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Targeting leukemia stem cells: which pathways drive self-renewal activity in T-cell acute lymphoblastic leukemia?

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ABSTRACT

T-Cell acute lymphoblastic leukemia (T-ALL) is a malignancy of white blood cells, characterized by an uncontrolled accumulation of T-cell progenitors. During leukemic progression, immature T cells grow abnormally and crowd into the bone marrow, preventing it from making normal blood cells and spilling out into the bloodstream. Recent studies suggest that only discrete cell populations that possess the ability to recreate the entire tumour might be responsible for the initiation and propagation of T-ALL. Those unique cells are commonly called "cancer stem cells" or, in the case of hematopoietic malignancies, "leukemia stem cells" (LSCs). Like normal hematopoietic stem cells, LSCs are thought to be capable of self-renewal, during which, by asymmetrical division, they give rise to an identical copy of themselves as well as to a daughter cell that is no longer capable of self-renewal activity and represents a more "differentiated" progeny. Here, we review the main pathways of self-renewal activity in LSCs, focusing on their involvement in the maintenance and development of T-ALL. New stem cell–directed therapies and LSC-targeted agents are also discussed.

Key Words Leukemia stem cells, T-ALL, leukemia, Notch, Wnt, β-catenin, HIF, mouse models, patient-derived xenografts

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is an aggressive tumour of the hematopoietic system. Most ALLs (85%) originate from the B-cell lineage (so-called B-ALL); the rest (15%) are of T-cell lineage (or T-ALL)[1]. By definition, ALL is an aggressive malignancy that can be fatal if left untreated. Most human cases of T-ALL occur in young children between 2 and 5 years of age, but T-ALL can occur at any age[2]. The cure rate depends primarily on the age of the patient. Outcomes in infants are better than those in children more than 10 years of age; most adults succumb quickly to the disease. The long-term survival rate is 30%–40%[3].

T-Cell ALL is the result of genetic mutations or chromosomal translocations (or both) that alter the functional pathways involved in the proliferation, survival, growth, and differentiation of hematopoietic progenitors during T-cell development[4]. During the period since the late 1990s, published reports have noted aberrant expression by selected groups of transcription factors in T-ALL as a result of various chromosomal rearrangements or small intrachromosomal deletions. More than 50% of human cases of T-ALL are characterized by genetic alterations in *NOTCH1* or other genes related to Notch1 signalling, resulting in constitutive activation of the Notch1 pathway[5,6]. Moreover, more than 70% of human T-ALL presents loss of *p16/INK4A* and *p14/ARF* suppressor genes as result of deletions in the *CDKN2A* locus (chromosome band 9p21)[7]. Additional oncogenic transcription factors include c-Myc[8], NKX2-1, NKX2-2[9]; the LIM domain only (*LMO*) genes, *LMO1* and *LMO2*[10]; the *HOXA* homeobox genes[11]; and the basic helix-loop-helix family members *TAL1*[12] and *TAL2*[13]. Aberrant expression of these regulatory genes leads to a block in the differentiation process and induces uncontrolled proliferative signals in T-ALL.

DISCOVERY OF LSCs IN T-ALL

Leukemia stem cells were first described in the mid-1980s by John Dick's group, when they demonstrated the existence of LSC-enriched subsets in the heterogeneous bulk populations of acute myeloid leukemia[14]. Those primitive cells had the capacity to be serially transplanted and could give rise to differentiated cells, recreating the complete phenotypic heterogeneity of primary leukemia[14].

In T-ALL, cell populations with enriched LSC activity were initially reported within the CD34+CD4– or CD34+CD7– subsets. Those cell populations were able to

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reinitiate disease in naïve immunocompromised non-obese diabetic and severe combined immunodeficiency (NOD/SCID) mice—so-called leukemia-initiating cell activity[15]. However, in a subsequent study, it was reported that the T-ALL development in NOD/SCID mice is not strictly correlated with CD34 surface expression[16]. After the foregoing reports, a third study emerged from John Dick's group. Looking at a cohort of 5 cases, they found that the CD7+ phenotype was a stronger marker of leukemia-initiating cell activity and that a lower number of CD7+CD1a– cells were transplantable in a more permissive host[17]. Notably, CD34 positivity was not a consistent finding across their cohort, and additional experiments suggested that those subsets performed better in a co-culture system with OP9 stroma cells expressing the Notch1 ligand delta-like 1 and were dependent on Notch1 signalling. In a very limited subset of cases, those authors also highlighted some correlation with dexamethasone resistance *in vivo*[17].

Further experimental evidence of LSC activity in T-cell leukemia was acquired using mouse models of T-ALL based on the genes most commonly affected in patients. In particular, Dr. Hong Wu's group showed that in a PTEN-null T-ALL mouse model (*PTEN* is mutated at a frequency of 5%–10% in T-ALL[18]), the c-Kit^{mid}CD3+Lin– subset was the most efficiently transplantable in syngeneic recipients[19]. In the transgenic *TAL1*/*LMO1* mouse model of T-ALL, an enrichment of LSC activity was reported in CD4–CD8–CD44–CD25+ (DN3) and CD4–CD8–CD44–CD25– (DN4) fractions[20,21]. Finally, it was shown that in Notch1- and KrasG12D-induced T-ALL mouse models, LSCs are enriched in the CD44+ROS^{low} subset[22] (Table I). Those findings suggest that T-ALL might consist of different subpopulations, reflecting the complex heterogeneity of T-cell leukemia[9]. A better comprehension of LSC distribution in human T-ALL awaits complementary studies.

New discoveries about the functional mechanisms of LSC maintenance and propagation and the key regulatory pathways involved in LSC self-renewal in human and mouse models of T-ALL are emerging. Those new findings could contribute to the development of therapies that will be more efficient in specifically targeting the malignant stem-cell population and in reducing cytotoxic effects on normal stem-cell subsets.

SELF-RENEWAL PATHWAYS OF LSC ACTIVITY

Self-renewal is the main feature that discriminates stem-cell from differentiated-cell populations. It is defined as the capacity to self-renew for indefinite periods while the entire differentiation potential is preserved.

The role of stem cells is indeed to generate differentiated progeny while maintaining the undifferentiated stem-cell pool. This unique property of stem cells results from their ability to divide asymmetrically: Of two daughter cells, one retains the stem-cell characteristics; the other is destined for a limited number of future divisions and will produce even-more-specialized cells[23].

The functional mechanisms that control self-renewal in LSCs are not clearly described. Increasing evidence shows that common pathways regulate the self-renewal capacity in both normal and cancer stem cells and promote cancer

TABLE I Leukemia cell subsets enriched with leukemia stem cells in T-cell acute lymphoblastic leukemia

Reference	Cell-surface markers	Tumour type	Minimal tumourigenic dose (cells)	Transplant technique	Strain of recipient mice
Cox *et al.*, 2007[15]	CD34+CD4– or CD34+CD7–	Human	10^4	Intravenous injection	Immunodeficient (NSG[a])
Guo *et al.*, 2008[19]	c-Kit^{mid}CD3+Lin–	Pten$^{-/-}$ induced mouse leukemia	10^2	Intravenous injection	Immunodeficient (SCID)
Armstrong *et al.*, 2009[17]	CD7+CD1a–	Human	10^3	Intrafemoral injection	Immunodeficient (NSG[a])
McCormack *et al.*, 2010[20], Tremblay *et al.*, 2010[21]	CD4–CD8–CD44–CD25+ (DN3), CD4–CD8–CD44–CD25– (DN4)	*TAL1/SCL* and *LMO1/2* induced mouse model	10^3	Intravenous injection	Immunodeficient (Rag1$^{-/-}$)
Giambra *et al.*, 2012[22]	ROS^{low}	Human	10^5	Intravenous injection	Immunodeficient (NSG[a])
	ROS^{low}CD44+	Notch1 ΔE-induced mouse leukemia	10^2	Intravenous injection	Syngeneic (C57BL/6)
	ROS^{low}CD44+	Kras^{G12D}-induced mouse leukemia	10^3	Intravenous injection	Syngeneic (C57BL/6)

[a] The Jackson Laboratory, Bar Harbor, ME, U.S.A.

NSG = NOD SCID gamma; SCID = severe combined immunodeficiency; DN3/4 = double-negative 3/4; TAL1 = T-cell acute lymphocytic leukemia 1; LMO1/2 = LIM domain only 1/2; ROS = reactive oxygen species.

progression when deregulated[24]. The most relevant examples include the Notch1, Wnt, and hypoxia-inducible factor (HIF) cellular signalling pathways that modulate the maintenance and activity of LSCs in T-ALL[16,19,22,25,26]. Interestingly, acquisition of mutations within those pathways contributes to leukemic progression by promoting self-renewal and survival within supportive niches. In the subsections that follow, we review those three functional pathways, underlining their role in the activity of LSCs and progression of T-cell leukemia.

Notch1 Signalling Pathway

Notch1 signalling is one of main pathways involved in the proliferation, maintenance, and survival of T-cell leukemia; it is activated when the transmembrane receptor Notch1 recognizes ligands from the Jagged family (for example, JAG1 and JAG2) or delta-like proteins DLL1, DLL3, and DLL4 on neighbouring cells[27]. The binding of the extracellular subunit of Notch1 (ECN1) to its ligand activates several proteolytic cleavages and allows for the release and translocation of the intracellular Notch1 domain (ICN1) to the nucleus. The intracellular Notch1 domain acts as a transcription factor and forms part of a transcriptional complex with coactivators such as mastermind-like protein 1 and the DNA-binding transcription factor CSL, leading to the activation or repression of Notch1 target genes[28] [Figure 1(A)].

The constitutive expression of Notch1 in hematopoietic stem cells (HSCs) leads to immortalized and cytokine-dependent cell lines that are able to generate cell progenitors with lymphoid and myeloid features both *in vivo* and *in vitro*[29]. Using transgenic Notch1 reporter mice, it was also shown that the Notch1 signalling pathway is downregulated and less active in the differentiated hematopoietic cells of peripheral lymphoid organs. Inhibition of Notch1 signalling causes accelerated differentiation of HSCs *in vitro* and depletion of HSCs *in vivo,* suggesting that the Notch1 pathway is important for the maintenance of HSCs in an undifferentiated state[30].

In cancer, deregulation of Notch1 signalling can induce the transcriptional expression of several oncogenes and inhibit various tumour suppressors, leading to the generation of strong oncogenic signals involved in the malignant transformation of immature T progenitors and the maintenance of established leukemia. Some examples of oncogenic Notch1 targets include pre-TCRa, CD28, Deltex-1, HEY1, HES1, and c-Myc, that activate programs such as the MTOR (mammalian target of rapamycin)–dependent PI2K–Akt/PKB pathway, supporting cell-cycle progression and growth[31]. More than 50% of human T-ALL cases carry alterations in the *NOTCH1* gene itself[5]; 15% have mutations in *FBW7*/Sel-10, the F-box protein 7 involved in Notch1 turnover[6]. In both cases, it leads to hyperactivation of Notch1 signalling. About one quarter of human T-ALL cases show mutations in the extracellular heterodimerization domain of Notch1 receptor that weaken the interaction between the extracellular subunit of Notch1 and the ICN1, reducing the minimal signal required by a ligand to activate the pathway[32]. Deletion of the PEST domain in the C-terminal portion of Notch1 receptor occurs in 12% of T-ALL patients and increases the stability of intracellular Notch1 (or ICN1) by lowering the protein turnover. Both mutations have been detected simultaneously in more than 18% of human cases[5]. Notably, the Notch1 signalling pathway has been instrumental in the development of mouse models of T-ALL[5,33], which have helped achieve a better understanding of the role played by Notch1 in the self-renewal activity of LSCs[16,22,25].

In T-cell leukemia, the Notch1 signalling pathway promotes both the growth and the survival of bulk cells[34], but it has also been shown to play a role in the self-renewal of LSCs, as assayed by serial transplantation of primary human T-ALL into immunocompromised NOD/SCID mice[16]. Notably, in the *Tal1*/*Lmo2* mouse model of T-ALL, *in vivo* treatment of leukemic mice with γ-secretase inhibitors (GSIs) significantly reduced LSC frequency and decreased cell survival[25]. Both observations support the idea that the Notch1 pathway is required for LSC maintenance *in vitro* and *in vivo*.

It was also reported that several Notch1 target genes modulate the leukemia-initiating activity in T-ALL[22,35,36] [Figure 1(B)]. The deletion of insulin-like growth factor 1 receptor in Notch1-induced mouse leukemia reduces the transplantability of cells to secondary recipients and, consequently, the LSC frequency. Moreover, pharmacologic inhibition of insulin-like growth factor 1 receptor impairs the viability and growth of human T-ALL cells *in vitro*[35].

Expression of c-Myc was also correlated with LSC activity in T-ALL. In Notch1-induced mouse leukemia harbouring the c-Myc^{GFP} fusion allele, c-Myc^{GFP}–positive cell subsets were enriched in LSCs and, in microarray gene expression profiling, showed a gene signature similar to that of embryonic and hematopoietic stem cells. Interestingly, the selective BET bromodomain inhibitors—for example, JQ1—that specifically target BRD4, a transcriptional c-Myc activator, impair the *in vitro* growth of human T-ALL, representing a potential therapeutic strategy for the treatment of T-cell leukemia[36].

Our group demonstrated that the downregulation of protein kinase C θ expression correlates with low levels of reactive oxygen species and further modulates the LSC activity of CD44+ subsets in T-ALL. Protein kinase C θ is an indirect target of Notch1 in T-ALL and is suppressed by a Notch1-induced transcriptional circuit that involves the induced runt-related transcription factor 3 (RUNX3) and RUNX1. In particular, the Notch1 signalling pathway activates RUNX3 expression, which then represses RUNX1, a tumour suppressor that positively modulates protein kinase C θ expression[22] [Figure 1(B)]. Finally, compared with wild-type *NOTCH1* pediatric T-ALL, mutated *NOTCH1* pediatric T-ALL has a higher leukemia-initiating cell frequency within the CD34 compartment[37].

Overall, the foregoing observations demonstrate that the Notch1 signalling pathway sustains the self-renewal activity of LSCs in human T-ALL and suggest that novel and Notch1-specific drugs might improve clinical outcomes, especially for patients with refractory or relapsed leukemia.

Wnt Signalling Pathway

The Wnt signalling pathway is a well-known regulatory mechanism of self-renewal activity in cancer and stem-cell biology.

In the canonical Wnt pathway, the signal transduction cascade is started by the secretion of glycoproteins called

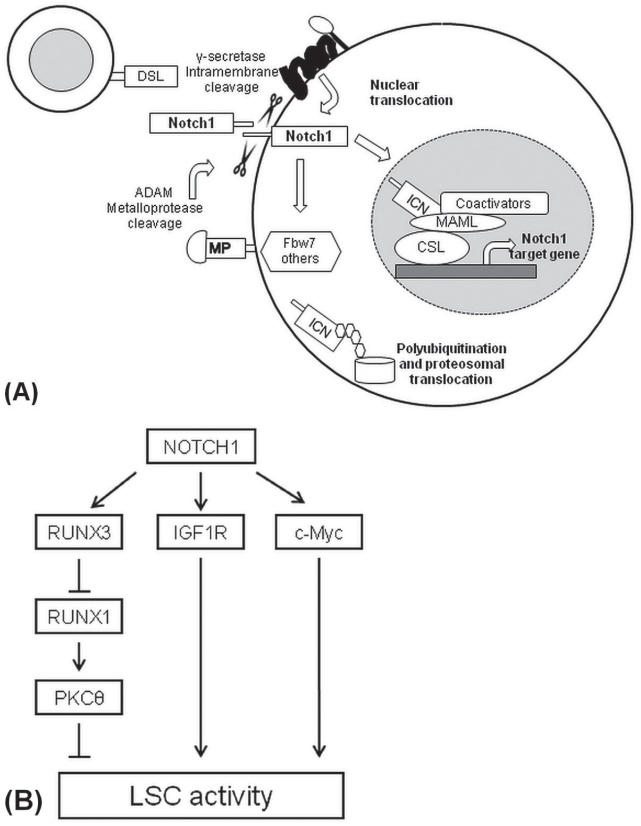

FIGURE 1 The Notch1 signalling pathway. (A) The binding of Notch1 ligand (DSL) with the Notch1 receptor activates two subsequent protease cleavages. Afterward, intracellular Notch1 (ICN1) translocates into the nucleus where it interacts with other coactivators in a protein complex for the induction of Notch1 target genes. ADAM = A disintegrin and metalloproteinase; MAML = mastermind-like protein; MP = metalloproteinase; CSL = CBF1/Su(H)/Lag-1 transcription factor complex. (B) The Notch1 signalling pathway promotes leukemia stem cell (LSC) activity by upregulation of *IGF1R* and *c-Myc* target genes and the indirect downregulation of protein kinase C–theta (PKCθ). RUNX3/1 = runt-related transcription factor 3/1.

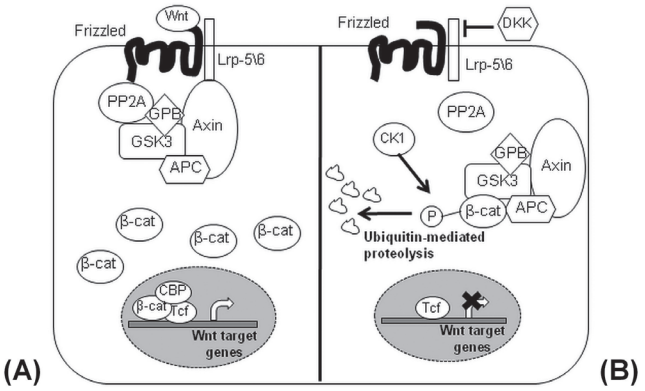

FIGURE 2 The canonical Wnt signalling pathway. (A) To activate the canonical Wnt signalling pathway, Wnt ligands bind the Frizzled and Lrp5/6 receptors and promote the stabilization of β-catenin after the recruitment and destruction of axin\APC\GSK3 complex. The activated β-catenin enters into the nucleus and induces the expression of Wnt target genes. APC = adenomatous polyposis coli; GSK3 = glycogen synthase kinase 3; PP2A = protein phosphatase 2A; GPB = GSK3 binding protein; CBP = CREB binding protein; Tcf = T-cell–specific transcription factor. (B) In the absence of Wnt ligands, the axin\APC\GSK3 complex leads to the proteasomal degradation of β-catenin after phosphorylation mediated by casein kinase 1α (CK1α). DKK = Dickkopf Wnt signalling pathway inhibitor; P = Phosphoryl group.

Wnts, members of a highly conserved family of 19 ligands that further modulate various cellular processes in the receptive cell, including growth, survival, polarity, and differentiation[38]. Wnt ligand binding to the Frizzled or LRP-5/6 receptors blocks the degradation of β-catenin that will then translocate into the nucleus and act as a coactivator alongside transcription factors from the T-cell factor/lymphoid enhancing factor (TCF/LEF) family. That complex induces the transcription of Wnt target genes such as cyclin D1 and c-Myc. When Frizzled or LRP-5/6 receptors are not engaged, Wnt signalling is not active, and β-catenin forms a protein complex with adenomatosis polyposis coli, axin, protein phosphatase 2A, casein kinase 1α, and glycogen synthase kinase 3, which promotes β-catenin phosphorylation by casein kinase 1α and glycogen synthase kinase 3 and its degradation after translocation to the proteasomal machinery[39] (Figure 2).

In normal hematopoietic development, the Wnt signalling pathway sustains the self-renewal activity of HSCs. Expression of activated β-catenin preserves HSCs in an undifferentiated state in long-term *in vitro* cultures, increasing their numbers by a factor of between 20 and 48[40]. Moreover, inhibition of Wnt signalling reduces the capacity of HSCs to reconstitute the hematopoietic system in secondary recipients, emphasizing the role of that pathway in lymphoid and myeloid lineage commitment and in the maintenance of HSCs[40]. Interestingly, the expression levels of HoxB4 and Notch1 increase in HSCs transduced with the activated form of β-catenin, suggesting that Wnt signalling might cooperate with the HoxB4 and Notch1 pathways to mediate its own functions[40,41].

Given the relevance of Wnt signalling in normal hematopoietic stem cells, the expectation is that, when deregulated, Wnt signalling might contribute to leukemia establishment and self-renewal activity by LSCs[26,42,43]. Aberrant pathway activation leads to the malignant transformation of mouse hematopoietic cells and produces both chronic and acute myeloid leukemia[39]. In mouse thymocytes, constitutive stimulation of the pathway by transduction with active forms of β-catenin generates T-cell leukemia within 60–80 days[44], and notably, LSCs of mouse and human T-ALL are characterized by elevated levels of β-catenin protein[19,26]. In approximately 85% of human cases of T-ALL, abnormally high expression of β-catenin is observed at the protein level in non-leukemic thymocytes[45]. Moreover, recent studies have demonstrated that Cre-mediated β-catenin stabilization in CD4Cre-*Ctnnb*^{A}*ex3* mice blocks normal T-cell development at the double-positive stage and predisposes the double-positive T-cells to malignant transformation. Notably, Notch1 activation was not required for β-catenin–induced lymphomagenesis, suggesting that activation of β-catenin might induce T-ALL

independently of Notch1[44]. Moreover, it was reported that, in human and mouse models of T-ALL, T-cell factor 1 (TCF-1) acts as a T-cell–specific tumour suppressor[46,47]. In particular, mice with a TCF-1–null genetic background developed T-cell leukemia and showed an aberrant upregulation of *Lef1* in pre-leukemic thymocytes and in established leukemic cells, supporting the idea that TCF-1 and LEF-1 have opposing but cooperative functions during the malignant transformation[47]. Those observations highlight the importance of TCF-1 and β-catenin as critical regulators of T-cell malignant transformation and leukemia maintenance.

The role of Wnt-signalling in leukemia stem-cell biology has been emphasized in human and mouse models of T-ALL[19,26]. In PTEN-null leukemia, the c-Kit^{mid}CD3+Lin– cell subsets, enriched in self-renewable LSCs, are characterized by high levels of unphosphorylated β-catenin and by an aberrant overexpression of *c-Myc,* a well-known Wnt target oncogene[48]. Moreover, the conditional deletion of the β-catenin gene impairs LSC frequency, suggesting that the Wnt signalling pathway might contribute to the maintenance of LSC activity in T-ALL and that the loss of PTEN might cooperate with β-catenin for LSC transformation[19].

Recently, using an integrated fluorescent reporter of Wnt signalling, it has been also reported that, in Notch1-induced mouse leukemia, only a small fraction of bulk leukemia cells shows active Wnt signalling. Those Wnt-active cells are highly enriched for LSC activity, and the deletion of β-catenin demonstrates a detrimental effect on LSC frequency[26]. Interestingly, blocking Wnt signalling in xenograft-expanded human T-ALL by transduction with lentivirus encoding dominant negative TCF[49] impairs the transplantability of human cells into immunocompromised secondary recipients, demonstrating their dependence on the canonical Wnt pathway. From a therapeutic perspective, inhibitors of Wnt signalling, such as the tankyrase inhibitor XAV-939[50] and indomethacin[42], reduce *in vitro* proliferation and survival of various human T-ALL cell lines and xenograft-expanded patient T lymphoblasts, suggesting that pharmacologic inhibitors of the Wnt pathway could potentially be used in therapies for the treatment of patients with aggressive T-ALL[26].

HIF-1 Signalling Pathway

Oxygen regulates major cellular processes, including proliferation, survival, and metabolism. In both normal and malignant hematopoietic compartments, conditions of restricted oxygen modulate the self-renewal activity and differentiation of stem subsets by the transcriptional activity of hypoxia inducible factors (HIFs)[51]. Those transcription factors are critical for the cellular response to low levels of oxygen or hypoxia (<3% O_2, 20 mmHg) and ensure cell growth in hypoxic microenvironments by mediating the switch from oxidative to glycolytic metabolism. The HIFs act as highly-conserved heterodimeric complexes composed of 2 subunits (α and β). The α subunits (HIF-1α, HIF-2α, HIF-3α) are sensitive to high concentrations of oxygen and are stable only under hypoxic conditions. The β subunit, known as HIF-1β or aryl hydrocarbon receptor nuclear translocator, is constitutively expressed and does not respond to oxygen levels[52] (Figure 3). Of the HIF-α isoforms, HIF-1α is the most studied and the most broadly expressed in various tissues.

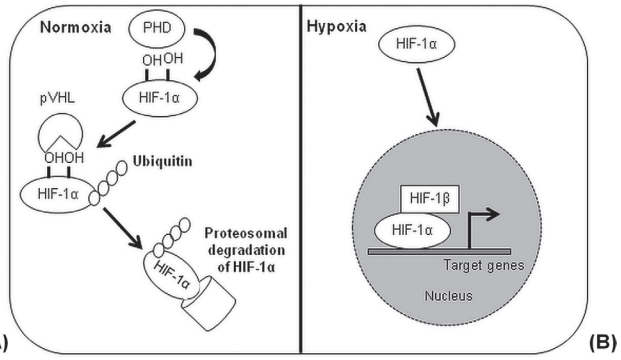

FIGURE 3 Regulation of the hypoxia-inducible factor 1α (HIF-1α) signalling pathway. (A) Under normoxic conditions, HIF-1α is degraded by the proteasomic machinery after hydroxylation and ubiquitination mediated by prolyl hydroxylases (PHDs) and the pVHL–E3–ubiquitin ligase complex. pVHL = von Hippel–Lindau tumour suppressor; E3 = ubiquitin-protein ligase. (B) In the presence of hypoxic microenvironments, HIF-1α is stable and translocates into the nucleus, where it forms a complex with the HIF-1β subunit for the activation of HIF target genes.

In presence of high oxygen concentrations (>3% O_2), HIF-1α is, like the other α subunits, hydroxylated at conserved proline sites by the HIF prolyl hydroxylases, triggering its degradation by the proteasome after ubiquitination by the von Hippel–Lindau ubiquitin E3 ligase complex[53]. The lack of oxygen under hypoxic or anoxic conditions inhibits the activity of HIF prolyl hydroxylase, which uses oxygen as a co-substrate and stabilizes HIF-1α, allowing the transcriptional activation of HIF-1α target genes[54].

Since the early 2000s, HIFs have been known for their importance for cancer stem cells in solid tumours[54]. However, emerging evidence suggests that HIF signalling plays a critical role in leukemogenesis and maintenance of LSCs. It was reported that the induction of HIF activity can promote a stem-like phenotype and increase the number of LSCs[51]. In acute myeloid leukemia, HIF-1α is overexpressed and selectively activated in CD34+CD38– LSC–enriched subsets, and notably, the pharmacologic inhibition of HIF-1α by echinomycin[55] affects the transplantability of human LSCs into immunocompromised mice[56]. In a mouse model of chronic myeloid leukemia, the deletion of HIF-1α in Bcr-Abl–expressing LSCs impairs the leukemogenic activity of LSCs after transplant into secondary recipients and induces the expression of p16(Ink4a) and p19(Arf), affecting the cell survival and promoting apoptosis[57].

In the Notch1-induced mouse model of T-ALL, it was recently reported that cell subsets enriched in LSCs and with active Wnt signalling reside preferentially within hypoxic niches *in vivo.* In particular, HIF-1α stabilization directly upregulates the expression of β-catenin at the transcriptional level, which potentiates Wnt signalling[26]. Moreover, HIF-1α deletion in mouse leukemia and HIF inhibition on patient-derived T-ALL xenografts by lentiviral small hairpin RNAs reduce the LSC frequency, suggesting that the HIF and Wnt/β-catenin signalling pathways cooperate together to support LSC function in T-ALL[26].

In human T-ALL, HIF-1α appears to be a critical regulator of the Notch1 pathway as well. The stabilization of HIF-1α potentiates Notch1 signalling and so alters the expression of two matrix metalloproteinases, MMP2 and MMP9, involved in the invasion and chemoresistance of leukemic cells[58]. In the LSC-enriched c-Kit+Sca1+ subsets of mouse T-cell lymphoma, generated by a mutant isoform of *Epm2a,* HIF-1α was also described to promote LSC activity by repressing *Hes1,* a Notch1-target gene and negative regulator of the Notch1 pathway[56]. Those observations indicate that HIF signalling is strictly connected with relevant pathways of LSC maintenance in T-cell leukemia and lymphoma (such as Notch1 and Wnt signalling) and that blocking HIF activity might improve conventional therapies by generating LSC-specific and less-detrimental treatments.

SUMMARY: TARGETING LEUKEMIA STEM CELLS

The idea that only restricted and self-renewing stem cells have the capacity to maintain and propagate malignant cell populations has strong implications about the way a tumour should be treated. Traditional therapies have been designed and applied to target the bulk of tumour mass, without any distinction between the various cell subsets. However, the failure of those therapies suggests that cancer stem cells might not be being efficiently targeted, even if the conventional remedies produce dramatic responses[59]. In modulating the Notch1, Wnt/β-catenin, and HIF pathways, stem-cell signalling pathway modifiers could thus hold promise as targeted agents in the treatment of refractory or relapsed T-ALL.

The pharmacologic drugs most widely known to suppress Notch signalling are GSIs that block the proteolytic cleavage mediated by γ-secretase and thus prevent the translocation of the intracellular Notch1 fragment ICN1 into the nucleus[60]. In phase I clinical trials, the antitumoural activities of several GSIs—for example, RO4929097 and MRK-003—have already been tested in mouse models of T-ALL and in relapsed T-ALL patients[61–63]. Those studies show that Notch1 signalling pathway inhibition by GSIs increases the sensitivity of leukemia cells to radiation and cytotoxic chemotherapy[64]. However, adverse side effects in leukemia patients and non-selectivity have been reported with GSIs[60]. To prevent gut toxicity, several approaches, such as treatment with glucocorticoids[65] or optimal dosing of GSIs, have been proposed[63]. Because gut toxicity is a result of Notch2 inhibition by GSI[66], another alternative is to use a Notch1-specific agent[67].

The effective inhibition of Wnt signalling is also a critical objective on the road to cure cancer. Negative physiologic regulators of Wnt signalling, such as the secreted Frizzled-related proteins and the Dickkopf-related protein 1, have been used as natural inhibitors of the Wnt pathway in cancer[68–70]. However, since about 2005, high-throughput screening of libraries containing small chemical compounds on cells transduced with Wnt reporters have identified various synthetic inhibitors of Wnt signalling—among them, the molecules XAV939 and pyrvinium. Specifically, XAV939 increases axin stability through the inhibition of tankyrase activity, and pyrvinium induces β-catenin phosphorylation through the activation of casein kinase[50,71]. Notably, alternative strategies such as blocking antibodies that target Wnt ligands or receptors have been also synthesized to hamper the Wnt signalling pathway[72,73].

A novel class of therapeutic agents, presently in clinical trials as anticancer agents, consists of drugs that specifically target HIF signalling[74]. Some effective inhibitors of the HIF pathway are echinomycin, a peptide antibiotic that blocks the binding of HIF-1α to DNA[55]; chetomin, which prevents the interaction of HIF-1α with the transcriptional coactivator p300–CBP[75]; the heat shock protein 90 inhibitor 17-allyl-aminogeldanamycin, which promotes a von Hippel–Lindau–independent degradation of HIF-1α[76]; and 2-methoxyestradiol, an inhibitor of microtubule polymerization[77]. Other small molecules, such as YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole], thioredoxin, and topoisomerase I inhibitors, have also been shown to reduce HIF-1α expression and xenograft growth[78,79]. However, ongoing screens are still in progress and might lead to the characterization of more selective and effective HIF-1 inhibitors in the near future.

In conclusion, observations suggest that leukemia stem cells might not be targeted using a single universal strategy. To achieve complete patient remission and to improve clinical outcomes by preventing disease relapse, future research must focus on the mechanisms of crosstalk by the self-renewal signalling pathways and the activity of other oncogenes and tumour suppressors.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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