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REVIEW

Mapping and targeting of the leukemic microenvironment

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Numerous studies support a role of the microenvironment in maintenance of the leukemic clone, as well as in treatment resistance. It is clear that disruption of the normal bone marrow microenvironment is sufficient to promote leukemic transformation and survival in both a cell autonomous and non–cell autonomous manner. In this review, we provide a snapshot of the various cell types shown to contribute to the leukemic microenvironment as well as treatment resistance. Several of these studies suggest that leukemic blasts occupy specific cellular and biochemical "niches." Effective dissection of critical leukemic niche components using single-cell approaches has allowed a more precise and extensive characterization of complexity that underpins both the healthy and malignant bone marrow microenvironment. Knowledge gained from these observations can have an important impact in the development of microenvironment-directed targeted approaches aimed at mitigating disease relapse.

Leukemia pathogenesis within the bone marrow niche

Leukemia is a clonal hematopoietic neoplasm characterized by the proliferation and accumulation of lymphoid or myeloid progenitor cells throughout the bone marrow. Acute lymphoblastic leukemia (ALL; Hunger and Mullighan, 2015), acute myeloid leukemia (AML; Cancer Genome Atlas Research Network, 2013; van Galen et al., 2019), chronic lymphocytic leukemia (CLL; Landau et al., 2015), and chronic myeloid leukemia (CML; Melo and Barnes, 2007) are heterogeneous diseases in which a variety of genomic alterations have biological and clinical relevance. Extensive genomic characterizations, including large genome-wide sequencing efforts, have uncovered multiple candidates for targeted therapy. Those include the utilization of tyrosine kinase inhibitor therapy in the treatment of *BCR-ABL1*$^+$ CML (Hochhaus et al., 2017) and *BCR-ABL1*$^+$ ALL (Bernt and Hunger, 2014), as well as the development of increasingly potent and selective kinase inhibitors for patients with *FLT-ITD* AML (Daver et al., 2019b), all trans retinoic acid to treat acute promyelocytic leukemia (Wang and Chen, 2008), DOT1L inhibitors in *KMT2A* fusion-positive childhood AML (Bernt et al., 2011; Campbell et al., 2017; Chen et al., 2015), and BH3-mimetic approaches, such as venetoclax, in AML (Knight et al., 2019) and CLL (Roberts et al., 2016). Unfortunately, many targeted therapies fail to elicit prolonged disease remission due to the emergence of preexistent or de novo therapy-resistant leukemic clones. Against this backdrop, there is compelling emerging evidence that cell nonautonomous contributions to leukemia play a pivotal role in disease development, propagation, and maintenance (MacLean et al., 2014; Schepers et al., 2015). These observations may hold promise for the development of new approaches to treat leukemia that focus on the microenvironment (also known as the niche), which supports the leukemia phenotype.

To understand the role of the niche in leukemia development or propagation, one must consider the various cellular components of the bone marrow microenvironment that form among them a network of molecular interactions. This array of cell types includes immune cells, adipocytes, bone-forming osteoblasts, mesenchymal stromal cells, and vascular endothelial cells. Distinct cell types in the bone marrow microenvironment regulate self-renewal or differentiation of hematopoietic stem cells (HSCs; Reagan and Rosen, 2016). Under healthy conditions, the bone marrow microenvironment has been proposed to instruct HSC fate via multiple mechanisms, including proximal interactions between HSCs and the stromal microenvironment, such as VE-cadherin$^+$ vascular endothelial cells and leptin receptor–expressing (Lepr$^+$) perivascular stroma cells that secrete stem cell factor (SCF; Ding et al., 2012) and CXCL12 (Ding and Morrison, 2013) to regulate HSC quiescence and survival. Osteolineage cells also contribute to regulation of HSC differentiation (Galán-Díez and Kousteni, 2017), as well as immune cell types, such as macrophages (Winkler et al., 2010) and

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megakaryocytes (Bruns et al., 2014; Zhao et al., 2014), which have been shown to regulate HSC bone marrow retention and quiescence via proximal interactions.

Numerous studies support a role of the microenvironment in maintenance of the leukemic clone, as well as in treatment resistance. It is clear that disruption of the normal bone marrow microenvironment is sufficient to promote leukemic transformation in a non–cell autonomous manner (Dong et al., 2016; Kode et al., 2014; Raaijmakers et al., 2010; Walkley et al., 2007a; Walkley et al., 2007b). In this review, we provide a snapshot of the various cell types shown to contribute to the leukemic microenvironment as well as treatment resistance (Table 1). Several of these studies suggest that leukemic blasts occupy specific cellular and biochemical niches. Effective dissection of critical leukemic niche components using single-cell approaches has allowed a more precise and extensive characterization of complexity that underpins both the healthy and malignant bone marrow microenvironment. Knowledge gained from these observations can have an important impact in the development of microenvironment-directed targeted approaches aimed at mitigating disease relapse.

Cellular components of the leukemic microenvironment

The vascularized stromal microenvironment

The vascular endothelium plays a pivotal role in regulating the development and function of multiple bone marrow cell types, including osteoblasts (Kusumbe et al., 2014), monocytes (Gamrekelashvili et al., 2016), chondrocytes (Ramasamy et al., 2014), and HSCs (Itkin et al., 2016). Within the bone marrow, the vascular endothelial compartment can be separated into two major cellular subsets, sinusoidal and arteriole, based on functional characteristics and cell surface marker expression (Sivaraj and Adams, 2016). For example, reduced SCF production by arteriole endothelial, but not sinusoidal endothelial, cells results in both reduced HSC numbers and diminished multilineage regenerative capacity (Xu et al., 2018). Moreover, recent single-cell studies have highlighted unique gene expression states that exist within the arteriole and sinusoidal compartments, further expanding our understanding of the heterogeneous biochemical and functional properties present throughout the vascular endothelium (Baryawno et al., 2019; Tikhonova et al., 2019).

Disruption of vascular endothelial integrity represents an important step in the progression of leukemia within the bone marrow. Primary human AML bone marrow biopsies display increased blood vessel formation when compared with healthy bone marrow (Hussong et al., 2000). This is supported by AML xenograft studies and preclinical B-cell ALL (B-ALL) studies, whereby leukemic bone marrow colonization induces bone marrow angiogenesis, a significant increase in nitric oxide production (e.g., NOS3), and increased vascular permeability and hypoxia (Benito et al., 2011; Passaro et al., 2017). A subset of HSCs localized to low-oxygen sinusoidal regions express stabilized hypoxia inducing factor 1α (HIF-1α) coinciding with increased HSC quiescence (Forristal et al., 2013; Parmar et al., 2007). In addition, induction of HIFs is associated with B-ALL and AML chemo-resistance (Muz et al., 2014; Wellmann et al., 2004).

Interactions occurring through the C-X-C chemokine receptor type 4 (CXCR4)/CXCL12 axis, as well as adhesive interactions mediated by leukemic blast expression of transmembrane glycoprotein CD44, are now recognized as a critical extrinsic signaling event promoting leukemic cell survival. Overexpression of chemokine receptor CXCR4 has also been shown to correlate with inferior patient outcome in pediatric B-ALL (Schneider et al., 2002) and AML (Spoo et al., 2007). Disrupting the vascular endothelial microenvironment-derived CXCL12/CXCR4 axis results in apoptosis of T-cell ALL (T-ALL) in vivo (Passaro et al., 2015; Pitt et al., 2015; Sison and Brown, 2011). CD44 expression on the surface of both myeloid and lymphoid blasts is critical for homing and survival within the bone marrow niche and, in some cases, associates with disease outcome (Fedorchenko et al., 2013; Godavarthy et al., 2019; Jin et al., 2006; Krause et al., 2006). Collectively, this suggests that homing to the vascular endothelium represents an important step in leukemic progression within the bone marrow.

Mesenchymal stem cells (MSCs) are capable of tri-lineage differentiation into chondrocytes, osteoblasts, and adipocytes. Extensive heterogeneity within the MSC compartment underpins this multilineage potential. In addition, perivascular cells enriched in MSC activity provide signals critical for HSC function, such as the secretion of SCF from sinusoid-associated Lepr$^+$ stromal cells (Ding et al., 2012), and Cxcl12 secretion produced by arteriole-associated NG2$^+$ perivascular cells (Asada et al., 2017). In leukemia, MSC function is dramatically perturbed by AML blasts leading to, for example, a block in normal osteogenesis and subsequent reduction in osteoblast formation (Baryawno et al., 2019; Duarte et al., 2018; Hanoun et al., 2014). In B-ALL, loss-of-function *IKZF1* mutations strongly associate with B-ALL disease relapse and show a greater dependence on stromal cell adhesion for tumor cell survival via, for example, up-regulation of cell surface integrins (Churchman et al., 2015; Joshi et al., 2014; Vitanza et al., 2014). This is consistent with xenograft studies suggesting that mesenchymal "supporting" cells provide a chemo-resistant niche for leukemic blasts (Duan et al., 2014). Contrary to these findings, the existence of a chemo-resistant stromal niche has been challenged using intravital imaging of transplanted murine T-ALL driven by an activating *NOTCH1* mutation. Using live calvarium imaging, dexamethasone monotherapy was shown to enhance T-ALL motility without evidence of specific stromal interactions (Hawkins et al., 2016). The implications of this live imaging approach may be important when considering what we describe as the "leukemic niche"; however, it must be noted that this study was limited to a highly aggressive NOTCH1-driven preclinical T-ALL model exposed to acute dexamethasone treatment, and used an intravital imaging technique that may be unable to quantify specific cell-to-cell interactions occurring via fine cytoplasmic projections (Gomariz et al., 2018). Many studies use static bone marrow imaging to infer localization of cellular components, including studies of hematopoietic stem and progenitor cell localization (Acar et al., 2015; Bruns et al., 2014; Coutu et al., 2018; Pinho et al., 2018); however, leukemic cells are highly motile, and therefore live imaging approaches and spatial transcriptomic approaches (Baccin et al., 2019 *Preprint*) may highlight unappreciated niche dependencies.

Table 1. **Summary of human and murine studies describing the function of specific niche components in both normal and malignant hematopoiesis**

Niche component	Function/role	Mouse studies	Human studies
Vascular endothelium	AML increases blood vessel formation, marrow angiogenesis, nitric oxide production, vascular permeability, and hypoxia	Hussong et al., 2000; Benito et al., 2011; Passaro et al., 2017	
	HIF induction associated with B-ALL and AML chemo-resistance	Muz et al., 2014; Wellmann et al., 2004	
	The CXCR4/CXCL12 axis and adhesive interactions through CD44 promote leukemic cell survival	Schneider et al., 2002; Spoo et al., 2007; Passaro et al., 2015; Pitt et al., 2015; Sison and Brown, 2011; Fedorchenko et al., 2013; Godavarthy et al., 2019; Jin et al., 2006; Krause et al., 2006	
MSCs			
MSCs	Support IKZF1-mutant B-ALL relapse and may promote chemoresistance, but not in T-ALL	Churchman et al., 2015; Joshi et al., 2014; Vitanza et al., 2014; Duan et al., 2014; Hawkins et al., 2016; Gomariz et al., 2018;	
	AML cells induce osteogenic differentiation	Battula et al., 2017	
	Associate with SNS to regulate HSC mobilization and regeneration following chemotherapy	Ho et al., 2019; Katayama et al., 2006; Lucas et al., 2013; Maryanovich et al., 2018; Méndez-Ferrer et al., 2010; Scheiermann et al., 2012	
CD271$^+$ MSCs	Increase in MDS and AML patients; favor blast expansion through CXCL12		Geyh et al., 2013, 2016
Sinusoid-associated Lepr$^+$ stromal cells	Secretion of SCF, critical for HSC function	Ding et al., 2012	
Arteriole-associated NG2$^+$ perivascular cells	Secretion of CXCL12, critical for HSC function	Asada et al., 2017	
Osteolineage cells			
Osteoblasts	AML blocks osteogenesis and decreases osteoblast numbers	Baryawno et al., 2019; Duarte et al., 2018; Krevvata et al., 2014; Hanoun et al., 2014	Krevvata et al., 2014; El-Ziny et al., 2005; Fitter et al., 2008; Haddy et al., 2001; Sala and Barr, 2007; Shalet, 1996; Sinigaglia et al., 2008
	Disease improvement following chemotherapy correlated with increased osteoblast activity		Crofton et al., 1998; El-Ziny et al., 2005; Fitter et al., 2008
	MDS, MPN, and CML cells remodel endosteal osteoblasts into a self-reinforcing leukemic niche	Medyouf et al., 2014; Schepers et al., 2013	Medyouf et al., 2014
	PTHR activation differentially affects *BCR-ABL1*$^+$ CML-like MPN and *MLL-AF9*$^+$ AML	Krause et al., 2013	
	Restricted by MLL-AF9-AML β2-adrenergic signaling	Hanoun et al., 2014	
Osx$^+$ cells	Dicer-1 deletion leads to MDS	Raaijmakers et al., 2010	
	SDS mutation drives MDS and predicts AML	Zambetti et al., 2016	Zambetti et al., 2016
Osteoblast precursors and osteoblasts	Activated β-catenin leads to MDS/AML	Kode et al., 2014; Bhagat et al., 2017; Stoddart et al., 2017	Kode et al., 2014; Bhagat et al., 2017;
Nestin$^+$ cells	SHP2 activating mutations lead to MPN progression	Dong et al., 2016	
	Expanded by MLL-AF9+AML–induced sympathetic neuropathy	Hanoun et al., 2014	

Table 1. **Summary of human and murine studies describing the function of specific niche components in both normal and malignant hematopoiesis (*Continued*)**

Niche component	Function/role	Mouse studies	Human studies
Adipocytes			
Adipocytes	Decreased chemotherapy responsiveness in obese pediatric B-ALL and adult AML patients		Castillo et al., 2016; Castillo et al., 2012; Orgel et al., 2016; Orgel et al., 2014
	Sequester and metabolize commonly used chemotherapeutic drugs	Behan et al., 2009; Pramanik et al., 2013; Sheng et al., 2017	Sheng et al., 2017
	Secrete glutamine: inhibits the activity of L-asparaginase, a common treatment for ALL	Ehsanipour et al., 2013	
	Fuel AML blasts survival by production of free fatty acids	Shafat et al., 2017; Ye et al., 2016; Li et al., 2018	Shafat et al., 2017
Lepr$^+$Esm1$^+$ perivascular cells	Adipocyte progenitor: negatively regulate HSC function	Tikhonova et al., 2019; Naveiras et al., 2009	
Lepr$^+$ cells	Following injury, accumulation of adipocytes in the bone marrow: critical for hematopoietic recovery	Tikhonova et al., 2019; Zhou et al., 2017	
Immune cells			
T cells	CML- and AML-specific T cell responses		Greiner et al., 2006; Molldrem et al., 2000
	Exhaustion contributes to failure of the graft vs. leukemia response in AML patients		Noviello et al., 2019; Toffalori et al., 2019,
	Exhausted subpopulations predict inferior outcome in pediatric B-ALL		Hohtari et al., 2019
CSF1R-expressing myeloid cells	Promote AML growth		Edwards et al., 2019
Macrophages	Promote CLL xenograft survival	Galletti et al., 2016	

Osteolineage cells

Recently, osteoblasts have emerged as critical regulators of the development of hematological myeloid malignancies. A single activating mutation in β-catenin signaling in osteoblasts is sufficient to lead to the development of myelodysplastic syndrome (MDS), eventually progressing to AML in mice. The disease is transplantable and characterized by clonal evolution at the cytogenetic level. Activated β-catenin signaling is present in the osteoblasts of approximately one third of MDS patients, suggesting that this pathway sustains dysplastic hematopoiesis and progression to MDS in humans (Kode et al., 2014). Recently, other laboratories have shown the malignancy-inducing signal of activated β-catenin in the stroma (Bhagat et al., 2017; Stoddart et al., 2017). Aberrant methylation in stromal cells from MDS patients leads to reduced expression of the Wnt pathway antagonists *FRZB* and *SFRP1*, leading to activation of β-catenin, which correlates with adverse prognosis in humans (Bhagat et al., 2017). Treatment with the DNA methylation inhibitor 5-azacytidine reinstates normal methylation in the stroma, including high *FRZB* levels, and renders MDS stromal cells capable of supporting hematopoiesis. In the second study, loss of one β-catenin allele in the MSCs of *Apc*-haploinsufficient mice prevents the development of MDS, delays the onset of anemia, and prolongs survival (Stoddart et al., 2017). Administration of pyrvinium, a Wnt–β-catenin inhibitor, rescues anemia and prolongs survival in mice due to reduced Wnt pathway activation in the stroma.

Other studies have shown myeloid disease-initiation mutations in cells of the bone marrow stroma. Deletion in osteolineage cells of *Dicer1*, a main regulator of the microRNA biosynthesis machinery, enables a disease that profoundly resembles human MDS (Raaijmakers et al., 2010); activating mutations of the protein tyrosine phosphatase SHP2 in mesenchymal stem/progenitor cells and osteoprogenitors leads to myeloproliferative neoplasm (MPN) in a CCL3-CCR1/5–dependent manner (Dong et al., 2016; Zambetti et al., 2016); and Schwachman-Diamond syndrome mutation in stromal cells drives MDS in mice through S100A8/9-TLR signaling that predicts AML progression in patients (Zambetti et al., 2016).

Healthy osteoblasts can also transmit signals that affect the fate of AML cells. Ablation of osteoblastic cells accelerates leukemia progression in several mouse models of AML and ALL (Frisch et al., 2012; Krevvata et al., 2014). Maintenance of osteoblast numbers during leukemia by pharmacological inhibition of the synthesis of duodenal serotonin through treatment with a tryptophan hydroxylase inhibitor stimulates normal hematopoiesis, delays disease engraftment, reduces tumor burden, and prolongs survival (Krevvata et al., 2014).

Conversely, MDS and leukemic cells in MPN models provide instructive signals that remodel osteoblasts in the endosteal bone marrow niche into a self-reinforcing leukemic niche (Medyouf et al., 2014; Schepers et al., 2013). MPN cells secrete inflammatory mediators such as CCL3 and thrombopoietin, driving differentiation of bone marrow MSCs to osteoblasts (Schepers et al., 2013). The remodeled stroma favors leukemic stem and progenitor cell function over normal hematopoiesis and becomes highly fibrotic. As a result of this pro-inflammatory milieu, CML remodels the endosteal niche so as to reinforce and self-perpetuate the blast crisis. In line with this notion, MDS patient-derived MSCs were able to instruct the stroma to overproduce N-cadherin, IGFBP2, VEGFA, and LIF, which in turn promoted MDS expansion (Medyouf et al., 2014).

AML progression in mice is accompanied by remodeling of endosteal vessels coupled to loss of osteoblasts, HSCs, and HSC niches. Interestingly, rescue of endosteal vessels preserves HSCs and improves chemotherapy efficacy (Duarte et al., 2018). In addition, leukemic cells, but not normal CD34$^+$ or CD33$^+$ cells, induce osteogenic differentiation of bone marrow MSCs via BMP-mediated signaling at the expense of adipocytic differentiation, hence suppressing the formation of an adipocyte niche (Battula et al., 2017; Boyd et al., 2017). AML cells also induce expression of genes involved in osteogenesis, such as CTGF, in bone marrow MSCs, which in turn enhances leukemic engraftment in mice (Battula et al., 2017).

Effects on the bone marrow microenvironment can differ in the context of different types of myeloid malignancies. For instance, it has been reported that activation of the parathyroid hormone receptor in osteoblasts, which leads to increased TGF-β1, exerts differential effects on *BCR-ABL1*$^+$ CML-like MPNs and *MLL-AF9*$^+$ AML; and although parathyroid hormone receptor activation prolonged survival in *BCR-ABL1*$^+$ CML-like MPNs, it acted to shorten survival in murine *MLL-AF9*$^+$ AML transplantation models (Krause et al., 2013).

In humans, although the phenotypic profile of cell surface expression markers defining stromal MSCs in the bone marrow niche does not change between healthy subjects and myeloid malignancy patients, specific subpopulation numbers, functions, or molecular profile appear to be altered. MDS and AML patients show a reduction in osteoblast numbers, reflecting a corresponding reduction in bone formation rate without any changes in osteoclast numbers (Krevvata et al., 2014). This agrees with clinical reports of osteopenia or osteoporosis, due to a decrease in osteoblast function, noted in newly diagnosed children or adults with acute leukemia (El-Ziny et al., 2005; Fitter et al., 2008; Haddy et al., 2001; Sala and Barr, 2007; Shalet, 1996; Sinigaglia et al., 2008). In several of these studies, improvement of disease burden following chemotherapy correlated with an increase in osteoblast activity and bone mass, in spite of whether corticosterone treatment was also used (Crofton et al., 1998; El-Ziny et al., 2005; Fitter et al., 2008). In stromal cells from MDS and AML patients, expression of cell surface molecules involved in interaction with HSCs is decreased (Geyh et al., 2013), whereas the population of CD271$^+$ MSCs, which favor blast expansion through up-regulation of CXCL12, is increased. In addition, colony formation capacity and osteogenic differentiation are significantly impaired (Geyh et al., 2016).

Sympathetic nervous system

Another means malignant cells employ to alter the bone marrow niche, to their advantage through actions in osteoblasts or osteoblast precursors, is induction of sympathetic nervous system damage. Under healthy conditions, MSCs have been shown to associate with the sympathetic nervous system to regulate multiple HSC functions, including mobilization and hematopoietic regeneration after chemotherapy (Ho et al., 2019; Katayama et al., 2006; Lucas et al., 2013; Maryanovich et al., 2018; Méndez-Ferrer et al., 2010; Scheiermann et al., 2012). In the MLL-AF9-AML model, leukemic cells induce sympathetic neuropathy, leading to aberrant expansion of *Nestin*$^+$ MSCs while restricting the numbers of mature osteoblasts through β2-adrenergic signaling (Hanoun et al., 2014). As a result, expression of HSC retention factors, including CXCL12, SCF, Ang-1, and VCAM1, is decreased. In a mouse model of MPNs harboring the JAK2V617 mutation, the production of IL-1β by mutant HSCs causes damage to sympathetic nerve fibers and death of Schwann cells, leading to loss of Nestin$^+$ MSCs and decreased CXCL12 production (Arranz et al., 2014). In turn, the impaired niche promotes expansion of mutant HSCs and facilitates MPN progression. These events are prevented by treatment with agonists of β3-adrenergic receptors, which are expressed in MSCs.

Adipocytic niches

Recent single-cell analysis approaches have shed light on the heterogeneity that exists within the bone marrow MSC compartment, including the presence of sinusoidal vessels expressing both the Lepr and endothelial specific molecule 1 (Esm1). These Lepr$^+$Esm1$^+$ perivascular cells contain a subset of stromal cells expressing high mRNA levels of adipocyte-associated markers, such as *Lpl* and *Adipoq* (Tikhonova et al., 2019). Under steady-state conditions, these mesenchymal progenitors give rise to adipocytes believed to negatively regulate HSC function (Naveiras et al., 2009). Following injury (e.g., 5-FU treatment and irradiation), however, the Lepr$^+$ stromal cell compartment is enriched with adipocyte-biased stromal progenitors followed by the accumulation of adipocytes within the bone marrow (Tikhonova et al., 2019). Here, adipocytes play a critical role in hematopoietic recovery, through the secretion of SCFs (Zhou et al., 2017).

With this in mind, the role of adipocytes in promoting tumor cell survival has also emerged. Obese leukemia patients display inferior chemotherapy responsiveness when compared with nonobese patients in pediatric B-ALL and adult AML (Castillo et al., 2016; Castillo et al., 2012; Orgel et al., 2016; Orgel et al., 2014). Adipocytes actively sequester and metabolize commonly used chemotherapeutic drugs, such as daunorubicin and vincristine (Behan et al., 2009; Pramanik et al., 2013; Sheng et al., 2017). Secretion of glutamine by adipocytes has also been shown to inhibit the activity of L-asparaginase, a common treatment agent in ALL therapy (Ehsanipour et al., 2013).

Fatty acid oxidation represents an important energy source for leukemic blasts. AML cells enhance the production of free fatty acids from bone marrow adipocytes, via up-regulation of FABP4 by adipocytes, thus fueling AML blast survival (Shafat et al., 2017). Recent studies of blast-crisis CML suggest that

leukemia-initiating cells expressing high levels of fatty acid transporter CD36 seed adipose tissue. Further, CD36$^+$ leukemic blasts enhance adipose tissue lipolysis to fuel fatty acid oxidation (Ye et al., 2016). Collectively, an adipocyte-rich microenvironment may provide an important protective reservoir for leukemic blasts, particularly with the increasing abundance of bone marrow adipocytes with age (Li et al., 2018).

Immune cell microenvironment

Immunosuppressive microenvironments may pose many challenges to effective leukemia therapy, particularly approaches that rely on effective immune function for leukemic blast clearance, such as chimeric antigen receptor (CAR)–T cell therapy. When compared with studies of solid tumors (Quail and Joyce, 2013), the functional state of leukemia-associated immune cells is largely unknown. Evidence for tumor-specific T cell responses have been reported in human CML and AML (Greiner et al., 2006; Molldrem et al., 2000). Further, T cell exhaustion is a significant contributor to failure of the graft versus leukemia response in AML patients relapsing after allogeneic HSC transplantation (Noviello et al., 2019; Toffalori et al., 2019), while exhausted T cell subpopulations are present and predict inferior patient outcome in pediatric B-ALL bone marrow (Hohtari et al., 2019).

Myeloid cells, such as macrophages, are often implicated in establishing immunosuppressive solid tumor microenvironments (Fleming et al., 2018); however, evidence of immunosuppressive myeloid cells within the leukemic microenvironment is limited (De Veirman et al., 2014). Non-transformed *CSF1R*-expressing myeloid cells have been shown to promote AML growth; however, the precise identity and prognostic impact of these myeloid cells are unknown (Edwards et al., 2019). Tumor-associated macrophages also promote CLL xenograft survival in vivo (Galletti et al., 2016). Collectively, the contribution of T cell–mediated responses and leukemia-associated myeloid cells to leukemic emergence and immunosuppression remains unclear.

Immune-based approaches have emerged as an effective treatment option in high-risk leukemia and rely on both efficient T cell function and a permissive extrinsic microenvironment for leukemic killing. These approaches include CAR–T cell therapy, Bispecific T cell Engager (BiTE) therapy, and immune checkpoint blockade (ICB) therapy, all of which differ significantly in drug design and mechanism of action, yet all rely on optimal effector T cell function to elicit anti-leukemic effects. Highly effective FDA-approved CAR-T therapeutic, Tisagenlecleucel, utilizes patient-derived autologous T cells, which are transduced with lentiviral vectors expressing a CAR comprised of single-chain fragment from a murine monoclonal antibody specific for CD19, a CD3-zeta domain for T cell activation, and a CD28 domain for TCR costimulation (Leahy et al., 2018; Lee et al., 2015; Maude et al., 2018; Milone et al., 2009; Mueller et al., 2018). Upon patient infusion, CAR–T cells engage CD19$^+$ leukemic cells to elicit a cytotoxic response in an MHC-independent manner. B cell–specific BiTE therapies, such as blinatumomab, comprise bispecific single-chain antibody constructs that promote direct engagement of CD3$^+$ T cells with CD19$^+$ B cells, resulting in cytolysis of proximal CD19$^+$ cells (Lee et al., 2016). In recent years, ICB therapy has been effective in solid tumors through the reinvigoration of T cells in the tumor microenvironment. One example of this is inhibition of the PD-1/PD-L1 interaction, a critical axis for T cell exhaustion in the tumor microenvironment whereby PD-1, expressed on T cells, interacts with its ligand PD-L1, expressed on tumor cells (Ribas and Wolchok, 2018).

Although these immune-based approaches converge upon T cell killing of leukemic blasts, many extrinsic factors may impact treatment efficacy. For example, excessive secretion of IL-1 and IL-6 from activated monocytes and macrophages during CAR-T therapy has been shown to drive cytokine release syndrome in preclinical B-ALL models (Giavridis et al., 2018; Norelli et al., 2018), consistent with human studies of blinatumomab cytokine release syndrome (Teachey et al., 2013). Further, increased regulatory T cell abundance negatively correlates with blinatumomab efficacy in the treatment of high-risk B-ALL patients (Duell et al., 2017). AML blasts have also been shown to express immune checkpoint inhibitors, such as PD-L1 (Chen et al., 2008; Krönig et al., 2014), which are further augmented in expression by hypomethylating agent therapy (Stahl et al., 2018; Yang et al., 2014). Collectively, this highlights the complex extrinsic interactions that occur throughout immune-based treatment; however, the magnitude to which the microenvironment regulates treatment responsiveness still requires further investigation.

Targeting critical components of the leukemic microenvironment

Inhibition of extrinsic signals required for leukemic survival

Eradication of the minimal residual disease that drives disease relapse remains an outstanding issue in high-risk subtypes of pediatric ALL. Minimal residual disease may hijack the bone marrow microenvironment to escape existing therapies. Additional mechanisms of leukemic treatment resistance have also emerged in relation to homing interactions with the bone marrow niche. Based on the greater dependence on cell adhesion for IKAROS-mutated tumor cell survival (Joshi et al., 2014), pharmacological inhibition of cell adhesion or focal adhesive kinase activity results in Ikaros mutant B-ALL cell death in preclinical models (Churchman et al., 2015; Joshi et al., 2014). Extending on this, targeted inhibition of specific surface integrins expressed on leukemic blasts, such as Integrin Beta 3 and Integrin Alpha 4, has proven an effective means of eliciting AML and B-ALL blast killing, respectively (Hsieh et al., 2013; Miller et al., 2013). Preclinical studies highlighting the importance of the CXCR4/CXCL12 axis on leukemic survival (Passaro et al., 2015; Pitt et al., 2015; Sison and Brown, 2011) have spawned early clinical trials of AML patients with CXCR4 inhibitor, plerixafor, which effectively mobilizes leukemic blasts to enhance their sensitivity to cytotoxic chemotherapy (Uy et al., 2012).

Vascular leakiness induced by AML xenografts is partially reversed through inhibition of the NOX4–NOS3 axis (Passaro et al., 2017). However, despite targeting of the hypoxic leukemic niche leading to inhibition of leukemic growth and drug resistance in preclinical systems (Benito et al., 2011), early clinical trials showed limited anti-leukemic activity (Konopleva et al., 2015).

Targeting adipocytic interactions, such as blocking free fatty acid transfer to AML blasts (Shafat et al., 2017), may also present a novel approach to targeted niche therapy. Adipocytes also produce high levels of the hormone leptin. In preclinical T-ALL and B-ALL models, fasting of leukemia recipient mice resulted in decreased leptin, which, in turn, was suggested to increase Lepr signaling. This increased Lepr signaling was shown to be selectively detrimental to T-ALL and B-ALL blast survival, but not AML (Lu et al., 2017). It remains to be seen whether these findings are applicable to the majority of leukemic subtypes; however in light of studies correlating obesity to therapeutic responsiveness, they do support the notion of a targetable adipocytic niche.

Immune-based therapeutic opportunities

Targeting components of the leukemic microenvironment may also present a novel means of enhancing immune therapeutic effectiveness, particularly in the face of an immunosuppressive microenvironment. For example, based on the observed increase in checkpoint inhibition expression on AML blasts following hypomethylating agent therapy, the combination of ICB with the hypomethylating agent azacytidine has shown remarkable clinical efficacy in AML (Daver et al., 2018; Daver et al., 2019a).

Immune evasion presents further challenges in the elimination of leukemia-initiating clones. For example, the ability of leukemia-initiating AML cells to evade phagocytosis by innate immune cells, such as that mediated by surface expression of CD47 on AML-initiating cells, is an independent poor prognostic in AML (Jaiswal et al., 2009; Majeti et al., 2009). To address this, CD47-inhibiting monoclonal antibody approaches have been developed to promote phagocytosis of leukemia-initiating AML cells (Majeti et al., 2009), with the use of the first-in-class anti-CD47 monoclonal antibody Hu5F9-G4 in early human AML clinical trials demonstrating promising antitumor responses as either a single agent or in combination with demethylating agent therapy (Sallman, D.A., W.B. Donnellan, A.S. Asch, D.J. Lee, M.A. Malki, G. Marcucci, D.A. Pollyea, S. Kambhampati, R.S. Komrokji, J.V. Elk, et al. 2019. 2019 ASCO Annual Meeting. Abstr. 7009). More recently, the importance of natural killer (NK) cells in mediating clearance of AML-initiating cells has become clear, primarily through the recognition of leukemic blasts by NK cells via NK group 2D (NKG2D) receptors. Here, "stem-like" AML cells lacked the expression of the NKG2D ligands, allowing the evasion NK-mediated clearance. Strikingly, inhibition of poly-ADP-ribose polymerase 1 (PARP1) was sufficient to induce expression of NKG2D ligands on AML-initiating cells and promote NK cell–mediated leukemic clearance. These studies, and many others (Austin et al., 2016; Curran et al., 2017), highlight the importance of the immune surveillance mechanisms exploited by leukemic blasts in evading clearance and, ultimately, allowing leukemic progression and treatment resistance.

To avoid CAR-T cell exhaustion during treatment of relapsed/refractory cases of B cell non-Hodgkin lymphoma or B-ALL, clinical trials are also underway in which the PD-1 inhibitors pembrolizumab or nivolumab will be combined with CD19 CAR–T cell therapy (Chong, E.A., J.J. Melenhorst, J. Svoboda, S. Dwivedy Nasta, D.J. Landsburg, A.R. Mato, L. Tian, H. Parakandi, S.F. Lacey, C.H. June, et al. 2017. 59th ASH Annual Meeting. Abstr. 4121; Maude, S.L., G.E. Hucks, A.E. Seif, M.K. Talekar, D.T. Teachey, D. Baniewicz, C. Callahan, V. Gonzalez, F. Nazimuddin, M. Gupta, et al. 2017. 2017 ASCO Annual Meeting. Abstr. 103). This also highlights recent preclinical developments, such as "Armored" CAR–T cells that are capable of manipulating the tumor microenvironment to favor efficient killing (Yeku et al., 2017). For example, CD19 CAR–T cells constitutively expressing CD40-L or IL-18 both promote a local pro-inflammatory microenvironment, which, in turn, enhances recruitment of antigen-presenting cells while increasing effective CAR-T killing of murine lymphoma in vivo (Avanzi et al., 2018; Kuhn et al., 2019).

Enhancing T cell homing and motility within the leukemic microenvironment may be important when attempting to eradicate leukemic blasts in previously inaccessible regions. For example, an increasingly hypoxic bone marrow niche driven by B-ALL causes a significant decrease in the motility of B-ALL–associated T cells in vivo (Benito et al., 2011; Rytelewski et al., 2019). This inability of T cells to home, adhere, and extravasate into these leukemic reservoirs may prove problematic, especially with the dramatic increase in bone marrow vascular permeability and decreased blood flow that coincide with leukemic growth (Passaro et al., 2017). Therefore, it is conceivable that restoring vascular integrity and reversing hypoxia may enhance T cell function at sights of leukemic colonization, hence improving the efficacy of immune-based therapies.

Generating a map of the leukemic bone marrow

Although knowledge of specific components of the leukemic microenvironment is still emerging, comprehensive mapping of the diverse interactions occurring throughout the bone marrow during disease pathogenesis and treatment would provide invaluable insights into targetable critical extrinsic interactions. Single-cell technologies allow prospective identification of multiple cell types from a highly complex tissue, such as the bone marrow, and valuable insights into the diverse functions of these heterogeneous cell populations. As an example, single-cell tracking of AML-associated FLT3-ITD mutations throughout the hematopoietic compartment found significant inter-patient variation in predicted transformed hematopoietic progenitor subpopulations (van Galen et al., 2019). Giustacchini et al. (2017) used single-cell approaches to dissect the heterogeneity existing within the HSC compartment of CML patients, where tyrosine-kinase inhibition-resistant, quiescent BCR-ABL1$^+$ stem cells were identified, as well as nonleukemic BCR-ABL1$^-$ exhibiting a distinct molecular signature that associated with disrupted hematopoiesis. More recently, single-cell RNA sequencing of bone marrow stroma isolated from AML-bearing recipient mice demonstrated the presence of AML significantly reduced normal osteolineage differentiation (Baryawno et al., 2019), consistent with previous studies (Krevvata et al., 2014). In addition, in the Lepr$^+$ mesenchymal stromal cell compartment, mRNA expression of pro-hematopoietic cytokines, such as *Kitl* and *Cxcl12*, is significantly reduced. In light of this, it is now possible to generate a complete map of the heterogeneity that exists at cell-intrinsic and -extrinsic levels of the leukemic process. Furthermore, using

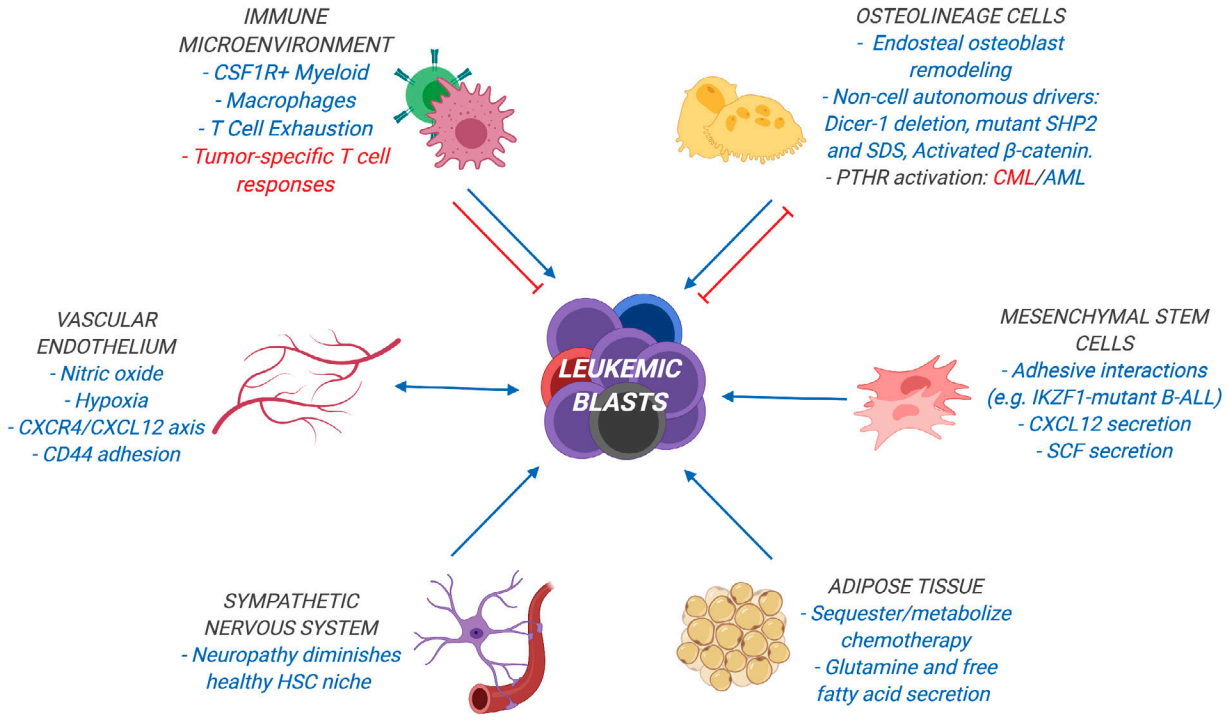

Figure 1. **Dynamic interactions between leukemic blasts and their niche.** Schematic depicts the multiple extrinsic regulatory mechanisms implicated in promoting leukemic blast survival, highlighting critical interactions that either promote (blue) or inhibit (red) leukemic cell growth. Upon disease transformation, remodeling of the leukemic bone marrow microenvironment involves various interactions between leukemia and its microenvironment that may be reciprocal and multifaceted. Non-hematopoietic components (such as osteolineage cells, adipose tissue, and mesenchymal and vascular endothelial cells), the immune microenvironment, and the sympathetic nervous system have all been previously implicated in cell-mediated regulation on leukemic survival.

rapidly improving technologies that provide spatial gene expression information (Baccin et al., 2019 *Preprint*; Ståhl et al., 2016) and real-time in vivo imaging, it may be possible to identify the precise proximal interactions and extrinsic signaling events required for leukemic blast survival within the bone marrow microenvironment.

Concluding remarks and future directions

Multiple critical studies have identified cell types present in the healthy and leukemic bone marrow microenvironment that contribute to cellular functions through a multitude of extrinsic mechanisms (Fig. 1). However, how these different interactions coalesce to promote tumor survival and affect treatment responsiveness remains unclear. Targeting the microenvironment has shown limited success in the clinical arena, which may be due to the unexplored extrinsic interactions that underpin leukemic blast survival. Furthermore, with the introduction of new therapeutics that capitalize on immune cell function (e.g., CAR-T therapy), creating a permissive microenvironment for optimal leukemic killing may be essential for optimal use of these agents. Recent developments in single-cell and imaging technologies have made it possible to assess the composition and diverse cellular and biochemical interactions present throughout complex tissue. Future studies aimed at identifying the diverse network of cellular and biochemical interactions underlying the leukemic niche have the potential to inform novel strategies targeting the niche dependencies and, thus, mitigating residual or chemo-resistance leukemic blasts.

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Fig. 1 was created using BioRender.

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