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Leukaemia: a model metastatic disease

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Abstract

In contrast to solid cancers, which often require genetic modifications and complex cellular reprogramming for effective metastatic dissemination, leukaemic cells uniquely possess the innate ability for migration and invasion. Dedifferentiated, malignant leukocytes retain the benign leukocytes' capacity for cell motility and survival in the circulation, while acquiring the potential for rapid and uncontrolled cell division. For these reasons, leukaemias, although not traditionally considered as metastatic diseases, are in fact models of highly efficient metastatic spread. Accordingly, they are often aggressive and challenging diseases to treat. In this Perspective, we discuss the key molecular processes that facilitate metastasis in a variety of leukaemic subtypes, the clinical significance of leukaemic invasion into specific tissues and the current pipeline of treatments targeting leukaemia metastasis.

Metastasis of solid tumours is defined as the emergence of secondary growth away from a primary site. For liquid tumours such as leukaemias that initially present with widespread disease, the precise location of origin in the bone marrow (BM), spleen or lymph node (LN) is, however, often unknown. This ambiguity, as well as the recognition that leukaemic cells 'inherit' rather than gradually acquire motility, has resulted in disagreement over whether leukaemia should be considered a metastatic disease. Despite this controversy, disseminated leukaemia cells share many of the properties of metastasizing solid tumour cells. These include derivation from a mutant clone or clones possessing disease-initiating potential[1–3], spread via a cascade of molecular events entailing intravasation, extravasation and tissue colonization[4–10], specific and reproducible patterns of organ involvement[11,12], adaptation to tissue microenvironments that are distinct from that of the cell of origin[13,14], and widespread disease that is challenging to treat and can fuel future relapse[13,14]. The unique selective pressures applied by different organs often lead to heterogeneous clonal evolution, compounding the difficulty in achieving complete treatment response. For these reasons, we consider the process of leukaemia cell dissemination to represent metastasis.

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Author contributions

All authors researched data for the article, substantially contributed to discussion of the content, and wrote sections of the article. A.E.W., T.T.P. and D.A.S. reviewed and edited the article before submission. A.E.W. and D.A.S. designed the figures.

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In this Perspective, we argue that leukaemia dissemination is indeed metastatic, and we describe the events in this multi-step process. We review the various sites of leukaemia metastasis and the molecular mechanisms governing this organotropism. Finally, we provide an overview of current and upcoming strategies to target leukaemia metastasis.

Classification and derivation

The term leukaemia is derived from the Greek words ‘leukos’, meaning white, and ‘haima, meaning blood. The name, which is used to reference a broad array of haematopoietic malignancies currently subcategorized according to their morphology, immunophenotype, cytogenetic and molecular abnormalities and clinical features, refers quite literally to the excess of white blood cells seen in the bloodstream of patients with these diseases[15]. Clinically, these diseases fall within four broad categories, myeloid or lymphoid lineage and acute or chronic, which help to dictate the approach to treatment. The latter categorization refers to the rapidity of clinical onset and disease progression. Although there are a multitude of specific diagnoses, the most commonly seen and studied leukaemias are acute myeloid leukaemia (AML), acute lymphocytic leukaemia/lymphoma (ALL), chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) and chronic myeloid leukaemia (CML). We therefore focus on these entities in this article.

Although circulating malignant white blood cells are a prominent feature of the leukaemias, it should be noted that blood-borne tumour cells can often be detected in epithelial malignancies and other haematopoietic disorders, including lymphomas and multiple myeloma, often in association with more aggressive disease[16,17]. Furthermore, leukaemia cell involvement is not limited to the bloodstream and haematopoietic organs (BM, spleen, LN and tonsils), and some subtypes show a particular predilection for specific non-haematopoietic tissues (for example, ALL and the central nervous system (CNS)[18,19]). These observations underscore the premise that wide-ranging, organotropic dissemination is a shared trait among the liquid and solid cancers.

Whereas solid tumour metastasis is a largely inefficient process with a high rate of attrition[20], leukaemia cells are motile and can easily traffic throughout the body without the need to acquire a heavy mutational burden and anchorage independence. Leukaemia metastasis is therefore an efficient and cyclical process of tissue homing, colonization and mobilization back into circulation, making leukaemia a deadly and challenging disease to treat, and in the case of the acute leukaemias, an ultimate example of aggressive metastasis.

The haematopoietic stem cell (HSC) is positioned atop the developmental hierarchy of all lymphoid, myeloid and erythroid lineage progenitor and mature haematopoietic cells[21]. Although the bulk of the HSC population is quiescent and ensconced within regulatory and protective BM niches at any given time, HSCs are in fact motile cells and possess the remarkable ability to swiftly enter circulation and traffic to both haematopoietic and non-haematopoietic organs in response to a variety of inflammatory cues[22]. Compelling data, recently reviewed[3], have shown that AML and CML cells arise from HSCs or myeloid progenitors in which key driver mutations have occurred (for example, the *BCR–ABL* translocation in CML). Despite the single cell origin of disease, significant heterogeneity

arises as mutations accumulate in daughter cells, resulting in what is referred to as subclonal evolution[2,23,24]. Yet, only a subset of leukaemic cells within a malignant population is believed to harbour the stem cell properties of self-renewal and the ability to initiate disease when engrafted into an immunocompromised host[1,3], and these are referred to as leukaemia stem cells (LSCs). LSCs, like other cancer stem cells, are particularly important in the context of metastasis as they are often therapy resistant[25–27]. Fuelled by the acquisition of new pro-survival and pro-proliferative mutations, they may emerge as a dominant subclone later in the disease course[28,29] and serve as a reservoir for disease relapse[27]. Although the precise cells of origin for CLL and ALL and the relevance of LSCs in these leukaemias remain unclear, the process of clonal evolution, occurring independently at different sites of metastasis, also contributes to the difficulty in curing these diseases[30–33].

Reflecting their haematopoietic cells of origin, leukaemia cells co-opt haematopoietic stem and progenitor cell (HSPC) and/or mature leukocyte trafficking mechanisms at almost every step of the metastatic cascade. For example, leukaemic cells respond to environmental cues similar to those that enable HSPCs and leukocytes to exit (‘mobilize’) and enter tissues from peripheral circulation[9,34]. After extravasating into a new tissue, leukaemia cells, like HSPCs, encounter a complex microenvironment composed of diverse niches capable of fostering either cellular proliferation or dormancy[35]. Further echoing the cellular origin of leukaemias, much of the microenvironmental crosstalk that regulates leukaemia metastatic growth recapitulates the niche signalling that regulates HSPC repopulation or quiescence[22,36].

Patterns of metastasis

One of the hallmarks of metastasis is organ tropism, dictated by the appropriateness of a particular organ’s ‘soil’ as host for the metastatic ‘seed’. Different leukaemia subtypes exhibit unique patterns of metastasis to various tissues including, but not limited to the BM, LN, liver, spleen, CNS, skin and testicles[12] (FIG. 1). Although leukaemias are primarily understood to be diseases of the haematopoietic tissues and circulatory system, their metastatic invasion of extramedullary (non-BM) sites holds significant importance for disease progression, severity and outcomes in patients. For example, unique interactions between leukaemic cells and the surrounding microenvironment of different organs may play a role in the heterogeneous responses to treatment observed in some patients. Rituximab, an anti-CD20 monoclonal antibody, has been reported to be more effective in clearing follicular lymphoma from BM than from LNs[37], and similarly, its mechanism of cytotoxicity in CLL is believed to vary depending on tumour site[38]. The metastasis of leukaemia to sanctuary sites such as the CNS and testes, where drugs and the immune system have more restricted penetration, is another important example of the impact of the tissue metastatic profile on disease treatment[19,39–41].

Underpinnings of leukaemia metastasis

The concept of leukaemia as a metastatic disease is bolstered by the recognition that leukaemic cells engage many of the same cell surface adhesion molecules, chemokines, matrix molecules and intracellular motility signalling pathways as solid cancers to invade

distant organs. Detailed investigations have revealed considerable molecular conservation between solid and liquid tumours in these processes. Some important molecular components in the leukaemic metastatic cascade are described below.

Selectins.

Selectins (E-, P- and L-selectins) are a family of cell surface glycoproteins expressed on inflamed endothelial cells (E- and P-selectin) and leukocytes (L-selectin), the importance of which in cell trafficking was first established in lymphocytes[42] (BOX 1). Later, recognition of selectin ligand expression by leukaemia[43] and solid tumour cells[44–46] and their association with advanced disease in patients led to investigations defining their important role in tumour metastasis[47].

Selectin ligands are known to be expressed by all major categories of leukaemia[43], and high levels of E-selectin ligand expression are associated with poor disease outcomes in patients with AML[48]. Early in vitro studies reported that inhibition of L-selectin and E-selectin binding decreased leukaemia cell adhesion to human umbilical vein endothelial cells, suggesting a functional role in haematogenous metastasis[49]. In upcoming sections, we highlight examples of selectin-mediated leukaemic metastasis to the BM and LN.

While E- and P-selectin expression is limited to the vasculature, L-selectin can be expressed on the surface of leukaemic cells. In ALL and CLL, L-selectin expression and shedding have been shown to correlate with more highly metastatic disease[7,50,51]. In fact, patients with CLL who were treated with the PI3Kδ inhibitor idelalisib exhibited decreased L-selectin expression, suggesting an additional pharmacological mechanism by which PI3K inhibition prevents CLL progression[7].

Integrins.

Integrins are a family of cell surface molecules that have been shown to have an important role in almost every step of the metastatic cascade from invasion to the activation of pro-survival signalling pathways[52]. In both solid tumours and leukaemias, integrins enable malignant cells to sense and respond to a variety of cell, extracellular matrix (ECM), cytokine and growth factor binding partners in their tissue microenvironments. During leukaemic cell dissemination, integrins play a key adhesive role, anchoring cells to the vessel lumen during haematogenous metastasis (FIG. 2) and tethering them to the metastatic niche after invasion[8,53–55]. Integrin activation also stimulates intracellular signalling pathways that augment leukaemia cell motility[56].

The integrin VLA-4 (also known as α4β1) is a receptor for vascular cell adhesion molecule 1 (VCAM1) and for ECM fibronectin, and the integrin VLA-5 (also known as α5β1) is a receptor for fibronectin and fibrinogen; both have important roles in tissue homing and retention for the lymphoid leukaemias[8,53–55]. Clinical investigations of patients with B cell ALL (B-ALL) have also shown a correlation between low-affinity VLA-4 binding and elevated peripheral blood blast numbers, further suggesting that VLA-4 has a crucial role in the retention of lymphoblasts in the BM microenvironment[53].

Clinical research has also revealed a role for integrins in leukaemic cell tethering within LNs. In CLL, VLA-4 clustering, which promotes adhesion to VCAM1 expressed on LN endothelium, is induced by B cell receptor activation[57]. Patients with CLL who were treated with ibrutinib, a small-molecule inhibitor of Bruton tyrosine kinase (BTK) that antagonizes B cell receptor signalling and is approved for treatment of CLL[58], displayed LN shrinkage concurrent with transient increases in circulating CLL cells that exhibited decreased adhesion to VCAM1 ex vivo[57].

Chemokines, cytokines and growth factors.

Soluble factors such as chemokines, cytokines and growth factors have an integral role in the establishment of metastatic disease. They form a complex intracellular signalling network that guides tumour cell chemotaxis and invasion and colonization in both liquid and solid tumours.

The interaction between stromal cell-derived factor 1 (SDF1; also known as CXCL12) and its receptor, CXCR4, is one of the most studied interactions in leukaemia, as CXCR4 is expressed in most haematological malignancies and is often associated with poor prognosis[59,60]. SDF1 is synthesized and secreted by a myriad of stromal cells in tissues commonly colonized by leukaemia including the spleen, BM, CNS and skin, where it serves as a potent chemoattractant for leukaemia cells[9,61,62] and LSCs[10]. CXCR4 expression can also be upregulated in response to niche factors such as stem cell factor (SCF; also known as Kit ligand)[63] and hypoxia[64] and in response to therapy[65,66]. Other chemokine receptors that have been implicated in leukaemia metastasis[67] include CCR7 in the CNS[68], spleen[69] and LN[70], and CCR4 and CCR2 (REFS[71,72]) in skin.

As in solid tumours, leukaemia metastasis can be enhanced by changes to the microenvironment induced by aberrant cytokine and growth factor secretion. Among the best-studied examples in solid malignancies are the vascular endothelial growth factor (VEGF) family-dependent processes of neoangiogenesis and vascular remodelling, which promote tumour growth and prime pre-metastatic niches[73]. Although not as intensively studied in the leukaemias, evidence of neoangiogenesis in the BM of patients with ALL[74] and AML[75] is well documented and will be discussed in upcoming sections.

Extracellular matrix.

Although epithelial cells critically depend on matrix adhesion to prevent anoikis, the ECM also has an important role in leukaemia metastasis as a scaffold to direct adhesion and migration in the metastatic niche. Fibronectin, a major structural component of the basement membrane of BM and LN blood vessels[76–78], has been shown to enhance the chemotaxis of AML cells towards SDF1 (REF.[79]). Similarly, laminin expressed in the basement membrane of CNS vessels serves as a scaffold for B-ALL cell migration towards cerebrospinal fluid (CSL) chemokines in the leptomeninges and also activates leukaemia cell actinomysin contractility pathways through its binding to ALL integrin α6 receptors[56]. Matrix binding interactions with osteopontin (OPN) and fibronectin have also been shown to anchor leukaemic cells in the nutrient-rich and protective environment of the BM, shielding them from therapeutic killing[35,80].

Motility modes.

Similarly to solid tumour cells, leukaemic cells adopt a variety of migratory strategies, including amoeboid-, invadopodia- and lamellipodia-driven movement, that each employ unique and complex molecular signalling pathways[81] (BOX 2).

Leukaemic cells, like normal leukocytes, use amoeboid-like migration to efficiently squeeze through tissues without having to degrade the ECM. The BCR–ABL fusion protein, present in CML cells as well as Philadelphia chromosome-positive ALL, can directly phosphorylate and activate cell motility machinery in this pathway. For example, ectopic expression of the p210 variant of the BCR–ABL fusion protein in murine Ba/F3 pro-B cells activates RHOA–ROCK signalling to myosin light chain (MLC) that works in conjunction with the actin-binding protein destrin (also known as actin depolymerizing factor) to promote spontaneous amoeboid motility[82,83]. The Dbl homology–pleckstrin homology domain in the p210 BCR–ABL chimera was identified to be the specific activator of RHOA, thus providing a further understanding of how BCR–ABL signalling activates cell motility pathways. Amoeboid-like migration may not be exclusive to BCR–ABL$^+$ leukaemia, however. Ongoing work in our group suggests that only some ECM components can induce amoeboid-like migration in BCR–ABL$^-$ B-ALL cells, suggesting that the microenvironment also plays an important role in migration mode selection (D.A.S., unpublished observations). Finally, the importance of actomyosin-driven motility in leukaemia progression has been highlighted in recent years by studies showing high expression of the actin-polymerization protein, cortactin, on blasts biopsied from the BM and CSF of patients with relapsed B-ALL[84].

Like solid tumour cells[85,86], leukaemia cells may transition from one migration mode to another. For example, by suppressing RHOA activity and activating CDC42, BCR–ABL$^+$ leukaemic cells can switch from amoeboid to invadopodia-based migration[87]. In CML, it has been reported that BCR–ABL constitutively activates the SRC family haematopoietic cell kinase (HCK), which contributes to invadopodia formation[88,89].

The process of invadopodia formation is also associated with the release into the extracellular space of matrix-degrading enzymes that facilitate disease spread. For example, in vitro confocal microscopy studies revealed that the fibronectin and VCAM1 degrading enzyme, matrix metalloproteinase 9 (MMP9), co-localizes with invadopodia in CLL cells[90]. A clinical study found that in patients with CLL, LN infiltrates had high MMP9 content and a substantial reduction in ECM. The authors reported that elevated MMP9 expression correlated with advanced stage and poor prognosis[91], suggesting the role of MMP9 in tissue invasion. Additional studies have reported that leukaemic cells can secrete MMP2, which degrades other ECM substrates, including collagen and elastin[92,93].

Finally, microenvironmental factors may influence the migration mode adopted by leukaemia cells. In vitro studies have shown that T cell ALL (T-ALL) cells form lamellipodia when plated on a fibroblast monolayer[94]. Upon contact with fibroblasts, the RAC-specific guanine nucleotide exchange factor, TIAM1, is recruited to the plasma membrane where it interacts with the adhesion molecule CADM1 and activates RAC-dependent actin reorganization and lamellipodia formation. However, leukaemia cell lamellipodia formation may not always depend on contact with an adhesive substrate. For

example, soluble extracellular galectins can drive lamellipodia formation in T-ALL cells by binding to integrin β1 and activating downstream PI3K–RAC1–ERK1/2 signalling[95].

Navigating bone marrow niches

Most clinicians and scientists are unaccustomed to viewing the BM as a leukaemia metastatic site, yet consideration of the anatomy and pathophysiology of the disease supports the concept that widespread leukaemia BM dissemination is indeed a metastatic process. The collective BM spaces within an individual are largely non-contiguous. For example, no anatomical connection exists between contralateral femurs, yet leukaemia typically presents with involvement of contralateral and distant BM cavities in the femurs, pelvis, sternum, vertebrae and elsewhere[96]. As leukaemic cells are derived from one mutant clone originating in a single BM space, disseminated BM disease must by necessity arise from leukaemic cells that have exited the primary BM site, travelled through the bloodstream, and recognized and successfully entered a secondary BM location. These processes mirror the metastatic cascade of solid tumour cells. In the solid malignancies, distant spread through a single organ system, for example, contralateral intrapulmonary disease in lung cancer[97], is also considered metastasis.

Most patients with leukaemia present at diagnosis with disseminated disease involving the BM, obviating the typical metastatic staging assessments used in solid tumours. As the BM is an important site of metastasis shared across the acute, chronic, myeloid and lymphoid leukaemias, as well as the most readily sampled tissue in patients with leukaemia apart from peripheral blood, much research has focused on the molecular mechanisms of disease spread to this organ. Despite the pervasiveness of disease spread in the BM, this process has been shown to be highly selective, involving specific molecular interactions between leukaemia cells and a subgroup of blood vessels[9,98–101].

Similar to circulating normal leukocytes that precisely identify and enter haematopoietic organs or inflamed tissues through vascular interactions[102,103], circulating leukaemic cells bind to and exit the vasculature to access new metastatic sites, a process generally known as extravasation and often referred to as diapedesis or transmigration in haematological malignancies[4–10]. Much of our early understanding of the molecular mechanisms underlying this metastatic process comes from studies of leukocyte trafficking, therefore, it is first helpful to understand the cascade of carefully choreographed events that leukocytes execute to home to secondary lymphoid organs or invade inflamed tissues[102,103] (BOX 1). One of the seminal discoveries of leukaemia co-opting normal leukocyte homing mechanisms used intravital microscopy to view the BM microenvironment in mice, in real time[9]. The authors showed that both circulating pre-B-ALL cells and normal HSPCs home to sinusoidal vessels in the BM that uniquely express SDF1 and E-selectin, molecules with expression typically limited to inflamed vasculature. Mirroring HSC and T lymphocyte interactions with the vessel wall, blood-borne leukaemic cells were observed rolling along, arresting and diapedesing through these BM vascular domains in a manner predominantly dependent on SDF1 and, to a lesser degree, E-selectin[9].

Subsequent studies in murine models have demonstrated that diverse leukaemias hijack E-selectin trafficking interactions to disseminate to the BM. Work in a murine CML-like neoplasia model has shown that loss or blockade of selectin–ligand interactions prevents leukaemia cell BM homing[5,104]. In a positive feedback loop, leukaemic cells that have colonized the BM may encourage additional homing events through secretion of inflammatory cytokines that upregulate endothelial E- and P-selectin expression[49]. CD44, a cell adhesion molecule and known E-selectin ligand expressed by HSPCs[105] and mature white blood cells[106], has been shown to play a part in CLL[107] and myeloid LSC BM dissemination[4,6]. Its function as a tissue homing receptor for cancer cells has also been widely documented in solid tumours[108,109].

A mounting body of literature has continued to unveil other BM homing mechanisms shared between leukaemia cells and normal leukocytes[98–101]. The expression of the integrin VLA-4 on leukaemia cells has been correlated with the increased capacity of patient-derived B-ALL cells to home to the BM in mice[8]. CD98 is another adhesion molecule expressed by HSPCs and leukocytes as well as leukaemic cells, the deletion of which preferentially decreases AML BM repopulation in vivo[110].

After diapedesis and entry into a tissue, leukaemic cells must navigate a new microenvironment, a process that involves continued co-option of HSC signalling pathways. It has long been recognized that HSCs occupy unique stem cell regulatory niches in the BM[22,36]; however, over the past decade it has become increasingly apparent that leukaemic cells also require specialized niches to thrive and that these often overlap with stem cell niches[111] (FIG. 2). As described above, the sinusoidal vascular niche serves as the main access point of leukaemic cells and HSPCs migrating in and out of the BM microenvironment[9]. Once inside the BM, leukaemic cells and stem cells surrounding these large, fenestrated sinusoidal vessels are thought to inhabit a pro-proliferative state supported by key microenvironmental interactions including SDF1–CXCR4 (REF.[101]) and VLA-4–VCAM1 (REFS[80,112]), as well as growth factors such as VEGF[113–116], and colony stimulating factor 1 receptor-positive ($CSF1R^+$) myeloid cells[117].

Leukaemic cells that have entered the BM at the sinusoidal vascular niche also migrate to alternative HSC BM niches, including peri-endosteal, or bony, niches. While the sinusoidal niche tends to be conducive to leukaemia proliferation, long-term, quiescent AML stem cells[118] and dormant ALL cells[119] encounter pro-dormancy signals in the endosteal niche. In a xenograft mouse model, B-ALL cells were shown to migrate to and persist in the endosteal niche through OPN engagement via the integrin VLA-4 (REF.[35]); leukaemic cell interaction with the endosteal niche promoted dormancy, resistance to chemotherapy and the persistence of minimal residual disease (MRD) in this model[35]. This mechanism is conserved from HSCs, which also exhibit OPN-dependent dormancy[120]. Beyond the endosteal niche, other safe-haven niches have been identified. For example, it was reported that human T-ALL cells transplanted into mice home to adipocyte-rich BM niches where they display decreased metabolic activity and cell-cycle progression, suggesting a dormant phenotype that is also observed in HSPCs occupying ‘fatty’ niches[121,122].

Insidiously, leukaemic cell occupation and accumulation in BM niches can induce molecular changes in the host microenvironment that support disease progression by providing a competitive growth advantage over HSPC populations. Our group documented that B-ALL cells disrupt the behaviour of haematopoietic progenitor cells (HPCs) in the BM by secreting SCF, which displaces HPCs from their normal regulatory niches, triggering their loss[123]. In this way, ALL cells obtain a survival advantage over naive HPCs, co-opting their niches to fuel tumour growth. High levels of leukaemia-derived IL-6 and IL-1 in the BM have also been shown to promote leukaemia cell proliferation while negatively impacting normal haematopoeisis[124–126]. Leukaemic cells can also disrupt sympathetic nervous system regulation of the HSC niche wherein AML-induced neuropathy impairs the differentiation status of mesenchymal stem/progenitor cells, promoting a pro-leukaemic, as opposed to HSC, niche[127].

Niche occupation can also result in vascular remodelling that favours metastatic growth. For example, studies in mouse syngeneic and patient-derived xenograft (PDX) models have demonstrated that AML expansion can cause degradation and remodelling of the BM vascular architecture through pro-inflammatory cytokine release[128] or the induction of hypoxia[129]. This leads to increased vascular permeability, altering levels of supportive growth factors in a manner that favours leukaemic cells over HSCs[130]. The VEGF family members VEGFA and VEGFC have also been reported to be overexpressed in the BM of patients with AML and to contribute to increased microvessel density[116,131]. These and other studies suggest that liquid tumours, like solid tumours, can use VEGF paracrine signalling to induce angiogenesis and stimulate endothelial cells to release growth factors that support leukaemia proliferation[132]. Leukaemic cells may further capitalize on elevated VEGF levels in hypoxic BM metastatic niche conditions through autocrine VEGF signalling[133–138].

Dynamic changes in the ECM during disease progression can also promote leukaemia cell invasion. For example, AML cells in the BM can remodel collagen IV, increasing its affinity for the collagen-activated receptor tyrosine kinase, DDR1, on AML cells and promoting their AKT-dependent migration[139]. B-ALL cells have also been reported to remodel the BM niche by releasing TNF, which stimulates mesenchymal stem cells (MSCs) to produce the ECM-degrading protease MMP9 and thereby enhances leukaemia cell infiltration[140].

After taking up residence in the BM, leukaemic cells can later re-enter the bloodstream and populate additional metastatic sites. This process, known generally as intravasation and often referred to as mobilization in haematological malignancies, is essential for disease progression. Disease colonization can promote leukaemia cell mobilization in syngeneic and PDX mouse models by altering the expression of niche molecules such as SDF1 that normally anchor leukaemic cells to the BM[9,34]. The selectin and integrin interactions that foster leukaemia BM homing have also been shown to have roles in tethering leukaemic cells to the BM niche. E-selectin blockade with the small-molecule inhibitor GMI-1271 was shown to rapidly mobilize leukaemic cells into peripheral circulation in preclinical mouse models of AML[141]. Similarly, VLA-4 deletion in B-ALL cells impairs both BM homing and retention[54]. In CML, recent work has shown that conditional deletion of the gene encoding

the integrin-binding adaptor protein, kindlin 3 (also known as FERMT3), mobilizes LSCs from the BM in mice[142].

Lastly, to promote their own metastatic spread, leukaemic cells can secrete proteases and matrix-degrading enzymes that allow for more efficient mobilization. For example, AML cells can express cell surface elastase that degrades vascular adhesion molecules such as VCAM1 and intercellular adhesion molecule 1 (ICAM1) (REF.[143]). MMP2 secretion by ALL cells has also been shown to degrade the vascular basement membrane, facilitating intravasation[93].

Extramedullary metastasis

All leukaemias possess the ability to metastasize to the BM to varying extents, although some subtypes exhibit a predilection for the extramedullary haematopoietic tissue of the spleen and lymph nodes[12] (FIG. 1). As these organs have important roles in haematopoiesis, leukaemia cell infiltration likely reflects the reactivation of haematopoietic programmes, as evidenced by the shared homing mechanisms of normal and malignant leukocytes. However, there are also unique patterns of metastasis to non-haematopoietic organs such as the skin and CNS that do not clearly recapitulate the behaviour of the leukaemia haematopoietic cell of origin. Interesting similarities and differences in the patterns of leukaemic and solid tumour metastasis to these organs exist, and some are highlighted below.

Spleen.

The spleen is an unusual organ for solid tumour metastasis but a frequent site of leukaemia metastasis (FIG. 3). It is unclear why circulating epithelial tumour cells lack the ability to navigate the splenic vasculature or to survive in its microenvironment, although theories based on the spleen's unique blood flow dynamics and immune composition have been posited[144]. Multiple studies in the leukaemias, however, have produced insights into specific molecular mechanisms used by leukaemic cells to invade the spleen.

As in the BM, leukaemic cells have been found to co-opt SDF1 and other haematopoietic niche chemotactic or adhesive factors to migrate to the spleen. SDF1–CXCR4 interactions have been revealed as key mediators of B-ALL splenic homing, and treatment with CXCR4 antagonist AMD3100 was shown to reduce splenic disease in mouse xenograft models[61,145]. Molecular mechanisms that specifically regulate spleen metastasis have also been elucidated. For example, a recent study using a syngeneic T-ALL mouse model demonstrated that $Ly6C^+$ leukaemia-associated macrophages that secrete CCL8 and CCL9 chemokines promote leukaemia cell invasion and survival in the spleen[146]. Inhibiting the respective chemokine receptors, CCR1 and CCR2, decreased splenomegaly but had no effect on BM disease burden[146]. The chemokine receptor CCR7 and its ligand CCL19 may also have a specific role in T-ALL splenic metastasis. Using a NOTCH1-driven mouse model of T-ALL in which leukaemia cells have a high propensity to metastasize to the spleen compared with BM, investigators showed that leukaemia cells migrate more avidly towards splenocytes than BM cells in vitro, and that migration towards splenocytes, but not BM cells, could be inhibited by CCR7 or CCL19 blockade[69]. Lastly, in mouse studies using a

syngeneic CLL-like cell line, hyaluronan-CD44 variant 6 (REF.[147]) interactions were shown to regulate splenic but not BM leukaemia homing[147].

Further investigations in CLL using murine genetic models have shown that contact with the splenic microenvironment promotes disease growth and survival, perhaps contributing to the preponderance of splenic disease in these patients[147–150] (FIG. 3). Interestingly, interactions between CLL cells and splenic stromal cells mediated by the CLL surface receptor, SLAM5 (also known as CD84), can increase the survival of both leukaemic cells and the involved stromal cells, indicating mutually beneficial crosstalk[149]. CLL cells can also secrete IL-10 and transforming growth factor-β (TGFβ), which promote the differentiation of splenic neutrophils into a helper B cell phenotype[148,150]. These 'bystander' leukocytes then support disease progression by providing pro-growth and anti-apoptotic stimuli[148,150].

Lymph nodes.

In dramatic contrast to the spleen, LNs are frequent hosts to solid tumour metastases. Although both are lymphoid tissues, their immune cell, stromal and vascular architecture differ considerably, which may lead to a more permissive immunological environment for solid tumour growth in LNs than in the spleen[151]. Moreover, solid tumour metastases generally occur in regional LNs draining the primary tumour site, while leukaemic LN metastases are typically widespread throughout the body[152,153]. This clinical observation suggests that solid tumours depend on lymphatic routes for metastasis, while leukaemias appear capable of distant, haematogenous LN metastasis.

Lessons learnt from the study of leukocyte migration have informed our understanding of the molecular mechanisms of leukaemic LN metastasis. The haematogenous trafficking of normal immune cells in and out of LNs is regulated by specialized post-capillary venules termed high endothelial venules (HEVs)[154]. HEVs express a myriad of chemokines, adhesion molecules and matrix components that are co-opted by leukaemic cells during the multi-step process of invasion[155]. As in the BM, intravital imaging of the mouse inguinal LNs has demonstrated that CLL cells roll and crawl on the HEV wall[7] prior to extravasation. L-selectin expression by CLL cells has been shown to crucially mediate these adhesive interactions in the HEV lumen[7]. Furthermore, the chemokines SDF1, CCL19 and CCL21, localized at HEVs, serve as potent chemoattractants for CLL cells entering the LNs[70,156,157]. CCR7, the receptor for CCL19 and CCL21, is highly expressed by CLL cells and is required for their migration towards these cytokines in vitro[158]. In mice, CCR7 antibody blockade decreased CLL LN invasion, supporting the importance of the CCR7–ligands axis in CLL LN metastasis[159].

Once in the LN tissue, CLL cell surface VLA-4 and CD44 may form a docking complex for MMP9 (REF.[160]), which may facilitate CLL invasion by degrading the HEV basement membrane and/or LN ECM. Just as leukaemias corrupt BM HSPC niches to promote metastasis, CLL cells have been shown to usurp the germinal centre microenvironment to form a malignant 'proliferation centre' where they co-opt stromal cells, chemokines, cytokines and other niche factors to multiply[161]. CLL cells in the LNs also secrete CCL3 and CCL4, directing the recruitment of T cells and other leukocytes that alter the immune repertoire in this microenvironment to support CLL survival[162,163]. Uniquely, CLL cells can

induce the differentiation of monocytes into large, adherent nurse-like cells that reside in the LNs and support CLL cell growth through the secretion of soluble factors such as SDF1, B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL)[164–167].

Although LN invasion is also common in ALL, there has not been intensive in vivo investigation into the molecular mechanism involved. However, as ALL cells express many of the molecular components observed in CLL such as VLA-4 (REFS[53,54]) and CCR7 (REF.[69]), it is likely that there are similar, shared mechanisms of LN metastasis between these diseases.

The central nervous system.

Although CNS metastasis is rare for most leukaemia subtypes, ALL is a highly neurotropic leukaemia that frequently involves the leptomeninges and CSF surrounding the brain and spinal cord, although rarely the brain parenchyma[168]. Metastasis to the CNS is found in approximately 5% of patients with ALL at diagnosis and occurs in 30–50% of patients over time, in the absence of prophylactic treatment[18,19]. Despite routine CNS-directed prophylactic chemotherapy, 5–15% of patients will develop CNS disease, and the prognosis for these patients is poor[169,170]. Although benign lymphocytes are known to ‘patrol’ the CNS, they are rarely present in the CSF of healthy individuals[171,172]. By contrast, the pronounced ability of ALL to invade and proliferate within the leptomeninges was shown to be an inherent property of ALL cells[173]. More recent work using high-throughput sequencing to analyse paired BM and CSF samples from patients with ALL at diagnosis and relapse supports the hypothesis that therapy can induce additional selective pressures that generate CNS-metastatic subclones[174].

Three primary mechanisms of CNS invasion have been identified (FIG. 4). First, several studies suggest that leukaemia cells cross the blood–brain barrier to enter the CNS by promoting their own extravasation through VEGF signalling[175,176]. Various chemokines and chemokine receptors have also been implicated in this process[177]. In mouse models of oncogenic NOTCH1-driven T-ALL, the CCR7 receptor was shown to play an important role in CNS metastasis by attracting leukaemia cells to CCL19 or CCL21 chemokines expressed by the brain vascular endothelium[68]. More recent work has demonstrated that the T cell-specific kinase ZAP70 enhances CCR7-dependent migration to the CNS and that ZAP70 expression correlates with CNS metastasis in patients with T-ALL[178].

Other work has highlighted the choroid plexus as a specific site of entry for leukaemic cells into the CNS from the peripheral circulation[179,180]. The choroid plexus is a secretory tissue that produces CSF within the ventricular space in the brain. Recent work using an in vitro model of the blood–CSF barrier demonstrated that paediatric ALL cells are capable of transmigrating across a layer of choroid plexus epithelial cells[179], suggesting the choroid plexus as a potential avenue for ALL CNS invasion. However, mouse xenograft studies have failed to find evidence of ALL cell diapedesis through the choroid plexus in vivo, suggesting that the role of the choroid plexus as an access point to the CNS requires further investigation[56].

Lastly, our group has reported on a novel avenue for leukaemia cell entry in the CNS that allows ALL to bypass the blood–brain barrier[56]. We showed that B-ALL cells bind to extracellular matrix laminin surrounding specialized emissary blood vessels in mice. These emissary vessels passage from the skull and vertebral BM through cortical bone fenestrations, emerging as leptomeningeal vasculature in the adjacent CNS, and serve as a direct scaffold for leukaemic cells to migrate from BM to leptomeninges along their surface. Leukaemia cells do not extravasate through these vessels but migrate along their abluminal (outer) surface using integrin α6 to bind to the laminin-positive basement membrane. In a small patient series, leukaemia integrin α6 expression at diagnosis was associated with subsequent CNS relapse[56]. Although not specifically examining patients with CNS relapse, a study of clinical samples found elevated integrin α5 expression on ALL cells from the CSF at the time of diagnosis compared with BM blasts[181], suggesting that additional integrins may have roles in ALL CNS metastasis.

Independently of their route of access, leukaemia cells that enter the CNS reside in a ‘sanctuary site’ of restricted immune cell and chemotherapy access, and we are only beginning to understand the mechanisms that govern this protective environment. In one study it was reported that activated IL-15-producing natural killer (NK) cells controlled peripheral disease in mice but were unable to penetrate the CNS, suggesting a model wherein leukaemic cells enter the CNS to evade NK-mediated cell death[182]. In another study, ALL cells in the meningeal microenvironment acquired chemoresistance through direct contact with meningeal cells[183] and by upregulation of PBX1 (REF.[184]), a transcription factor known to regulate HSC quiescence.

Skin.

Although there are many forms of cancer that originate in the skin, leukaemias and lymphomas are among the handful of malignancies that infiltrate the skin as a metastatic site. In contrast to solid tumours, which typically demonstrate locoregional skin involvement suggesting local invasion and/or lymphatic spread, leukaemic skin metastases are usually widespread, consistent with blood-borne dissemination[185–187]. Leukaemic skin metastasis, referred to as leukaemia cutis, is hypothesized to share the mechanisms of benign leukocyte skin infiltration, which is mediated by P-selectin glycoprotein ligand 1 (PSGL1; also known as cutaneous lymphocyte antigen) adhesion to the E-selectin receptor on endothelial cells[188]. Recently, the molecular pathways responsible for leukaemic cell skin infiltration have been the subject of more intense scrutiny. Foundational work in benign lymphocytes showed that CCR4 deletion impaired $CD4^+$ T cell homing to inflamed skin but not to inflamed peritoneum in a murine transgenic adoptive transfer model, implicating its specific role in cutaneous lymphocyte trafficking[189]. Although in vivo mechanistic work has not yet been conducted in leukaemia, the observation that CCR4 expression significantly correlates with skin metastasis in patients with T cell leukaemia/lymphoma suggests a skin homing mechanism shared with lymphocytes[72].

Data have also shown a role for the chemokine receptor CXCR4 in myelomonocytic AML and lymphoblastic leukaemia invasion into $SDF1^+$ skin niches[61,190]. Other chemokine receptors have also been identified as important mediators of AML skin infiltration,

including CCR5, CXCR7 and CX3CR1 (REF.[71]). AML blasts isolated from the skin of patients with leukaemia cutis had higher expression of these chemokine receptors than blasts isolated from the BM and peripheral blood, suggesting that local expression of ligands CCL3, SDF1 and CX3CL1 in the skin mediates blast homing and retention.

Targeting metastasis vulnerabilities

The majority of adult leukaemias are not cured by standard therapies. Novel approaches to control disease metastasis could conceivably convert fatal diagnoses into chronic illnesses. In addition, strategies to physically and/or molecularly uncouple leukaemia cells from their protective tissue niches could improve responses to current regimens[35,80,112,119,121,191]. Below are some examples of the more advanced translational efforts to apply the emerging knowledge of leukaemia metastatic mechanisms to the clinic (TABLE 1).

Targeting chemokine receptors.

Molecular interventions to detach leukaemia cells from anti-apoptotic tissue niches, and in the most extreme sense, mobilize them into circulation, could render the disease more susceptible to systemic therapy. The SDF1–CXCR4 axis is the most studied in terms of leukaemia cell mobilization and chemosensitization. In AML in particular, high surface expression of CXCR4 correlates with poor prognosis[59]. AMD3100 is a specific CXCR4 antagonist that was first approved to mobilize HSPCs into the peripheral blood prior to collection for autologous BM transplant in non-Hodgkin lymphoma and multiple myeloma[192,193]. Years of work in mouse xenograft models of ALL and AML have demonstrated that blocking the SDF1–CXCR4 axis with AMD3100 mobilizes leukaemic cells into circulation, where they enter active phases of the cell cycle and are susceptible to chemotherapy[9,34,194,195]. Even in the absence of overt mobilization, inhibiting leukaemia cell interactions with SDF1 has been reported to be pro-apoptotic, further emphasizing the potential of CXCR4 blockade to treat leukaemias[196,197].

Following an initial encouraging phase I/II clinical trial of AMD3100 combined with chemotherapy in patients with relapsed or refractory AML[198], a phase I/II clinical trial investigated AMD3100 combined with an additional mobilizing agent, granulocyte colony-stimulating factor (G-CSF) in this population[199]. Although the precise molecular mechanisms regulating G-CSF-mediated mobilization remain unclear, stromal cell production of G-CSF has been shown to decrease SDF1 levels in the BM niche and induce proteases that cleave VCAM1 (REFS[200,201]). Some studies have reported a desirable effect of G-CSF priming to enhance chemotherapy in standard-risk patients with AML[202,203], while other trials have failed to show a clinical benefit[204,205]. The dual use of G-CSF and AMD3100 to mobilize leukaemic cells during salvage chemotherapy of patients with relapsed or refractory AML did not, however, improve treatment efficacy, potentially due to the induction of counteractive pro-survival signalling effects by G-CSF[199]. Despite this initial discouragement, more potent approaches to inhibit leukaemia interactions with SDF1 and thus promote leukaemia cell apoptosis remain in development, including monoclonal antibodies, SDF1 protein mimetics, peptide inhibitors and new small-molecule inhibitors of CXCR4 (REFS[10,196,206–208]). For example, in a recent phase II clinical trial, the

small-molecule CXCR4 inhibitor, dociparstat, was shown to increase event-free survival in combination with chemotherapy in elderly patients with AML[208], paving the way for a phase III clinical trial (NCT04571645)[209].

In addition to targeting of CXCR4, therapeutic CCR7 and CCR4 chemokine receptor antagonists have advanced to the clinic. Anti-CCR7 monoclonal antibodies were reported to decrease LN invasion in mouse models of CLL as well as inducing CLL antibody-dependent cytotoxicity with minimal effects on normal haematopoietic cells[158]. This led to the clinical development of the anti-CCR7 monoclonal antibody, CAP-100, which is currently being tested in phase I trials in patients with relapsed or refractory CLL (NCT04704323)[210]. An antibody–drug conjugate combining anti-CCR7 with the microtubule inhibitor JBH492 is also being tested in patients with CLL in a phase I trial (NCT04240704)[211].

As discussed above, CCR4 is often highly expressed by T cell leukaemias and lymphomas and is thought to have a role in skin metastasis[72,212]. As such, a CCR4 monoclonal antibody is currently being tested in a phase I study in adult T cell leukaemia/lymphoma (ATLL) and cutaneous T cell lymphomas (NCT04185220)[213].

Targeting adhesion molecules.

Other promising approaches to target the metastatic niche involve disrupting alternative adhesion molecules, including the selectins and integrins, that contribute to leukaemia blast migration to and retention at metastatic sites.

Two specific E-selectin small-molecule inhibitors that have been studied in leukaemia are GMI-1271 (REFS[141,214]) and GMI-1359 (REF.[215]) (a dual E-selectin and CXCR4 inhibitor). In syngeneic murine models, treatment with GMI-1271 mobilized leukaemia cells into circulation and suppressed pro-survival signalling pathways, sensitizing AML blasts to chemotherapy[141]. The results from a phase I/II clinical trial testing GMI-1271 in combination wth standard chemotherapy in patients with relapsed or refractory AML were recently presented at a conference and reported promising survival outcomes[214]. This prompted the initiation of an ongoing phase III trial testing GMI-1271 in combination with chemotherapy to evaluate overall survival as a primary end point (NCT03616470)[216]. In CML, a preclinical study has demonstrated that GMI-1271 administration decreases CML cell contact with the protective BM endothelium and prolongs survival in mice when combined with imatinib, suggesting that investigation of this compound in patients with CML is warranted[217].

The dual E-selectin and CXCR4 inhibitor GMI-1359 has also been shown to have potent anti-leukaemia activity in mouse xenograft models of AML[215]. The ability of GMI-1359 to mobilize cancer cells from the BM and sensitize them to therapy may also be shared across solid tumours, as suggested by preclinical studies in breast cancer[218]. A phase Ib proof-of-concept clinical trial to test the safety and pharmacodynamics of GMI-1359 in patients with bone-metastatic breast cancer is ongoing[219].

Another category of adhesion molecule that has been targeted in leukaemia is the integrin family. In mouse xenograft models of acute leukaemia, administration of the VLA-4

monoclonal antibody natalizumab has been shown to mobilize leukaemic cells from the BM and sensitize them to chemotherapy[220]. Despite encouraging preclinical studies, clinical trials targeting integrin–ligand interactions have not been as successful[221]. A more promising approach, however, may be to target integrin receptor expression or downstream effectors indirectly. Ex vivo analysis of peripheral blood samples from patients with CLL showed that treatment with BTK inhibitor ibrutinib abrogates VLA-4-dependent adhesion of CLL cells to fibronectin, suggesting an ability to modulate crosstalk between BTK–integrin signalling pathways[222].

A promising target for direct or indirect therapeutic intervention is integrin α6. Integrin α6 is highly expressed in ALL and is associated with CNS metastatic relapse and MRD within the BM[56,223]. Our lab reported that targeting integrin α6 directly using monoclonal antibodies, or indirectly by decreasing its expression through PI3K inhibition, reduced CNS metastasis and prolonged survival in mouse ALL xenograft models[56]. Moreover, a recent ALL xenograft study showed that integrin α6 blockade sensitized cells to chemotherapy by blocking SRC family kinase activation of anti-apoptotic signalling[224]. A window-of-opportunity clinical trial to assess the effect of PI3K inhibition with copanlisib on pharmacodynamic markers of CNS metastasis is currently in development[225].

Conclusions

The data reviewed here illustrate the manifold mechanisms of tissue migration employed by leukaemia cells. Careful examination of the anatomy and pathophysiology of leukaemias and of the molecular pathways involved in their spread supports the concept that leukaemia dissemination, as in the solid tumours, is a metastatic process. Indeed, many discoveries of the mechanisms by which tumour cells breach the basement membrane using integrins, selectins and MMPs, then extravasate into the stroma and navigate tissues with amoeboid-like actin dynamics, were first extensively described in normal and malignant leukocytes. Although the multiple basic science discoveries in this space over the past decade have not yet translated to novel, targeted therapies for leukaemias, we are optimistic that, similar to the work culminating in successful cancer immunotherapies, perseverance in this area will reap clinical rewards[226]. Finally, it is likely that the solid and liquid tumour fields will continue to benefit each other as continued research unveils new mechanisms of metastasis that may be shared between these two disease entities.

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Competing interests

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Glossary

Choroid plexus
A secretory tissue in the brain that produces cerebrospinal fluid.

Diapedesis
The process of extravasating out of a vessel into the surrounding stroma.

Endosteal niche
The niche in the bone marrow adjacent to the bone lining (endosteum).

Germinal centre
Site within the spleen and lymph node where B cells proliferate and differentiate.

High endothelial venules (HEVs)
Specialized post-capillary venules in the lymph node that allow for the trafficking of immune cells in and out of this lymphoid organ.

Intravital microscopy
High-resolution imaging of a living organism to study biological events at the cellular level.

Leptomeninges
The inner two meningeal layers that surround the brain and spinal cord and contain cerebrospinal fluid.

Minimal residual disease (MRD)
A subclinical amount of disease remaining after therapy that can fuel relapse.

Nurse-like cells
Monocyte-derived cells that support the survival and growth of chronic lymphocytic leukaemia.

Sinusoidal vessels
Large vessels found in the bone marrow, spleen, lymph node and liver that contain fenestrations allowing the trafficking of cells across the vascular endothelium.

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Box 1 |

Leukocyte migration mechanisms

Tethering and rolling

Leukocyte extravasation begins with tethering (also known as ‘capture’; see figure), the process through which leukocytes first establish contact with the endothelial cells that make up the surface of blood vessels. Leukocytes engage endothelial E- and P-selectin via cell surface carbohydrate ligands such as sialyl Lewis x (SLeX) and P-selectin glycoprotein ligand 1 (PSGL1)[244]. These adhesions are further promoted by VLA-4, engaging intercellular adhesion molecule 1 (ICAM1) on vascular endothelial cells[245].

Slow rolling

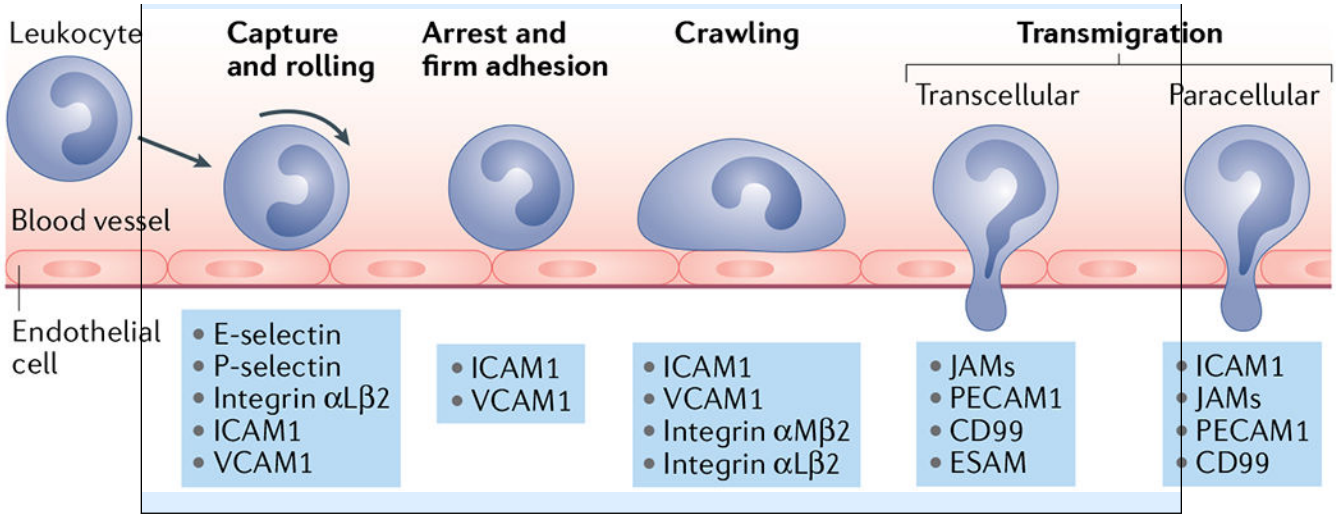
As adhesions become stronger, rolling slows down in a phase identified as ‘slow rolling’ wherein leukocyte adhesion to endothelial P- and E-selectin induces an affinity conformation of integrin αLβ2 (also known as lymphocyte function-associated antigen 1 (LFA1)). This allows for the transient binding of ICAM1 on the vascular endothelium and ligand-induced adhesion strengthening[246–248].

Firm adhesion

Slow rolling eventually results in leukocytes stopping and firmly adhering to the surface of endothelial cells, a process widely known as ‘arrest’. Arrest is triggered through a chemokine-driven process known as activation, in which endothelial cells are stimulated to express higher levels of adhesive molecules such as ICAM1 and vascular cell adhesion molecule 1 (VCAM1) (REF.[249]). Thereafter, adhesion molecules are reorganized and clustered to reinforce adhesion[250]. Before diapedesis, leukocytes crawl along the blood vessel wall via the integrins αMβ2 (also known as MAC-1) and αLβ2 to probe the endothelium and identify sites permissive for crossing[251,252].

Transmigration

In most cases, leukocytes extravasate through the endothelial monolayer via a paracellular (diapedesis) route by using membrane protrusions to probe the endothelial surface and identify endothelial cell–cell junctions. This process is mediated by a complex array of integrins, proteases and signalling pathways to ‘squeeze’ through the endothelium[253]. On rare occasions, leukocytes can extravasate transcellularly (going through endothelial cells) by forming small pockets in the plasma membrane (caveolae) that eventually fuse to form an intracellular channel[254].



Box 2 |

Leukaemia cell migration modes

Amoeboid

Leukocytes and leukaemic cells have been shown to migrate using what is known as amoeboid motility. This mode of migration is a highly efficient method employed by leukocytes and cancer cells to squeeze through tissues without having to degrade the extracellular matrix (ECM). Amoeboid migration relies on membrane blebs that are formed when the plasma membrane detaches from the underlying actin cortex allowing cytoplasm to form herniations in the membrane and generate a motile force[255]. This high contractile force generated through myosin II contractility followed by hydrostatic pressure in the cytoplasm enables the cell to push its way forwards through connective tissues and the ECM[256].

Invadopodia

Invadopodia and their close relatives podosomes are F-actin-rich membrane protrusions that play an essential part in degrading the ECM to drive invasion[257,258]. Invadopodia formation relies on the coordination of CDC42 and tyrosine kinases to form actin bundles that extend into the plasma membrane, aiding cellular invasion. Vesicles carrying matrix-degrading molecules such as matrix metalloproteinases (MMPs) are delivered specifically to invadopodia[259], where they can be released into the ECM and break it down[260].

Lamellipodia

Lamellipodia are leading edge membrane protrusions that drive directional cell migration in most motile cell types and are occasionally found in migrating leukaemic cells. Lamellipodia consist of thin, flat protrusions that drive the cell forwards by generating a branched actin filament network[81]. As they migrate, leukocytes activate the RAC–WAVE–ARP2/3 signalling axis that drives actin polymerization, forming a retrograde flow of actin branches that pushes the cell forwards[261].

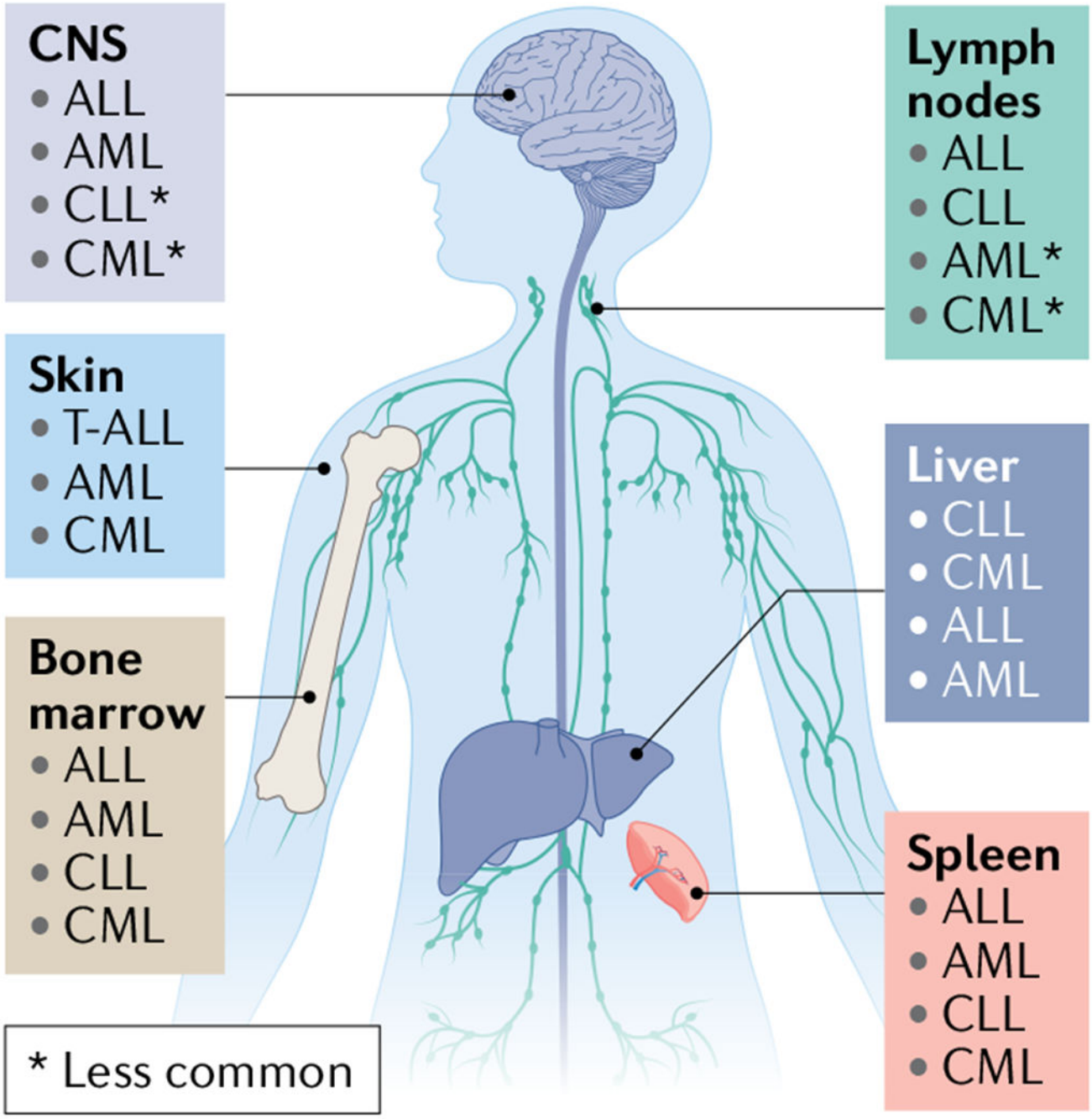


Fig. 1 |. Primary metastatic profiles of leukaemia metastasis.

Acute lymphoblastic leukaemia (ALL) cells are typically initially found in the peripheral vascular system and the haematopoietic organs, including the bone marrow (BM)[15], lymphatic system and spleen; however, central nervous system (CNS) involvement occurs in 5–10% of adult patients at diagnosis[230] and can occur in up to 30–50% of patients in the absence of CNS-directed therapy. WHO classifies ALL and lymphoblastic lymphoma as a spectrum disorder and distinguishes these diseases according to clinical presentation favouring BM (involvement of >20% of blasts) or lymphatic tissue involvement,

respectively[15]. Acute myeloid leukaemia (AML) primarily develops and spreads within the BM and less frequently involves other haematopoietic organs. It may invade non-haematopoietic organs including the gingiva, skin, muscle and CNS, although uncommonly. Chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is notable for its highly stereotyped clinical evolution: at the earliest stages, bloodstream lymphocytosis is the sole clinical finding, followed over time by the development of lymph node enlargement, then splenomegaly and in advanced stages, progressive BM involvement[231]. CLL and SLL are considered the same entity, but with differing disease presentation of bloodstream (CLL) or nodal involvement (SLL)[232,233]. Chronic myeloid leukaemia (CML) primarily involves the BM, peripheral circulation and spleen[234]. In rare cases including during evolution to blast crisis, CML expansion can result in infiltration into other less common sites, including the lymph nodes, liver, skin or CNS[235–237]. T-ALL, T cell ALL.

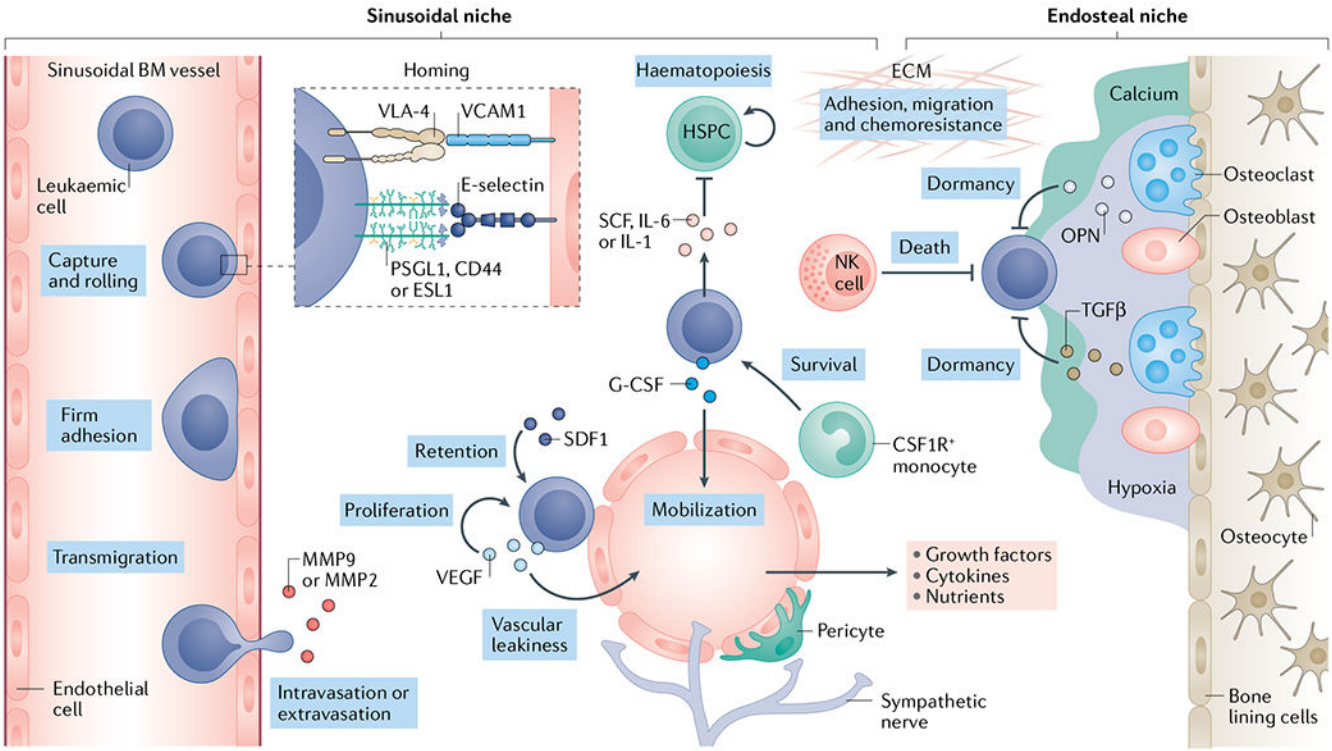


Fig. 2 |. The leukaemia bone marrow microenvironment.

Leukaemia cell homing is mediated by the chemokine stromal cell-derived factor 1 (SDF1)[9,99–101] and adhesion molecules such as the integrin VLA-4[8,53–55] and E-selectin[9] expressed by the vascular endothelium of fenestrated, sinusoidal bone marrow[153] vessels. In this niche, growth factors, cytokines, extracellular matrix (ECM) components as well as stromal cells such as $NG2^+$ cells, pericytes and colony stimulating factor 1 receptor-positive ($CSF1R^+$) monocytes[117] all serve to promote leukaemia cell growth and survival. As leukaemia cells colonize the bone marrow (BM), they deplete resident haematopoietic stem and progenitor cells (HSPCs) through the secretion of stem cell factor (SCF), creating a malignant niche that disrupts normal haematopoiesis[123]. Leukaemia cells may also migrate to pro-dormancy endosteal niches where osteopontin (OPN)[35], transforming growth factor-β (TGFβ)[238] and hypoxic conditions support a quiescent state, thus protecting them from chemotherapy. Niche factors such as granulocyte colony-stimulating factor (G-CSF) can mobilize leukaemia cells from the metastatic niche into circulation where they can seed distant sites[201–203]. Matrix-degrading enzymes such as elastase[143], matrix metalloproteinase 2 (MMP2)[92] and MMP9 (REFS[90,239]) can facilitate the extravasation process. Finally, immune cells such as natural killer (NK) cells may limit the survival of leukaemia cells in the BM[240,241]. PSGL1, P-selectin glycoprotein ligand 1; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

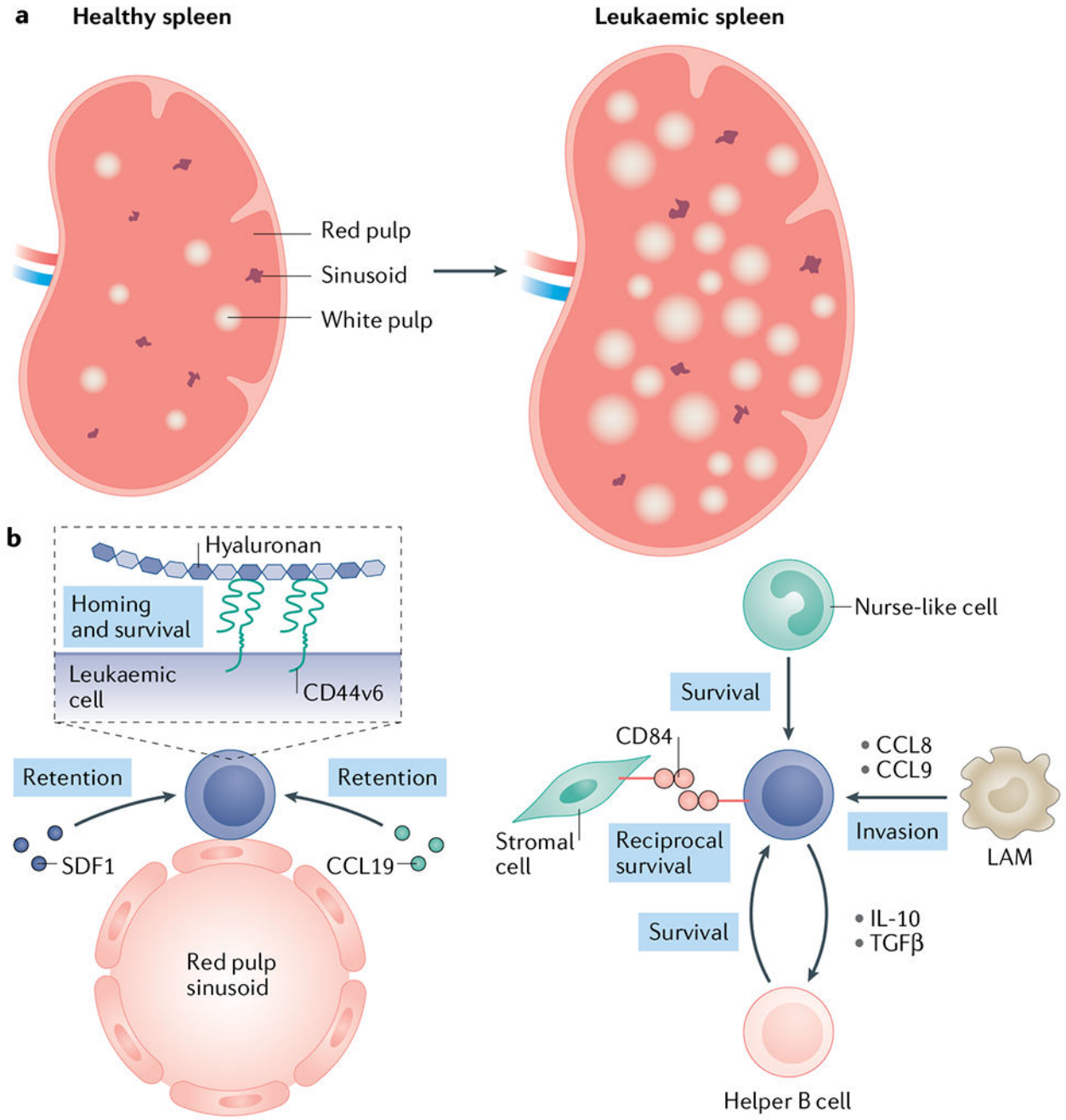


Fig. 3 |. The leukaemia splenic microenvironment.

The spleen is a site of leukaemic progression primarily in chronic leukaemias and contains specific microenvironmental niches that can actively promote leukaemia cell homing and support disease expansion. **a** | As disease progresses, the white pulp expands, leading to a hypertrophic phenotype seen in many patients[242,243]. **b** | Leukaemia cells enter the spleen through large, fenestrated sinusoidal vessels in the red pulp. This is mediated by stromal cell-derived factor 1 (SDF1)–CXCR4 (REFS[61,145]) and CCL19–CCR7 (REF.[69]) expression in the splenic sinusoidal niche. Ly6C^{+} leukaemia-associated macrophages (LAMs) may also

promote leukaemia cell migration to the spleen through the secretion of the CCL8 and CCL9 chemokines[146]. Leukaemia proliferation and survival in the spleen is supported by various stromal components such as hyaluronan[147], monocyte-derived nurse-like cells[167] and helper B cells[148]. For example, the reciprocal interaction between CD84 expressed by both leukaemia cells and stromal cells can promote the survival of either[149]. TGFβ; transforming growth factor-β.

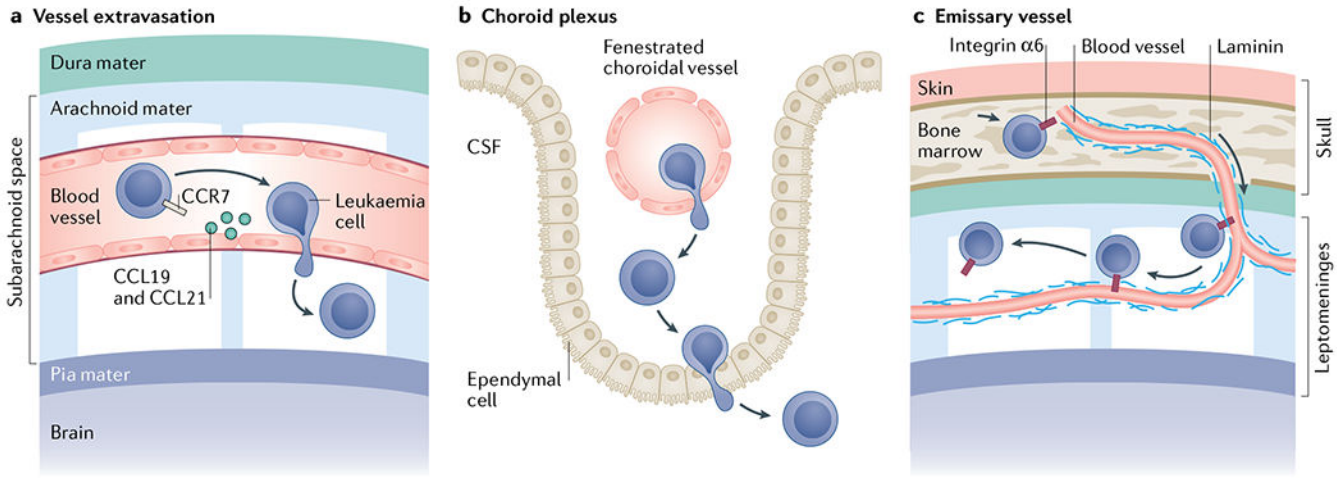


Fig. 4 |. Routes of leukaemia central nervous system invasion.

Leukaemia cell invasion of the central nervous system (CNS) mostly relates to acute leukaemias. Leukaemia cells have been shown to invade the CNS by breaching the blood–brain barrier of parenchymal or leptomeningeal vessels[68] (part **a**), crossing the blood–cerebrospinal fluid (CSF) barrier of the choroid plexus[179] (part **b**) or travelling along the abluminal surface of emissary blood vessels that exit the bone marrow through fenestrations in the bone and transition into leptomeningeal vessels (part **c**)[56]. The choroid plexus is a secretory tissue in the brain that is responsible for producing CSF. It contains fenestrated vessels and a monolayer of ependymal cells with tight junctions, ion pumps and transporters that filter out many cells, ions and proteins to produce CSF. The meninges comprise three membranous layers–the dura, the arachnoid and the pia mater–that form a continuous physical barrier surrounding the brain parenchyma and spinal cord. The dura mater is the outermost layer that is adjacent to the calvarium (skull) or vertebral bone. The subsequent arachnoid and pia mater are connected and form the leptomeninges. Between these two layers that form the leptomeninges is the subarachnoid space, which is filled with an acellular CSF that physically cushions the brain and spinal cord.

Table 1 |

Examples of therapeutics targeting leukaemia engagement with the metastatic niche

Target	Type	Therapy	Condition	Clinical trial number	Phase	Status	Refs
CXCR4	Small molecule	AMD3100 + MEC	R/R AML	NCT00512252	I/II	Completed	198
CXCR4	Small molecule	AMD3100 + G-CSF (filgastrim)	R/R AML	NCT00906945	I/II	Completed	199
CXCR4	Small molecule	BL-8040 + AraC	R/R AML	NCT01838395	II	Completed	227
CXCR4	Small molecule	Dociparstat + chemotherapy	AML	NCT04571645	III	Not yet recruiting	206,208,209
CXCR4	Small molecule	BL-8040 + nelarabine	R/R T-ALL	NCT02763384	II	Recruiting	228
CCR7	Monoclonal antibody	CAP-100	R/R CLL	NCT04704323	I	Not yet recruiting	159,210
CCR7	Antibody–drug conjugate	JBH492	CLL + NHL	NCT04240704	I	Recruiting	211
CCR4	Monoclonal antibody	Mogamulizumab	R/R ATLL	NCT04185220	I	Active, not recruiting	213
G-CSF	Recombinant protein	Recombinant G-CSF (filgastrim) + chemotherapy	AML	#NTR230	III	Completed	203
E-selectin	Small molecule	GMI-1271 + chemotherapy	R/R AML	NCT03616470	III	Recruiting	214
BTK	Small molecule	Ibrutinib	CLL	NCT02048813	III	Active	58
PI3Kδ	Small molecule	Idelalisib	CLL	NCT01539512	III	Completed	229
PI3Kα/δ/γ	Small molecule	Copanlisib	R/R B-ALL	NCT04803123	Window of opportunity	Active, not recruiting	225

ALL, acute lymphocytic leukaemia/lymphoma; AML, acute myeloid leukaemia; AraC, cytarabine; ATLL, adult T cell leukaemia/lymphoma; B-ALL, B cell ALL; BTK, Bruton tyrosine kinase; CLL, chronic lymphocytic leukaemia; G-CSF, granulocyte colony-stimulating factor; MEC, mitoxantrone, etoposide and cytarabine; NHL, non-Hodgkin lymphoma; R/R, relapsed/refractory; T-ALL, T cell ALL.