The interplay of leukemia cells and the bone marrow microenvironment

Delfim Duarte[1,2,*] Edwin D Hawkins[3,4,*], and Cristina Lo Celso[1,2]

[1] *Department of Life Sciences, Sir Alexander Fleming Building, Imperial College London, SW7 2AZ, London, UK.*

[2] *The Francis Crick Institute, WC2A 3LY, London, UK.*

[3] *The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3052, Australia.*

[4] *Department of Medical Biology, The University of Melbourne, Parkville, Victoria 3010, Australia.*

[*] *Both authors contributed equally to this manuscript.*

Correspondence to:
Cristina Lo Celso
E-mail: c.lo-celso@imperial.ac.uk
Address:
Imperial College London
Department of Life Sciences
Sir Alexander Fleming Building
London SW7 2AZ
UK
Phone: +44 2075945359

Abstract

The interplay of cancer cells and surrounding stroma is critical in disease progression. This is particularly evident in hematological malignancies that infiltrate the bone marrow and peripheral lymphoid organs. Despite clear evidence for the existence of these interactions, the precise repercussions on the growth of leukemic cells are poorly understood. Recent development of novel imaging technology and preclinical disease models have advanced our comprehension of leukemia-microenvironment crosstalk and have potential implications for development of novel treatment options.

Introduction

Leukemias are characterized by the aggressive nature of disease and poor response to therapy. Patients with leukemia often present with cytopenias resulting from disruption of normal hematopoiesis. This leads to complications due to bleeding and recurrent infections. Hematopoiesis is maintained by self-renewing hematopoietic stem cells (HSC) that reside in niches within the bone marrow (BM)[1]. The precise composition and function of these niches is still under intense investigation. Regardless, the biology of HSC is thought to be regulated by a complex array of cell populations, including arteriolar[2] and sinusoidal[3] endothelial cells, mesenchymal stem cells (MSC)[4], perivascular stromal cells[2,5], osteoblasts[6], sympathetic neurons[7], non-myelinating Schwann cells[8], adipocytes[9] megakaryocytes[10,11] and regulatory T cells[12]. It has been speculated that leukemia cells highjack[13,14], and destroy[15] HSC-supportive microenvironments potentially shifting the equilibrium of microenvironments from a state that supports steady state hematopoiesis in favour of conditions that instead lead to accelerated expansion of leukemic cells or even to leukemogenesis and development of chemoresistance. Thus, understanding the role of microenvironments in leukemia initiation, progression and development of chemoresistance (Figure 1) is critical for development of novel therapeutic interventions.

Leukemia initiation

It has been suggested that changes to the steady state HSC niche can promote leukemogenesis. In mouse models, onset of pre-leukemic myeloproliferative-like disease has been observed after manipulation of the microenvironment. Specifically, loss of retinoic acid receptor gamma in non-hematopoietic cells[16], defective Notch signaling (either endothelial-specific[17] or global[18]) and

targeted expression of *Ptpn11* activating mutations in MSCs and osteoprogenitors (a positive regulator of RAS signaling found in Noonan syndrome)[19] have been implicated in disease development. Furthermore, a condition similar to myelodysplastic syndrome with sporadic progression to acute myeloid leukemia (AML)/myeloid sarcoma was observed after specific deletion of the endoribonuclease *Dicer1* in osteprogenitors[20]. In these mice, mutated osteprogenitors expressed lower levels of *Sbds*, the gene mutated in Shwachman-Bodian-Diamond Syndrome (a condition characterized by BM failure, occasional myelodysplastic syndrome development and secondary AML). Interestingly, deletion of *Sbds* in osteoprogenitors mimicked the phenotype observed in *Dicer* loss. Additional support for the role of osteolineage cells in leukemogenesis is demonstrated by mouse models in which overexpression of β-catenin was targeted to osteoblasts. This was shown to induce transformation of HSCs and promote AML development mediated by the downstream overexpression of the Notch ligand Jagged-1 in osteoblasts[21]. These data provide evidence that in mouse models the microenvironment can initiate leukemogenesis or promote the growth of mutant hematopoietic cells that do not usually expand under normal homeostatic conditions. However, it is still uncertain whether similar changes in the microenvironment alone are causative of human leukemias[22]. Clinically, this hypothesis is best supported by donor cell leukemia[23], where leukemia originates from engrafted donor cells after allogeneic HSC transplantation. These cases strongly suggest that the microenvironment can initiate leukemogenesis in healthy cells. However the contribution of drug-induced effects on the stroma and transplanted hematopoietic cells are still being questioned. Furthermore, the prevalence of donor cell leukemia is rare (reviewed in [24]). Therefore, it is still unclear whether these cases result from rare germline mutations or alternatively are driven by alterations in the recipient BM microenvironment.

Leukemia propagation and development of chemoresistance

There is an increasingly popular view that development of cancer follows a Darwinian-like evolution, in which microenvironmental changes contribute to the selection and expansion of adapted malignant clones[25]. It is not clear whether the microenvironment facilitates the propagation of pre-leukaemic clones. Clonal hematopoiesis is a recently described entity in which clonally expanded hematopoietic cells harbouring somatic mutations are found in persons with no history of hematological malignancy[26-29]. It is present in more than 10% of individuals over 70 years old and it is associated with increased all-cause mortality and with a 10-fold higher incidence of hematologic cancer[26,27]. The methyltransferase *DNMT3A* is the most commonly mutated gene in clonal hematopoeisis[26-28] and is commonly mutated in leukemia (reviewed in [30]). Consistently, *Dnmt3a*-null HSCs have increased self-renewal capacity and expand preferentially in competitive

transplantation assays[31]. Moreover, *DNMT3A*[mut] pre-leukaemic HSCs were shown to outcompete wild-type HSCs and to survive in AML patients in remission[32]. There is evidence that an aged BM microenvironment favours the expansion of single dominant HSPCs clones[33]. Whether the competitive fitness of *DNMT3A*[mut] pre-leukaemic is purely driven by cell intrinsic mechanisms or whether the microenvironment is also taking part is currently unexplored.

Through a series of xenotransplantation studies[34,35], it was shown that once overt disease is established AML cells are hierarchically organized and are descendants of rare transformed leukemic stem cells (LSCs) that have the ability to self-renew and differentiate into highly proliferative progeny. The resultant leukemic cell mass is the result of clonal evolution and is organized in a complex architecture where dominant clones co-exist with minor subclones. This complexity is illustrated by genomic analyses of leukemic samples showing that AML relapse can be driven by either the dominant clone or by minor subclones, upon acquisition of new mutations during chemotherapy[36]. However, and despite the genomic complexity, it was recently shown that leukemic cells with a "stem-like" transcriptional signature initiate disease relapse in AML[37]. Although consensus regarding the role of LSCs has not been reached[38], the similarities in phenotype and biology between LSCs and HSCs[39] have propagated the idea that LSCs (much like HSC) reside in niches that support the expansion, survival and relapse of leukemia[13,14]. It is likely that BM niches act differently on LSCs and blasts. For example, the chemokine CXCL12 (also known as SDF-1α) secreted specifically by osteoblastic cells was shown to be irrelevant for the maintenance of HSCs but key for early lymphoid progenitors[40]. An analogous differential regulation might exist in the maintenance of LSCs *versus* blasts during leukemia propagation. Importantly, the relationship between malignant cells and the microenvironment is also specific to both disease stage and subtype. LSCs in AML[34] and chronic myeloid leukemia (CML)[41] are well characterised and have previously been suggested to have an altered dependency on the endosteal niche, and specifically osteoblastic cells after parathyroid hormone treatment[42]. In this context, the expansion of osteoblastic cells promotes the propagation of MLL-AF9 driven AML while it halts BCR-ABL CML-like disease[42].

In contrast to factor-specific microenvironments within the tissue, the BM as a whole can also be viewed as having its own unique factor/environmental identity when compared to other organs. Perhaps the most frequently studied of these "globally distributed" BM leukemia-stroma interactions is the CXCR4/CXCL12 axis. These interactions support leukemia cells that express high levels of CXCR4 and bind CXCL12, secreted by multiple BM stromal cells. Using genetic models and CXCR4 antagonists (e.g. AMD3100/plerixafor), it was shown that CXCL12 promotes the homing, residence and survival of leukemic cells in the BM[43-46]. These studies provided the

rationale for clinical trials (proved safe in AML[47]) and for the development of new, more potent, CXCR4 antagonists[48]. However, it is not well understood how short-acting CXCL12 gradients may control the behaviour of leukemic cells (e.g. cell migration) within the BM. In addition to CXCR4, there are other molecules expressed by leukemic cells crucial for their adhesion to, and survival in the BM microenvironment. Chemoresistance is enhanced in leukemic cells expressing the integrin VLA-4, which binds fibronectin in the extracellular matrix[49] and VCAM-1 on BM stroma[50]. Another key adhesion molecule on leukemia cells is the glycoprotein CD44, which binds hyaluronic acid in the extracellular matrix. LSCs in both CML[51] and AML[52] require CD44 for homing and engraftment efficiency, while this molecule seems dispensable for healthy HSC.

More recently, we challenged the view that all leukemic cells depend on specific niches. Using intravital microscopy, we tracked T-cell acute lymphoblastic leukemia (T-ALL) cells from early stages of BM infiltration to development of chemoresistance[53]. Imaging the whole BM tissue revealed that seeding and chemoresistant T-ALL cells are stochastically distributed in relation to Col2.3$^+$ osteoblasts, Nestin$^+$ MSCs and blood vessels. Contrarily to the popular view that leukemic cells are immotile and evade chemotherapy by nesting in specific hot spots, we observed that T-ALL cells are highly motile and exploratory. The mechanisms driving this motility and whether this migratory phenotype is a feature of other types of leukemia is still unresolved.

Bone Marrow remodeling

The interplay of leukemic cells with the BM microenvironment has been demonstrated to be a two way street, with malignant cells able to remodel the microenvironment. This is well illustrated by the destruction of BM microenvironments induced by xenotransplanted ALL cell lines[15,54]. Importantly, the leukemia-driven remodeling can promote the loss of bone homeostasis and healthy hematopoieis and also lead to the expansion and survival of the leukemia itself. For example, precursor B-cell ALL cells were shown to secrete CCL3, recruit Nestin$^+$ MSCs from sinusoidal niches and promote their transition into α-SMA$^+$ cells (through TGF-β) to form chemo-protective islands[54].

Perhaps the best-studied example of bone marrow remodeling in hematological malignancies is multiple myeloma (MM). MM is characterized by the accumulation of malignant antibody secreting plasma cells in the BM. The severe buildup of BM plasma cells results in both elevated serum immunoglobulin and significant bone loss. Bone disease remains one of the most significant issues in management of MM. Patients with bone disease have a significant increase in morbidity and the number of bone lesions (which is a reflection malignant plasma cell infiltration in the BM) directly

correlates with a poorer prognosis for patients[55,56]. Bone remodeling is driven by factors intrinsic to MM cells as well as extrinsic sources from additional hematopoietic cell populations recruited to foci of malignant cells. These include RANKL, MIP-1α, IL-3, IL-6, IL-7, SDF-1α, and VEGF (reviewed in [57]). Combined, they disrupt the balance between healthy bone production and resorption by osteoblasts and osteoclasts.

Recently, we[53] and others[55] have reported that osteoblastic remodeling is also a characteristic of T-ALL. We observed T-ALL cells cause dramatic remodeling of bone tissue through induction of apoptosis in osteoblastic cells. This remodeling is aggressive, and can lead to complete loss of healthy endosteal niches in less than 48 hours once the BM is fully infiltrated by T-ALL cells[53]. Although the mechanisms, exact consequences and prevalence of osteoblastic remodeling in other subtypes of ALL are not well understood, therapeutic interventions that protect these endosteal cells are a potentially exciting option for management of bone pain observed in pediatric ALL (and less frequently in adult cases)[57,58].

The role of remodeling in leukemia cell propagation is also exemplified by models of CML where modification of MSC differentiation into an aberrant pro-fibrotic osteoblast lineage promotes leukemia growth at the expense of normal hematopoiesis[60]. Similarly, in the MLL-AF9 model of AML, sympathetic neuropathy develops and limits the differentiation of Nestin$^+$ MSCs into NG2$^+$ peri-arteriolar cells that normally support HSCs[61]. In $JAK2^{V617F}$-induced myeloproliferative neoplasms (MPN), HSC-supporting Nestin$^+$ MSCs are critically reduced. Interestingly, the specific depletion of Nestin$^+$ MSCs causes expansion of hematopoietic progenitors and an MPN-like phenotype, highlighting the interplay between niche and leukemic cells[62]. In MPN patients and MPN mice there is also a loss of sympathetic nerve fibers and nonmyelinating Schwann cells next to Nestin$^+$ cells[62]. This remodeling is mediated by IL-1β and can be partially reverted by pharmacological treatment[62]. More recently, we showed that AML selectively remodels endosteal blood vessels and osteoblasts at later disease progression[63]. Endosteal areas have been described as the major site for initiation of AML relapse[13], and disruption of this major osteovascular HSC niche[64] is emerging as a key mechanism allowing AML to inhibit healthy hematopoiesis. Interestingly, T-ALL patients rarely develop cytopenias, and in this disease model loss of osteoblastic cells was not accompanied by loss of endosteal vessels[63]. Altogether, these studies show the decay of HSC-supportive niches in several types of leukemia and support the view that leukemic cells outcompete HSCs[59] by re-shaping the BM microenvironment.

The factors driving remodeling of the BM microenvironment in leukemia are not well understood. Yet, inflammation (a hallmark of cancer[65]) seems likely to be a key player in the leukemic niche and candidate remodeling factors observed in different leukemia types perhaps reflects lineage specific immune function left over from their pre malignant state. The pro-inflammatory

environment is driven by cytokines that depend on the model used and leukemia subtype studied, and include TNF[17,63,66] (in myeloproliferative neoplasm and AML), IL-1β[60,62] (in myeloproliferative neoplasm), CCL3[54,60] (in myeloproliferative neoplasm and ALL) and CXCL2[63] (in AML). In addition, non-immunomodulatory factors such as exosomes that transport microRNAs[67] have also been implicated in leukemia-induced remodeling of the microenvironment[68].

Conclusions

Here, we have summarized examples of leukemia-microenvironment crosstalk. The emerging picture is that although redundancy is observed and common pathways are potentially shared across multiple leukemias, it is likely that most microenvironment interactions are leukemia subtype specific[42]. Understanding how pre-leukemic and leukemic cells co-opt and disrupt HSC niches will help with designing new therapies that target the microenvironment to restore healthy hematopoiesis, improve HSC transplantation and limit disease relapse. The combination of chemotherapy with novel approaches that target cell-intrinsic mechanisms with new CXCR4 antagonists[46,48], small molecules targeting cell adhesion[51,52] and anti-inflammatory therapies[66] has the potential to improve disease outcomes in leukemia. Furthermore, changes of the BM stroma in leukemic patients have potential for use as less-invasive prognostic factors[69] that could revolutionise disease management in the future.

Acknowledgements

Authorship

All authors contributed to the writing of the manuscript.

Conflict of interest disclosure

CLC is consultant for Onkaido Therapeutics.

References

1.   Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014;505(7483):327-334.
2.   Kunisaki Y, Bruns I, Scheiermann C, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*. 2013;502(7473):637-643.
3.   Acar M, Kocherlakota KS, Murphy MM, et al. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature*. 2015;526(7571):126-130.
4.   Asada N, Kunisaki Y, Pierce H, et al. Differential cytokine contributions of perivascular haematopoietic stem cell niches. *Nat Cell Biol*. 2017;19(3):214-223.
5.   Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity*. 2006;25(6):977-988.
6.   Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003;425(6960):841-846.
7.   Mendez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. *Nature*. 2008;452(7186):442-447.
8.   Yamazaki S, Ema H, Karlsson G, et al. Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell*. 2011;147(5):1146-1158.
9.   Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature*. 2009;460(7252):259-263.
10.   Bruns I, Lucas D, Pinho S, et al. Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion. *Nat Med*. 2014;20(11):1315-1320.
11.   Zhao M, Perry JM, Marshall H, et al. Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. *Nat Med*. 2014;20(11):1321-1326.
12.   Fujisaki J, Wu J, Carlson AL, et al. In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature*. 2011;474(7350):216-219.
13.   Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol*. 2007;25(11):1315-1321.
14.   Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. *Blood*. 2009;114(6):1150-1157.
15.   Colmone A, Amorim M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells. *Science*. 2008;322(5909):1861-1865.
16.   Walkley CR, Olsen GH, Dworkin S, et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell*. 2007;129(6):1097-1110.
17.   Wang L, Zhang H, Rodriguez S, et al. Notch-dependent repression of miR-155 in the bone marrow niche regulates hematopoiesis in an NF-kappaB-dependent manner. *Cell Stem Cell*. 2014;15(1):51-65.
18.   Kim YW, Koo BK, Jeong HW, et al. Defective Notch activation in microenvironment leads to myeloproliferative disease. *Blood*. 2008;112(12):4628-4638.
19.   Dong L, Yu WM, Zheng H, et al. Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. *Nature*. 2016;539(7628):304-308.
20.   Raaijmakers MH, Mukherjee S, Guo S, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*. 2010;464(7290):852-857.
21.   Kode A, Manavalan JS, Mosialou I, et al. Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. *Nature*. 2014;506(7487):240-244.
22.   Sanchez-Aguilera A, Mendez-Ferrer S. The hematopoietic stem-cell niche in health and leukemia. *Cell Mol Life Sci*. 2017;74(4):579-590.

23.   Wiseman DH. Donor cell leukemia: a review. *Biol Blood Marrow Transplant.* 2011;17(6):771-789.

24.   Reichard KK, Zhang QY, Sanchez L, Hozier J, Viswanatha D, Foucar K. Acute myeloid leukemia of donor origin after allogeneic bone marrow transplantation for precursor T-cell acute lymphoblastic leukemia: case report and review of the literature. *Am J Hematol.* 2006;81(3):178-185.

25.   DeGregori J. Connecting Cancer to Its Causes Requires Incorporation of Effects on Tissue Microenvironments. *Cancer Res.* 2017;77(22):6065-6068.

26.   Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med.* 2014;371(26):2477-2487.

27.   Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371(26):2488-2498.

28.   Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med.* 2014;20(12):1472-1478.

29.   McKerrell T, Park N, Moreno T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Rep.* 2015;10(8):1239-1245.

30.   Brunetti L, Gundry MC, Goodell MA. DNMT3A in Leukemia. *Cold Spring Harb Perspect Med.* 2017;7(2).

31.   Challen GA, Sun D, Jeong M, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet.* 2011;44(1):23-31.

32.   Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature.* 2014;506(7488):328-333.

33.   Vas V, Senger K, Dorr K, Niebel A, Geiger H. Aging of the microenvironment influences clonality in hematopoiesis. *PLoS One.* 2012;7(8):e42080.

34.   Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature.* 1994;367(6464):645-648.

35.   Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med.* 1997;3(7):730-737.

36.   Ding L, Ley TJ, Larson DE, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature.* 2012;481(7382):506-510.

37.   Shlush LI, Mitchell A, Heisler L, et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. *Nature.* 2017;547(7661):104-108.

38.   Kelly PN, Dakic A, Adams JM, Nutt SL, Strasser A. Tumor growth need not be driven by rare cancer stem cells. *Science.* 2007;317(5836):337.

39.   Dick JE. Stem cell concepts renew cancer research. *Blood.* 2008;112(13):4793-4807.

40.   Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature.* 2013;495(7440):231-235.

41.   Sirard C, Lapidot T, Vormoor J, et al. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. *Blood.* 1996;87(4):1539-1548.

42.   Krause DS, Fulzele K, Catic A, et al. Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nat Med.* 2013;19(11):1513-1517.

43.   Zeng Z, Shi YX, Samudio IJ, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. *Blood.* 2009;113(24):6215-6224.

44.   Nervi B, Ramirez P, Rettig MP, et al. Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. *Blood.* 2009;113(24):6206-6214.

45.   Tavor S, Petit I, Porozov S, et al. CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res.* 2004;64(8):2817-2824.

46.   Pitt LA, Tikhonova AN, Hu H, et al. CXCL12-Producing Vascular Endothelial Niches Control Acute T Cell Leukemia Maintenance. *Cancer Cell*. 2015;27(6):755-768.

47.   Uy GL, Rettig MP, Motabi IH, et al. A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood*. 2012;119(17):3917-3924.

48.   Abraham M, Klein S, Bulvik B, et al. The CXCR4 inhibitor BL-8040 induces the apoptosis of AML blasts by downregulating ERK, BCL-2, MCL-1 and cyclin-D1 via altered miR-15a/16-1 expression. *Leukemia*. 2017;31(11):2336-2346.

49.   Matsunaga T, Takemoto N, Sato T, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. *Nat Med*. 2003;9(9):1158-1165.

50.   Jacamo R, Chen Y, Wang Z, et al. Reciprocal leukemia-stroma VCAM-1/VLA-4-dependent activation of NF-kappaB mediates chemoresistance. *Blood*. 2014;123(17):2691-2702.

51.   Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med*. 2006;12(10):1175-1180.

52.   Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med*. 2006;12(10):1167-1174.

53.   Hawkins ED, Duarte D, Akinduro O, et al. T-cell acute leukaemia exhibits dynamic interactions with bone marrow microenvironments. *Nature*. 2016;538(7626):518-522.

54.   Duan CW, Shi J, Chen J, et al. Leukemia propagating cells rebuild an evolving niche in response to therapy. *Cancer Cell*. 2014;25(6):778-793.

55.   Wang W, Zimmerman G, Huang X, et al. Aberrant Notch Signaling in the Bone Marrow Microenvironment of Acute Lymphoid Leukemia Suppresses Osteoblast-Mediated Support of Hematopoietic Niche Function. *Cancer Res*. 2016;76(6):1641-1652.

56.   Hameed A, Brady JJ, Dowling P, Clynes M, O'Gorman P. Bone disease in multiple myeloma: pathophysiology and management. *Cancer Growth Metastasis*. 2014;7:33-42.

57.   Jonsson OG, Sartain P, Ducore JM, Buchanan GR. Bone pain as an initial symptom of childhood acute lymphoblastic leukemia: association with nearly normal hematologic indexes. *J Pediatr*. 1990;117(2 Pt 1):233-237.

58.   Maman E, Steinberg DM, Stark B, Izraeli S, Wientroub S. Acute lymphoblastic leukemia in children: correlation of musculoskeletal manifestations and immunophenotypes. *J Child Orthop*. 2007;1(1):63-68.

59.   Boyd AL, Campbell CJ, Hopkins CI, et al. Niche displacement of human leukemic stem cells uniquely allows their competitive replacement with healthy HSPCs. *J Exp Med*. 2014;211(10):1925-1935.

60.   Schepers K, Pietras EM, Reynaud D, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell*. 2013;13(3):285-299.

61.   Hanoun M, Zhang D, Mizoguchi T, et al. Acute myelogenous leukemia-induced sympathetic neuropathy promotes malignancy in an altered hematopoietic stem cell niche. *Cell Stem Cell*. 2014;15(3):365-375.

62.   Arranz L, Sanchez-Aguilera A, Martin-Perez D, et al. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature*. 2014;512(7512):78-81.

63.   Duarte D, Hawkins ED, Akinduro O, et al. Inhibition of Endosteal Vascular Niche Remodeling Rescues Hematopoietic Stem Cell Loss in AML. *Cell Stem Cell*. 2018;22(1):64-77 e66.

64.   Ramasamy SK, Kusumbe AP, Itkin T, Gur-Cohen S, Lapidot T, Adams RH. Regulation of Hematopoiesis and Osteogenesis by Blood Vessel-Derived Signals. *Annu Rev Cell Dev Biol*. 2016;32:649-675.

65.   Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
66.   Mead AJ, Neo WH, Barkas N, et al. Niche-mediated depletion of the normal hematopoietic stem cell reservoir by Flt3-ITD-induced myeloproliferation. *J Exp Med*. 2017;214(7):2005-2021.
67.   Kumar B, Garcia M, Weng L, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. *Leukemia*. 2017.
68.   Skog J, Wurdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470-1476.
69.   Kim JA, Shim JS, Lee GY, et al. Microenvironmental remodeling as a parameter and prognostic factor of heterogeneous leukemogenesis in acute myelogenous leukemia. *Cancer Res*. 2015;75(11):2222-2231.
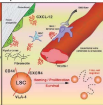
Figure Legend

Figure 1 – The crosstalk between leukemic cells and the microenvironment.

*Several studies suggest a causative role of the BM microenvironment in leukemogenesis (**Initiation**), mediated by alterations of signalling pathways in specific cell types, involving for example β-catenin, Jagged1, Ptpn11, Dicer1 in osteoblastic cells, RBPJ in endothelial cells, RARγ and Notch in stroma. Additionally, Leukemic Stem Cells (LSC) co-opt existing strategies normally used by HSC to interact with the microenvironment and proliferate and survive (**Expansion and Chemoresistance**). For example, LSC use adhesion molecules (CD44 and VLA-4) to bind the extracellular matrix and stroma cells, and CXCR4 to bind the abundantly secreted CXCL12. Both mechanisms enable leukemia cell migration. Leukemia also shapes the microenvironment (**Remodeling**) by creating a pro-inflammatory milieu, impairing MSC differentiation and destroying key HSC-supportive niches. As a result, HSCs intravasate while leukaemia cells remain within the parenchyma.*
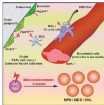
Initiation — **Expansion and Chemo-resistance** — **Remodeling**