REVIEW

Shutting the gate: targeting endocytosis in acute leukemia

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Endocytosis entails selective packaging of cell surface cargos in cytoplasmic vesicles, thereby controlling key intrinsic cellular processes as well as the response of normal and malignant cells to their microenvironment. The purpose of this review is to outline the latest advances in the development of endocytosis-targeting therapeutic strategies in hematological malignancies.

HIGHLIGHTS

- Niche signals support leukemia development, while driving progression and relapse.
- Endocytosis controls cellular processes that are essential in leukemia.
- Targeting endocytosis represents a novel therapeutic strategy for acute leukemia.

RECENT FINDINGS

Signals from the microenvironment play a key role in the development and survival of leukemic cells. Furthermore, these signals protect leukemic cells from chemotherapy, suggesting that inhibition of these signals has therapeutic potential. Given the essential role of endocytosis in regulating cellular responses to stimuli from the microenvironment, endocytosis-targeting small molecules could impair multiple key cellular processes driving therapeutic resistance. Despite the development of several inhibitors to study endocytic routes, their use as anticancer drugs remains very limited, with a single preclinical study demonstrating the antileukemic activity of endocytosis inhibitors in several models of acute leukemia [1].

UNMET CLINICAL NEEDS IN ACUTE LEUKEMIA

Although uncommon, acute leukemia (both myeloid and lymphoid) comes with significant morbidity and mortality. In children, acute lymphoblastic leukemia (ALL) is the most common cancer and is curable in most, but requires prolonged chemotherapy that is associated with long-term morbidities [2,3]. In adults, in whom acute myeloid leukemia (AML) is more common with aging, chemotherapy provides cure rates lower than 20% in those >65 years of age [4]. Some inroads have been made over the last 5 years, with the introduction of targeted therapies including FMS-like tyrosine kinase 3 (FLT3) inhibitors and venetoclax for AML [5–7] and immunotherapies such as the bispecific T-cell engager blinatumomab for B-cell ALL [8].

Despite these advances, it is unlikely these therapies will have large impacts on cure rates because they either fail to target or do not completely eliminate leukemia-regenerating cells (LRCs), which are responsible for relapse [9–12].

IMPORTANCE OF LRCS FOR THERAPY RESISTANCE

Acute leukemias arise from either hematopoietic stem cells (HSCs) or downstream progenitors following the acquisition of an initial "founding" mutation [10]. These leukemia-initiating cells carry some but not all the mutations required to generate leukemia, and are thus termed preleukemic stem cells (pre-LSCs) [13]. With acquisition of additional

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collaborative mutations, pre-LSCs evolve into LSCs, which are functionally defined as cells capable of generating overt leukemia when transplanted into immunocompromised animals [12,14]. Genomic studies of matched samples from diagnosis and relapse in acute leukemia suggest that relapse arises from clonal evolution of either pre-LSCs or LSCs present in the diagnostic sample [10]. The term *leukemia-regenerating cells* encompasses all relapse-inducing cells, including both pre-LSCs and LSCs, recognizing that they are often derived from progenitors rather than HSCs.

An inherent ability to resist eradication by high-dose therapy may reflect the LRC stem cell-like properties, which are exhibited by both normal and malignant stem cells. Potential stem cell-like properties that protect normal HSCs from genotoxic stress include increased expression of ABC drug transporters, enhanced DNA repair, reduced metabolic activity and reduced cell cycle (quiescence) [15]. A recent study using single-cell RNA sequencing analysis of human B-cell ALL (B-ALL) revealed that quiescence and stemness were essential properties of chemoresistant relapse-inducing cells [16]. Both AML and B-ALL enriched for gene stemness signature have worse outcomes [17,18], confirming the clinical importance of LRCs. Using a genetic model that enables tracking of cell cycle kinetics in stem cells, we have experimentally determined that quiescence is also crucial for sustaining stem cell-like properties and promoting chemoresistance of LRCs in *Lmo2*-driven T-cell ALL (T-ALL) [19]. Quiescence of both HSCs and LRCs has been reported to be integrally linked to specialized local tissue microenvironments (niches) in the bone marrow [16,20–22]. Although HSCs remain in close proximity to bone marrow (BM) endothelial cells in perivascular niches [20,21], quiescent and chemoresistant LRCs have been found to preferentially localize to the BM endosteal region in close proximity with osteoblasts [16,23], suggesting key differences between the specific microenvironments nurturing normal and malignant stem cells. However, the experimental models used for assessing the microenvironment of malignant cells were suboptimal because the immunophenotype of LRCs is ill-defined and functional studies that define LRCs use xenografts where the supportive cell niche is not physiological.

PROTECTIVE NICHE: A KEY PLAYER IN THERAPY RESISTANCE

Although the functional capabilities of malignant and normal stem cells are conceptually similar [15,24], LRCs appear more reliant on their microenvironment than their normal somatic counterparts. For example, malignant stem cells are more difficult to maintain in in vitro cultures, often requiring maintenance through serial passage in animals [15]. Specific niche factors including nutrients, cytokines, adhesion molecules, chemokines and cellular interactions have been reported for the survival and chemoresistance of ALL and AML (Table 1).

Several mechanisms leading to chemoresistance of ALL mediated by extrinsic protective cures transmitted by the microenvironment have been described, with the functional and metabolomic abnormalities of bone marrow niche cells most extensively characterized in patients with B-ALL [25,26]. Most knowledge on the protective role of bone marrow cells in the biological context of B-ALL has emerged from investigating how mesenchymal, endothelial, or osteoblastic cells support normal HSC self-renewal, differentiation, or resistance to genotoxic stress. For example, the discovery of endosteal niches as a preferential site of residence for HSCs [27,28] led to the subsequent characterization of B-ALL endosteal protective niches where blasts are anchored to osteoblastic and malignant cells through interaction with osteopontin, which also promotes LRC chemoresistance in B-ALL [29]. Further investigation on the localization of malignant cells within the medullar microenvironment have highlighted the diverse cellular niches that control B-ALL progression and response to chemotherapy through secretion of growth factors and direct cell−cell interactions [25]. In B-ALL, protective cell niches are dynamic, because malignant cells actively shape their own supportive microenvironment under the pressure of chemotherapeutic treatments [25,26]. A similar phenomenon has been described in different subtypes of ALL [30], although knowledge of the protective niches in T-ALL remains relatively limited [31,32]. Although the niches of LRCs during the early stages of T-ALL development are currently unknown, recent studies on the BM microenvironment at overt leukemia have revealed that T-ALL expands in various BM niches that play a key role in promoting chemoresistance [33,34]. Although several growth factors produced by stromal cells within the BM niche, such as interleukin-7 (IL-7) [35–37], have been associated with progression and therapeutic resistance in ALL, many other components of the leukemic microenvironment (e.g., adhesion molecules, chemokines, and cellular interactions) are likely to be important.

One of the most widely studied factors of LRC niches in the bone marrow is CXC-motif ligand 12 (CXCL12), a chemokine expressed by endothelial and mesenchymal stromal cells that binds to the heterodimeric G protein-coupled transmembrane CXC-motif receptor 4 (CXCR4) and plays a key role in the tropism of various normal and malignant cell types [38]. Binding of CXCL12 to CXCR4 triggers G-protein-dependent and -independent downstream signaling cascades, inducing MAPK, AKT, and ERK pathway activation, which ultimately regulate chemotaxis and homing, as well as cell survival and proliferation [39,40]. The prognostic impact of the CXCL12/CXCR4 signaling pathway has been extensively described in acute leukemia, with high CXCR4 expression in malignant cells associated with decreased overall and relapse-free survival [41,42]. CXCL12 expression is essential for normal lymphoid development [38], but dispensable for development of leukemia [43]. Inflammatory effects of CXCR4-expressing ALL cells lead to loss of CXCL12 expression [44], which leads to an imbalance in the CXCL12/CXCR4 axis that favors tumor progression by enabling malignant cells to dominate over normal cells. First reported to play a key role in the dynamic interaction of LRCs with the BM niche in the biological context of ALL [45–48], CXCR4 has also been reported to promote chemoresistance of relapse-inducing cells in several blood cancers, including AML [43,49,50]. AML blasts also trigger dynamic changes within the BM microenvironment that promote progression and chemoresistance through upregulation of cell-extrinsic factors that protect and support LRCs [51,52]. AML cells remodel the vascular niche by releasing inflammatory cytokines that stimulate expression of the endothelial cell adhesion molecule E-selectin, which in turn promotes chemoresistance [53]. Together, these findings support the notion of niche-mediated chemoresistance in acute leukemia, with malignant cells not only altering the immune landscape in the BM [51], but also mesenchymal cells [54] and endothelial cells [55], which are key components of both HSC and LSC niches [56].

Table 1 Cellular and acellular components of the microenvironment in acute leukemia

Leukemia	Interacting cells	Cytokine, chemokine, and soluble factors	Role	Reference
B-ALL	Adipocytes	FAT/CD36	Promote fatty acid oxidation and leukemic cell survival	Ehsanipour EA, et al. Cancer Res. 2013;73:2998–3006
	Endothelial cells	CD31	Adhesion and resistance of leukemic cells	Ahsberg J, et al. Haematological. 2020;96:e102–e106
		CX3CL1		Dander E, et al. Br J haematol. 2021;193:1157–1171
	Fibroblasts	GDF15	Chemoprotection of B-ALL cells and marrow adipocyte remodeling	Duan C-W, et al. Cancer Cell. 2014;25:778–793
		IL-8	Proliferation and survival of leukemic cells	Polak R, et al. Blood. 2015;126:2404–2414
	Granulocyte–myeloid-derived suppressor cells	CX3CR1	Promotes chemoresistance	Dander E, et al. Br J haematol. 2021;193:1157–1171
		CXCR4	Promotes chemoresistance	Crazzolara R, et al. Br J Haematol. 2001;115:545–553
	Mesenchymal stem cells and CXCL12-abundantreticular cells	Activin A (ACTA)		Portale F, et al. Exp Hematol. 2019;73:7–12.e4
		BMP4	Promotes disease progression and modulation of antileukemic immunosurveillance	Lopez V, et al.PLoS One. 9:e84496
		CCL2	Chemotaxis and proliferation of leukemic cells	de Vasconcellos JF, et al. Pediatr Blood Cancer. 2011;56:568–577
				Ma Z, et al. Cell Rep. 2019;26:1533–1543.e4
				Dander E, et al. Br J Haematol. 2021;193:1157–1171
		CCL3	Chemoresistance of B-ALL cells	Duan CW, et al. Cancer Cell. 2014;25:778–793
		CCL22	Chemotaxis and proliferation of leukemic cells	de Rooji B, et al. Haematologica. 2017;102:e389–e393
		CXCL1	CXCR2-dependent attraction of B-ALL cells	Dander E, et al. Br J haematol. 2021;193:1157–1171
		CXCL8	CXCR2-dependent attraction of B-ALL cells	Ma C, et al. Sci. Adv. 2020;6 doi: 10.1126/sciadv.aba5536
		CXCL10	Chemotaxis and proliferation of leukemic cells	Polak R, et al. Blood. 2015;126:2404–2414
		CXCL12	Survival, migration, and adhesion of leukemic cells	de Rooji B, et al. Haematologica. 2017;102:e389–e393
				van der Berk LC, et al. Br J Haematol. 2014;166:240–249
		Galectin-3	Positive feedback loop that promotes chemoresistance	Fei F, et al. Oncotarget. 2015;6:11378–11394
		IL-2		Polak R, et al. Blood. 2015;126:2404–2414
		MPP-9	Remodeling of the extracellular matrix	Verma D, et al. Leukemia. 2020;34:1540–1552

(continued)

Table 1 (*Continued*)

Leukemia	Interacting cells	Cytokine, chemokine, and soluble factors	Role	Reference
		N-cadherin	Promotes chemoresistance	Nygren MK, et al. Exp Hematol. 2009;37:225–233
		Notch3/Notch4	Promotes chemoresistance	Nwabo Kamdje AH, et al. Blood. 2011;118:380–389
		VCAM1	Facilitates adhesion and promotes chemoresistance of B-ALL cells	Jacamo R,et al. Blood. 2014;123:2691–2702
	Osteoclasts and osteoblasts	RANK	Bone remodeling by promoting osteoclast development and survival, leukemia-induced destruction	Cheung LC, et al. Leukemia. 2018;32:2326–2338
		Osteopontin (OPN)	Adhesion and quiescence of B-ALL cells	Boyerinas B, et al. Blood. 2013;121:4821–4831
	T cells	TGFb	Modulation of angiogenesis in B-ALL niche	Li X, et al. Leuk Res. 2018;67:60–66.
	Stromal cells (multiple origin)	I	Proinflammation cytokine	Balandrán JC, et al. Front Immunol. 2016;7:666
		IL-1b	Mediation of both innate and adaptive immune responses	Beneforti L, et al. Br J Haematol. 2020;190:262–273.
		IL-6	Proliferation, survival, differentiation, and migration	Ma C, et al. Sci Adv. 2020;6. doi: 10.1126/sciadv.aba5536
		IFN α/β	Antileukemic function	Balandrán JC, et al. Front Immunol. 2016;7:666
		IFN g	Proinflammatory cytokine	Polak R, et al. Blood. 2015;126:2404–2414
		TGFb		Ford AM, et al. J Clin Invest. 2009;119:826–836
		TNFa	Inflammatory response, antileukemic factor	Beneforti L, et al. Br J Haematol. 2020;190:262–273.
T-ALL	Adipocytes	FAT/CD36	Promote fatty acid oxidation and leukemic cell survival	Tucci J, et al. Front Oncol. 2021;11:665763
	Endothelial cells	CXCL12	Survival, migration, adhesion of T-ALL cells	Passaro D, et al. Cancer Cell. 2015;27:769–779
				Pitt LA, et al. Cancer Cell. 2015;27:755–768
		DLL1/DLL4	Adhesion, proliferation and survival of leukemic cells	Indraccolo S, et al. Cancer Res. 2009;69:1314–1323
		E selectin	Adhesion of T-ALL cells to endothelium	Winter SS, et al. Br J Haematol. 2001;115:862–871
	Mesenchymal stem cells and CXCL12-abundant reticular cells	Notch1	Promotes proliferation and chemoresistance	Ma W, et al. PLoS One. 2012;7: e39725
		CXCL12	Survival, migration, and adhesion of leukemic cells	Medo Rde C, et al. PLoS One. 2014;9:e85926
		Galectin-3	Positive feedback loop that promotes chemoresistance	Fei F, et al. Oncotarget. 2015;6:11378–11394
		ICAM-1	Adhesion and survival of T-ALL cells	Winter SS, et al. Br J Haematol. 2001;115:862–871

(*continued*)

Table 1 (*Continued*)

Leukemia	Interacting cells	Cytokine, chemokine, and soluble factors	Role	Reference
		JAG1	Promotes proliferation and chemoresistance	Yuan Y, et al. Oncol Lett. 2013;6:1000–1006
		VCAM1	Facilitates adhesion and promotes chemoresistance of T-ALL cells	Winter SS, et al. Br J Haematol. 2001;115:862–871
	Osteoclasts and osteoblasts	DLL4	Promoting proliferation, survival, and chemoresistance	Cheung LC, et al. Leukemia. 2018;32:2326–2338
		JAG1	Adhesion and suppression of osteoblast function	Wang W, et al. Cancer Res. 2016;76:2847
	Stromal cells (multiple origin)	IL-7	Proliferation, survival, and chemoresistance of T-ALL cells	Silva A, et al. Cancer Res. 2011;71:4780–4789
				Scupoli MT, et al. Haematologica. 2027;92:264–266
		IL-8	Proliferation and survival of leukemic cells	Scupoli MT, et al. Haematologica. 2018;93:524–532
		IL-18	Proliferation, survival, differentiation, and migration	Uzan B, et al. EMBO Mel Med. 2014;6:821–834
		IGF1	Proliferation, survival, and stem cell properties	Medyouf H, et al. J Exp Med. 2011;208:1809–1822
		CCL19 (MIP-3b)	Promotes migration and survival of T-ALL cells	Ma S, et al. J Hematol Oncol. 2014;7:71
				Buonamici S, et al. Nature. 2009;459:U1000–U1129
		CCL25	Promotes migration and chemoresistance of T-ALL cells	Zhou B, et al. Leuk Res. 2010;34:769–776
				Deng X, et al. Oncotarget. 2017;8:39033–39047
AML	Adipocytes	FAT/CD36	Promote fatty acid oxidation and leukemic cell survival	Ye H, et al. Cell Stem Cell. 2016;19:23–37
	Endothelial cells	CD31	Adhesion and resistance of AML cells	Gallay N, et al. Cancer Res. 2007;67:8624–8632
		E selectin	Adhesion of leukemic cells to endothelium, through interaction with CD44	Cavenagh JD, et al. Br J Haematol. 1993;85:285–291
				Barbier V et al. Nat Commun. 2020;11:2042
		P Selectin	Adhesion of leukemic cells to endothelium	Cavenagh JD, et al. Br J Haematol. 1993;85:285–291
		VCAM1	Facilitates adhesion of AML cells	Cavenagh JD, et al. Br J Haematol. 1993;85:285–291
		Fibronectin	Adhesion of AML cells to stromal extracellular matrix	Matsunaga T, et al. Nat Med. 2003;9:1158–1165
		GM-CSF	Secretion regulated by VEGF, mitogen for AML cells	Fiedler W, et al. Blood. 1997;89:1870–1875
	Fibroblasts	GDF15	Chemoprotection of AML cells	Zhai Y, et al. J Exp Clin Cancer Res. 2016;35:147

(*continued*)

Table 1 (*Continued*)

Leukemia	Interacting cells	Cytokine, chemokine, and soluble factors	Role	Reference
		IL-8	Proliferation and survival of leukemic cells	Ryningen A, et al. Leuk Res. 2005;29:185–196
	MSCs and CAR cells	CCL2	Chemotaxis and proliferation of AML cells	Macanas-Pirard P, et al. PLoS One. 2017;12:e0168888
		CXCL8	Survival and proliferation of AML cells	Cheng J, et al. FASEBJ. 2019;33:4755–4764
		CXCL12	Survival, migration, and adhesion of AML cells promoting disease progression	Zeng Z, et al. Blood. 2009;113:6215–6224
				Möhle R, et al.Blood. 1998;91:4523–4530
				Kondo M, et al. Annu Rev Immunol. 2003;21:759–806
		VCAM1	Adhesion of AML cells through interaction with VLA-4	Jacamo R,et al. Blood. 2014;123:2691–2702
	Osteoclasts and osteoblasts	RANK	Bone remodeling by promoting osteoclast development and survival, impairment of NK antileukemic activity	Schmiedel BJ, et al. Oncoimmunology. 2013;2: e23850
		CXCR4	Chemokine receptor, adhesion of AML cells	Roodman GD. Leukemia. 2009;23:435–441
		Osteopontin (OPN)	Adhesion of leukemic cells through interaction with CD44	Liersch R, et al. Blood. 2012;119:5215–5220
	Sympathetic neural cells	TGR-b	Promotes maintenance and repopulation activity	Yamazaki S, et al. Cell. 2011;147:1146–1158
				Hanoun M, et al. Cell Stem Cell. 2014;15:365–375
	T cells	PD-1	Antitumor immune response	Zhang L, et al. Blood. 2009;114:1545–1552
		TIM-3	T-Cell exhaustion	Zhou Q, et al. Blood. 2011;117:4501–4510
	Stromal cells (multiple origin)	IL-1b	Mediation of both innate and adaptive immune responses	Yang J, et al. Int J Cancer. 2013;133:1967–1981
		IL-6	Proliferation, survival, differentiation, and migration	Burger R. Transfus Med Hemother. 2013;40:336–343
		ICAM-1	Proliferation, survival, and stem cell properties	Liu YF, et al. Stem Cell Reports. 2018;11:258–273
		IFN a/b	Antileukemic function	Hemmati S, et al. Front Oncol. 2017;7:265.
		IFN g	Proinflammatory cytokine	Hemmati S, et al. Front Oncol. 2017;7:265.
		TNFa	Inflammatory response, antileukemic factor	Zhou X, et al. Exp Hematol. 2017;45:17–26

IFN=Interferon; *IL*=interleukin; *TNF*=tumor necrosis factor.

TARGETING THE LRC MICROENVIRONMENT

Given the importance of the cell niche in acute leukemia, targeting the niche signals that support LRCs is an attractive treatment strategy in acute leukemia although it remains unclear which factor or factors would be the optimal therapeutic target (Table 2). Moreover, therapy exposure has been reported to alter the microenvironment in different hematopoietic organs [57–59], thereby adding to the complexity of targeting particular niche signals in acute leukemia.

One of the most studied is the CXCL12/CXCR4 axis, where disruption with the CXCR4 antagonist Plerixafor promoted cell cycle and mobilization and inhibited pro-survival factors in LRCs [60–62]. Initially developed as an HIV blocking agent, Plerixafor is now approved for mobilization of autologous stem cell transplantation (HSCT) in patients with blood cancers when administered alone or in combination with granulocyte colony-stimulating factor (G-CSF) [63,64]. Other CXCR4 inhibitors including monoclonal antibodies have been found to impair the beneficial interaction between malignant cells and their protective niche, resulting in delayed leukemia progression as well as increased sensitivity of relapse-inducing cells to chemotherapy [45–47,61]. Early clinical trials of these inhibitors with chemotherapy reveal tolerability but efficacy remains unknown.

E-Selectin is another attractive therapeutic target in acute leukemia, because this adhesion molecule plays a key role in malignant cell survival, adhesion and tropism within their microenvironment (Table 1). E-Selective small molecule inhibitors have been reported to successfully mobilize normal HSCs with the highest self-renewal, leading to significantly improved engraftment and reconstitution [65,66]. Administration of the E-selectin antagonist uproleselan (GMI-1271) promoted mobilization of AML blasts by blocking adhesion to endothelial cells within the BM niche, with consequent impaired malignant cell regeneration and improved efficacy of chemotherapy, as combination therapy doubled the duration of mouse survival over chemotherapy alone in preclinical models of AML [55]. A phase I/II trial of GMI-1271 in combination with high-dose chemotherapy in AML patients was quite promising (Table 2), hence progressing to two large randomized phase III trials (NCT03616470, NCT03701308).

Although inhibitors of singular niche signals display promising anticancer effects, there is evidence of treatment resistance through activation of alternative proliferation and survival pathways [67–70]. Consistent with these compensatory changes, inhibition of both CXCR4 and E-selectin may be more effective than single agents [71,72], thereby supporting the rationale for targeting multiple niche signals.

POTENTIAL ROLES OF ENDOCYTOSIS IN LEUKEMIA

Endocytosis is the active process by which cells internalize surface proteins and molecules provided by the surrounding niche across the plasma membrane [73]. Endocytosis tightly regulates both the initiation and termination of the signaling cascade and thereby controls the magnitude of the cellular response (Figure 1A) [74–77]. Depending on the type of cargo, route of internalization, and mechanism of endocytic scission, endocytosis of specific receptors can take place by clathrin-mediated (CME), caveola-mediated (CavME), or clathrin-independent (CIE) pathways.

CME is integral for cell migration, cytokinesis, signal transduction, nutrient uptake, and recycling or degradation of proteins [75]. In CME, cargos are internalized via clathrin-coated pits, assembly of which is initiated by complexes of adaptor proteins, such as adaptor protein 2 (AP2) lipids [78–80]. As the nascent plasma membrane invagination grows, AP2, clathrin, and other cargo-specific adaptor proteins recruit, internalize, and concentrate the cargo within the endocytic vesicle. CME is a tightly orchestrated homeostatic process in which aberrant expression or malfunction of key endocytic components (for example, the AP2A2 subunit of AP2 complex was reported to be involved in HSC self-renewal [81]) could have regulatory roles during malignant transformation and disease progression [82].

The caveola pathway is crucial for the endocytosis of ligands (e.g., albumin, glycosphingolipids, integrins) and signaling receptors, as well as fatty acids and cholesterol, all important for regulation of key cellular processes such as proliferation, metabolism, and migration. CavME involves the formation of small bulb-shaped plasma membrane invaginations called caveolae that are driven by both integral (i.e., caveolins) and peripheral (i.e., cavins) membrane proteins [83,84]. Once CavME is initiated, ligand-bound cargo receptors are concentrated into the budding caveolae, which is regulated by kinases and phosphatases (e.g., Src tyrosine kinases, serine/threonine phosphatases PP1 and PP2A). As with CME, dynamin (DNM) is also recruited around the neck of budding vesicles to pinch off caveola endocytic vesicles from the plasma membrane [85]. Genomic mutations leading to aberrant expression or malfunction of the CavMe pathway have been linked with metabolic alterations that contribute to oncogenesis and relapse [86,87].

CIE has been found to regulate the internalization of different adhesion molecules (i.e., ALCAM) [88] as well as cellular blebbing, which involves the formation of actin-based membrane protrusions involved in cell movement, cytokinesis, cell spreading, and apoptosis [89]. CIE encompasses several endocytic pathways, and unlike CME or CavME, the endocytic vesicles involved in CIE have no distinct coat [90,91]. The clathrin- and dynamin-independent (CLIC/GEEC) pathway involves the GTPases RAC1 and CDC42, which regulate the actin-dependent formation of clathrin-independent carriers (CLICs) and the subsequent production of glycosylphosphatidylinositol (GPI)-AP-enriched endosomal compartments (GEECs). The endophilin-, dynamin-, and RhoA-dependent endocytic pathway is essential for the internalization of several receptors, including interleukin-2 receptors (IL-2Rs) and T-cell receptors (TCRs). CIE has been reported to suppress blebbing in malignant cells and impairs their invasiveness [92], suggesting a tumor suppressor role for specific key components of the CIE pathway in disease progression and dissemination.

Endocytosis plays a key role in many integral cellular processes that support stemness and contribute to oncogenesis, including cell cycle, differentiation, survival, and metabolism [81,93,94]. Several components of the different endocytic routes are mutated in different cancers, highlighting the importance of derailed endocytosis in the pathogenesis of acute leukemia [73]. Accordingly, we have found that loss of function of dynamin 2 (DNM2) promoted progression by impairing growth factor receptor internalization, which enhanced receptor-mediated signaling in LRCs and promoted clonal expansion in the *Lmo2*-driven model of T-ALL [95]. Given its central role in regulating several endocytic routes and its involvement in the pathophysiology of several cancers including acute leukemia [96], DNM appears to be a promising therapeutic target.

Table 2 Therapeutics targeting the microenvironment in acute leukemia

Niche factor	Malignant cell interaction	Therapeutics	Mechanism of action	Pathway	Leukemia	Status	References
Activin A (ACTA)	Activin receptors (ACVRs)	AZD3463	Inhibitor of activin receptor-like kinases (ALKs)	ALK-Smad	AML	Pre-clinical	Moharram SA, et al. Blood Cancer J. 2019;9:5
BMP4	BMP receptors (BMPRs)	K02288	Inhibitor of activin receptor-like kinases (ALKs)	BMP-Smad	AML	Pre-clinical	Long X, et al. Blood. 2019;134 (Supplement_1):3731
		LDN-193189	Inhibitor of activin receptor-like kinases (ALKs)	BMP-Smad	AML	Pre-clinical	Raymond A, et al. Oncotarget. 2014;5:12675–12693
CCL2	CCR2	SC202525	CCR2 antagonist	CCL2/CCR2	AML	Pre-clinical	Macanas-Picard P, et al. PLoS One. 2017;12:e0168888
CCL3	CCR5	Maraviroc	CCR5 antagonist	CCL3/CCR5	ALL	Pre-clinical	Zi J, et al. Am J Cancer Res. 2017;7:869–8803
CCL19 (MIP-3b)	CCR7	CAP-100	CCR7 blocking antibody	CCL19/CCR7	T-ALL	Pre-clinical	Cuesta-Mateos C, et al. Cancer Res. 2019;79(13 Supplement):4849
CCL25	CCR9	92R	CCR9 blocking antibody	CCL25/CCR9	T-ALL	Pre-clinical	Somovilla-Crespo B, et al. Front Immunol. 2018;9:77
CXCL10	CCR3	AMG487	CCR3 antagonist	CXCL10/CCR3	B-ALL	Pre-clinical	Gomez AM, et al. Blood Cells Mol Dis. 2015;555:220–227
CXCL12	CXCR4	Plerixafor (AMD3100)	CXCR4 antagonist	CXCL12/CXCR4	AML	Phase 1/2	Uy GL, et al. Blood. 2012;119:3917–3924
					AML, ALL	Phase 1	Cooper TM, et al. Pediatr Blood Cancer. 2017;64:e26414
					T-ALL	Pre-clinical	Walker KL, et al. Leuk & Lymp. 2021;62:1167–1177
					AML	Phase 1/2	NCT0090645
		AMD3465	CXCR4 antagonist		AML	Pre-clinical	Zeng Z, et al. Blood. 2009;113:6215–6224
		BL-8040 (formerly BTK140)	CXCR4 antagonist		AML	Phase 2a	Borthakur G, et al. Blood. 2016;128:2745
					AML	Pre-clinical	Abraham M, et al. Leukemia. 2017;31:2336–2346
		LY2310924	CXCR4 antagonist		AML	Pre-clinical	Cho BS, et al. Blood. 2015;126:222–232.
		Ulocuplumab (BMD-936564/MDX-1338)	CXCR4 blocking antibody		AML	Phase 1	Becker P, et al. Blood. 2014;124:386
E selectin	CD162	Uproleselan (GMI-1271)	E selectin antagonist	E selectin/ CD162	AML	Pre-clinical	Barbier V et al. Nat Communs. 2020;11:2042
					AML		Erbani J, et al. Front Cell Dev Biol. 2020;8:668

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Table 2 (*Continued*)

Niche factor	Malignant cell interaction	Therapeutics	Mechanism of action	Pathway	Leukemia	Status	References
					AML	Phase 1/2	DeAngelo DJ, et al. Blood. 2018;132(Supplement 1):331.
					AML	Phase 3	NCT03616470, NCT03701308
DLL1/DLL4/ JAG1	NOTCH1-4	GSI-XII	Gamma secretase inhibitor (GSI)	Notch	B-ALL	Pre-clinical	Kamga PT, et al. Cancer Res. 2019;79:639−649
		GSI-I	Gamma secretase inhibitor (GSI)		B-ALL	Pre-clinical	Meng X, et al. Leukemia. 2011;25:1134−1146
		BMS-906024	Gamma secretase inhibitor (GSI)		T-ALL	Phase 1	Zweidler-McKay PP, et al. Blood. 2014;121:Abstract 968
Galectin-3	Diverse	GCS-100	Galectin inhibitor	Galectin	AML	Pre-clinical	Ruvolo PP, et al. Biochim Biophys Acta. 2016;1863:562 −571
					CLL	Phase 2	NCT00514696
		KB1019.7	Galectin inhibitor		ALL	Pre-clinical	Tarighat SS, et al. Blood. 2015;126:2047
IGF1	IGF-1R	NVP-AEW541	IGF-1R tyrosine kinase inhibitor	IGF1/IGF-1R	AML	Pre-clinical	Tazzari PL, et al. Leukemia. 2007;21:889−896
IL-1a/b	IL-1R1	Kineret (Anakinra)	Il-1 R antagonist	IL-1a/b/IL-1R1	AML	Pre-clinical	Arranz L, et al. Nature. 2014;512:78-81
					CLL	Phase 1	NCT04691765
IL-2	IL-2R	BNZ-1	g-chain cytokine inhibitor	IL-2/IL-2R	T-ALL	Pre-clinical	Wang TT, et al. Leukemia. 2019; 33:1243−1255
					CTCL	Phase 1/2	Querfeld C, et al. Blood. 2020; 136(Supplement 1):37
IL-6	IL-6R	Tocilizumab	IL-6R blocking antibody	IL-6/IL-6R	B-ALL	Phase 1	Kadauke S, et al. J Clin Oncol. 2021;39:920−930
					AML	Phase 1	NCT04547062
IL-7	IL-7R	Ruxolitinib	JAK1/2 inhibitor	IL-7/JAK/STAT	ALL	Phase 2	Tasian SK, et al. Blood. 2018;132(Supplement 1):555
					AML	Phase 2	NCT01348490
		GSK2618960	IL-7R blocking antibody	IL-7/JAK/STAT	B-ALL	Pre-clinical	Abdelrasoul H, et al. Nat Communs. 2020;11:3194
IL-8	CXCR2	SB-332235	CXCR2 antagonist	IL-8/CXCR2	AML	Pre-clinical	Schinke C, et al. Blood. 2015;125:3144−3152
N-cadherin	N-cadherin	GC-4	N-cadherin blocking antibody	N-cadherin	AML	Pre-clinical	Marjon KD, et al. Oncogene. 2016;35:4132−4140

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Table 2 (*Continued*)

Niche factor	Malignant cell interaction	Therapeutics	Mechanism of action	Pathway	Leukemia	Status	References
Osteopontin (OPN)	Integrin		OPN blocking antibody	OPN/integrin	T-ALL	Pre-clinical	Maeda N, et al. Retrovirology. 2015;12:99
PD-1	PDL-1	Pembrolizumab	PD-1 antagonist	PD-1/PDL-1	ALL	Phase 2	Cassaday RD, et al. Blood Adv. 2020;4:3239–3245
					AML	Phase 1/2	NCT02996474
RANK	RANKL	OPG-Fc	RANKL antagonist	RANK/RANKL	B-ALL	Pre-clinical	Rajakumar SA, et al. Sci Transl Med. 2020;12:561
TGFb	TGFbR	Galunisertib	TGFbR kinase inhibitor	TGFb/TGFbR	ALL	Pre-clinical	Maier A, et al. Call Oncol. 2015;38:131–144
					AML	Phase 2	Santini V, et al. Clin Cancer Res. 2019;25:6976–6985
TIM-3	Diverse	MGB453	TIM3 antagonist	TIM3	AML	Phase 1/2	Ahn M. J Thor Onc. 2018;13(10, supplement):S299–S300
					AML	Phase 2	NCT04150029
VCAM1	VLA-4	Natalizumab	VLA-4 blocking antibody	VCAM1/VLA-4	B-ALL	Pre-clinical	Hsieh YT, et al. Blood. 2013;121:1814–1818

DYNAMINS: A NOVEL THERAPEUTIC TARGET IN ACUTE LEUKEMIA

Dynamins are a family of large GTPases required budding and scission of endosomes for CME, CavME, and some CIE pathways [73,97]. The mammalian genome encodes three classic DNM proteins, which share 80% overall homology and play at least partially redundant roles in endocytosis [98]. DNM1 is neuron specific and plays a key role in synaptic vesicle endocytosis with *DNM1*-null mice dying in early postnatal life [99] DNM2 is ubiquitously expressed and its loss causes embryonic lethality [100]. DNM3 is found prominently in the brain, heart, lung, and testes, yet mice lacking DNM3 are viable and fertile [101]. Recent work has highlighted the key role of DNM in completing the endocytosis process by orchestrating the multivalent protein interactions that enable successful membrane scission in stimulated cells [102]. Accordingly, DNM dysfunction or overexpression in malignancies has been associated with increased receptor-mediated signaling, thus promoting cell migration, invasion, and metastasis [96].

Genetic and pharmacologic studies revealed that dynamin-dependent endocytosis (DDE) is essential for internalization of ligand-bound receptors and downstream activation of signaling pathways (Figure 1A), as well as the internalization and recycling of surface proteins in stimulated cells (Figure 1B) [75,103]. Although previous studies have determined the in vitro efficacy of DNM inhibitors in blocking endocytosis of ligand-bound receptors in cancer cells [95,104–106], our recent work provided the first in vivo evidence that small molecule inhibition of DDE significantly impaired LRC activity (self-renewal as measured by serial transplantation assays, and clonal expansion as defined by repopulation capacity and leukemogenicity) and promoted chemosensitivity by blocking multiple signaling pathways (Figure 1C) [1]. In T-ALL, treatment with the specific DNM inhibitor Dynole 34-2 prevented the internalization of receptors for IL-7 and stem cell factor (SCF), as well as Notch1, whereas in AML, inhibition of DDE impaired signal transduction from IL-3, SCF, and granulocyte–macrophage colony-stimulating factor (GM-CSF) [1]. Administration of Dynole 34-2 significantly reduced leukemic burden in association with conventional chemotherapy in different models of acute leukemia, including patient-derived xenograft (PDX) models of both T-ALL and AML. Importantly, Dynole 34-2 significantly delayed the onset of disease [1], alone and in combination with the chemotherapy regimens commonly used for these hematological cancers in all models of leukemia tested, suggesting antileukemic activity of DDE inhibitors independent of the mutational profile. Together, these preclinical data illustrate the importance of DDE for survival of LRCs that are responsible for relapse in acute leukemia and, most importantly, that a safe and controlled manipulation of endocytosis may enhance the clinical response of combination treatment. Given the incredible diversity of niche signals, targeting multiple pathways is likely to be more effective and could overcome the rapid resistance-promoting adaptive responses to chemotherapies and targeted therapies [107]. Although these studies focused on endocytosis of ligand-bound receptors and signal transduction (Figure 1A), the effects of blocking DDE on the uptake of nutrients and cell–cell interactions within the microenvironment may also be important. An improved understanding of the effects of DNM inhibitors on LRCs and their surrounding niche might inform the development of superior combination therapies for refractory acute leukemias.

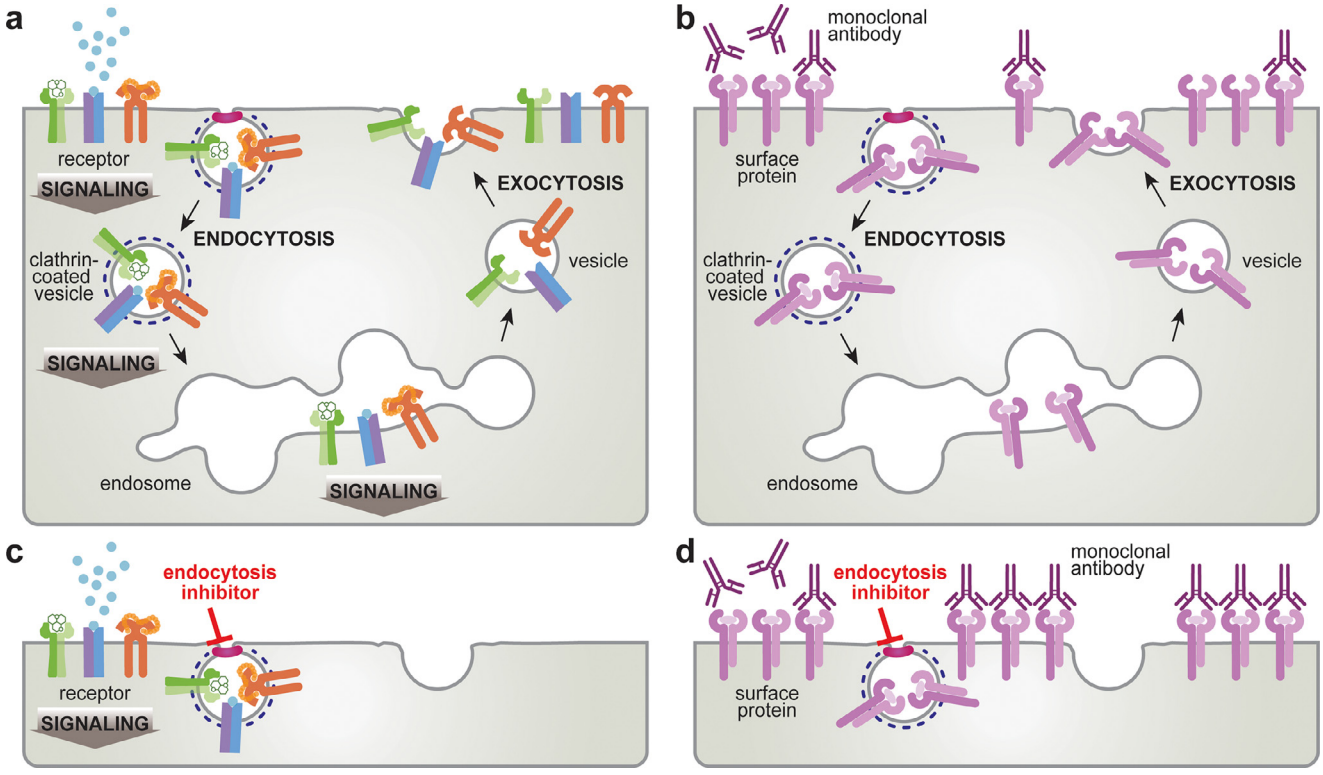

Figure 1 Consequences of targeting endocytosis in combination therapies. **(A)** Schematic model of the transduction of niche-induced signaling pathways. Stimulation by ligands such as hormones (*green*) and cytokines (*blue*) or immunogenic factors (*orange*) triggers downstream signaling pathways, as well as the dynamin-dependent endocytosis (DDE) of ligand-bound receptors in clathrin-coated vesicles. **(B)** Signal block: inhibition of endocytosis with small molecule inhibitors, such as Dynole 34-2, prevents the internalization of ligand-bound receptors and limits downstream activation of signaling pathways, leading to decreased survival and synergy with stress-inducing therapies such as chemotherapy [1]. **(C)** Schematic model of the immunogenic recognition of cell-specific antigens. Stimulation of diverse surface proteins by ligands (*pink*) triggers DDE, which limits the recognition of specific epitopes displayed on these surface proteins by circulating monoclonal antibodies. **(D)** Surface trap: Inhibition of endocytosis with small molecule inhibitors prevents the internalization of surface proteins, leading to increased exposure to circulating monoclonal antibodies and synergy with immunotherapy targeting these specific cell surface proteins [108].

A recent breakthrough study revealed that reversible inhibition of DDE improved the efficiency of natural killer cell-mediated antibody-dependent cellular toxicity (ADCC) by promoting the accumulation of surface proteins targeted by antitumor monoclonal antibodies (Figure 1C,D) [108]. A pilot study determined that inhibition of endocytosis synergized with immunotherapies in vivo and significantly improved the clinical response in humans [108]. Given the key role of immune escape in facilitating leukemia initiation, progression and dissemination of malignancies [109,110], enhancing the anti-tumor immunity response with endocytosis inhibitors might be of great therapeutic value. Immune selection of tumor subclones devoid of the antigen targeted by anti-tumor immunotherapies represents another mechanism of therapeutic resistance, which might be counteracted by using DNM inhibitors in combination therapies. Given the increasing interest in immunotherapies based on anti-tumor monoclonal antibodies or engineered immune cells (e.g., chimeric antigen receptor [CAR] T cells) for the treatment of acute leukemia and several other cancers, the accessibility of the specific tumor cell antigens is crucial for effective targeting, tumor clearance, and clinical response (Figure 1B). Therefore, reversible inhibition of endocytosis represents an attractive strategy to enhance tumor-antigen presentation at the surface of malignant cells, increase immune response, and ultimately improve the clinical benefit of immunotherapies in patients.

Therapeutic doses of DNM inhibitors appear well tolerated in vivo in combination with chemotherapy, with no obvious myelosuppression or gut toxicity, despite inhibiting multiple signaling pathways that are important for homeostasis [1,105], suggesting a suitable therapeutic window for targeting DDE. Unlike chemotherapy, which impairs HSC activity [111–113], Dynole 34-2 had no detrimental effect on HSC fitness and differentiation potential, as assessed in long-term transplantation assays [1]. On the contrary, DDE inhibition had a significant protective effect on normal HSCs, because Dynole 34-2 prevented the chemotherapy-induced decrease in HSC activity and restored normal differentiation in recipients [1]. Furthermore, pilot clinical data in patients with solid tumors indicated the safety of using DNM inhibitors as combination therapies in humans [108]. This represents a paradigm shift on the clinical application of specifically and reversibly inhibiting endocytosis in combination therapies.

Apart from inhibitors of DNM [114,115], small molecules that selectively target other key regulators of the major endocytic routes have recently been reported [116]. The Pitstop series of small molecules were among the first class of CME-specific inhibitors to be described, and were subsequently used for investigating the dynamics of clathrin-coated pits and more broadly the many steps involved in endocytosis and vesicle recycling [117,118]. Together, these tool compounds have been instrumental in improving our understanding of endocytosis and characterize the key factors regulating the different endocytic routes. However, their anticancer activity remains poorly documented, with a single study indicating that inhibition of CME by Pitstop 2 induces apoptosis in malignant cells [119].

CONCLUSIONS AND PERSPECTIVES

Adaptive therapeutic resistance relies on the inherent plasticity of LRCs that enables the acquisition of point mutations affecting the binding site of small molecule inhibitors, as well as the activation of alternative pathways [67,68]. Although targeting multiple signaling pathways by inhibiting key components of specific endocytic routes represents a promising approach to limit therapeutic resistance, the antileukemic activity of endocytosis-targeting drugs could be limited by LRC plasticity, which might allow relapse-inducing cells to use alternative endocytic routes. Selection of clones harboring genetic abnormalities decreasing the affinity of specific therapies for binding pockets on their targets represents another potential mechanism of therapeutic resistance, which would enable the emergence of resistant LRC clones. Protection by the cell niche is another proposed mechanism of chemoresistance, becuase the tumor microenvironment is a critical regulator of immune escape, progression, and dissemination of malignancies. Extrinsic-adaptive resistance lies in the treatment process rather than the intrinsic properties of relapse-inducing cells, and is thereby influenced by signals from the microenvironment. Simultaneously targeting LRC stem cell-like properties (e.g., self-renewal, plasticity, therapy resistance) and the cellular interactions from the niche that protects malignant cells from killing by high-dose therapy might represent a superior therapeutic strategy to eradicate LRCs and improve the outcome for poor-prognosis malignancies. Therefore, targeting endocytosis may be advantageous as it straddles both the microenvironment and LRC intrinsic adaptive resistance pathways.

Despite encouraging proof-of-concept data with dynamin inhibitors such as Dynole 34-2 and other small molecule inhibitors of key components of endocytosis [1,105], inherent limitations (e.g., unsuitability for optimization, limited potency, little evidence of on-target activity) have prevented their clinical application, with only one study describing the safety and efficacy of endocytosis inhibitors in patients [108]. Although the merit of targeting key components of specific endocytic routes is yet to be fully explored in clinical trials, encouraging preclinical data for Dynole 34-2 revealed the in vivo efficacy of endocytosis inhibitors in targeting LRCs in acute leukemia [1], thereby suggesting that blocking endocytosis might limit the adaptive changes or selection for clones that drive relapse. Although endocytosis inhibition has emerged as a promising target in hematological malignancies, validation and translational studies have been hindered by the lack of clinical-grade small molecule inhibitors of endocytosis. Therefore, the development of suitable pharmacologic inhibitors has the potential to significantly benefit patients, because endocytosis-targeting therapies are expected to improve the efficacy of current chemotherapy and/or immunotherapy regimens while limiting life-threatening side effects caused by prolonged exposure to genotoxic drugs, improving response to treatment, and ultimately improving survival for acute leukemia and other poor-prognosis cancers.

Conflict of interest disclosure

The authors declare no competing interests.

REFERENCES

1. Tremblay CS, Chiu SK, Saw J, et al. Small molecule inhibition of dynamin-dependent endocytosis targets multiple niche signals and impairs leukemia stem cells. Nature communications 2020;11:6211.
2. Testi AM, Canichella M, Vitale A, et al. Adolescent and young adult acute lymphoblastic leukemia: final results of the phase II pediatric-like GIMEMA LAL-1308 trial. Am J Hematol 2021;96:292–301.
3. Olivier-Gougenheim L, Arfeuille C, Suciu S, et al. Pediatric randomized trial EORTC CLG 58951: Outcome for adolescent population with acute lymphoblastic leukemia. Hematol Oncol 2020;38:763–72.
4. Martinez-Cuadron D, Serrano J, Gil C, et al. Evolving treatment patterns and outcomes in older patients (>/=60 years) with AML: changing everything to change nothing? Leukemia 2020.
5. Wei AH, Montesinos P, Ivanov V, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. Blood 2020;135:2137–45.
6. Perl AE, Martinelli G, Cortes JE, et al. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. N Engl J Med 2019;381:1728–40.
7. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. N Engl J Med 2020;383:617–29.
8. Wyatt KD, Bram RJ. Immunotherapy in pediatric B-cell acute lymphoblastic leukemia. Hum Immunol 2019;80:400–8.
9. Boyd AL, Aslostovar L, Reid J, et al. Identification of chemotherapy-induced leukemic-regenerating cells reveals a transient vulnerability of human AML Recurrence. Cancer Cell 2018;34:483–498.e485.
10. Mullighan CG, Phillips LA, Su X, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science 2008;322:1377–80.
11. Pollyea DA, Stevens BM, Jones CL, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. Nat Med 2018;24:1859–66.
12. Shlush LI, Mitchell A, Heisler L, et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. Nature 2017;547:104–8.
13. Tremblay CS, Curtis DJ. The clonal evolution of leukemic stem cells in T-cell acute lymphoblastic leukemia. Curr Opin Hematol 2014;21:320–5.
14. Vedi A, Santoro A, Dunant CF, Dick JE, Laurenti E. Molecular landscapes of human hematopoietic stem cells in health and leukemia. Ann NY Acad Sci 2016;1370:5–14.
15. Rossi F, Noren H, Jove R, Beljanski V, Grinnemo KH. Differences and similarities between cancer and somatic stem cells: therapeutic implications. Stem Cell Res Ther 2020;11:489.
16. Ebinger S, Ozdemir EZ, Ziegenhain C, et al. Characterization of rare, dormant, and therapy-resistant cells in acute lymphoblastic leukemia. Cancer cell 2016;30:849–62.
17. Yan F, Wong NC, Powell DR, Curtis DJ. A 9-gene score for risk stratification in B-cell acute lymphoblastic leukemia. Leukemia 2020;34:3070–4.
18. Ng SW, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. Nature 2016;540:433–7.

19. Tremblay CS, Saw J, Chiu SK, et al. Restricted cell cycle is essential for clonal evolution and therapeutic resistance of pre-leukemic stem cells. Nat Commun 2018;9:3535.
20. Itkin T, Gur-Cohen S, Spencer JA, et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. Nature 2016;532:323–8.
21. Kunisaki Y, Bruns I, Scheiermann C, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. Nature 2013;502:637–43.
22. Saito Y, Kitamura H, Hijikata A, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med 2010;2:17ra19.
23. 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;25:1315–21.
24. Schepers K, Campbell TB, Passegue E. Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell Stem Cell 2015;16:254–67.
25. Dander E, Palmi C, D'Amico G, Cazzaniga G. The bone marrow niche in B-cell acute lymphoblastic leukemia: the role of microenvironment from pre-leukemia to overt leukemia. Int J Mol Sci 2021;22:4426.
26. Delahaye MC, Salem KI, Pelletier J, Aurrand-Lions M, Mancini SJC. Toward therapeutic targeting of bone marrow leukemic niche protective signals in B-cell acute lymphoblastic leukemia. Front Oncol 2020;10:606540.
27. Tay J, Levesque JP, Winkler IG. Cellular players of hematopoietic stem cell mobilization in the bone marrow niche. Int J Hematol 2017;105:129–40.
28. Levesque JP, Helwani FM, Winkler IG. The endosteal 'osteoblastic' niche and its role in hematopoietic stem cell homing and mobilization. Leukemia 2010;24:1979–92.
29. Boyerinas B, Zafrir M, Yesilkanal AE, Price TT, Hyjek EM, Sipkins DA. Adhesion to osteopontin in the bone marrow niche regulates lymphoblastic leukemia cell dormancy. Blood 2013;121:4821–31.
30. Mendez-Ferrer S, Bonnet D, Steensma DP, et al. Bone marrow niches in haematological malignancies. Nat Rev Cancer 2020;20:285–98.
31. Calvo J, Fahy L, Uzan B, Pflumio F. Desperately seeking a home marrow niche for T-cell acute lymphoblastic leukaemia. Adv Biol Regul 2019;74:100640.
32. Hawkins ED, Duarte D, Akinduro O, et al. T-Cell acute leukaemia exhibits dynamic interactions with bone marrow microenvironments. Nature 2016;538:518–22.
33. Cahu X, Calvo J, Poglio S, et al. Bone marrow sites differently imprint dormancy and chemoresistance to T-cell acute lymphoblastic leukemia. Blood Adv 2017;1:1760–72.
34. Passaro D, Quang CT, Ghysdael J. Microenvironmental cues for T-cell acute lymphoblastic leukemia development. Immunol Rev 2016;271:156–72.
35. Silva A, Laranjeira AB, Martins LR, et al. IL-7 contributes to the progression of human T-cell acute lymphoblastic leukemias. Cancer Res 2011;71:4780–9.
36. Bednarz-Misa I, Bromke MA, Krzystek-Korpacka M. Interleukin (IL)-7 signaling in the tumor microenvironment. Adv Exp Med Biol 2021;1290:9–49.
37. Lodewijckx I, Cools J. Deregulation of the interleukin-7 signaling pathway in lymphoid malignancies. Pharmaceuticals (Basel) 2021;14:443.
38. Karpova D, Bonig H. Concise review: CXCR4/CXCL12 signaling in immature hematopoiesis—lessons from pharmacological and genetic models. Stem Cells 2015;33:2391–9.
39. Guo F, Wang Y, Liu J, Mok SC, Xue F, Zhang W. CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. Oncogene 2016;35:816–26.
40. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. Biochim Biophys Acta 2007;1768:952–63.
41. Matsuo H, Nakamura N, Tomizawa D, et al. CXCR4 overexpression is a poor prognostic factor in pediatric acute myeloid leukemia with low risk: A report From the Japanese Pediatric Leukemia/Lymphoma Study Group. Pediatr Blood Cancer 2016;63:1394–9.
42. Ko SY, Park CJ, Park SH, et al. High CXCR4 and low VLA-4 expression predicts poor survival in adults with acute lymphoblastic leukemia. Leuk Res 2014;38:65–70.
43. Ramakrishnan R, Pena-Martinez P, Agarwal P, et al. CXCR4 Signaling has a CXCL12-independent essential role in murine MLL-AF9-driven acute myeloid leukemia. Cell Rep 2020;31:107684.
44. Balandran JC, Purizaca J, Enciso J, et al. Pro-inflammatory-related loss of CXCL12 niche promotes acute lymphoblastic leukemic progression at the expense of normal lymphopoiesis. Front Immunol 2016;7:666.
45. Passaro D, Irigoyen M, Catherinet C, et al. CXCR4 is required for leukemia-initiating cell activity in T cell acute lymphoblastic leukemia. Cancer Cell 2015;27:769–79.
46. Pitt LA, Tikhonova AN, Hu H, et al. CXCL12-producing vascular endothelial niches control acute t cell leukemia maintenance. Cancer cell 2015;27:755–68.
47. Juarez J, Dela Pena A, Baraz R, et al. CXCR4 antagonists mobilize childhood acute lymphoblastic leukemia cells into the peripheral blood and inhibit engraftment. Leukemia 2007;21:1249–57.
48. Sison EA, Rau RE, McIntyre E, Li L, Small D, Brown P. MLL-Rearranged acute lymphoblastic leukaemia stem cell interactions with bone marrow stroma promote survival and therapeutic resistance that can be overcome with CXCR4 antagonism. Br J Haematol 2013;160:785–97.
49. Agarwal P, Isringhausen S, Li H, et al. Mesenchymal niche-specific expression of Cxcl12 controls quiescence of treatment-resistant leukemia stem cells. Cell Stem Cell 2019;24:769–784.e766.
50. Sison EA, McIntyre E, Magoon D, Brown P. Dynamic chemotherapy-induced upregulation of CXCR4 expression: a mechanism of therapeutic resistance in pediatric AML. Mol Cancer Res 2013;11:1004–16.
51. Guo R, Lu M, Cao F, et al. Single-cell map of diverse immune phenotypes in the acute myeloid leukemia microenvironment. Biomark Res 2021;9:15.
52. Borella G, Da Ros A, Borile G, et al. Targeting mesenchymal stromal cells plasticity to reroute acute myeloid leukemia course. Blood 2021;138:557–70.
53. Erbani J, Tay J, Barbier V, Levesque JP, Winkler IG. Acute myeloid leukemia chemo-resistance is mediated by E-selectin receptor CD162 in bone marrow niches. Front Cell Dev Biol 2020;8:668.
54. von der Heide EK, Neumann M, Vosberg S, et al. Molecular alterations in bone marrow mesenchymal stromal cells derived from acute myeloid leukemia patients. Leukemia 2017;31:1069–78.
55. Barbier V, Erbani J, Fiveash C, et al. Endothelial E-selectin inhibition improves acute myeloid leukaemia therapy by disrupting vascular niche-mediated chemoresistance. Nat Commun 2020;11:2042.
56. Kokkaliaris KD, Scadden DT. Cell interactions in the bone marrow microenvironment affecting myeloid malignancies. Blood Adv 2020;4:3795–803.
57. Gilbert LA, Hemann MT. DNA damage-mediated induction of a chemoresistant niche. Cell 2010;143:355–66.
58. Tikhonova AN, Dolgalev I, Hu H, et al. The bone marrow microenvironment at single-cell resolution. Nature 2019;569:222–8.
59. Di Grande A, Peirs S, Donovan PD, et al. The spleen as a sanctuary site for residual leukemic cells following ABT-199 monotherapy in ETP-ALL. Blood Adv 2021;5:1963–76.
60. Cho BS, Zeng Z, Mu H, et al. Antileukemia activity of the novel peptidic CXCR4 antagonist LY2510924 as monotherapy and in combination with chemotherapy. Blood 2015;126:222–32.
61. Kuhne MR, Mulvey T, Belanger B, et al. BMS-936564/MDX-1338: a fully human anti-CXCR4 antibody induces apoptosis in vitro and shows antitumor activity in vivo in hematologic malignancies. Clin Cancer Res 2013;19:357–66.

62. Parameswaran R, Yu M, Lim M, Groffen J, Heisterkamp N. Combination of drug therapy in acute lymphoblastic leukemia with a CXCR4 antagonist. Leukemia 2011;25:1314–23.
63. Kouroukis CT, Varela NP, Bredeson C, Kuruvilla J, Xenocostas A. Plerixafor for autologous stem-cell mobilization and transplantation for patients in Ontario. Curr Oncol 2016;23:e409–30.
64. Shaughnessy P, Uberti J, Devine S, et al. Plerixafor and G-CSF for autologous stem cell mobilization in patients with NHL, Hodgkin's lymphoma and multiple myeloma: results from the expanded access program. Bone Marrow Transplant 2013;48:777–81.
65. Winkler IG, Wiercinska E, Barbier V, Nowlan B, Bonig H, Levesque JP. Mobilization of hematopoietic stem cells with highest self-renewal by G-CSF precedes clonogenic cell mobilization peak. Exp Hematol 2016;44:303–314.e301.
66. Winkler IG, Barbier V, Nowlan B, et al. Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. Nat Med 2012;18:1651–7.
67. Brand TM, Iida M, Wheeler DL. Molecular mechanisms of resistance to the EGFR monoclonal antibody cetuximab. Cancer Biol Ther 2011;11:777–92.
68. Zhang H, Savage S, Schultz AR, et al. Clinical resistance to crenolanib in acute myeloid leukemia due to diverse molecular mechanisms. Nat Commun 2019;10:244.
69. Abdelrahman RA, Begna KH, Al-Kali A, Hogan WJ, Litzow MR, Tefferi A. Revised assessment of response and long-term discontinuation rates among 111 patients with myelofibrosis treated with momelotinib or ruxolitinib. Leukemia 2015;29:498–500.
70. Soulieres D, Senzer NN, Vokes EE, Hidalgo M, Agarwala SS, Siu LL. Multicenter phase II study of erlotinib, an oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with recurrent or metastatic squamous cell cancer of the head and neck. J Clin Oncol 2004;22:77–85.
71. Muz B, Azab F, Fiala M, et al. Inhibition of E-selectin (GMI-1271) or E-selectin together with CXCR4 (GMI-1359) re-sensitizes multiple myeloma to therapy. Blood Cancer J 2019;9:68.
72. Zhang W, Ly C, Zhang Q, et al. Dual E-selectin/CXCR4 antagonist GMI-1359 exerts efficient anti-leukemia effects in a FLT3 ITD mutated acute myeloid leukemia patient-derived xenograft murine model. Blood 2016;128:3519.
73. Mosesson Y, Mills GB, Yarden Y. Derailed endocytosis: an emerging feature of cancer. Nat Rev Cancer 2008;8:835–50.
74. Grandal MV, Madshus IH. Epidermal growth factor receptor and cancer: control of oncogenic signalling by endocytosis. J Cell Mol Med 2008;12:1527–34.
75. Sorkin A, von Zastrow M. Endocytosis and signalling: intertwining molecular networks. Nat Rev Mol Cell Biol 2009;10:609–22.
76. Reis CR, Chen PH, Srinivasan S, Aguet F, Mettlen M, Schmid SL. Crosstalk between Akt/GSK3beta signaling and dynamin-1 regulates clathrin-mediated endocytosis. EMBO J 2015;34:2132–46.
77. Reis CR, Chen PH, Bendris N, Schmid SL. TRAIL-death receptor endocytosis and apoptosis are selectively regulated by dynamin-1 activation. Proc Natl Acad Sci USA. 2017;114:504–9.
78. Chen Z, Schmid SL. Evolving models for assembling and shaping clathrin-coated pits. J Cell Biol 2020: 219.
79. Kovtun O, Dickson VK, Kelly BT, Owen DJ, Briggs JAG. Architecture of the AP2/clathrin coat on the membranes of clathrin-coated vesicles. Sci Adv 2020;6:eaba8381.
80. Traub LM, Bonifacino JS. Cargo recognition in clathrin-mediated endocytosis. Cold Spring Harb Perspect Biol 2013;5:a016790.
81. Ting SB, Deneault E, Hope K, et al. Asymmetric segregation and self-renewal of hematopoietic stem and progenitor cells with endocytic Ap2a2. Blood 2012;119:2510–22.
82. Schmid SL. Reciprocal regulation of signaling and endocytosis: Implications for the evolving cancer cell. J Cell Biol 2017;216:2623–32.
83. Parton RG, Tillu V, McMahon KA, Collins BM. Key phases in the formation of caveolae. Curr Opin Cell Biol 2021;71:7–14.
84. Parton RG, McMahon KA, Wu Y. Caveolae: Formation, dynamics, and function. Curr Opin Cell Biol 2020;65:8–16.
85. Henley JR, Krueger EW, Oswald BJ, McNiven MA. Dynamin-mediated internalization of caveolae. J Cell Biol 1998;141:85–99.
86. Nwosu ZC, Ebert MP, Dooley S, Meyer C. Caveolin-1 in the regulation of cell metabolism: a cancer perspective. Mol Cancer 2016;15:71.
87. Singh V, Lamaze C. Membrane tension buffering by caveolae: a role in cancer? Cancer Metastasis Rev 2020;39:505–17.
88. Renard HF, Tyckaert F, Lo Giudice C, et al. Endophilin-A3 and galectin-8 control the clathrin-independent endocytosis of CD166. Nat Commun 2020;11:1457.
89. Thottacherry JJ, Kosmalska AJ, Kumar A, et al. Mechanochemical feedback control of dynamin independent endocytosis modulates membrane tension in adherent cells. Nat Commun 2018;9:4217.
90. Hemalatha A, Mayor S. Recent advances in clathrin-independent endocytosis. F1000Res. 2019;8. F1000 Faculty Rev-138.
91. Mayor S, Parton RG, Donaldson JG. Clathrin-independent pathways of endocytosis. Cold Spring Harb Perspect Biol 2014;6:a016758.
92. Holst MR, Vidal-Quadras M, Larsson E, et al. Clathrin-independent endocytosis suppresses cancer cell blebbing and invasion. Cell Rep 2017;20:1893–905.
93. Antonescu CN. The intricate relationship between metabolism and endocytic membrane traffic. Traffic 2019;20:887–8.
94. Hinze C, Boucrot E. Endocytosis in proliferating, quiescent and terminally differentiated cells. J Cell Sci 2018;131:jcs216804.
95. Tremblay CS, Brown FC, Collett M, et al. Loss-of-function mutations of dynamin 2 promote T-ALL by enhancing IL-7 signalling. Leukemia 2016;30:1993–2001.
96. Trochet D, Bitoun M. A review of dynamin 2 involvement in cancers highlights a promising therapeutic target. J Exp Clin Cancer Res 2021;40:238.
97. Antonny B, Burd C, De Camilli P, et al. Membrane fission by dynamin: what we know and what we need to know. EMBO J 2016;35:2270–84.
98. Ramachandran R, Schmid SL. The dynamin superfamily. Curr Biol 2018;28:R411–6.
99. Nakata T, Iwamoto A, Noda Y, Takemura R, Yoshikura H, Hirokawa N. Predominant and developmentally regulated expression of dynamin in neurons. Neuron 1991;7:461–9.
100. Cook TA, Urrutia R, McNiven MA. Identification of dynamin 2, an isoform ubiquitously expressed in rat tissues. Proc Natl Acad Sci USA 1994;91:644–8.
101. Vaid KS, Guttman JA, Babyak N, et al. The role of dynamin 3 in the testis. J Cell Physiol 2007;210:644–54.
102. Rosendale M, Van TNN, Grillo-Bosch D, et al. Functional recruitment of dynamin requires multimeric interactions for efficient endocytosis. Nat Commun 2019;10:4462.
103. Ferguson SM, Raimondi A, Paradise S, et al. Coordinated actions of actin and BAR proteins upstream of dynamin at endocytic clathrin-coated pits. Dev cell 2009;17:811–22.
104. Luwor RB, Chin X, McGeachie AB, Robinson PJ, Zhu HJ. Dynamin II function is required for EGF-mediated Stat3 activation but not Erk1/2 phosphorylation. Growth Factors 2012;30:220–9.
105. Luwor R, Morokoff AP, Amiridis S, et al. Targeting glioma stem cells by functional inhibition of dynamin 2: A novel treatment strategy for glioblastoma. Cancer Invest 2019;37:144–55.
106. Hill TA, Gordon CP, McGeachie AB, et al. Inhibition of dynamin mediated endocytosis by the dynoles—Synthesis and functional activity of a family of indoles. J Med Chem 2009;52:3762–73.
107. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. Nat Rev Cancer 2013;13:714–26.

108. Chew HY, De Lima PO, Gonzalez Cruz JL, et al. Endocytosis inhibition in humans to improve responses to ADCC-mediating antibodies. Cell 2020;180. 895–914 e827.
109. Swatler J, Turos-Korgul L, Kozlowska E, Piwocka K. Immunosuppressive cell subsets and factors in myeloid leukemias. Cancers 2021;13:1203.
110. Wang W, Thomas R, Sizova O, Su DM. Thymic function associated with cancer development, relapse, and antitumor immunity— A mini-review. Front Immunol 2020;11:773.
111. Shao L, Wang Y, Chang J, Luo Y, Meng A, Zhou D. Hematopoietic stem cell senescence and cancer therapy-induced long-term bone marrow injury. Transl Cancer Res 2013;2:397–411.
112. Winkler IG, Pettit AR, Raggatt LJ, et al. Hematopoietic stem cell mobilizing agents G-CSF, cyclophosphamide or AMD3100 have distinct mechanisms of action on bone marrow HSC niches and bone formation. Leukemia 2012;26:1594–601.
113. Zhao M, Tao F, Venkatraman A, et al. N-Cadherin-expressing bone and marrow stromal progenitor cells maintain reserve hematopoietic stem cells. Cell Rep 2019;26:652–669.e656.
114. Robertson MJ, Deane FM, Robinson PJ, McCluskey A. Synthesis of Dynole 34-2, Dynole 2-24 and Dyngo 4a for investigating dynamin GTPase. Nat Protoc 2014;9:851–70.
115. McCluskey A, Daniel JA, Hadzic G, et al. Building a better dynasore: the Dyngo compounds potently inhibit dynamin and endocytosis. Traffic 2013;14:1272–89.
116. Rennick JJ, Johnston APR, Parton RG. Key principles and methods for studying the endocytosis of biological and nanoparticle therapeutics. Nat Nanotechnol 2021;16:266–76.
117. Robertson MJ, Deane FM, Stahlschmidt W, et al. Synthesis of the Pitstop family of clathrin inhibitors. Nat Protoc 2014;9:1592–606.
118. von Kleist L, Stahlschmidt W, Bulut H, et al. Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. Cell 2011;146:471–84.
119. Smith CM, Haucke V, McCluskey A, Robinson PJ, Chircop M. Inhibition of clathrin by pitstop 2 activates the spindle assembly checkpoint and induces cell death in dividing HeLa cancer cells. Mol Cancer 2013;12:4.