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Acute myelogenous leukemia stem cells: From Bench to Bedside

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Abstract

Despite reaching remission with traditional chemotherapy, most adult patients with acute myeloid leukemia (AML) will relapse and die of their disease. Numerous studies have identified a rare subset of leukemia cells that evade traditional chemotherapy and are capable of self-renewal and initiating leukemia. These cells are thought to be responsible for relapse and are termed leukemia stem cells (LSCs). This article will review the current LSC translational research and focus on new approaches to detect LSC burden and its prognostic implications, as well as the identification and development of therapeutic agents active against LSCs.

Keywords

AML; Leukemia; LSC; Leukemia stem cell; Acute myelogenous leukemia; Therapies; Chemoresistance

1. Introduction

Despite much advancement in the treatment of acute myelogenous leukemia (AML) over the past three decades, the prognosis remains poor. New cytogenetic markers such as the Flt-3/ITD and NPM1 mutations now aid prognosis, but these discoveries have not translated to significant advancements in survival for the majority of patients with AML. Current chemotherapy regimens employing a backbone of cytarabine combined with an anthracycline have remained largely unchanged since 1973. This treatment regimen is capable of reducing the tumor burden (i.e. the total blast population) and produces a

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complete remission (CR) in almost 70% of patients. Despite this and the addition of allogeneic stem cell transplant in certain high-risk groups or in those with a matched sibling, most patients will ultimately relapse and approximately 60% of patients will succumb to their disease [1]. Pediatric patients below 15 years of age, while faring better, only have a survival rate approaching 60% [2].

Due to the high incidence of relapse in AML, it is clear that that there is a rare subset of malignant cells that are not effectively eliminated by current treatment regimens [3]. There is a growing opinion that these cells have stem cell-like properties and have been termed leukemia stem cells (LSCs). Independent of the specific cell that gives rise to LSCs, LSCs may share properties with normal hematopoietic stem or progenitor cells (HSCs/HPCs) that make them difficult to target with conventional cancer treatments [4]. Fundamental to their function is their ability to self-renew and give rise to more differentiated progeny. This allows them to maintain a small population of cells capable of recapitulating disease. Like HSCs, LSCs tend to be quiescent, remaining in G0. Furthermore, increasing evidence indicates that LSCs displace normal HSCs from their niche, appropriating its resources to maintain themselves [3,5,6]. Targeting LSCs has the promise to eradicate AML and will involve novel therapies that will exploit the differences between these cells and HSCs. This review will discuss strategies used to identify LSCs and developing therapies that selectively target them.

2. Identifying LSCs and their clinical implications

LSCs were initially characterized in human AML by Bonnet and Dick when they isolated subpopulations of cells from AML patient samples based on their immunophenotype, and xenotransplanted them into NOD/SCID mice. They discovered that the CD34+CD38− expressing subpopulation was exclusively capable of serially transplanting these immunodeficient mice [7]. This finding supported previous evidence of a functional leukemia initiating cell (LIC) defined by its ability to engraft immunocompromised mice [8]. However, this phenotype is not absolute. Taussig et al. discovered that certain antibodies against CD38, including those used previously to determine whether CD34+CD38+ AML cells could engraft [7], were themselves sufficient to block engraftment [9]. Other experiments demonstrated that certain populations of CD34+CD38+AML cells were capable of serially engrafting NSG mice [10]. Additional work in AML samples with the nucleophosmin gene mutation (NPM1) showed that the major subpopulation of these cells capable of engrafting immunocompromised mice were CD34− [11]. All of these observations have led to a re-evaluation of the phenotypic definition of a LSC.

While the above studies demonstrate a lack of a definitive phenotype for the LSC, there is an increasing body of evidence that supports the functional and clinical importance of LSCs. In one study, the ability of a primary patient sample to engraft NOD/SCID mice correlated with both worse clinical outcomes in intermediate risk patients and a poor risk karyotype [12]. In another study, it was found that AML patient samples with greater than 3.5% of their AML cells demonstrating CD34+CD38− correlated with a median relapse free survival of 5.6 months versus 16 months in those with a lower percentage of CD34+CD38− cells ($p = 0.02$) [13]. Gene expression profiles of LSCs have also been created and linked to clinical

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outcome. Gentles and colleagues [14], in a retrospective study obtained the gene expression profile from the CD34+CD38− subset of 15 AML patient samples. The more highly expressed genes were then summarized as a LSC gene expression signature, which included genes previously implicated in AML such as HOPX and GUCY1A3 and some with no previous association with AML such as GIMAP2, GIMAP6, and GIMAP7. The LSC signature was then evaluated in AML gene expression profiles generated from four publicly available sources (n = 1047). High LSC gene expression resulted in worse clinical outcomes independent of karyotype and cytogenetics. Interestingly, the LSC signature obtained in this study correlated with the HSC gene signature obtained from normal bone marrow in the control group. These results are supported by a recent study from John Dick's group [15] where 18 AML patient samples were sorted based on their CD34 and CD38 immunophenotype, obtaining four distinct populations (CD34+CD38−, CD34+ CD38+, CD34−,CD38+, CD34−CD38−). Each of these subpopulations was then tested for engraftment potential into a NSG xenograft model to determine the functional LSC population for each sample. A LSC gene signature was then generated based on the functional LSC subpopulations. In addition, a gene signature based on normal HSCs was also generated. The results demonstrated that patient samples exhibiting a LSC or HSC gene expression signature had worse clinical outcomes. This study indicates the importance of the pre-clinical xenotransplant model in studying human LSCs while emphasizing its clinical value. A summary of these studies is available in Table 1.

Unfortunately, at this point in time, there is only limited consensus regarding a phenotype that accurately detects LSCs with flow cytometry across all AML patients. Likewise, there are neither gene sets nor quantitative PCR markers yet available that can be used to determine LSC MRD in the clinic. Thus, increasing efforts have been placed on evaluating putative LSC populations in the context of remission and MRD. To date, several markers have been reported to aid in the isolation and identification of LSCs such as CD47, CD96, CD44, CD32, CD25, CD133, CD90, CD117, CD123, TIM3, CLL-1, and ALDH1 [16–21]. The identification of these markers has motivated investigations into how the presence of such AML subpopulations impacts clinical outcome. One study by Terwijn et al. [22] used CD34+CD38−CLL-1+ as a marker to detect LSCs by flow cytometry. Using this as a marker for LSCs, the authors found that high levels of LSCs after the first cycle of chemotherapy predicted poor survival in this cohort and that those with LSC negative results had the best prognosis. Another study by Gerber et al. used flow cytometry to assess aldehyde dehydrogenase (ALDH) activity in CD34+ cells using Aldefluor [23]. ALDH activity and CD38 were determined in the CD34+ compartment of 27 AML samples and compared to 10 normal bone marrow samples. In normal bone marrow samples, there were two distinct populations: CD34+CD38−ALDH^{low} and CD34+CD38−ALDH^{high}. The CD34+ CD38−ALDH^{high} group was capable of engrafting NSG mice and creating normal hematopoiesis. In AML, there was a subpopulation of CD34+CD38− cells with intermediate ALDH activity (CD34+CD38− ALDH^{int}) that was 89% leukemic by FISH and which generated AML when transplanted into NSG mice. Importantly, 6 out of 7 patients with the CD34+CD38−ALDH^{int} subpopulation relapsed while none of the patients without it relapsed. While this and other assays need to be further validated before entering clinical use, the above studies demonstrate the potential utility in measuring LSC burden in the

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clinical setting in order to stratify patients into different prognostic groups and to tailor treatment accordingly. These studies are summarized in Table 1.

3. Targeting LSCs

Due to the relative resistance of LSCs to traditional cancer therapies such as chemotherapy, radiation and HSCT, new approaches are currently in development that specifically target the LSC population. LSCs have many attributes that distinguish them from the general blast population and make them more similar to stem cells. In order to eradicate LSCs, it is crucial to overcome the properties that can make them resistant to therapy such as their quiescence, ability to self-renew, and modify the HSC niche to promote LSC maintenance. At the same time, it is also crucial to select properties that discriminate LSCs from HSCs in order to minimize toxicity, an important consideration for the older patient population that AML affects most greatly. This review will highlight the current preclinical research relevant to targeting LSCs and emphasize the translational projects that are in current development. Table 2 gives a brief summary of various relevant ongoing clinical trials.

4. Disrupting the LSC microenvironment

Cells rely on interactions with other cells in order to function properly and LSCs are no exception. There is evidence from murine xenotransplant models that leukemic cells infiltrate the bone marrow niche, displace HSCs, and use the niche to create an environment hospitable for themselves [24]. Within this niche, LSCs are able to send and receive signals that influence their ability to self-renew, maintain their quiescence, activate pro-growth signals, and inactivate apoptosis pathways. These signals include angiotensin-1, Flt3-ligand, thrombopoietin, wnt/beta-catenin, CXCL12/SDF-1alpha, osteopontin and various cytokines such as bFGF, IGF, IL-6 and VEGF [3,25]. In addition, the bone marrow niche is characterized with both hypoxic and well-vascularized zones that likely influence LSC cycling [25]. Thus, blocking the interaction of LSCs with the bone marrow niche represents a promising strategy to disrupt LSC homeostasis and make them more susceptible to cell death.

One such strategy is to interrupt the homing of LSCs to the bone marrow niche. Normal HSCs rely on the interaction between CXC chemokine receptor-4 (CXCR4) and stromal cell derived factor-1 (SDF-1alpha/CXCL12) in order to migrate into and remain within the bone marrow niche [25]. Treatment with antagonists of this interaction such as anti-CXCR4 and/or G-CSF leads to expansion of HSCs to the periphery, a method clinically employed to mobilize stem cells for autologous HSCT [26,27]. There is evidence that LSCs also employ the CXCR4 and CXCL12 interaction in order to home into their niche. High levels of CXCR4 in AML blasts confer a poor prognosis [28]. Additionally, AMD3465, a CXCR4 antagonist, blocked the chemoprotective effects of SDF-1. Antibodies directed against CXCR4 or SDF-1 decreased AML cell survival and engraftment in NOD/SCID mice and treatment with the proteasome inhibitor, bortezomib, inhibited the migration of AML blasts to CXCL12 [29–32]. Currently, there are several phase 1 clinical trials testing the use of anti-CXCR4 agents with or without G-CSF and proteasome inhibition in AML [33].

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In vivo murine studies with antibodies directed at cell surface markers on LSCs have demonstrated anti-homing effects in mice receiving human AML xenografts. The monoclonal antibody, H90, targets CD44, a cell surface marker differentially expressed on LSCs [21]. Administration of H90 to mice engrafted with AML led to decreased homing into the bone marrow and spleen in secondary transplant-recipients and increased differentiation of these cells [21]. Likewise, the monoclonal antibody, 7G3, which targets CD123 inhibited AML engraftment into immunodeficient mice when administered to AML cells ex vivo and then transplanted. Analysis following the experiment indicated that 7G3 inhibited AML LSC homing to the bone marrow [34]. These studies demonstrated that there is therapeutic potential in preferentially disrupting LSC homing to the bone marrow while sparing normal HSC interactions with the bone marrow niche.

Another strategy involves blocking the ability of LSCs to thrive within the bone marrow niche. The transcription factor, hypoxia inducible factor-1 alpha (HIF-1α), is induced in hypoxic conditions such as those found in the bone marrow [35,36]. In an experiment by Wang et al. [37], echinomycin, a HIF-1α inhibitor with minimal hematopoietic toxicity in humans, eliminated acute leukemia stem cells in a serial xenograft transplant model [37–39]. While echinomycin had dose limiting gastrointestinal toxicities in prior clinical trials, other HIF-1α inhibitors such as EZN-2968 and PX-478 are currently being tested in solid tumors [33]. HIF-1α may represent another target to disrupt the leukemic niche, eradicate leukemia stem cells and decrease chemoresistance.

5. Eliminating LSC self-renewal mechanisms

There are a variety of signaling pathways that allow HSCs to self-renew such as Notch, homeobox (HOX), hedgehog, and the Wnt/beta-catenin signaling pathways [40]. While the details of these signaling pathways in both HSCs and leukemia are still not completely known, there are indications that LSCs rely on these pathways for self-renewal. In a recent report by Wang et al. [41], the authors transformed either HSCs or granulocyte–macrophage progenitors (GMP) with either HOXA9 and MEIS1a oncogenes or with the fusion protein MLL-AF9 and studied them in a xenograft mouse model. In both cases, the Wnt/beta-catenin pathway was necessary for the self-renewal of LSCs. This dependence is despite the fact that Wnt/beta-catenin signaling is normally absent from GMPs, suggesting that LSC rely on acquisition of this pathway to proliferate. Interestingly, the HOX and MEIS1a genes were both highly expressed in both the LSC and HSC gene signatures obtained in the previously mentioned study by John Dick’s group [15]. This makes the Wnt/beta-catenin pathway an attractive future target against LSCs [41].

Additionally, other pathways such as Hedgehog have been described in other malignant stem cells such as CML, but their role in AML has yet to be established. Zhao et al. [42] demonstrated that absence of smoothened protein (Smo) led to decreased transformation of HSCs to CML when transduced with a BCR-ABL retrovirus in a xenograft model of primary and secondary transplant. The inhibition of Smo with the agent cyclopamine had a similar effect. Currently, Smo inhibitors are in clinical trials for CML [33].

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6. Targeting LSC growth factors and regulators of apoptosis

Leukemia stem cells appear to be dependent on several survival pathways within the cell leading to aberrant growth and escape from apoptosis. It was previously demonstrated that nuclear factor kappa B activity is elevated in LSCs [43]. NF-κB is a transcription factor active in promoting growth and anti-apoptotic activity within the cell. NF-κB is normally inhibited by the Inhibitor of kappa B alpha (IkBa) family of proteins, which sequester the protein in the cytoplasm not allowing it to enter the nucleus [44]. Bortezomib, the proteasome inhibitor, prevents the degradation of IκB creating an anti-NF-κB effect. When NF-κB was inhibited in LSCs using parthenolide, a sesquiterpene lactone, it led to cell death and decreased engraftment of leukemic cells into NOD/SCID mice [45]. HSCs, which do not exhibit elevated levels of NF-κB were not killed by parthenolide and engrafted NOD/SCID mice. Co-treatment of N-acetyl-cysteine (NAC) with parthenolide abrogated this anti-leukemia effect suggesting that parthenolide may act by sensitizing these cells to oxidative stress. Currently, various clinical trials are in process assessing the efficacy of bortezomib in combination with various other agents in AML [33].

The PI3K/AKT/mTOR pathway appears to be another major player in AML stem cell survival. This signaling pathway has links to numerous transcription factors and survival processes in the cell such as: the FOXO family of proteins that are important in regulating ROS, the BCL-2 family of proteins which regulate apoptosis, the Wnt/β-catenin signaling pathway implicated in LSC self-renewal and it is also involved in activating the transcription factor NF-κB [46]. In a study by Hassane et al. [47], the PI3K/mTOR pathway inhibitors, temsirolimus and wortmannin, potentiated the effects of parthenolide on LSCs with concomitant inhibition of Nrf2, a transcription factor responsible for the upregulation anti-oxidant genes. Regulation of ROS through FOXO proteins and ATM appear to be required for stem cell quiescence and repopulating ability [48,49]. It is possible that LSCs are dependent on factors that mediate ROS and other end products of the PI3K/AKT/mTOR pathway in order to maintain themselves and that altering these mechanisms through the PI3K/AKT/mTOR pathway will sensitize these cells to other agents. Many inhibitors of the PI3K/AKT/mTOR pathway are available and are currently in clinical trials in AML and in various other tumors.

Inhibition of apoptosis via the Bcl-2 family of proteins appears to be another method used by leukemia cells to perpetuate disease. This family encompasses 25 individual proteins that both promote or inhibit apoptosis. Family members Bcl-2, Bcl-xL, Bcl-w and Mcl-1 appear to prevent apoptosis while proteins such as Bax, Bim, and Bad appear to promote apoptosis [50–52]. Elevated levels of Bcl-2 are noted in various malignancies as well as AML and an increased ratio of Bcl-2 to Bax has been associated with poor prognosis in AML [52,53]. Studies performed by Glaser et al. showed that the inactivation of Mcl-1 in transformed AML cells induces apoptosis [54]. Konopleva et al. were able to demonstrate that AML samples enriched for the CD34+CD38−CD123+phenotype were more sensitive to the anti Bcl-2/BH3 mimetic, ABT-737, than to cytarabine while normal HSCs showed less sensitivity to this agent [55]. Various inhibitors of the anti-apoptotic Bcl-2 proteins targeting different family members are now in clinical trials for AML and other malignancies [33]. These agents include ABT-263, obatoclax mesylate and oblimersen sodium.

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7. Targeting cell surface antigens

Recently, many cell surface antigens have been described showing increased expression in LSCs as compared to HSCs, making these antigens potential therapeutic targets. These antigens include CD123, CD44, CLL-1, CD47, CD25 and CD32 [56]. Various ways of targeting cell surface antigens currently exist and are currently in clinical trials in many illnesses. These include monoclonal antibodies (Ab), immunotoxins, chimeric antigen receptor modified T-cells (CAR T-cells) and nanoparticles containing medication specifically directed at these cell surface markers. Monoclonal antibodies are directed against specific antigens and allow for targeted immune-cytoxicity. Adjuncts such as toxins and radioisotopes have also been attached to monoclonal antibodies to deliver these agents directly to their target of interest [57].

While not specifically targeting LSCs, Gemtuzumab ozogamicin, an anti-CD33 antibody, was the first monoclonal antibody approved by the FDA in the year 2000 for the treatment of AML after it showed some complete remissions in elderly patients [58]. Unfortunately, post-marketing trials revealed no additional benefit when gemtuzumab was given during AML induction or in maintenance therapy while adding hepatic toxicity. In June 2010, Pfizer voluntarily withdrew the drug from the market due to those results. Recently, though, a European group demonstrated that with a reduced dose regimen, patients between 50 and 70 years of age could benefit from Gemtuzumab [59]. Further studies are necessary to see the role of this monoclonal antibody in leukemia therapy.

CD123 is the LSC antigen most targeted to date. It was found to be preferentially expressed in the CD34+CD38− compartment of AML samples, as compared to normal HSC samples and correlated with the engraftment of immunocompromised mice [20]. In a follow-up study, AML cells were pretreated with the anti-CD123 monoclonal Ab, 7G3, and subsequently injected into a xenograft model leading to decreased engraftment [34]. On the basis of this pre-clinical work, two biologic agents have been developed and tested in phase 1 trials. The first, CSL360, is a monoclonal antibody. A phase 1 study with CSL360 in 26 patients with relapsed, refractory or high risk AML showed minimal toxicity. There was one CR [60]. The second, DT388IL3 (SL-401), is a recombinant immunotoxin created by the fusion of diphtheria toxin with a ligand targeting the IL-3 receptor that underwent phase 1 trials in AML and MDS. Toxicity included fever, chills, and transaminitis with vascular leak being the dose limiting toxicity. CRs were noted in both trials for this agent [61,62].

Preclinical trials targeting other LSC antigens also show clinical promise. Studies by Majeti et al. [17] demonstrated that the anti-CD47 monoclonal antibody, B6H12.2, eliminated engraftment after pretreatment and transplantation into a xenograft model. It also reduced tumor burden and eliminated secondary engraftment in NSG mice. Preclinical studies demonstrated that a CLL-1 monoclonal antibody was reported to induce cellular toxicity in AML blasts [63–65]. Furthermore, another group has created a CLL-1-targeted nanoparticle containing daunorubin, which is preferentially absorbed by CD34+CLL-1 expressing AML samples as compared to normal HSCs [66]. Studies such as these offer great promise in eradicating LSCs while minimizing toxicity to normal functioning cells.

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8. Genomic approaches to targeting LSCs

The process of devising new drugs to target LSCs is hindered by the lack of a defined LSC phenotype. While surface markers can be used to identify populations enriched in LSC activity, the LSC potential of any one cell is unknown and the proportion of cells demonstrating LSC activity in functional assays is rather low. Thus, given the tremendous effort required to find a single compound, it is of interest to capture the molecular changes caused by known anti-LSC compounds to accelerate the discovery of compounds with previously unknown anti-LSC activity. Recent and ongoing efforts at the Broad Institute have sought to capture the molecular changes induced by a variety of drugs thereby enabling an understanding of the connections between drugs using the common language of gene expression signatures, implemented in the Connectivity Map [67–69]. Thus, if a transcriptional signature that captures the transcriptional changes associated with an anti-LSC compound such as parthenolide is obtained, compounds previously unknown to exhibit anti-LSC activity would be revealed. Hassane and colleagues used and extended this approach by capturing the transcriptional signature of CD34+AML cells exposed to parthenolide using it to interrogate the gene expression space of the Connectivity Map and Gene Expression Omnibus to identify compounds with previously unknown anti-LSC activity using a pattern matching and machine learning strategy [70]. This study yielded two novel drugs, celastrol and 4-hydroxy-2-nonenal. Celastrol is an inhibitor of Hsp90 and by extension NF-κB[71,72], as Hsp90 chaperoning is essential for proper NF-κB signaling. 4-hydroxy-2-nonenal is a product of lipid peroxidation, inhibiting the proteasome and NF-κB function [73–75]. Both compounds targeted LSCs at the stem, progenitor, and bulk level while sparing normal HSCs.

Of particular note, gene expression-based drug discovery led to the unexpected finding that parthenolide, while inhibiting NF-κB, induced a secondary cytoprotective response involving activation of the PI3K/mTOR pathway and anti-oxidant genes controlled by Nrf2 [47]. This led to the hypothesis that inhibitors of this cytoprotective response could make parthenolide more effective at lower doses. As predicted, the combination of parthenolide and PI3K/mTOR-inhibitors potentiated the effects of parthenolide in a synergistic manner, while continuing to spare normal cells. While this finding was instrumental in highlighting the power of gene expression-based drug discovery, it also suggests a new paradigm in which genomic approaches enable the detection of secondary cytoprotective responses, allowing existing drugs to work at lower doses. Perhaps more significantly, it suggests that ascertaining these unexpected cytoprotective responses in the pre-clinical stages of drug development may allow more drugs to succeed in the development pipeline.

9. Conclusion

For decades, it has been known that there is a subpopulation of AML cells that are resistant to traditional cancer therapies and lead to relapse in the majority of patients with this disease. Over the past two decades, the ability to identify and sort different subpopulations of cells and engraft them into immunocompromised mice has allowed for the ability to define these cells as LSCs. These cells exhibit many qualities found in HSCs such as the ability to both self-renew and to give rise to differentiated progeny as well as quiescence.

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Importantly, recent studies have shown that high levels of LSCs in patients correlates with poor prognosis signifying the need to effectively eradicate these cells in order to cure AML. The ability to isolate these cells has allowed for the identification of numerous cell surface antigens such as CD123, CLL-1, CD44, CD25, and CD32 that are preferentially expressed on LSCs as compared to HSCs. This has led to the development of novel agents such as CSL360 and SL-401 that are currently being tested in clinical trials. These targets have also demonstrated promise in determining prognosis and MRD in patients with AML.

Numerous studies have identified mechanisms employed by LSCs to maintain themselves, including the manner in which they interact with the bone marrow niche, their self-renewal mechanisms, their reliance on growth factors and their ability to evade apoptosis. Pre-clinical trials using xenograft models have led to numerous ongoing phase 1 studies in AML targeting the Wnt/β-catenin pathway, the BCL-2 family of proteins, the PI3K/AKT/mTOR pathway and proteasome inhibitors. This is likely only the beginning as future studies will continue to provide more therapeutic targets and lead us to potential synergistic therapeutic options.

Additionally, high throughput drug screens optimized to target LSCs will continue to identify available established agents. Ciclopirox olamine and tigecycline were recently discovered through such drug screens and demonstrate anti-LSC activity through iron chelation and inhibition of mitochondrial translation respectively [76,77]. Both tigecycline and an oral formulation of ciclopirox olamine are currently being tested in phase 1 trials [33]. Overall, there is much reason to be optimistic for new targeted therapies aimed, not just at inducing remission, but in eradicating AML.

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Table 1

Clinical implications of leukemia stem cells.

Study	Results	Reference
Presence of LSCs correlates with MRD		
Van Rhenen et al. (2005)	Patients with greater than 3.5% CD34+CD38− leukemic blasts at diagnosis had relapse free survival of 5.6 months compared to 16 months in those with a lower percent. ($p = 0.02$). This cutoff also predicted MRD after 3rd cycle of chemotherapy ($p = 0.03$)	[13]
Terwijn et al. (2009)	CD34+CD38− frequency of greater than 1×10^{-3} in leukemic blasts after first cycle chemotherapy led to lower relapse free survival (RFS) of 5 months vs. 56 months. ($P = 0.00003$) and LSC frequency combined with blast MRD was a better predictor of RFS than blast MRD alone	[22]
Gerber et al. (2012)	MRD in AML patients in morphologic complete remission was relatively enriched for CD34+CD38− $ALDH^{int}$. Presence of this immunophenotype both predicted a sample's ability to engraft NSG mice and clinical relapse	[23]
LSC gene signatures indicates poor prognosis		
Gentles et al. (2010)	High expression of a LSC gene signature generated from the CD34+CD38− subpopulations of primary patient AML samples was associated with worse overall, event free and relapse free survival	[14]
Eppert et al. (2011)	A gene expression signature was created based on the subpopulations of 16 primary AML samples capable of initiating leukemia in a xenograft model. This gene signature correlated with that of HSCs and both LSC and HSC gene signatures were predictors of patient survival	[15]

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Table 2

Anti-leukemic stem cell agents in clinical trials.

Target	Name of inhibitor	Clinical trial for AML
Cell surface antigen targeting therapy		
CD123	DT388IL-3/SL-401, CSL360	Phase I/II
CD33	Gemtuzumab ozogamicin	Phase III trials
Molecular targets		
NF-κB	Bortezomib	Phase I/II/III
PI3K	BKM120, CAL-101	Phase I
AKT	GSK21110183, MK-2206, perifosine	Phase I/II
mTOR	Sirolimus, everolimus, temsirolimus	Phase I/II
BCL-2	Olimersen sodium, obatoclax mesylate	Phase I/II/III
Microenviroment		
CXCR4	Plerixafor, G-CSF	Phase I/II
HIF-1α	Inhibitors in clinical trials for other malignancies	N/A
Self-renewal, differentiation and other pathways		
Wnt	CWP232291	Phase I
Hedgehog	Inhibitors in clinical trials for other malignancies	N/A
Other mechanisms		
Iron chelation	Ciclopirox olamine	Phase I
Mitochondrial translation	Tigecycline	Phase I