Table 2
Strategies to limit microenvironment–leukemia crosstalk.

Biochemical interactions in the BM niches	Mechanism of BM mediated resistance to chemotherapy	Potential therapeutic approaches	References
CXCR4–CXCL12	CXCR4 and CXCL12 interaction retains HSCs in BM niche inaccessible by the drug. Increased HSCs' affinity to integrins (VLA-4, VLA-5) increasing adhesion to BM niche CXCR4 and CXCL12 interaction activates PI3K/Akt and MAPK pathways inducing HSCs' survival and proliferation.	CXCR4 inhibitors, inhibitors of the CXCR4–CXCL12 interaction	[10,69,70,185]
G-CSF–G-CSFR	The plasma levels of G-CSF, which induces HSCs' mobilization, are normally low to undetectable.	Treatment with G-CSF analogues to induce HSCs' mobilization	[186]
PTH–PTH1R	Parathyroid hormone (PTH)/Parathyroid Hormone Receptor (PTH1R) [G-protein coupled receptor] ligation induces HSC expansion and increased survival.	Inhibition of PTH/PTHR1R interaction	[187]
VLA-4–VCAM-1, fibronectin	Very Late Antigen 4 (VLA-4) plays an important role in the homing and retention of HSPCs within the bone marrow microenvironment.	Disruption of the interaction of VLA-4 with its ligands to induce rapid and reversible mobilization of HSCs into the peripheral circulation and reduce BM chemoprotective effect	[7,104–107,188]
c-kit–SCF	c-kit binding to SCF activates proliferation, migration, and differentiation of HSCs.	SCF inhibition leads to restoration of normal HSC activity.	[71,72]
Tie2–Ang-1	The receptor tyrosine kinase (Tie2)/Angiopoietin-1 signaling pathway maintains HSCs in quiescence state and induces HSCs' adhesion to the BM endosteal niche thereby conferring chemoresistance to HSCs.	Tie-2-blocking antibodies inhibit growth of AML cells.	[189]
ASNS	Asparagine synthetase (ASNS) expression in ALL cells is low rendering them sensitive to asparagine depletion. ASNS expression levels in bone marrow mesenchymal cells (MSCs) are high thereby protecting HSCs from asparagine depletion.	L-Asparaginase to deplete L-asparagine	[62,64]
Efflux pumps, ABC transporters	Low oxygen concentration in BM niche induces upregulation of ABC transporters' gene expression.	Targeting specific ABC transporters known to be upregulated in resistant leukemic cells	[55]
TGF-β	TGF-β induces HSCs' quiescence in the niche.	TGF-β neutralizing antibodies	[14,84]
IL-6	MSCs are rich in IL-6 and other cytokines known to induce HSC expansion and inhibit apoptosis.	Blockage of IL-6 with monoclonal antibodies	[75–78,131]
Microvesicles (exosomes)	BM-MSCs derived exosomes contain proteins, lipids, mRNA, and miRNA which they secrete into the niche creating a chemoresistant environment.	Preventing exosome formation	[90–99,190]

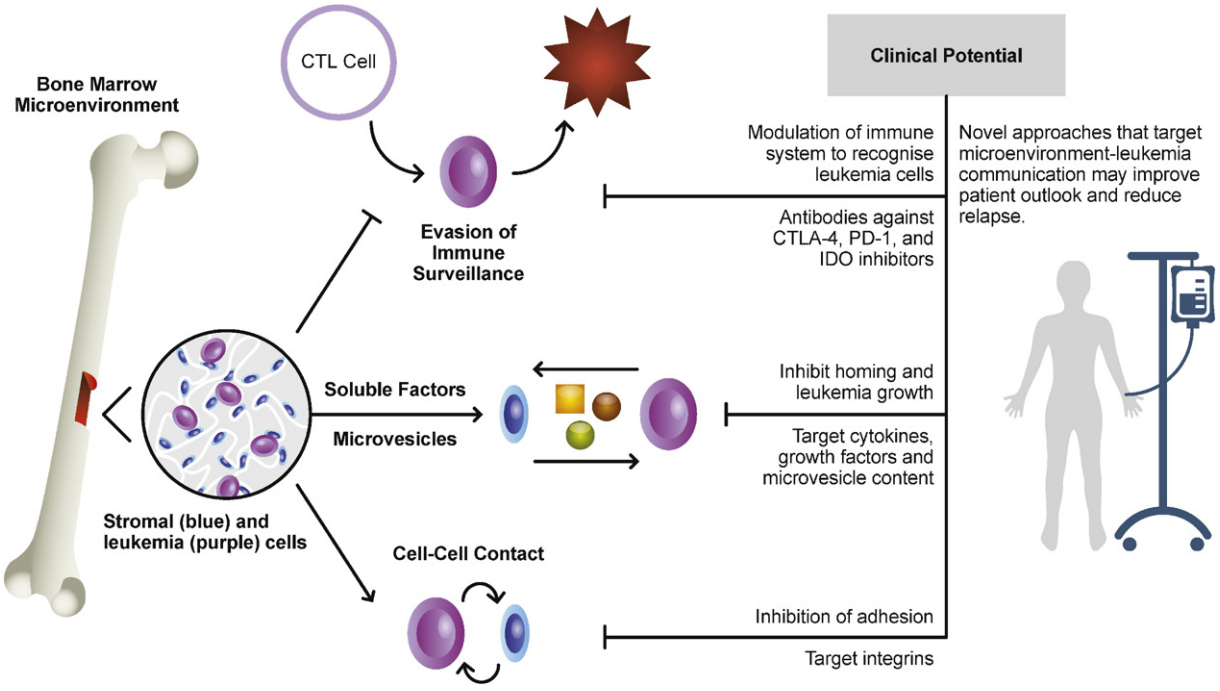

Fig. 2. The role of the microenvironment in chemoresistance. Three main mechanisms of chemoprotective effect of the microenvironment on leukemia cells include cell–cell contact, soluble factors and immune evasion. Key regulators of cell–cell contact are mediators such as integrins. Soluble factors are another way that the microenvironment may exert its effect. This two-way exchange is mediated through a variety of factors such as cytokines, growth factors, and microvesicles. The immune surveillance theory posits that CTLs (cytotoxic T lymphocytes) target leukemia and induce cell death. Signaling from the microenvironment may abrogate this. Novel therapies directed towards the limiting the microenvironment–leukemia crosstalk may improve chemotherapy. Possibilities include targeting stromal cells to reduce adhesion and the resultant protective effect, in addition to interfering with the soluble factors that are exchanged between stroma and leukemia. The immune system may also be trained to recognize leukemic cells, thus limiting immune evasion and improving therapy. Figure key: cytokines (yellow square); microvesicles (brown circles) and growth factors (green circles).

that novel cancer therapies could be developed that improve patient outcome.

4. Future directions

The emerging understanding of the complex crosstalk between the bone marrow microenvironment and leukemic cells has raised new challenges. Effective pharmacological targeting of leukemia cells necessitates combinatory targeting of chemotherapeutics targeting leukemic cells themselves and at the same time their interaction with the bone marrow microenvironment. Prerequisite therefore is to identify the specific characteristics of the BM niches and of leukemia cells that facilitates disease progression and drug resistance. More detailed insight in the similarities and differences of the cellular and biochemical components of the BM niches in different types of leukemias and between patients suffering from the same type of leukemia are important areas to investigate further.

Moreover recent studies have identified particular biochemical factors secreted by cells located in the microenvironment, changing the physiology of the white blood cells, thereby favoring clonal expansion and drug resistance. This, as well as signals released by leukemia cells that alter the architecture of the microenvironment, need to be further explored.

Another area requiring intensive research effort is the role of the BM microenvironment in the evasion of immunosurveillance by leukemia cells. In particular, the detailed understanding of the pathways that leukemia cells use to prevent tumor antigen presentation will allow the development of ex vivo methodologies to “educate” the immune system to effectively detect neoplastic leukemic cells. Furthermore this approach could lead to reduced chemoresistance and improved therapies.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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