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REVIEW ARTICLE

Current Concepts of Leukemic Stem Cells: Origin, Characteristics, and its Clinical Implications in Acute Myeloid Leukemia

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ABSTRACT:
Despite significant advancements in therapy, patients diagnosed with acute myeloid leukemia (AML) continue to face poor outcomes, often experiencing relapses even after achieving initial complete remission. The occurrence of relapse is attributed to the inability of conventional treatment to eliminate a specific subset of cells within the bone marrow known as leukemic stem cells (LSCs). These specialized cells exhibit self-renewal capacity and have the ability to proliferate and differentiate into leukemic blasts. The accumulation of multiple genetic mutations in LSCs makes them resistant to standard chemotherapy. Several studies have been conducted to identify the phenotypic characteristics and genetic signatures of LSCs, with the aim of differentiating them from normal hematopoietic stem cells (HSCs). Understanding the role of LSCs in AML treatment resistance has paved the way for the development of targeted and more precise treatments, especially for relapsed AML patients, without affecting the healthy HSCs. This review elaborates on the origin, phenotypic and genotypic characteristics of LSCs, and their role in the biology of AML, with a brief note on therapies targeting LSCs.

Keywords: acute myeloid leukemia, leukemic stem cells, immunophenotype, targeted therapy, relapsed AML

Abbreviations:

ADC – Antibody drug conjugate
AML – Acute myeloid leukemia
ARCH - Age related clonal hematopoiesis
BiTE – Bispecific T-cell engager molecules
BM – Bone marrow
CAR T cells – Chimeric antigen receptor T cells
CD – Cluster of differentiation
CLL-1 – Human C-type lectin-like molecule-1
CSCs – Cancer stem cells
DART – Dual affinity retargeting proteins
GM-CSF – Granulocyte macrophage colony-stimulating factor
GO – Gemtuzumab ozogamycin
GPR56 – G protein-coupled receptor 56
HiDAC – High dose cytarabine
HSCs – Hematopoietic stem cells
HSCT – Hematopoietic stem cell transplant
IL-1β – Interleukin 1β
IL-3 – Interleukin 3
IL1-RAP – IL-1 receptor accessory protein
ITGA6 – Integrin subunit alpha 6
LICs – Leukemia initiating cells
LPCs – Leukemia propagating cells
LSCs – Leukemic stem cells
mAb – Monoclonal antibody
MDS – Myelodysplastic neoplasm
MFC – Multiparametric flow cytometry
MPCs – Multipotent progenitor cells
MRD – Measurable/ minimal residual disease
MSCs – Mesenchymal stem cells
NGS – Next generation sequencing
NOD/SCID – Non-obese diabetic/ severe combined immunodeficient
PCR – Polymerase chain reaction
PTH2R – Parathyroid hormone 2 receptor
RNA – Ribonucleic acids
SCF – Stem cell factor
TGF-β – Transforming growth factor-β
Tie-2 – Tyrosine-protein kinase receptor 2
TIM3 – T-cell immunoglobulin and mucin domain-containing 3
TLC – Total leucocyte count
TNF-α – Tumour necrosis factor α
VEGF – Vascular endothelial growth factor
WT-1 – Wilms tumour protein 1

Introduction:

The incidence of acute myeloid leukemia (AML) in India varies from 0.9 to 1.5 per 100,000 general population.[1–3] AML represents a group of clonal hematopoietic stem cell (HSC) disorders, in which, there is a failure to differentiate along with increased proliferation potential in the stem cell compartment, resulting in the accumulation of non-functional immature cells termed as myeloblasts. It is associated with a variety of cytogenetic abnormalities and genetic mutations.[4,5] Even though a good number of patients with AML achieve complete remission with induction therapy, more than half of these cases subsequently relapse and eventually die of the disease. This relapse is thought to occur because of the inability of the conventional therapy to eradicate a distinct type of self-renewing cells in the bone marrow (BM) compartment which are called as leukemic stem cells (LSCs) or leukemia-initiating cells (LICs) or leukemia-propagating cells (LPCs).[6]

Leukemic stem cells (LSCs) are specialised type of hematopoietic stem cells (HSCs) which harbour a variety of mutations and have the ability to exhibit self-renewal capacity as well as differentiate into leukemic blasts.[7] Upon transplantation, these cells have the capacity to initiate and propagate leukemia in a host. The concept of LSCs in AML were brought to light by Dick and his colleagues in 1997.[8] These cells were isolated using cell sorting techniques to study their immunophenotypic characteristics and molecular signatures. Multiparametric flow cytometry (MFC) has shown that LSCs were immunophenotypically distinct from the more mature leukemic blasts. LSCs were also identified to be an independent poor prognostic factor in AML patients.[9,10]

In addition, LSCs have specific molecular signatures that make them more resistant to conventional chemotherapeutic regimens for AML.[11–13] Understanding the role of LSCs in treatment resistance has led to the development of new therapeutic strategies tailored to target these cells. However, a major challenge has been that the targeted immunophenotypic markers are not exclusive to LSCs and are also expressed in normal hematopoietic cells, leading to unwanted side effects with some drugs. Hence, there is a need to identify new markers that are unique to LSCs to develop novel therapeutic drugs in the future.

In this context, the aim of this review is to concisely discuss the origin, phenotypic and genotypic characteristics of LSCs, their role in the biology of AML, and provide a brief overview of therapies targeting LSCs.

1. Normal hematopoiesis and bone marrow niche for hematopoietic stem cells:

The hematopoietic stem cells (HSCs) form the apex in the hierarchy of all the hematopoietic elements formed in the BM. These cells undergo differentiation towards the uncommitted progenitors, which will undergo further differentiation towards fully functional hematopoietic cells (i.e. red cells, granulocytes and platelets) in our body. In addition, they also possess the unique ability of self-renewal to maintain its

number in sufficient quantity. In order to balance both these properties, there should be striking control over its dormancy, self-renewal and lineage differentiation.[14]

Under normal physiological conditions, these HSCs reside in a specialized BM microenvironment called as 'niche' and this concept was first described by Schofield et al.[15] Previous studies have proposed the existence of an 'osteoid niche,' comprising osteoblasts, as a key cellular component responsible for maintaining the characteristic properties of HSCs.[16,17] However, some subsequent studies examining the effects of gene deletion of two crucial cytokines required for hematopoiesis i.e. stem cell factor (SCF) and CXCL-12 in osteoblasts, did not demonstrate any drastic effects on HSCs maintenance within the bone marrow niche.[18,19] Nonetheless, these osteoblasts form a niche for early lymphoid progenitors and are implicated in the progression of leukemias.[20]

The other compartment which lies in close proximity to the HSCs are the blood vessels and perivascular cells, which are collectively termed as the 'vascular niche'. The gene deletion in the endothelial cells showed a substantial effect on BM recovery following myeloablative therapy.[7,20] Bone marrow has another crucial type of support cells, known as mesenchymal stem cells (MSCs), which possess self-renewal capabilities and can differentiate into osteoblasts, chondrocytes, and adipocytes. The mouse models with deletions of SCF and CXCL-12 in the MSCs directly affected the HSCs number and localization within the BM niche.[21,22] Adipocytes in the BM generally serve as important negative regulators of normal hematopoiesis.[23] But it is interesting to note that the effect of the adipocytes on the HSCs may vary depending on their location. In fact, the adipocytes support the regeneration of blood cells and also the maturation of myeloid and erythroid cells, if they are situated in the active red BM region.[24,25]

In addition, the sympathetic nerves indirectly help in HSCs mobilization following adrenergic signals and in response to G-CSF.[26] Non-myelinating Schwann cells are involved in HSCs quiescence by activating the transforming growth factor (TGF-β).[27] Moreover, the other normal hematopoietic cells such as megakaryocytes, macrophages, regulatory T-cells, neutrophils and other myeloid cells also influence the micro-environment of HSCs either directly or indirectly.[20]

2. Malignant hematopoiesis:

Leukemias originate from transformed HSCs called as LSCs or LICs/ LPCs.[6] Leukemic stem cells (LSCs) were first identified in AML and gained prominence through studies conducted by Bonnet, Dick, and Lapidot et al.[28] These LSCs are phenotypically and biologically distinct cell populations from leukemic blasts. During the course of malignant transformation, LSCs exhibit properties of self-renewal capacity, proliferation and differentiation through continuous genetic and epigenetic alteration and clonal diversification. Age related clonal hematopoiesis (ARCH) or other factors result in accumulation of mutations in HSCs and transform them to preleukemic stem cells (pre LSCs). Pre LSCs undergo clonal expansion and acquire further mutations like ASXL1, DNMT3A, E2H2, IDH1, IDH2, IKZF1, JAK2 and NPM1 to get converted to LSCs, which has malignant characteristics.[29] Due to accumulation of multiple mutations that impede further maturation, these LSCs continue to produce immature cells/ blasts in the marrow, which becomes one of the hallmarks of AML.[6] When compared to HSCs, LSCs tend to exhibit some degree of hierarchical similarities.[8,12,13]

3. Heterogeneity model of leukemic stem cells in acute myeloid leukemia:

Studies using xenotransplantation models involve transplanting human leukemia cells into immunodeficient mice to assess the ability of these cell populations to initiate and propagate the disease. The successful engraftment and serial transfer of leukemia in these models highlight that these LSCs have higher self-renewal capacity and differentiation potential than the non-malignant cells.[8,28]

In order to understand how these LSCs acquire both the properties could be explained in two different ways. The first way is that normal HSCs gain a variety of mutations, some of which become 'driver mutations' and convert them to LSCs with enhanced proliferation potential.[14,28] Another mechanism involves the multipotent progenitor cells (MPCs), which, normally loses its self-renewal capacity during the normal maturation sequence. These MPCs acquire mutations and regain the property of self-replication.[30] Hence, it is evident that regardless of whether they originate from HSCs or MPCs, the final phenotype of LSCs differs remarkably from their cell of origin.

Moreover, these LSCs can acquire additional mutations, making them highly heterogenous within the common pool of LSCs.[31] Whole genome/ exome sequencing had demonstrated that hardly two AML samples share the same combination of mutations. The Cancer Genome Atlas Research Network analysed 200 AML samples and found that about 30 genes were mutated in about 2% of AML

patients.[32] It has been well documented by multiple studies that there is a remarkable heterogeneity in AML both at the genetic and epigenetic level, particularly in relapsed cases.[13,31,33] Similar cells have been identified in other non-hematological malignancies termed as cancer stem cells (CSCs). However, not all CSCs in the solid tumours adhere to the concept of heterogeneity model of LSCs.[34]

4. Remodelling of bone marrow niche by leukemic stem cells in acute myeloid leukemia:

Several studies have demonstrated that LSCs induce remodelling of the BM niche.[25,35–40] The 'vascular niche' is altered in AML by production of pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) which increases the micro-vessel density.[41] There is selective remodelling of central and endosteal vasculature in the BM of AML resulting in the progressive loss of endosteal stromal cells.[35] This hypoxia related death of these stromal cells leads to the production of reactive oxygen species and nitric oxide which further increases vascular permeability and are associated with the AML progression.[36]

There are few important cytokine receptors and adhesion molecules namely CXCR4, VLA-4 and CD44, that help in linking the 'vascular niche' to nearby MSCs. The chemosensitivity of AML relies on the homing and retention of LSCs in the 'vascular niche', which is facilitated by higher expression of these adhesion molecules.[37] In relapsed AML cases, it was found that the loss of these interactions played a substantial role in the chemoresistance of LSCs.[38]

The MSCs are reprogrammed in such a way to create a pro-tumoral niche in the BM micro-environment. This reprogramming is mediated through various factors secreted by them or through exosomes by direct cell-to-cell contact.[39] This provides LSCs with a survival advantage in AML. Nanotubes are formed in MSCs through which mitochondria are transferred to LSCs, thereby providing additional bio-energetics and increased capacity to withstand the reactive oxygen species formed due to chemotherapy.[42] This fact is supported by additional mouse models, which have shown that MSCs protect the LSCs by inhibiting apoptosis through Notch and Wnt signalling pathways.[40,43]

Another significant alteration observed is the establishment of an autocrine loop within the LSCs niche. Tumour necrosis factor α (TNF-α) is secreted by the LSCs which acts on itself via the nuclear factor kappa B (NF-κB) pathway, thereby creating a vicious cycle. Activation of Notch signalling also contributes to the NF-κB pathway activation.[37,44] A complex interplay between the immune cells and LSCs results in production of several cytokines by the LSCs such as interleukin 1β (IL-1β), interleukin 3 (IL-3), granulocyte macrophage colony-stimulating factor (GM-CSF) and TNF-α. These cytokines act on LSCs through autocrine/ paracrine loops, creating a pro-inflammatory milieu that further promotes their cell survival.[37,45]

In AML, LSCs repress maturation of adipocytes and thereby impairing the myeloid-erythroid differentiation.[23,25] These LSCs create an inflammatory environment within them and induce lipolysis of triglyceride to free fatty acids, which are utilised for further proliferation and survival. There is also an increased expression of fatty acid transporter (CD36) on the surface of LSCs which also plays a role in chemoresistance in AML.[24]

One of the critical regulatory components of the BM niche which gets altered due to aging is the sympathetic nervous system. This controls the plasticity of MSCs, which gets lost due to sympathetic neuropathy associated with aging leading to increased risk of myelodysplastic neoplasm (MDS) and AML.[26,46] It was demonstrated in mouse models that the neuropathy was associated with exhaustion of HSCs followed by expansion of MSCs and LSCs expressing the β2-adrenergic receptors.[47]

5. Characterisation of leukemic stem cells:

Leukemic stem cells (LSCs) are functionally defined by their self-renewal capacity, and upon transplantation into a new host, they have the ability to induce new leukemia.[8,28] Given the impossibility of conducting these studies in humans, the gold-standard method to characterize these LSCs involves experimental design in severely immunocompromised mouse models to demonstrate their BM engraftment. The studies performed by Dick and their colleagues in 1997 demonstrated their existence in non-obese diabetic/ severe combined immunodeficient (NOD/ SCID) mice.[8] Various studies also showed that the replicative potential of LSCs improved when cultured in semi-solid medium enriched with various constitutively expressed genes for 'stemness', cells or factors mimicking the BM micro-environment.[48,49] However, the propagation of LSCs in mouse models could not be demonstrated in every AML sample.

Many studies have been conducted to identify the unique immunophenotype of LSCs. Phenotype identification would pave the way for the development of targeted and more precise treatments, especially for relapsed AML patients.

The majority of LSCs expressed CD34, which is a normal HSCs surface marker. They found that LSCs were detected in CD34+CD38− compartment. However later studies observed that LSCs were more heterogeneous and were also found in the CD34+CD38+ compartment and more rarely in the CD34− compartment.[13,47] Several critical surface markers unique to AML LSCs have been identified. For example, it has been revealed that CD90 and CD117 are deficient in AML LSCs, while CD44, CD47, CD96, CD123, Human C-type lectin-like molecule-1 (CLL-1), G protein-coupled receptor 56 (GPR56), N- cadherin, T-cell immunoglobulin and mucin domain-containing -3 (TIM3), tyrosine-protein kinase receptor 2 (Tie-2) and IL-1 receptor accessory protein (IL1-RAP) are highly expressed in AML LSCs.[50–53] Various studies conducted to identify the immunophenotype of LSCs in AML patients are summarised in **Table 1**.

Table 1: List of some studies conducted to identify leukemic stem cells immunophenotype in acute myeloid leukemia

S. No.	Immunophenotype of LSCs	Sample size	References
01	CD34+, B220+, CD43+, CD93/AA4.1+, CD24+, CD90+	13 murine models	Deshpande et al., 2006[80]
02	CD34+, CD38-	55 cases	Van Rhenen et al., 2007[81]
03	CD34 negative compartment	15 cases	Taussig et al., 2010[47]
04	CD34+, CD135+, CD47+, ITGA6+, CD96+, PTH2R+	60 cases	Bonardi et al., 2013[82]
05	CD34+, CD38-, CD123+, N-cadherin +, Tie2+	22 cases	Qiu et al., 2014[51]
06	CD34+, CD38-, CD44+, CD96+, CD90+, WT-1+, CD123+, CD25+	31 cases	Garg et al., 2015[83]
07	CD34+, CD38-, CD123+	34 cases	Al-Mawali et al., 2016[11]
08	CD34+, CD38-, CD123+, CD33+, CLL-1+, TIM-3+, CD7+, CD11b+, CD22+, CD56+	131 cases	Zeijlemaker et al., 2016[61]
09	CD25+, CD96+, CD123+	80 cases	Yabushita et al., 2018[84]

Key: CLL-1 – Human C-type lectin-like molecule-1; ITGA6 – Integrin subunit alpha 6; LSCs – Leukemic stem cells; PTH2R – Parathyroid hormone 2 receptor; Tie-2 – Tyrosine-protein kinase receptor 2; TIM3 – T-cell immunoglobulin and mucin domain-containing 3; WT-1 – Wilms tumour protein 1

CD123 (IL-3 receptor α chain) is normally strongly expressed on plasmacytoid dendritic cells and expressed at lower levels on other more mature CD34+ subsets including common myeloid precursors and granulocyte macrophage precursors. It is completely absent on nucleated red blood cells.[54] CD123 is overexpressed in AML, B-cell acute lymphoblastic leukemia, hairy cell leukemia and blastic plasmacytoid dendritic cell neoplasm.[55,56] Studies have shown that CD123 is found on almost all AML specimens and is preferentially expressed in the CD34+/CD38− AML cells. Moreover, CD123 is not expressed by normal HSCs, making it a potential marker for identifying the malignant clone of LSCs.[10,11]

Nevertheless, the major issue faced while conducting studies on LSCs is identification of a unique immunophenotype for LSCs. Due to the marked intra and interpatient heterogeneity in LSCs, it is difficult to identify a specific marker that is expressed only in LSCs and not in the normal hematopoietic cells.[14,57]

6. Different methods of assessing leukemic stem cells:

In virtually all AML patients, diagnosis and follow-up monitoring can be effectively done by using mutation specific immunohistochemical markers in BM biopsy, multiparametric flow cytometry (MFC), cytogenetics and molecular analysis by polymerase chain reaction (PCR).[14] But, the results of cytogenetics and molecular analyses are usually time-consuming and will be obtained 2-3 weeks after starting induction chemotherapy. Hence, these tests are helpful to physicians only at the time of completion of induction therapy, to determine the type of consolidation to proceed with (e.g. allogeneic transplantation or not).[58] So, new diagnostic tools need to be developed that must be accurate and easily available in a clinical setting.

Multiparametric flow cytometry (MFC) can significantly contribute to the risk assessment of AML patients during and after treatment by aiding in quantification of LSCs. Early prediction of the percentage of LSCs can assist in patient categorization and allow for modification of treatment plans and follow-up accordingly. It is the

best technology for detecting subpopulations of LSCs, as it is reliable and can easily discriminate less than 0.01% of cells with a high degree of sensitivity and lesser turn-around time.[59–61]

Specifically, these LSCs can be isolated by sorting techniques either based on immunophenotyping or with the help of magnetic beads using MFC sorters. After isolation, these LSCs can be used in experimental studies such as NOD/ SCID/IL-2 mouse model and inoculated directly into the BM compartment.[13,28] Their BM microenvironment could be enriched with the necessary growth factors or cytokines to produce 'niche-like' environment for inducing AML. Several subcultures can be studies by inducing the mutations step by step to see whether the particular clone is propagating with self-renewal capacity or not.[48] Subsequently, the mutations in these subclones can be analyzed using next-generation sequencing (NGS) to determine their role as either 'driver mutations' or 'non-driver mutations'.

7. Role of leukemic stem cells in predicting the prognosis and therapeutic outcome in acute myeloid leukemia (AML):

Various clinical, laboratory, and genetic parameters significantly impact the prognosis of AML including age, performance status of the patient, high total leucocyte count (TLC), secondary AML, adverse cytogenetics, high risk molecular mutations, and poor response to induction chemotherapy.[62] Despite achieving morphological remission, a considerable proportion of AML patients harbour residual blasts, which are responsible for relapse. Therefore, assessment of this minimal/ measurable residual disease (MRD) is crucial and can be performed using techniques such as immunophenotyping by MFC or molecular methods like PCR or NGS.[59,60] The presence of >0.1% of residual cells in the BM is considered as MRD in AML. In the current era, MRD has emerged as a pivotal biomarker in AML prognostication and effective monitoring of therapy.[63]

Despite advancements in AML therapy, a majority of elderly patients and approximately 40% of younger patients remain refractory to treatment or may experience relapse.[64] Some of these patients relapse even after undergoing allogenic hematopoietic stem cell transplant (HSCT). Hence, various emerging parameters have been investigated to identify relapse in AML and have shown promising results. These include analysis of apoptotic index using annexin V, propidium iodide, pro-apoptotic protein 'bax', and anti-apoptotic protein 'bcl-2', as well as assessment of specific micro-ribonucleic acid (miRNA) expression levels and evaluation of LSCs.[65,66]

It has come to light that LSCs not only contribute to leukemogenesis, but also play a vital role in disease relapse by being resistant to routine chemotherapy. Houthuijzen et al. described that the LSCs secrete certain chemicals like platinum-induced fatty acids (PIFAs), which are by products in arachidonic acid pathway. These chemical compounds confer resistance to chemotherapy-induced apoptosis of tumor cells.[67] Another concept suggests that LSCs populations are enriched with quiescent transcriptional signatures. Quiescence is an adaptive effect observed in AML, which is associated with the upregulation of anti-apoptotic factor BCL2 and a reduction in metabolic rate by relying on the glycolytic pathway, rendering LSCs resistant to a wide variety of chemotherapeutic agents.[68]

In GOELAMS study, Vergez et al. attempted to quantify the LSCs (CD34+/CD38-/CD123+ events) using MFC in 111 AML patients and found that patients with LSCs more than 15%, had lower rate of response to induction therapy. It was also observed that the presence of LSCs more than 1% was associated with poor outcome.[10] The chemo-resistant nature of LSCs could be the root cause for poor prognosis and relapse in AML patients after induction. Witte et al. studied the proportion of LSCs in 17 pediatric AML cases treated with AML-BFM 98/04 protocol using MFC. Their analysis revealed that patients with low LSCs level exhibited significantly higher event-free survival rates.[69] Conversely, those with higher LSCs level at diagnosis showed increased chemotherapy resistance and higher rates of relapse, thereby confirming their prognostic significance in pediatric AML cases. Additionally, several other studies have identified LSCs as an independent prognostic factor in AML, and demonstrated its association with induction failure, high MRD levels, poor survival outcomes, and therapy resistance in AML patients.[70–72]

8. Leukemic stem cells as therapeutic targets:

The standard therapy which is the 7+3 regimen includes continuous infusion of cytarabine 200mg/m^2 for 7 days with daunorubicin 60mg/ m^2 for 3 days. The daunorubicin can be replaced with idarubicin or mitoxantrone. Reinduction is done using high dose cytarabine (HiDAC) regimen.[73] Low-intensive therapy for elderly patients over 75 years includes azacitidine/ decitabine and venetoclax combination.[74] The treatment regime is entirely based on the performance status and other targetable markers. Despite advances in therapy, there was no significant improvement in the survival of AML patients. This is attributed to relapses occurring during the follow-up period despite

achieving initial complete remission.

In the modern era, novel targeted therapies have been identified to prevent relapse in AML, with LSCs emerging as a crucial therapeutic target. The different types of therapeutic agents developed against LSCs are chimeric antigen receptor (CAR) T cells, bispecific T-cell engager molecules (BiTEs), monoclonal antibodies (mAb), and antibody-drug conjugates (ADC). These therapies target cell surface markers and signaling pathways of LSCs or act as pro-apoptotic agents or epigenetic regulators.[75]

CD33 and CD123 were identified as the most reliable surface markers for LSCs identification. They act as potential therapeutic targets both at diagnosis and at relapse.[76] Gemtuzumab ozogamycin (GO) which has been used as a target against CD33 was found to have a low risk of relapse and improved overall survival in AML patients.[77] However, the first CD123 targeted therapy named 'Talacotuzumab' showed increased toxicity and only limited efficacy.[78,79] Numerous novel drugs targeting surface markers and transcription factors specific to LSCs are currently under clinical trials, promising to pave the way for personalized care in AML patients. Some of the novel LSC directed drugs intended to use in AML therapy are tabulated in **Table 2**.

Table 2: List of leukemic stem cells targeted therapeutic agents in acute myeloid leukemia

Therapeutic target	Name of the drug	Type of drug	Remarks	References
A. Drugs targeting LSC specific surface markers				
CD33	Gemtuzumab ozogamycin	mAb	FDA approved	Goldenson et al., 2021[85]
	Lintuzumab-Ac225 (Ab conjugated with alpha particle emitting radionuclide 'Actinium-225' (Ac-225)	mAb	Phase I trial	Abedin et al., 2022[86]
	Vadastuximab talirine (SGN-CD33A)	ADC	Discontinued due to safety concerns	Stein et al., 2018[87]
	AMG 330	BiTE (Binds CD33 and CD3)	Phase I trial	Ravandi et al., 2020[88]
	CD33CART	CAR T-Cells	Phase I/II trial	Shah et al., 2023[89]
CD123	Talacotuzumab	mAb	Phase II/III trial	Motesinos et al., 2020[90]
	IMGN632	ADC	Phase I/II trial	Daver et al., 2020[91]
	MB-102	CAR T-Cells	Phase I/II trial	ClinicalTrials.gov ID # NCT02159495
	Flotetuzumab (Bispecific for CD3ε and CD123)	DART	Phase I/II trial	Uy et al., 2021[92]
CD96	MSH-TH111e	mAb	Not in trial	Gramatzki et al., 2016[93]
TIM-3	Sabatolimab (MBG453)	mAb	Phase I trial	Brunner et al., 2024[94]
CLL-1	CLL-1 CAR-T cells	CAR T-Cells	Phase I trial	Jin et al., 2022[95]
B. Drugs targeting LSC intracellular pathways				
JAK/STAT	OPB-111077 (STAT3 inhibitor)	Small-molecule inhibitor	Phase I trial	Wilde et al., 2019[96]
NF-κB	Micheliolide	Small-molecule inhibitor	Not in trial	Ji et al., 2016[97]
WNT/ β-catenin	CWP232291	Small-molecule inhibitor	Phase I trial	Lee et al., 2020[98]
CXCR4	Plerixafor	Small-molecule inhibitor	Phase I/II trial	Uy et al., 2017[99]

Key: ADC – Antibody drug conjugate; BiTE – Bispecific T-cell engager molecules; CAR T cells – Chimeric antigen receptor T cells; DART - Dual affinity retargeting proteins; LSCs – Leukemic stem cells; mAb – Monoclonal antibody

An ideal therapeutic agent should be able to effectively eliminate LSCs, in addition to targeting the leukemic blasts. However, the challenge faced while targeting LSCs is the unintended elimination of HSCs and other healthy hematopoietic cells, due to shared expression of surface markers between LSCs and these cells. This has led to the development of toxicities, ultimately resulting in the withdrawal of some drugs from the market.

Conclusion:

Leukemic stem cells (LSCs) are indeed a unique type of cells with stem cell properties, which play a vital role in AML leukemogenesis and disease relapse. They possess distinct phenotypic and biological characteristics compared to leukemic blasts. Isolating and analysing LSCs have remarkably enhanced our understanding of their biological behaviour, characteristic marker expression and molecular signatures, making them a potential therapeutic target in AML. Targeting the molecular pathways of LSCs holds promise for opening up multiple avenues for personalized care in AML patients in the future.

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