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REVIEW

A rare subgroup of leukemia stem cells harbors relapse-inducing potential in acute lymphoblastic leukemia

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After initially successful chemotherapy, relapse frequently jeopardizes the outcome of patients with acute leukemia. Because of their adverse characteristics of self-renewal and dormancy, leukemia stem cells have been hypothesized to play a critical role in resistance to antiproliferative chemotherapy and the development of relapse. The high abundance of stem-like cells in acute lymphoblastic leukemia (ALL), however, suggests that not all leukemia-initiating cells carry these adverse characteristics, complicating the biological characterization of relapse-inducing cells in this malignancy. Here, we review sources of therapy resistance and relapse in acute leukemias, which include tumor cell plasticity and reversible characteristics. We discuss the development of patient-derived mouse models that are genetically engineered to mimic long-term dormancy and minimal residual disease in patients. These models allow the tracking and functional characterization of patient-derived ALL blasts that combine the properties of long-term dormancy, treatment resistance, and stemness. Finally, we discuss possible therapeutic avenues to target the functional plasticity of leukemia-initiating cells in ALL.

Cancer stem cells as a source for therapy resistance and relapse

Acute leukemias of lymphoid (acute lymphoblastic leukemia; ALL) or myeloid (acute myeloid leukemia; AML) lineage arise from hematopoietic stem or progenitor cells in the bone marrow. Patients with acute leukemia often achieve complete remission with induction chemotherapy, yet many will experience relapse, which poses a major obstacle to cure [1,2]. Leukemia relapse has been suggested to arise from a small subpopulation of cells that survive chemotherapy, persist as minimal residual disease (MRD) in complete remission, and ultimately reinitiate malignant growth. Although MRD detection in acute leukemia has a strong prognostic value [1,2], MRD is not an absolute marker of relapse because not all residual cells may have the functional capability to proliferate into relapse [3]. Accordingly, relapse-inducing cells are a "subpopulation" of MRD cells with important functional properties, including the ability to repopulate the tumor. One possible explanation for this clinical behaviour can be provided by the cancer stem cell model, which is based on the idea that cancers, like normal tissues, are maintained by a rare, biologically distinct subpopulation of cells that have the capacity for long-term tumor propagation and self-renewal and give rise to progeny that lack these characteristics. Such cancer stem cells are thought to be highly resistant to therapeutic regimens, survive chemotherapy, and ultimately lead to relapse [4,5].

The conceptual framework of a leukemia stem cell model is based on a putative analogy between normal and malignant hematopoiesis. Normal hematopoiesis is maintained by hematopoietic stem cells (HSCs), a rare subpopulation of bone marrow cells with multilineage differentiation and self-renewal capacity. HSCs give rise to highly proliferating

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hematopoietic progenitor cells, which generate all mature blood cells [6]. Different cell types within the bone marrow, including mesenchymal stem cells (MSCs, cells that form adipocytes, osteoblasts, and chondrocytes), endothelial cells, and nerve fibers, form the stem cell niche, a specialized microenvironment that allows HSC maintenance [7,8]. At the top of the hematopoietic hierarchy are extremely slow cycling, long-term dormant HSCs that carry the highest reconstitution potential [9–14]. Their contribution to steady-state blood formation may be limited because it is mediated by more actively cycling HSCs with restricted long-term propagation potential. The dormant HSC subsets can readily be activated upon stress, for example, by interferons, lipopolysaccharides, or chemotherapy among others, and significantly contribute to repair processes [8,15]. Importantly, the transition from dormant to active HSCs is a reversible process and HSCs can return into a deep quiescent state following stress [9,14], at least for a limited number of cell divisions [12]. Dormancy of adult stem cells has been suggested as a means to survive chemotherapeutic and other DNA-damaging challenges [9,16], limit the accumulation of mutations, and thus serve as a reserve stem cell pool to maintain normal hematopoiesis following stress [8].

Xenotransplantation of AML cells in immunodeficient mice has significantly contributed to the formal verification of a hierarchical organization of cancer because these studies demonstrated that rare stem-like cells with self-renewal capacity sustain AML. Such stem-like cells are termed leukemia-initiating cells (LICs) and they are enriched within the $CD34^+CD38^-$ immunophenotype [17–19]. Although more sophisticated mouse models demonstrated that other fractions ($CD34^+CD38^+$ and also $CD34^-$) can also contain LICs in AML patient samples, it is now widely accepted that AML and numerous other malignancies are hierarchically organized [4]. In analogy to HSCs, AML-LICs are functionally heterogeneous in terms of self-renewal potential [20] and heterogeneous in terms of growth kinetics; at least a subset of LICs is maintained in a quiescent state [21]. Of therapeutic relevance is the fact that AML-LICs display a similar transcriptional profile as HSCs, which is a powerful indicator of resistance to standard therapy [22]. Furthermore, AML-LIC frequency at diagnosis is indicative of a poor outcome [23] and frequency of LICs also increases following therapy, providing an explanation for the notorious drug resistance of relapsed AML [24]. In agreement, experiments in preclinical models strongly support the notion that AML-LICs are the source of relapse in AML [25,26]. In particular, the quiescence of AML-LICs has been associated with resistance to anti-proliferative chemotherapy [27–29]. Therefore, efforts to break the dormancy of these cells in order to sensitize them to anti-proliferative chemotherapy were suggested as a valuable means to eradicate LICs and achieve long-term remissions [28,29].

Although various similarities in the functional behavior of AML-LICs and HSCs have been established, these results are not readily transferable to all hematological malignancies. In ALL, no markers could be identified to prospectively isolate LICs and a high proportion of cells among various immunophenotypes display leukemia-initiating potential in xenotransplantation experiments [30–33]. Therefore, the hierarchical stem cell model of AML does not apply to ALL, in which tumor progression/relapse may be a stochastic process. Although highly similar regarding the clinical course of treatment response, MRD, and relapse, the two sister malignancies ALL and AML show major differences concerning LIC biology.

Sources for therapy resistance and relapse in ALL

Because stemness is an insufficient criterion to define the subpopulation of relapse-inducing cells in ALL, the basic biological conditions that determine relapse remain to be defined.

Genomic and functional analysis of ALL patient samples revealed a branching evolutionary pattern and multiple subclones coexist at the time of diagnosis and show functional diversity in terms of leukemia-initiating potential [34–36]. Such genetic diversity can provide a basis for the clonal selection of chemoresistant clones by therapeutic regimens. In agreement, genomic studies of paired diagnosis and relapse samples demonstrated that, at relapse, ALL blasts are derived from minor subclones present at diagnosis or evolve from ancestral diagnostic clones, but rarely from the main diagnostic clone [37–40]. Multiple genetic variants that confer resistance to standard therapy are enriched in relapsed ALL samples. These include *NT5C2* [41,42] and *PRPS1* [43], which mediate resistance to genotoxic agents; Alterations in *CREBBP* [44] contribute to glucocorticoid resistance and CDKN2A/B [1,36,39] or RAS pathway [40,45] alterations that are associated with resistance to multiple drugs. However, sequence analysis alone may not reveal all features of relapse-inducing cells in ALL. For example, although RAS pathway mutations are associated with poor prognosis [40,45], their presence at diagnosis does not strictly predict relapse; some mutations in this pathway can be lost at relapse or are present in diagnostic samples that never relapse [37,45,46]. This, together with the facts that pediatric and haematological cancers have the lowest somatic mutation rate and most cases of ALL are genetically stable [47,48] and that not all relapses are derived from minority subclones but can sometimes derive from the major diagnostic clone [37–40,49,50], strongly suggests that nongenetic factors significantly contribute to relapse. Although hardwired genetic mechanisms may account for refractory disease and early relapse, different mechanisms may account for late relapses that can occur even decades after the initial diagnosis [49,51]. Therefore, both clonal architecture and stemness remain insufficient criteria to define relapse-inducing cells in ALL.

Cellular plasticity in epigenetic programs (which is influenced by the genetic background as well as the tumor microenvironment) is considered the underlying cause of functional heterogeneity in cancer [5,52,53]. In response to appropriate environmental stimuli, tumor cells may be temporarily endowed with certain traits, including tumor-/leukemia-initiating capacity and therapy resistance, as has been suggested in diverse cancers [54–59]. The reversibility of drug resistance, for example, has been demonstrated in various cancer cell lines in response to targeted EGFR or BRAF inhibitors or cisplatin [58], as well as in T-cell ALL (T-ALL) xenografts following y-secretase inhibition [54]. Interestingly, the number of these drug-tolerant persisting cells can be decreased and establishment of resistance prevented in vitro by simultaneous treatment with histone deacetylase (HDAC) inhibitors [58]. Similarly, in patient-derived xenografts of pediatric T-ALL, combined inhibition of NOTCH and BRD4, a chromatin regulator upregulated in persistent T-ALL cells, significantly prolonged survival compared with single-agent treatment [54]. The interaction of tumor cells with the microenvironment has been suggested as a major regulator of such reversible traits [5,52,53]. Evidence that environmentally mediated drug resistance may protect ALL blasts from therapy comes from in vitro experiments, wherein the interaction of ALL blasts with stromal cells is essential for their survival in vitro and can protect blasts against asparaginase and prednisolone [60,61]. Accordingly, safe-haven niches that protect leukemic blasts from chemotherapy have been identified in the bone marrow in vivo, wherein mesenchymal stem cells [62], osteoblasts [63], or other stromal cell types in the microenvironment [64] protect leukemic blasts from therapy. In response to chemotherapy, ALL blasts can also actively remodel their environment via the recruitment of mesenchymal cells and the establishment of such a protective niche can be found in patient samples of partial responders or nonresponders [62].

Interestingly, regardless of the model tested, quiescence of cancer cells seems to be a common characteristic of therapy-resistant subpopulations: Quiescent glioblastoma stem cells survive chemotherapy and reinitiate malignant growth following remission [65] and, in diverse cancer cell lines, quiescent, drug-tolerant cells persist during the course of therapy [58]. Additionally, quiescent cells survive oncogene ablation in a mouse model of pancreatic cancer [59], and in colorectal cancer xenografts, a previously dormant cell population becomes dominant following chemotherapy [55]. Similarly, slow-cycling melanoma cells display a general drug-resistant phenotype [56,57] and a subpopulation of slow-cycling transforming growth factor-beta-responsive squamous cell carcinoma stem cells survive chemotherapy and induce relapse in xenotransplantation experiments [66]. These studies suggest that, regardless of the presence or absence of a strict hierarchical organization, cancer cells can temporarily adapt a quiescent and drug-resistant state, which, similar to adult stem cells, may be regulated by microenvironmental cues, including therapeutic regimens [5,29]. Evidence that ALL blasts survive chemotherapy by adapting a quiescent state in response to microenvironmental cues has been provided in preclinical models [63]. The hypothesis that quiescent therapy-persisting ALL cells may be the source for relapse is further supported by the fact that therapy-resistant cells isolated from patients at MRD are predominantly quiescent [67]. Given the accumulating evidence that quiescence may be an attribute of relapse-inducing cells, we set out to develop a model that allowed us to functionally characterize quiescent cells in primary ALL patient samples [68]. We hypothesized that such a model would allow us to study biological characteristics of relapse-inducing cells in ALL and help to resolve questions of translational importance: Do these traits already exist at diagnosis or are they acquired during therapy? Are these traits reversible or permanent?

Patient-derived xenograft model to track quiescent ALL cells over the course of therapy

To be able to track and isolate small numbers of viable patient-derived xenograft (PDX) ALL cells from the bone marrow of mice, we utilized our previously established lentiviral transduction protocols and ectopically expressed luciferase [69], a truncated nerve growth factor receptor and a red fluorophore [70] in PDX ALL blasts. This triple labeling of ALL PDX cells allows us to track their in vivo growth in mice and enables the isolation of minute numbers of viable human ALL cells from the murine bone marrow [68]. Following one passage in mice for enrichment of transgene-expressing cells, lentivirally transduced PDX ALL cells were stained with carboxyfluorescein succinimidyl ester (CFSE). This fluorescent dye has been useful to track cell divisions of HSC in mice over several months [14]. CSFE labels cytoplasmic proteins and is thus independent of the cell cycle status, is equally distributed to daughter cells with each cell division, and, importantly, unlike other proliferation dyes (e.g. BrdU), does not require permeabilization of cells for staining [14]. Therefore, the combined use of lentiviral transduction and CSFE labelling allows for tracking and isolation of nondividing viable cells from the in vivo environment.

Upon transplantation into immunodeficient recipient mice, we identified ALL cells that retained the CSFE dye up to 3 weeks, shortly before mice had to be sacrificed due to high leukemia burden. We could identify a rare subpopulation of these label-retaining cells (LRCs)

that had undergone no more than three cell divisions among all nine transgenic ALL-PDX samples analyzed [68]. Immunohistochemical analysis revealed that LRCs, unlike non-LRCs, are preferentially found close to the endosteum, a niche that has previously been suggested to regulate quiescence and resistance to therapy of LIC in vivo [27,28,63,71].

Functional characterization of LRC in ALL-PDX

Given the putative link among quiescence, stemness, and chemoresistance, we first characterized the leukemia-initiating potential of LRCs and evaluated how they respond to chemotherapy [68]. LIC frequency was very similar between the LRC and non-LRC compartments, which is in agreement with the stochastic stem cell model proposed for ALL, wherein most cells are capable of propagating the tumor [33]. However, these results demonstrate phenotypic heterogeneity within the LIC compartment, wherein a rare subpopulation of LICs is quiescent. To test whether treatment resistance and dormancy are linked, we treated leukemia-bearing mice with chemotherapy, which strongly selected for LRC, whereas most non-LRCs were eradicated [68]. Importantly, surviving LRCs retained the ability to propagate the leukemia upon retransplantation into secondary mice and formed tumors with similar growth kinetics as untreated LRC. Therefore, LRCs share the combined features of dormancy, drug resistance, and stemness, indicating that these cells may serve as preclinical surrogates for relapse-inducing cells in ALL [68] (Figure 1).

To further characterize molecular features of LRCs and to strengthen the link to MRD, LRCs and MRD cells established from our PDX model (PDX-MRD cells) were subjected to single-cell RNA sequencing. Gene expression profiles of single or bulk leukemia cells of untreated LRCs and PDX-MRD cells demonstrated that overall RNA content was decreased in LRCs, which is indicative of a low metabolic activity, a defining characteristic of dormant cells. Furthermore, in both LRCs and PDX-MRD cells, cell cycle and DNA replication were the most downregulated pathways. Therefore, transcriptomic analysis reinforced the presence of a dormancy phenotype in LRCs and PDX-MRD cells (Figure 1). Interestingly, the most upregulated gene network in LRCs and PDX-MRD cells was cell adhesion, indicating that the interaction with the microenvironment might mediate the observed phenotypes [68] (Figure 1). The established LRC signature was also identified in primary MRD cells isolated from adult or pediatric patients and differentially regulated genes of published transcriptomes of CD34-positive chronic myeloid leukemia cells, acute leukemia stem cells, hematopoietic stem cells, and pediatric ALL cells with high risk of relapse were significantly enriched in LRCs [68]. Importantly, the phenotypes of both dormancy and therapy resistance of LRCs were reversible: when LRCs and non-LRCs were transplanted into secondary mice, LRCs gave rise to tumors with similar growth kinetics as non-LRCs and vice versa; non-LRCs gave rise to tumors that contained a quiescent subpopulation at a similar frequency as observed following transplantation of unsorted leukemic cells (Figure 1). When LRCs or non-LRCs were treated with chemotherapeutic drugs in vitro, they demonstrated similar cell death rates, indicating that the release from the bone marrow microenvironment sensitizes LRCs to therapy. Accordingly, coculture of LRCs, non-LRCs, or unsorted bulk cells with feeder cells reduced drug sensitivity, strongly suggesting a role for the bone marrow microenvironment in determining drug resistance.

In summary, our studies demonstrated that there is significant functional heterogeneity within the abundant LIC population in ALL, wherein only a rare subpopulation of ALL-LICs displays the adverse phenotypes of quiescence and drug resistance. These LRCs exist before the onset of therapy and their adverse phenotypes are reversible. Additionally, the high similarity of LRCs and MRD cells isolated from pediatric and adult patients strongly suggests that LRCs can be used as surrogates for relapse-inducing cells in patients [68,71]. The presence of a quiescent and therapy-resistant subpopulation of LICs in ALL has important implications for therapy. In particular, the fact the adverse phenotypes of LICs are reversible once released from the protective bone marrow environment offers therapeutic opportunities.

Therapeutic implications

Environment-mediated drug resistance provides an explanation for the clinical observation that maintenance therapy with low-dose oral chemotherapy for about 2 years is required, even in low-risk patient groups, to prevent relapse in ALL [72]. However, maintenance therapy is not always sufficient and relapse might occur under treatment. Because even patients who maintained complete remission over several years can relapse, targeting long-term dormant residual cells represents an important unmet clinical need to increase the chance for long-term remission and cure (Figure 2).

Multiple routes can be envisioned to target LRCs (Figure 2). It has long been known that leukemic cells depend on stromal support for growth and survival, which is mediated by direct adhesion and soluble factors that can affect treatment response [60–64,73–77] and our data also strongly suggest that the release of ALL cells from the bone marrow environment sensitizes leukemic blasts to conventional therapies [68]. Inspired by the discovery of agents that can activate dormant HSCs [29], blocking the CXCL12/CXCR4 axis,

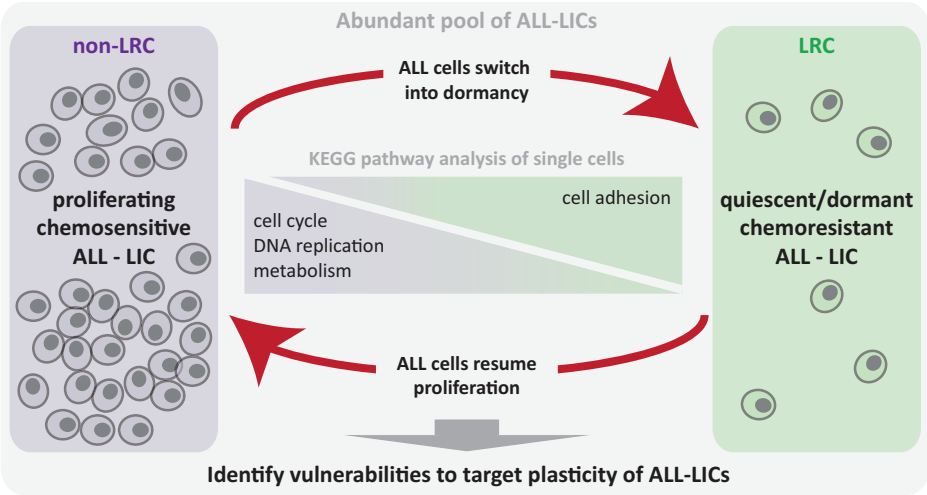

Figure 1. Plasticity of ALL-LICs. Characterization of LRCs in ALL PDXs revealed that LICs harbor functional plasticity and exist in at least two states within the bone marrow microenvironment: a proliferative, chemosensitive state and a dormant, chemoresistant state. The adverse phenotypes of dormancy and therapy resistance are reversible upon release from the bone marrow microenvironment, whereas stemness remains a constant feature. Single-cell RNA sequencing analysis demonstrated that the rare subpopulation of LRCs is quiescent and the most upregulated pathway is cell adhesion, indicating that the interaction with the microenvironment might influence the dormant phenotype.

inhibition of osteopontin, cell adhesion molecules, and treatment with granulocyte colony-stimulating factor (G-CSF) have been demonstrated to affect the homing of leukemic cells to the bone marrow and influence therapeutic response in experimental models [28,63,76,78–80] (Figure 2). Plerixafor and G-CSF, which are approved for HSC mobilization, or natalizumab, a monoclonal antibody that blocks integrin α_4, are promising candidates to evaluate for LRC-specific combination therapy [81–85]. However, because leukemic growth and chemotherapy induces massive remodeling of the stromal bone marrow environment [62,86–89], signals that regulate LIC homing likely differ from those regulating HSCs. The remodeled niches can furthermore be specific for distinct leukemia subtypes [89,90] and may change over the course of disease progression or following therapy. Accordingly, leukemia-specific signals will need to be identified to efficiently induce cell cycle entry of all leukemic blasts. In this context, CRISPR-Cas9-edited NGS mice may serve useful in elucidating the role of individual niche-derived factors [91]. Another point to consider is that real-time in vivo monitoring of T-ALL cells within the bone marrow demonstrated a highly motile phenotype of ALL blasts, suggesting that leukemic cells may not permanently engraft in a specific niche [90]. Therefore, interactions between leukemic blasts and their environment are versatile and multiple leukemia-specific factors may need to be targeted therapeutically to achieve an efficient release of all blasts from the bone marrow. Alternatively, one may envision targeting leukemia-cell-intrinsic adhesion pathways, with one putative target being focal adhesion kinase (FAK) (Figure 2). Small-molecule FAK inhibitors such as defactinib or GSK2256098 are currently under clinical investigation in solid tumors [92,93] and can be tested for their ability to sensitize LRCs to chemotherapy. Inhibition of FAK signaling has recently been demonstrated to disrupt cell adhesion in highly aggressive Philadelphia-chromosome-positive, IKZF1-mutated ALL mouse models and FAK inhibition combined with a BCR-ABL1 inhibitor effectively abrogated leukemic cell growth in vivo [94].

Instead of addressing the interaction between leukemic blasts and the environment, direct targeting of quiescent cells is another possibility to eradicate long-term persisting LICs (Figure 2). Metabolic vulnerabilities of quiescent drug-tolerant persisting cells have been identified and targeting, for example, their reliance on either oxidative phosphorylation (OXPHOS) [56,57,59] or an increased antioxidant capacity [66] may eradicate these cells or render them sensitive to conventional therapy. The bone marrow microenvironment also mediates metabolic adaptions of ALL blasts that support their survival [74] and microenvironment-mediated redox adaptions in ALL blasts reportedly mediate therapy resistance in cell culture models [95]. A multitude of drugs that target either OXPHOS or inhibit antioxidant production are available and have been approved for cancer treatment or non-oncological indications [96]. Possible drugs to test for their capacity to deplete LRCs can include the OXPHOS inhibitors metformin [97,98] and arsenic trioxide [96]. Furthermore, the anti-rheumatic drugs sulfasalazine and auranofin, together with the GSH-depleting drug buthionine sulfoximine, may serve to sensitize therapy-resistant cells to genotoxic agents [99]. The antibiotic tigecycline and inhibition of Bcl-2 antiapoptotic

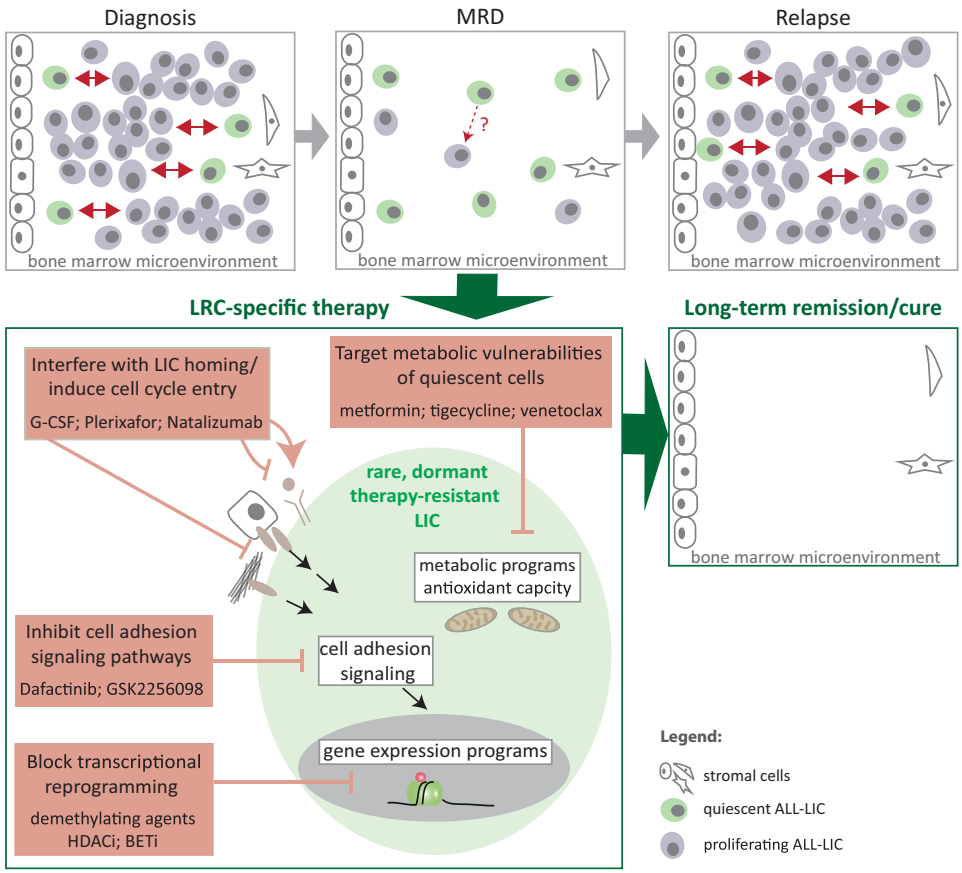

Figure 2. Rationale and possible approaches for targeting dormant, therapy-resistant ALL-LICs. The characterization of ALL-PDX models during the course of therapy indicated that quiescent LRCs preferably survive chemotherapy and persist at MRD. Dormant cells are already present at the time of diagnosis. Occasional transitions from the quiescent into a chemosensitive, proliferative state (dashed arrow) may explain that long-term maintenance therapy is required to prevent relapse in ALL patients. However, reversibility of the quiescent phenotype (red arrows) allows these LICs to resume proliferation at later time points and proliferate into relapse. Accordingly, therapeutic targeting of this dormant subpopulation of LICs may be necessary and sufficient to prevent the development of relapse in ALL. Possible routes to target therapy-persisting LICs are depicted in the lower left box. Inhibition of LIC stroma, a LIC–extracellular matrix (ECM) interaction, downstream adhesion signaling, or interfering with soluble factors that home LICs into the bone marrow microenvironment may release cells from the protective niche and render them sensitive to standard chemotherapy. Metabolic vulnerabilities of quiescent LICs provide an alternative target, which may allow eradication of LICs without the need to induce their proliferation. To prevent the de novo generation of quiescent, drug-resistant LICs during the course of therapy, epigenetic programs could be targeted. Some putative drugs to achieve each goal are depicted.

proteins have also been shown to excerpt their antitumor activity by impairing mitochondrial function and increasing oxidative stress [100–102]. These findings provide a rationale for testing tigecycline or the BH3 mimetics navitoclax, venetoclax, and S63845 [103–105] for their use as LRC-specific therapies.

Finally, interfering with the epigenetic programs that allow persisting cells to reversibly enter/escape a quiescent and therapy resistant state may be of therapeutic merit (Figure 2), as demonstrated in diverse cancer cell lines and a T-ALL xenograft model [54,58]. Several epigenetic therapeutics are either approved or under clinical evaluation, including BET bromodomain inhibitors [106–108].

One important consideration regarding the plasticity of the therapy-resistant state is the onset and timing of therapeutic approaches. Although our study has clearly demonstrated that a quiescent and therapy-resistant subpopulation of ALL cells exists before the onset of therapy, our experimental setup did not allow us to monitor the de novo emergence of quiescent cells upon treatment initiation [68]. However, because LRCs can be derived from non-LRCs upon retransplantation into secondary recipients, it is likely that the quiescent and therapy-resistant population can be continuously replenished. If innovative therapeutic strategies succeed in retrieving resistant leukemia cells from protective niches, then sensitive leukemia cells might take over empty slots and acquire a resistant phenotype. Therefore, therapeutic regimens will likely be most effective when administered throughout the course of therapy, as was also suggested by in vitro experiments in

non-small-cell lung cancer cell lines, where only continuous administration of HDAC inhibitors prevented the onset of resistance to EGFR inhibitors [58].

Concluding remarks

After initially successful chemotherapy, relapse frequently jeopardizes the outcome of cancer patients. To improve the prognosis of ALL patients, treatment strategies that eliminate tumor cells at MRD and prevent relapse are urgently required. Although ALL cells lack a clear hierarchical organization, several adverse phenotypes, including quiescence and chemoresistance, are maintained in a rare subset of ALL-LICs, which are otherwise generally attributed to stem cells. The fact that dormancy and resistance are reversible upon release from the microenvironment indicates that transcriptional reprogramming of ALL-LICs is an early event in the generation of therapy resistance. This process may precede the onset of hardwired irreversible resistance and relapse and a similar multistep mechanism of the emergence of resistant disease has recently been suggested under treatment with the immunotoxin moxetumomab pseudotox [109]. The in vivo model described in our recent study [68], in combination with CRISPR-Cas9-edited NGS mice [91], can facilitate study of the regulatory factors within the bone marrow microenvironment that endow LICs with adverse characteristics. Our models can thus serve as preclinical tools with which to test therapeutic strategies that aim to interfere with the interaction between leukemic blasts and their niche to prevent relapse and ultimately improve the prognosis and cure rate of patients with ALL.

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Conflict of interest disclosure

The authors declare no competing financial interests.

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