Journal: Expert Opinion on Drug Discovery

Title: Approaches for targeting self-renewal pathways in cancer stem cells: implications for haematological treatments

Abstract:

Introduction: Self-renewal is considered a defining property of stem cells. Self-renewal is essential in embryogenesis and normal tissue repair and homeostasis. However, in cancer, self-renewal pathways, e.g. WNT, NOTCH, Hedgehog and BMP, frequently become de-regulated in stem cells, or more mature progenitor cells acquire self-renewal properties, resulting in abnormal tissue growth and tumorigenesis.

Areas Covered: This review considers the conserved embryonic self-renewal pathways, including WNT, NOTCH, Hedgehog and BMP. The article describes recent advances in our understanding of these pathways in leukaemia and, more specifically, leukaemia stem cells (LSC), how these pathways cross-talk and interact with the LSC microenvironment, and discusses the clinical implications and potential therapeutic strategies, both preclinical and in clinical trial for haematological malignancies.

Expert Opinion: The conserved embryonic self-renewal pathways are frequently de-regulated in cancer stem cells (CSC), including LSCs. There is significant cross-talk between self-renewal pathways, and their downstream targets, and the microenvironment. Effective targeting of these pathways is challenging due to cross-talk, and importantly, because these pathways are important for normal stem cells as well as CSC, adverse effects on normal tissues may mean a therapeutic window cannot be identified. Nonetheless, several agents targeting these pathways are currently in clinical trials in haematological malignancies.
(200 words)

Article Highlights:

- Self-renewal pathways, including WNT, NOTCH, Hedgehog and BMP are frequently de-regulated in haematological malignancies
- There is significant cross-talk between self-renewal pathways and their downstream targets
- Self-renewal pathways remain active in normal tissues and are important for tissue repair, and often targeting these conserved embryonic self-renewal pathways results in toxicity to normal cells *in vitro* and significant side effects in patients
- Small molecule inhibitors targeting the NOTCH and Hedgehog pathways are being used clinically in some solid tumours (e.g. breast cancer and basal cell carcinoma, respectively) and are in clinical trial in haematological malignancies
- SMO inhibitors, which target the Hedgehog pathway have recently shown some promise in acute myeloid leukaemia and myelofibrosis

Key words:
Self-renewal, cancer stem cell, leukaemia stem cell, microenvironment, Hedgehog, Wnt, Notch, bone morphogenic protein

1. Introduction:

Cancer stem cells (CSC) offer the concept that a small population of cells sharing characteristics of differentiation, self-renewal and homeostatic control, allow for the maintenance and dissemination of disease [1, 2]. Within haematological disease, these are referred to as leukaemic stem cells (LSCs). LSCs are mostly quiescent, in G0-phase and out of the cell cycle, and home to the bone marrow microenvironment, in which they are protected from apoptosis and conventional treatments [3-5]. Within a variety of haematological malignancies, including acute myeloid leukaemia (AML) [6], chronic myeloid leukaemia (CML) [5, 7], and multiple myeloma [8], the existence of LSCs has been identified. *In vivo* and *in vitro* experimental models have demonstrated that LSCs share many features with haemopoietic stem cells (HSCs) [9], including self-renewal and engraftment potential, but have also offered critical differences in functional properties [10], which allows for a therapeutic index of intervention, that would permit targeting of LSCs, whilst maintaining normal haematopoiesis.

AML LSCs were one of the first CSC populations to be characterised within haematological and solid malignancy [6, 11]. The complexity in morphological, genetic, and epigenetic heterogeneity within AML makes identifying an appropriate therapeutic target challenging, but may allow for LSC characterisation if the AML LSCs have distinct features according to their subtype. However, the immunophenotypic identification of the AML LSC remains a persistent area of debate [6, 12-14]. Historically, LSCs (both in AML and CML) have been characterised within the CD34+CD38- population, where their presence has been shown to be capable of generating leukaemic primary, secondary and tertiary engraftment in non-obese diabetic severe combined immunodeficient mice [6, 11]. However, recent studies have suggested that LSC activity can extend into the CD34+CD38+ population, with data indicating that leukaemia-initiating capacity could be present in a mature CD34- population [15, 16]. This suggests that LSCs can either be derived from HSCs or from progenitor cells that acquire self-renewal properties. Additional markers of AML LSCs have been proposed, including CD123 [17, 18], CD33 [18], CD45RA [19], CD47 [20], CD96 [21], and CD93 [22]. To date, none have been translated into the clinical setting for identification or targeting of the LSC. This is, in part, due to the intra-patient and inter-patient heterogeneity identified within AML LSC populations [16, 23] and has been particularly noted within relapse, where AML LSC frequency and phenotypic diversity has been shown to be much greater compared to the initial diagnostic LSC [24, 25].

Regardless of these complexities, the importance of AML LSCs clinically is well established. Most pertinently, within the identification of minimal residual disease (MRD), which resembles that of the diagnostic disease population, allows response to therapy to be followed over time, and offers a prognostic indicator for adverse outcome [26, 27]. It seems reasonable that LSCs reside within this population, having only been minimally impacted by conventional chemotherapy that targets dividing cells, and therefore, it stands that treatments focussing on the elimination of LSCs will reduce MRD and ultimately improve disease outcomes. Furthermore, it has been shown that AML patients with a greater number of LSCs or a more prevalent stem cell phenotype at diagnosis have inferior clinical outcomes compared to those who have fewer LSCs or a less prevalent stem cell phenotype [23, 28].

Although the most historic evidence of LSCs within haematological disease arise within AML, much insight into potential therapeutic strategies can be acquired from CML, where the chronic-phase disease represents a classic example of the stochastic CSC hypothesis model, without the molecular, epigenetic and genetic heterogeneity seen within AML. Furthermore, it is known that as the disease progresses to the acute phase, termed blast crisis (BC), committed progenitors gain self-renewal function; again highlighting the potential for LSC to be derived from a more mature progenitor population [29-31]. Therefore, understanding the LSC in CML may be clinically transferable to other stem cell driven diseases, including AML.

In CML, low-level *BCR-ABL* positive LSCs, as defined within a CD34+CD38- population, have been identified in the bone marrow of tyrosine kinase inhibitor (TKI)-treated CP-CML patients in deep molecular response (i.e. those that achieve a 4-log or greater reduction of quantitative *BCR-ABL* expression from standardised baseline over a prolonged period) [32-35]. These cells have been shown to be capable of growth in long-term culture-initiating cell (LTC-IC) assays and have murine engraftment potential, demonstrating their self-renewal capability. The demonstration that CML LSCs persist in the presence of a targeted therapy demonstrates the phenomenon of disease persistence, and highlights that LSCs are *BCR-ABL* independent, relying on other pathways to sustain their survival [36, 37]. The concept of disease persistence through quiescent LSCs has been further highlighted in recent years by trials exploring the discontinuation of TKIs in CP-CML patients with sustained deep molecular response [38-41]. These trials have demonstrated that discontinuation of therapy can be selectively achieved, with the most recent update of the 'Stop IMatinib' (STIM) trial data stating the cumulative incidence of molecular relapse at 60 months was 61% (CI 52-57%), with few cases of late relapse being observed [42]. This suggests that if molecular relapse is to occur, it happens early. It seems reasonable then, that targeting both the LSC population and *BCR-ABL* will lead to superior curative potential within CML.

A number of challenges remain within both the scientific and clinical communities in the eradication of LSCs, in both AML and CML. Firstly, in the identification of an appropriate specific target, or pan-target, that will enable eradication of the LSCs without a transient response. Secondly, when an appropriate target is identified that is clinically justifiable, the timings of intervention must be deduced, as well as the evaluation of disease persistence. This will remain a huge clinical challenge, especially in view that therapies that are only effective against the stem cell compartment (which represents 1-2% of bulk) may be difficult to evaluate due to the persistence of bulk malignant cells and concordant chemotherapy-based treatment. This review aims to evaluate scientific and clinical approaches for targeting self-renewal pathways in LSCs.

2. Signalling pathways as a target for LSC eradication

As discussed, self-renewal is considered to be the integral property of the LSC, and its deregulation is known to affect the development, maintenance, and persistence of LSCs in both AML and CML. To date, a number of aberrant signalling pathways have been proposed to contribute to the LSC phenotype [43-47]. These pathways, including hedgehog, Notch and Wnt, are known regulators of cell survival, and are often differentially expressed following genetic events. A number of deregulated proteins within these pathways may represent a broadly applicable therapeutic strategy, however, it is well known that these pathways rarely work in isolation, and rely on a web of activity leading to

disease maintenance, persistence, and progression. The complexities in interaction between these pathways are well documented and will be discussed within each heading below (figure 1). To add to this complexity in AML is the genetic variation that is seen, as this can mediate the proliferative and anti-apoptotic signals. Expression of specific oncogenes, such as *FLT3*, *RAS*, and *MLL*, may create new dependencies on specific signalling pathways in LSCs, and activate signalling pathways in isolation or simultaneously [48, 49]. Therefore, developing a therapeutic intervention that is efficacious in all activated pathways remains unachievable. Our best option remains to understand the acquired vulnerability in the mechanisms of the signalling pathways, which may offer a therapeutic window to eradicate the LSCs and plan for clinical translation.

3. Evolutionary conserved self-renewal pathways

Hedgehog signalling

The Hedgehog (Hh) signalling pathway has been shown to be inappropriately activated in a number of malignancies, including AML and CML, where it is intrinsically involved in the maintenance and expansion of the LSC compartment [50-52]. The Hh signalling pathway is an evolutionary conserved signalling pathway that is critical for embryonic development and adult homeostasis [53, 54]. Within haematological malignancy, activation of the pathway has been shown to be linked to primary immotile cilia [55, 56], where the receptor-ligand interaction causes the internalisation of PTCH1 and subsequent activation of Smoothened (SMO). This enables SMO to move into the cilium allowing for the accumulation of the active forms and the activation of key downstream targets [57]. SMO is the critical mediator in the canonical pathway and, therefore, represents a key therapeutic target to prevent the pathway's activation. SMO can be readily targeted with pharmacological agents, including cyclopamine, and clinical grade agents, such as LDE225 (sonidegib) [58] or PF-04449913 (glasdegib) [57, 59].

However, the pathway's complexity lies in its interactions and dependency with other key survival pathways. It has been suggested that the survival of CML progenitor cells is maintained by both the auto-activation of Hh and β-catenin [60]. Furthermore, Hh activation modulates NUMB-p53 responses, therefore, Hh suppression will subsequently alter p53 target genes; p53 is referred to as the guardian of the genome, therefore, careful evaluation of modulation of its function needs to be gained [61, 62]. p53 as a modulatory target in leukaemia is an area which is becoming of increasing interest; both in CML [63] and AML [64].

The importance of Hh signalling in CML is well established, with SMO inhibition leading to reduced self-renewal capacity of CML LSCs in both *in vitro* models with clinical grade SMO antagonists, and *in vivo*¸ where Smo-deficient mice have reduced leukaemogenesis in primary and secondary transplantation models [50, 52, 58]. Furthermore, combination of SMO antagonists with TKIs has been demonstrated to lead to a synergistic reduction of chronic phase (CP) CML LSCs in patient samples *in vitro* and CP and BC CML xenograft transplantation models [58, 65]. Our group has recently demonstrated that LDE225, a small molecule clinically investigated SMO inhibitor, used alone and in combination with the TKI, nilotinib, inhibited the Hh pathway in CD34+ CP-CML cells, reducing the number and self-renewal capacity of CML LSC *in vitro* [58]. The combination had no effect on normal HSCs and when combined, these agents reduced CD34+ CP-CML cell engraftment in NSG mice.

Furthermore, upon administration to EGFP+/SCLtTA/TRE-BCR-ABL mice, the combination enhanced survival with reduced leukaemia development in secondary transplant recipients.

Importantly, deregulation of Hh may potentially contribute to disease progression, with differential Hh activity increasing as CML progresses to BC [60], and increased gene expression of *PTCH1* has been observed in BC samples [46]. Therefore, early targeting of the pathway may be therapeutically viable to reduce disease progression.

Whilst preclinical data appears promising to support the Hh pathway as a therapeutic target in CML, clinical trials utilising SMO antagonists as a therapeutic option have halted in early phases. Within solid tumors, these inhibitors have successfully been translated into clinical practice [66, 67], however, within CML when combined with TKI, toxicity has been a major limitation [68, 69].

In AML, the role of Hh has not been fully elucidated, with limited data available on the implications of Hh deregulation on disease biology, perhaps due to the vast heterogeneity seen within the disease. Activation of Hh, through *SMO* and *GLI* expression, has been described within primary AML samples, particularly acute promyelocytic leukaemia (APML) [70, 71]; no *SMO* mutations have been identified to account for this increase in activity [55]. It is clear, however, that there is a prognostic significance in its expression, with increased *GLI1* and *GLI2* being associated with reduced overall survival and as a marker of prognosis, respectively [72].

Studies suggest that HHIP, a membrane-associated or soluble glycoprotein that functions to bind Hh ligands, can suppress leukaemic cell proliferation [73]; furthermore, within the same study, reduced stromal HHIP expression was shown to contribute towards the development of AML. *HHIP* can be modulated through standard chemotherapy agents, where it has been demonstrated that 5-azacitadine-mediated amplification of stromal cell *HHIP* expression led to attenuated leukaemic cell proliferation potential.

The biological significance of pathway modulation has yet to be fully understood, with varying results available. Genetic inactivation of *SMO* in MLL-AF9-transformed LSCs does not affect AML development in primary recipient mice [74]. Conversely, SMO inhibition with cyclopamine has been shown to reduce proliferation in myelomonocytic cell lines [55]. AML, as a disease, shows great heterogeneity and, therefore, focused evaluation through each of the classified disease entities needs to be undertaken, but it appears that there may be a therapeutic role for Hh inhibitors within a myelomonocytic phenotype. It is more clear that pharmacologic inhibition of Hh signalling appears to enhance AML 'gold-standard' therapy by sensitising LSCs to chemotherapy within the bone marrow microenvironment [75], which may lead to clinical advances in the eradication of AML LSCs. Currently, clinical trials are under way to investigate Hedgehog inhibitors in AML, and early phase 1/2 results appear promising [59, 76]. Of note, a subsequent phase 2 clinical trial of LDE225 in AML, NCT01826214, recently closed due to lack of efficacy as a single agent; perhaps highlighting the limited activity of SMO inhibition on bulk disease.

Wnt signalling

The Wnt signalling pathway plays an essential role in the maintenance and differentiation of LSCs and the propagation of malignancies [29, 77]. Its activation has been demonstrated in acute disease, namely AML LSCs and within myeloid BC CML [29, 78]. Furthermore, it has been suggested that a

deletion within β-catenin reduces the ability of mice to develop *Bcr-Abl* positive leukemias, which is suggestive of a role in the pathogenesis of chronic disease [47]. Therefore, targeting Wnt is a viable option in eradication of the LSC and in the prevention of disease progression and dissemination.

Within CML, loss of β-catenin in a murine model of CML impaired the development of the disease by inhibiting LSC self-renewal, and genetic and pharmacological inhibition of β-catenin activity synergised with TKI to target the loss of CML LSCs [29, 79]. CBP/catenin antagonists have demonstrated efficacy in eliminating the CML and acute lymphoblastic leukaemia (ALL) LSC population *in vitro* and *in vivo* [80, 81]. A similar importance is seen within AML, where high expression of Ctnnb1 has been reported to correlate with poor prognosis [82]. Deletion of β-catenin within murine models has been shown to significantly reduce development and transplantation of AML driven by MLL-AF9 or HoxA9 [83, 84]. In turn, within murine models of MLL-rearrangement AML it has been shown that self-renewal of LSCs is mediated, in part, by Ctnnb1, suggesting that Ctnnb1 may represent a therapeutic target within this subtype. Genetic and pharmacological inhibition of Ctnnb1 leads to decreased leukaemia formation. Interference with prostaglandin signalling has been shown to target the Wnt/β-catenin axis in HSCs [85], and treatment with COX inhibitors, such as indomethacin, has been shown to lead to a decrease of LSCs in secondary recipients [83]. This is mediated through Ctnnb1, although translating this clinically would be a challenge in view of adverse risk of bleeding. Inhibitors of canonical Wnt signalling are currently undergoing phase I clinical trials in AML (NCT01398462) [86].

Again, the interactions with other survival pathways, complicates antagonising Wnt/β-catenin. For example, within CML, TKI exposure has been shown to upregulate CD27 signalling, resulting in activation of Wnt target genes, which include Notch and c-Myc [87, 88]. Wnt-Notch interaction is well documented, particularly within the bone marrow microenvironment, where mutations of Ctnnb1 have been found in osteoblasts resulting in overexpression of Notch ligands and activation of the Notch pathway in HSCs [89].

The non-canonical Wnt (i.e. β-catenin independent) signalling pathways are diverse and can be initiated by WNT interaction with Frizzled receptors, or RYK and ROR receptor tyrosine kinases, to regulate small GTPases, as well as calcium flux and kinase cascades [90]. This area is not as well characterised in LSC maintenance as the canonical pathway [91]. Non-canonical signalling has been shown to exert an antagonistic effect on canonical signalling, with Wnt5a promoting GSK3β independent degradation of β-catenin and competing with Wnt3a for binding to the receptor complex [92]. A greater understanding of the non-canonical pathway may decipher an interesting therapeutic approach in β-catenin inhibition.

Notch signalling

Notch signalling is involved in a variety of cell-fate decisions that influence the development and function of many organs, including stem cell maintenance, cell proliferation, haemopoiesis and apoptosis [93, 94].

Its role in malignancy has been shown to be cell and tissue-dependent, with the pathway playing both oncogenic and tumour suppressive roles depending on cell and cancer type [95-98]. In haemopoietic malignancies, accumulating evidence demonstrates its importance in growth, differentiation, and apoptosis [96, 97, 99-102], with its role in T-ALL, chronic lymphocytic leukaemia (CLL), and B cell leukaemias and lymphomas well documented [100, 101, 103, 104]. Improved understanding of the

Notch signalling pathway in these malignancies suggests that the Notch pathway may be a prime drug target; however, the therapeutic role of Notch inhibition may be directly dictated by the effects of its inhibition on other cell lineages, including the myeloid lineage [105, 106].

Reports about the role which Notch plays in myeloid disease are conflicting, as Notch activation in myeloid precursors has been shown to promote self-renewal, induce and inhibit differentiation to monocytes, or induce apoptosis [107-110]. Early observations suggested that Notch signalling may play a role in myeloid progression [110-112], with its role best characterised within AML. Importantly, it has been shown that exposing AML cells to plate-bound Notch ligands led to a full range of responses from proliferation to growth arrest that varied with patient sample, suggesting again the difficultly evaluating signalling pathways due to inter-patient heterogeneity [113]. More recently, observations have supported a tumour suppressive role for Notch signalling in immature LSC compartments of AML disease models [113-117]. Furthermore, in AML cell lines and primary patient blasts, downregulation of Notch1 expression was associated with a decrease in PU.1-mediated differentiation capacity, indicating a pivotal role in maintenance of an immature state [114].

Within CML, the data is limited. In another myeloproliferative disorder, chronic myelomonocytic leukaemia, a tumour suppressor role for the Notch pathway was again described, supporting a loss-of-function hypothesis [116]. Conversely, however, a recent paper has identified an antagonistic role between Notch and TKIs within primitive samples; although the mechanisms have not been fully elicited, this perhaps is representative of cross-talk between signalling pathways [118]. Recent data from our group has suggested the importance of Notch activation within LTC-IC assays, where activation of the CD34+38- population through *Jagged1* led to a statistically significant reduction in colonies [119]. It remains to be seen if this pathway has a functional and, indeed, therapeutic role, within CML biology. There are no clinical trials underway evaluating Notch modulation in myeloid disease.

BMP signalling

The bone morphogenic proteins (BMPs) belong to the transforming growth factor-beta (TGF-β) superfamily and have been shown to be involved in diverse cellular functions, from apoptosis to self-renewal, in embryonic and adult phenotypes. Dysregulation within the BMP-TGF-β pathway is critical in LSC survival [120-122], particularly mediated by its downstream target genes in the Cdx-Hox axis.

Interaction between Wnt and BMP signalling regulate the Cdx family of homeobox transcription factors – the master regulators of Hox gene expression [123]. Cdx2 is aberrantly expressed in AML and promotes leukaemia propagation through deregulation of Hox genes [124], with its overexpression demonstrated in 90% of AML patients and overexpression *in vivo* leading to increased engraftment in NSG murine models. Aberrant expression of HOX genes has been linked to both AML and CML [124, 125], with overexpression of HoxB3 [126], HoxB8 [127], or HoxA10 [126] leading to the generation of acute leukaemia in murine models, as well as being associated with expansion of the HSC compartment in *in vitro* and *in vivo* models [128, 129]. Although this represents an interesting target of both Wnt and BMP, no translational evidence is available for an antagonistic effect in LSC regression.

Genomic studies within primary CD34+ CML samples suggest that components of the pathway, including target genes, are downregulated [31, 130, 131]. This raises the possibility that the pathway can be activated through extrinsic mechanisms, and emphasises the role of the bone marrow microenvironment in the protection of LSCs against TKI-mediated apoptosis. It has been shown that type 1 receptors are present on LSCs in primary CML samples, with an associated downregulation of BMP ligands [120, 131]. CML aspirate and trephine bone marrow samples had significantly higher levels of BMP2 and BMP4 compared to normal donors. This suggests that there is the ability to upregulate the BMP pathway and that it is via extrinsic mechanisms within the diseased bone marrow microenvironment/niche. Laperrousaz *et al* [120] demonstrated that expression of BMP2 and BMP4 varied depending on niche cell type, with BMP2 and BMP4 being more highly expressed in polymorphonuclear cells and endothelial sinusoid cells, respectively. In response to increased levels of soluble BMP2 and BMP4, they showed that CML LSCs maintained their primitive phenotypes and enhanced long-term colony formation potential, indicating that the BMP pathway can suppress differentiation and potentiate LSC survival.

The Bone Marrow Microenvironment

Because LSCs home to the bone marrow microenvironment, it seems pertinent that the bone marrow is considered within cell-to-cell interactions and activation of aberrant self-renewal signalling. The interactions between LSCs and the bone marrow remain an important area of research and may determine the best strategy for eradication of the LSC. In many myeloid leukaemias' there is enhanced osteoblastic proliferation and a marked increase in LSCs and progenitor expansion [132]. LSCs rely on the bone marrow niche for their survival and modulate it to enhance survival, and a number of key interactions with self-renewal pathways contribute to the chemo-resistance that is seen. Deregulation of BMP has been shown in murine models where Bmpr1a/Alk3 conditional knockout mice have impaired BMP signaling, which leads to increased niche size and thereby enhanced numbers of HSCs [133]. Furthermore, a number of ligand-receptor mediated pathways regulate CML LSC, and in turn alter signaling pathways responsible for their maintenance, including MPL which regulates JAK/STAT signaling [134]. MPL has been shown in high levels to lead to reduced TKI sensitivity in CML, although, in turn, a higher sensitivity to JAK inhibitors. Expansion of the osteoblast layer of the CML bone marrow microenvironment can contribute to creating a hostile environment for HSC, mediated through alterations in TGFβ, NOTCH and pro-inflammatory signalling [132, 135]. However, there are difficulties in utilising the bone marrow microenvironment therapeutically – how should the pathways within the marrow be targeted? Would peripheral injections suffice to a large enough concentration of blockade without leading to adverse effects?

4. Conclusion

Aberrant signalling proteins have been extensively identified and evaluated within LSC biology. These represent a smaller number of signalling pathways. Preclinical data suggests that targeting these unifying pathways may offer an attractive, and more broadly applicable, therapeutic strategy to eradicate the LSC compared to chemotherapy alone. However, these signalling pathway interactions make them inherently complex, and modulating one may down- or up-regulate another, with biologically significant consequences. Furthermore, there is limited understanding of the biology of the LSC following treatment, whether that be with systemic or targeted chemotherapy, with most preclinical studies utilising treatment-naïve patient samples, or murine models. Further work is

needed to understand these intricacies. With increased scientific understanding, the next question will relate to translating these targets from bench to bedside, and with this many questions will unfold.

5. Expert Opinion:

The above suggests that as LSCs retain dependency on self-renewal pathways, they could be selectively targeted if the complexities in their interactions are fully understood. Disappointingly, despite strong *in vitro* and *in vivo* preclinical data, drugs against these targets have yet to be implemented in the clinical setting as a standard of care. This is, in part, due to trial design, as well as toxicities that are generated, particularly in areas with high cell turnover, such as the gastrointestinal tract.

The clinical need for alternative and more effective therapies in AML and CML are different. AML represents a disease where there has been little progress in therapeutic strategies, with improvement in survival likely secondary to better supportive measurements rather than improvements in standard chemotherapeutics. The need for targeted and individualised treatments is a necessity in the disease for improved outcome and eventual cure. However, the targeting of LSCs is likely only to be effective in a minority of patients and will vary, dependent on the sub-class of disease. The difficulty remains in 1) identification of LSC in bulk samples with no established immunophenotype, and 2) understanding the differences in signalling pathways within an epigenetic, genetic, and morphologically heterogeneous disease.

Within CML, there has been an overall improvement in survival of approximately ten-fold since the introduction of targeted therapy against *BCR-ABL* [136]. This has led to the majority of patients achieving close to normal life expectancy, when treated with TKIs. The need for eradication of the LSC is necessary to enable complete eradication of CML and subsequent cure, allowing patients to stop therapy, and to alleviate a financial burden within healthcare systems. Like AML, a biomarker of the LSC needs to be identified and sensitive enough to identify CML LSCs at low level following treatment. A number of potential biomarkers have been described, including CD26 [137], and IL1-RAP [138]. Our group has described the role of CD93 as a potential biomarker of the quiescent LSC population within CML [139]. Although identification of the CML LSC appears to be within reach, little is known about the biology of the LSC in those patients that are in deep molecular response, with much of our understanding and the previous data within a drug-naïve population. Clinically, the therapeutic need will be in those that are on TKI therapy.

The biological understanding of aberrant self-renewal pathways within the LSC and potential targets is well underway, however the preparation for translation into the clinical setting needs to be considered. Firstly, as stated above, within each disease the immunophenotypic characterisation of the LSC needs to be established. When this is verified and supported internationally, there are considerations that need to be addressed to translate targeted inhibition or activation clinically.

Firstly, decisions regarding evaluation of response to self-renewal modulation. Within an *in vivo* and *in vitro* setting, these responses are often generated by utilising immature populations of sorted cells or genetically manipulating murine models. Within a clinical setting, the response of bulk disease cannot be used as a surrogate for the clinical effect on the LSC population as there are differential sensitivities between the bulk and LSC populations, nor can evaluation of survival be used as a surrogate in treatment evaluation. Clearly, established markers of the LSC (e.g. murine engraftment,

LTC-IC assessment, replating efficiency) may be used as a surrogate for response, or an MRD evaluation could be used to generate some understanding of the proportion of LSC at diagnosis and then following treatment.

Secondly, comparing new targeted approaches to standardised chemotherapy regimens needs to be carefully executed. The use of gemtuzumab ozogamicin in AML has directed understanding of the evaluation of a targeted response compared to standard treatments [140, 141], with a realisation that often a targeted therapeutic approach within bulk samples will require a longer treatment period to achieve a clinical response. Can overall survival be used as an endpoint if standard chemotherapy eradicates bulk disease, whereas targeted approaches are acting as an adjunctive therapy that requires longer duration to see an effect?

Thirdly, treatment timings of intervention will be essential in the understanding of disease biology. The treatments could be started simultaneously to allow for the likely longer duration needed for the targeted therapy to achieve a response. But this would cause difficulties in an appropriate primary clinical end-point and evaluation of the LSC response to therapy. Alternatively, the treatments could be sequenced to allow for identification of those patients, particularly with CML, where targeted therapy is not needed, and in AML, in those that have MRD positivity despite chemotherapy. This would, however, open the potential for clonal evolution of the disease, that may render it more difficult to treat and skew results of an LSC-targeted approach. With a concurrent approach, it is likely that drug toxicities may be a clinical issue, rendering the approach undeliverable as a standard-of-care.

Therefore, not only is an in-depth understanding of aberrant signalling pathway biology within LSCs required for generation of appropriate and justifiable therapeutic targets in the eradication of LSCs, careful consideration in the isolation and identification of LSCs, endpoint response, and timing of therapy is needed to enable translation of the therapeutic targets into a clinical setting.

Figure Legends

Figure 1. **Complex interactions between self-renewal pathways**. The interconnectivity between self-renewal pathways is well documented within the LSC. It is known that proteins within each of the pathways, namely hedgehog, Wnt, and Notch, can both antagonise and agonise the other pathways by cross talk leading to both up- and downregulation of downstream targets.

REFERENCES

1. Alison MR, Lim SML, Nicholson LJ. Cancer stem cells: problems for therapy? Journal of Pathology. 2011;223(2):147-61.
2. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. Nature. 2001;414(6859):105-11.
3. Busfield SJ, Biondo M, Wong M, et al. Targeting of acute myeloid leukemia in vitro and in vivo with an anti-CD123 mAb engineered for optimal ADCC. Leukemia. 2014;28(11):2213-21.
4. Crews LA, Jamieson CHM. Chronic Myeloid Leukemia Stem Cell Biology. Current Hematologic Malignancy Reports. 2012;7(2):125-32.
5. Zhang B, Li M, McDonald T, et al. Microenvironmental protection of CML stem and progenitor cells from tyrosine kinase inhibitors through N-cadherin and Wnt-beta-catenin signaling. Blood. 2013;121(10):1824-38.
6. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730-7.
7. Graham SM, Vass JK, Holyoake TL, et al. Transcriptional analysis of quiescent and proliferating CD34+human hemopoietic cells from normal and chronic myeloid leukemia sources. Stem Cells. 2007;25(12):3111-20.
8. Yaccoby S, Barlogie B, Epstein J. Primary myeloma cells growing in SCID-hu mice: A model for studying the biology and treatment of myeloma and its manifestations. Blood. 1998;92(8):2908-13.
9. Bhatia M, Wang JCY, Kapp U, et al. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(10):5320-5.
10. Jordan CT, Guzman ML, Noble M. Mechanisms of disease - Cancer stem cells. New England Journal of Medicine. 2006;355(12):1253-61.
11. Lapidot T, Sirard C, Vormoor J, et al. A Cell Initiating Human Acute Myeloid-Leukemia after Transplantation into Scid Mice. Nature. 1994;367(6464):645-8.
12. Blair A, Hogge DE, Ailles LE, et al. Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. Blood. 1997;89(9):3104-12.
13. Blair A, Hogge DE, Sutherland HJ. Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34(+)/CD71(-)/HLA-DR-. Blood. 1998;92(11):4325-35.

14. Jordan CT, Upchurch D, Szilvassy SJ, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia. 2000;14(10):1777-84.
15. Sarry JE, Murphy K, Perry R, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2R gamma c-deficient mice. Journal of Clinical Investigation. 2011;121(1):384-95.
16. Taussig DC, Vargaftig J, Miraki-Moud F, et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34(-) fraction. Blood. 2010;115(10):1976-84.
17. Al-Mawali A, Gillis D, Lewis I. Immunoprofiling of leukemic stem cells CD34+/CD38-/CD123+delineate FLT3/ITD-positive clones. Journal of Hematology & Oncology. 2016;9.
18. Ehninger A, Kramer M, Rollig C, et al. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. Blood Cancer Journal. 2014;4.
19. Kersten B, Valkering M, Wouters R, et al. CD45RA, a specific marker for leukaemia stem cell sub-populations in acute myeloid leukaemia. British Journal of Haematology. 2016;173(2):219-35.
20. Majeti R, Chao MP, Alizadeh AA, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009;138(2):286-99.
21. Hosen N, Park CY, Tatsumi N, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(26):11008-13.
22. Iwasaki M, Liedtke M, Gentles AJ, et al. CD93 Marks a Non-Quiescent Human Leukemia Stem Cell Population and Is Required for Development of MLL-Rearranged Acute Myeloid Leukemia. Cell Stem Cell. 2015;17(4):412-21.
23. Eppert K, Takenaka K, Lechman ER, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. Nature Medicine. 2011;17(9):1086-U91.
24. Mendler JH, Balys M, Sivagnanalingam U, et al. Distinct Properties of Leukemia Stem Cells in Primary Refractory Acute Myeloid Leukemia. Blood. 2015;126(23).
25. van Rhenen A, Moshaver B, Kelder A, et al. Aberrant marker expression patterns on the CD34+CD38-stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. Leukemia. 2007;21(8):1700-7.
26. Feller N, van der Pol MA, van Stijn A, et al. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia. Leukemia. 2004;18(8):1380-90.
27. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. New England Journal of Medicine. 2016;374(5):422-33.
28. van Rhenen A, Feller N, Kelder A, et al. High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clinical Cancer Research. 2005;11(18):6520-7.
29. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med. 2004;351(7):657-67.
30. Kinstrie R, Karamitros D, Goardon N, et al. Heterogeneous leukemia stem cells in myeloid blast phase chronic myeloid leukemia. Blood Advances. 2016;1(3):160-9.
31. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2006;103(8):2794-9.

32. Bhatia R, Holtz M, Niu N, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood. 2003;101(12):4701-7.
33. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. Blood. 2011;118(13):3657-60.
34. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients in deep molecular response induced by tyrosine kinase inhibitors and the impact of therapy discontinuation. Oncotarget. 2016;7(23):35293-301.
35. Chu S, McDonald T, Lin A, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. Blood. 2011;118(20):5565-72.
36. Corbin AS, Agarwal A, Loriaux M, et al. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest. 2011;121(1):396-409.
37. Hamilton A, Helgason GV, Schemionek M, et al. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. Blood. 2012;119(6):1501-10.
38. Mahon FX, Rea D, Guilhot J, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029-35.
39. Richter J, Mahon FX, Guilhot J, et al. Stopping Tyrosine Kinase Inhibitors in a Very Large Cohort of European Chronic Myeloid Leukemia Patients: Results of the Euro-Ski Trial. Haematologica. 2016;101:22-3.
40. Ross DM, Branford S, Seymour JF, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. Blood. 2013;122(4):515-22.
41. Takahashi N, Kyo T, Maeda Y, et al. Discontinuation of imatinib in Japanese patients with chronic myeloid leukemia. Haematologica-the Hematology Journal. 2012;97(6):903-6.
42. Etienne G, Guilhot J, Rea D, et al. Long-Term Follow-Up of the French Stop Imatinib (STIM1) Study in Patients With Chronic Myeloid Leukemia. Journal of Clinical Oncology. 2017;35(3):298-+.
43. Liao HF, Su YC, Zheng ZY, et al. Sonic hedgehog signaling regulates Bcr-Abl expression in human chronic myeloid leukemia cells. Biomedicine & Pharmacotherapy. 2012;66(5):378-83.
44. Martelli AM, Evangelisti C, Chiarini F, et al. The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in normal myelopoiesis and leukemogenesis. Biochimica Et Biophysica Acta-Molecular Cell Research. 2010;1803(9):991-1002.
45. Naughton R, Quiney C, Turner SD, et al. Bcr-Abl-mediated redox regulation of the PI3K/AKT pathway. Leukemia. 2009;23(8):1432-40.
46. Sengupta A, Banerjee D, Chandra S, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. Leukemia. 2007;21(5):949-55.
47. Zhao C, Blum J, Chen A, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell. 2007;12(6):528-41.

48. Somervaille TCP, Matheny CJ, Spencer GJ, et al. Hierarchical Maintenance of MLL Myeloid Leukemia Stem Cells Employs a Transcriptional Program Shared with Embryonic Rather Than Adult Stem Cells. Cell Stem Cell. 2009;4(2):129-40.
49. Stirewalt DL, Kopecky KJ, Meshinchi S, et al. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. Blood. 2001;97(11):3589-95.
50. Dierks C, Beigi R, Guo GR, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell. 2008;14(3):238-49.
51. Long B, Zhu H, Zhu C, et al. Activation of the Hedgehog pathway in chronic myelogeneous leukemia patients. J Exp Clin Cancer Res. 2011;30:8.
52. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature. 2009;458(7239):776-9.
53. Hui CC, Angers S. Gli Proteins in Development and Disease. Annual Review of Cell and Developmental Biology, Vol 27. 2011;27:513-37.
54. Ingham PW, Placzek M. Orchestrating ontogenesis: variations on a theme by sonic hedgehog. Nature Reviews Genetics. 2006;7(11):841-50.
55. Campbell VL, Tarafdar A, Dobbin E, et al. Unravelling the complexities of hedgehog mediated signal transduction in acute myeloid leukaemia and normal haematopoiesis. Blood. 2016;128:2881.
56. Singh M, Chaudhry P, Merchant AA. Primary cilia are present on human blood and bone marrow cells and mediate Hedgehog signaling. Experimental Hematology. 2016;44(12):1181-7.
57. Irvine DA, Copland M. Targeting hedgehog in hematologic malignancy. Blood. 2012;119(10):2196-204.
58. Irvine DA, Zhang B, Kinstrie R, et al. Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. Scientific Reports. 2016;6:25476.
59. Martinelli G, Oehler VG, Papayannidis C, et al. Treatment with PF-04449913, an oral smoothened antagonist, in patients with myeloid malignancies: a phase 1 safety and pharmacokinetics study. Lancet Haematology. 2015;2(8):E339-E46.
60. Su W, Meng F, Huang L, et al. Sonic hedgehog maintains survival and growth of chronic myeloid leukemia progenitor cells through beta-catenin signaling. Exp Hematol. 2012;40(5):418-27.
61. Colaluca IN, Tosoni D, Nuciforo P, et al. NUMB controls p53 tumour suppressor activity. Nature. 2008;451(7174):76-U11.
62. Sheng WW, Dong M, Zhou JP, et al. Cooperation among Numb, MDM2 and p53 in the development and progression of pancreatic cancer. Cell and Tissue Research. 2013;354(2):521-32.
63. Abraham SA, Hopcroft LEM, Carrick E, et al. Dual targeting of p53 and c-MYC selectively eliminates leukaemic stem cells. Nature. 2016;534(7607):341- 6.
64. Latif AL, Cole JJ, Campos JM, et al. Dual Inhibition of MDM2 and BET Cooperate to Eradicate Acute Myeloid Leukemia. Blood. 2015;126(23):674.
65. Sadarangani A, Pineda G, Lennon KM, et al. GLI2 inhibition abrogates human leukemia stem cell dormancy. J Transl Med. 2015;13:98.
66. Rudin CM, Hann CL, Laterra J, et al. Brief Report: Treatment of Medulloblastoma with Hedgehog Pathway Inhibitor GDC-0449. New England Journal of Medicine. 2009;361(12):1173-8.

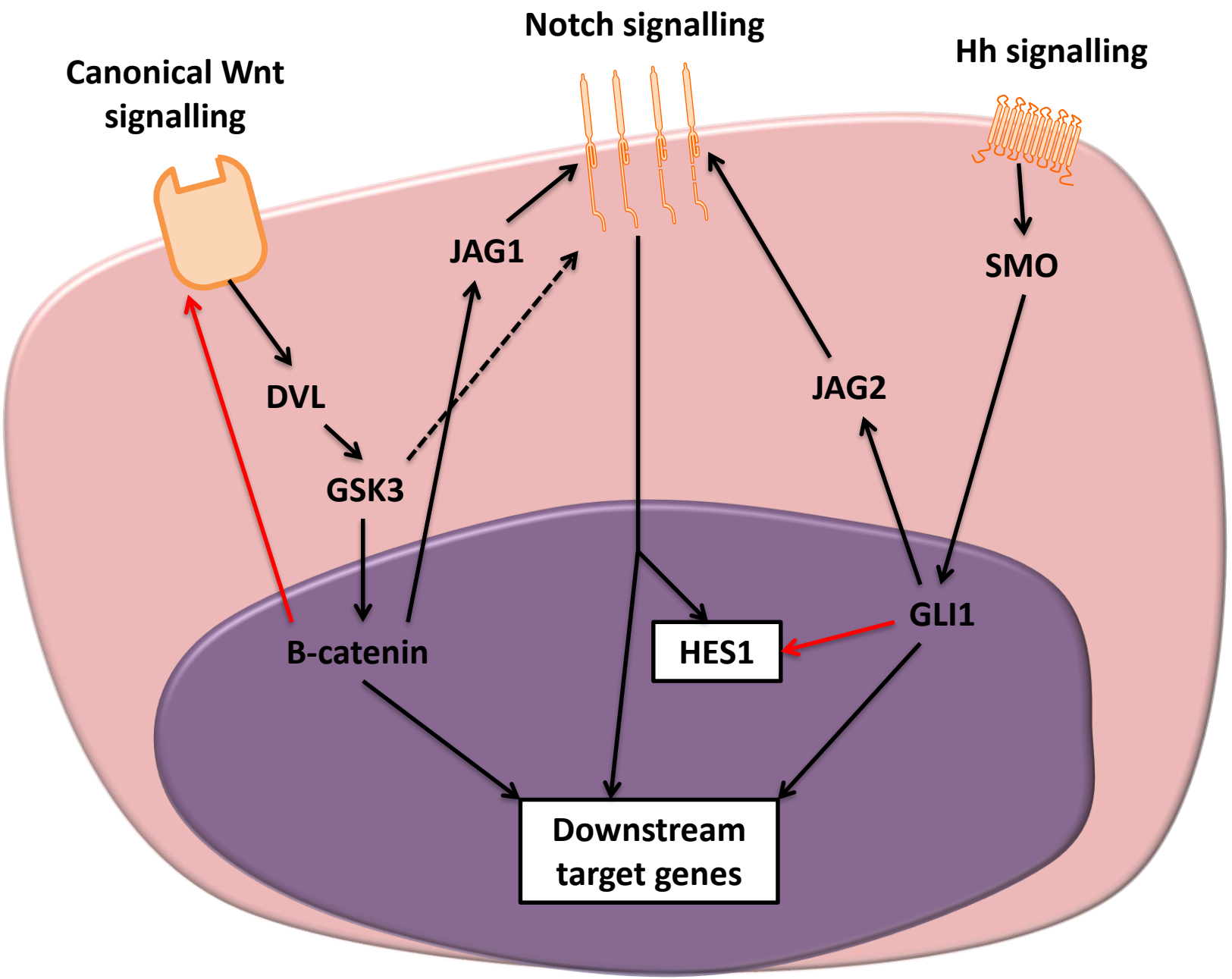
67. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the Hedgehog Pathway in Advanced Basal-Cell Carcinoma. New England Journal of Medicine. 2009;361(12):1164-72.
68. Ottmann O, Charbonnier A, Stegelmann F, et al. Smoothened (Smo) Inhibitor Lde225 Combined with Nilotinib in Patients with Chronic Myeloid Leukemia (Cml) Resistant/Intolerant (R/I) to at Least 1 Prior Tyrosine Kinase Inhibitor: A Phase 1b Study. Haematologica. 2015;100:62-3.
69. Shah NP, Cortes JE, Martinelli G, et al. Dasatinib Plus Smoothened (SMO) Inhibitor BMS-833923 in Chronic Myeloid Leukemia (CML) with Resistance or Suboptimal Response to a Prior Tyrosine Kinase Inhibitor (TKI): Phase I Study CA180323. Blood. 2014;124(21).
70. Bai LY, Chiu CF, Lin CW, et al. Differential expression of Sonic hedgehog and Gli1 in hematological malignancies. Leukemia. 2008;22(1):226-8.
71. Yang DG, Cao FL, Ye XM, et al. Arsenic Trioxide Inhibits the Hedgehog Pathway Which Is Aberrantly Activated in Acute Promyelocytic Leukemia. Acta Haematologica. 2013;130(4):260-7.
72. Wellbrock J, Latuske E, Kohler J, et al. Expression of Hedgehog Pathway Mediator GLI Represents a Negative Prognostic Marker in Human Acute Myeloid Leukemia and Its Inhibition Exerts Antileukemic Effects. Clin Cancer Res. 2015;21(10):2388-98.
73. Kobune M, Iyama S, Kikuchi S, et al. Stromal cells expressing hedgehog-interacting protein regulate the proliferation of myeloid neoplasms. Blood Cancer J. 2012;2:e87.
74. Hofmann I, Stover EH, Cullen DE, et al. Hedgehog Signaling Is Dispensable for Adult Murine Hematopoietic Stem Cell Function and Hematopoiesis. Cell Stem Cell. 2009;4(6):559-67.
75. Fukushima N, Minami Y, Kakiuchi S, et al. Small-molecule Hedgehog inhibitor attenuates the leukemia-initiation potential of acute myeloid leukemia cells. Cancer Science. 2016;107(10):1422-9.
76. Cortes J, Heidel FH, Heuser M, et al. A phase 2 randomized study of low dose ara-C with or without glasdegib (PF-04449913) in untreated patients with acute myeloid leukemia ir high-risk myelodysplastic syndrome. Blood. 2016;128:99.
77. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127(3):469-80.
78. Ho TC, LaMere M, Stevens BM, et al. Evolution of acute myelogenous leukemia stem cell properties after treatment and progression. Blood. 2016;128(13):1671-8.
79. Heidel FH, Bullinger L, Feng ZH, et al. Genetic and Pharmacologic Inhibition of beta-Catenin Targets Imatinib-Resistant Leukemia Stem Cells in CML. Cell Stem Cell. 2012;10(4):412-24.
80. Gang EJ, Hsieh YT, Pham J, et al. Small-molecule inhibition of CBP/catenin interactions eliminates drug-resistant clones in acute lymphoblastic leukemia. Oncogene. 2014;33(17):2169-78.
81. Zhao Y, Masiello D, McMillian M, et al. CBP/catenin antagonist safely eliminates drug-resistant leukemia-initiating cells. Oncogene. 2016;35(28):3705-17.
82. Ysebaert L, Chicanne G, Demur C, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. Leukemia. 2006;20(7):1211-6.
83. Wang YZ, Krivtsov AV, Sinha AU, et al. The Wnt/beta-Catenin Pathway Is Required for the Development of Leukemia Stem Cells in AML. Science. 2010;327(5973):1650-3.
84. Yeung J, Esposito MT, Gandillet A, et al. beta-Catenin Mediates the Establishment and Drug Resistance of MLL Leukemic Stem Cells. Cancer Cell. 2010;18(6):606-18.

85. Goessling W, North TE, Loewer S, et al. Genetic Interaction of PGE2 and Wnt Signaling Regulates Developmental Specification of Stem Cells and Regeneration. Cell. 2009;136(6):1136-47.
86. Cortes JE, Carter BZ, Quintas-Cardama A, et al. A Phase I Dose-Escalation Study of Pri-724, a Cbp/B-Catenin Modulator in Patients with Advanced Acute Myeloid Leukemia (Aml). Haematologica. 2014;99:222-3.
87. Riether C, Schurch CM, Flury C, et al. Tyrosine kinase inhibitor-induced CD70 expression mediates drug resistance in leukemia stem cells by activating Wnt signaling. Science Translational Medicine. 2015;7(298).
88. Schurch C, Riether C, Matter MS, et al. CD27 signaling on chronic myelogenous leukemia stem cells activates Wnt target genes and promotes disease progression. Journal of Clinical Investigation. 2012;122(2):624-38.
89. Kode A, Manavalan JS, Mosialou I, et al. Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. Nature. 2014;506(7487):240-+.
90. Ford CE, Ma SSQ, Quadir A, et al. The dual role of the novel Wnt receptor tyrosine kinase, ROR2, in human carcinogenesis. International Journal of Cancer. 2013;133(4):779-87.
91. Shen YL, Luo Q, Guo YX, et al. Bone marrow mesenchymal stem cell-derived Wnt5a inhibits leukemia cell progression in vitro via activation of the non-canonical Wnt signaling pathway. Oncology Letters. 2014;8(1):85-90.
92. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nature Reviews Cancer. 2013;13(1):11-26.
93. Chiba S. Notch signaling in stem cell systems. Stem Cells. 2006;24(11):2437-47.
94. Bray SJ. Notch signalling in context. Nature Reviews Molecular Cell Biology. 2016;17(11):722-35.
95. Capaccione KM, Pine SR. The Notch signaling pathway as a mediator of tumor survival. Carcinogenesis. 2013;34(7):1420-30.
96. Pancewicz J, Nicot C. Current views on the role of Notch signaling and the pathogenesis of human leukemia. Bmc Cancer. 2011;11.
97. Schwanbeck R, Just U. The Notch signaling pathway in hematopoiesis and hematologic malignancies. Haematologica-the Hematology Journal. 2011;96(12):1735-U21.
98. Yugaw T, Nishino K, Ohno SI, et al. Noncanonical NOTCH Signaling Limits Self-Renewal of Human Epithelial and Induced Pluripotent Stem Cells through ROCK Activation. Molecular and Cellular Biology. 2013;33(22):4434-47.
99. Chiang MY, Shestova O, Xu LW, et al. Divergent effects of supraphysiologic Notch signals on leukemia stem cells and hematopoietic stem cells. Blood. 2013;121(6):905-17.
100. Etet PFS, Vecchio L, Kamdje AHN. Interactions between bone marrow stromal microenvironment and B-chronic lymphocytic leukemia cells: Any role for Notch, Wnt and Hh signaling pathways? Cellular Signalling. 2012;24(7):1433-43.
101. Hannon MM, Lohan F, Erbilgin Y, et al. Elevated TRIB2 with NOTCH1 activation in paediatric/adult T-ALL. British Journal of Haematology. 2012;158(5):626-34.
102. Kamdje AHN, Mosna F, Bifari F, et al. Notch-3 and Notch-4 signaling rescue from apoptosis human B-ALL cells in contact with human bone marrow-derived mesenchymal stromal cells. Blood. 2011;118(2):380-9.
103. Ellisen LW, Bird J, West DC, et al. Tan-1, the Human Homolog of the Drosophila Notch Gene, Is Broken by Chromosomal Translocations in T-Lymphoblastic Neoplasms. Cell. 1991;66(4):649-61.

104. Kamdje AHN, Krampera M. Notch signaling in acute lymphoblastic leukemia: any role for stromal microenvironment? Blood. 2011;118(25):6506-14.
105. Deangelo DJ, Stone RM, Silverman LB, et al. A phase I clinical trial of the notch inhibitor MK-0752 in patients with T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) and other leukemias. Journal of Clinical Oncology. 2006;24(18):357s-s.
106. Krop I, Demuth T, Guthrie T, et al. Phase I Pharmacologic and Pharmacodynamic Study of the Gamma Secretase (Notch) Inhibitor MK-0752 in Adult Patients With Advanced Solid Tumors. Journal of Clinical Oncology. 2012;30(19):2307-13.
107. Carlesso N, Aster JC, Sklar J, et al. Notch1-induced delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. Blood. 1999;93(3):838-48.
108. Li LH, Milner LA, Deng Y, et al. The human homolog of rat Jagged1 expressed by marrow stroma inhibits differentiation of 32D cells through interaction with Notch1. Immunity. 1998;8(1):43-55.
109. Sarmento LM, Huang H, Limon A, et al. Notch1 modulates timing of G(1)-S progression by inducing SKP2 transcription and p27(Kip1) degradation. Journal of Experimental Medicine. 2005;202(1):157-68.
110. Schroeder T, Kohlhof H, Rieber N, et al. Notch signaling induces multilineage myeloid differentiation and up-regulates PU.1 expression. Journal of Immunology. 2003;170(11):5538-48.
111. Milner LA, Bigas A, Kopan R, et al. Inhibition of granulocytic differentiation by mNotch1. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(23):13014-9.
112. Tan-Pertel HT, Walker L, Browning D, et al. Notch signaling enhances survival and alters differentiation of 32D myeloblasts. Journal of Immunology. 2000;165(8):4428-36.
113. Tohda S, Nara N. Expression of Notch1 and Jagged1 proteins in acute myeloid leukemia cells. Leukemia & Lymphoma. 2001;42(3):467-72.
114. Chen PM, Yen CC, Wang WS, et al. Down-regulation of Notch-1 expression decreases PU.1-mediated myeloid differentiation signaling in acute myeloid leukemia. International Journal of Oncology. 2008;32(6):1335-41.
115. Kannan S, Sutphin RM, Hall MG, et al. Notch activation inhibits AML growth and survival: a potential therapeutic approach. Journal of Experimental Medicine. 2013;210(2):321-37.
116. Klinakis A, Lobry C, Abdel-Wahab O, et al. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. Nature. 2011;473(7346):230-+.
117. Lobry C, Ntziachristos P, Ndiaye-Lobry D, et al. Notch pathway activation targets AML-initiating cell homeostasis and differentiation. Journal of Experimental Medicine. 2013;210(2):301-19.
118. Aljedai A, Buckle AM, Hiwarkar P, et al. Potential Role of Notch Signalling in CD34(+) Chronic Myeloid Leukaemia Cells: Cross-Talk between Notch and BCR-ABL. Plos One. 2015;10(4).
119. Horne GA, Morrison H, Campbell VL, et al. Notch Pathway Activation Targets Leukemic Stem Cells in Chronic-Phase Chronic Myeloid Leukemia (CP-CML). Blood. 2016;128:3057.
120. Laperrousaz B, Jeanpierre S, Sagorny K, et al. Primitive CML cell expansion relies on abnormal levels of BMPs provided by the niche and on BMPRIb overexpression. Blood. 2013;122(23):3767-77.

121. Miyanari Y, Torres-Padilla ME. Control of ground-state pluripotency by allelic regulation of Nanog. Nature. 2012;483(7390):470-U123.
122. Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature. 2010;463(7281):676-U111.
123. Lengerke C, Schmitt S, Bowman TV, et al. BMP and Wnt specify hematopoietic fate by activation of the Cdx-Hox pathway. Cell Stem Cell. 2008;2(1):72-82.
124. Scholl C, Bansal D, Dohner K, et al. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. Journal of Clinical Investigation. 2007;117(4):1037-48.
125. Grier DG, Thompson A, Kwasniewska A, et al. The pathophysiology of HOX genes and their role in cancer. Journal of Pathology. 2005;205(2):154-71.
126. Sauvageau G, Thorsteinsdottir U, Hough MR, et al. Overexpression of HOXB3 in hematopoietic cells causes defective lymphoid development and progressive myeloproliferation. Immunity. 1997;6(1):13-22.
127. Perkins A, Kongsuwan K, Visvader J, et al. Homeobox Gene-Expression Plus Autocrine Growth-Factor Production Elicits Myeloid-Leukemia. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(21):8398-402.
128. Antonchuk J, Sauvageau G, Humphries RK. HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell. 2002;109(1):39-45.
129. Thorsteinsdottir U, Mamo A, Kroon E, et al. Overexpression. of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. Blood. 2002;99(1):121-9.
130. Gerber JM, Gucwa JL, Esopi D, et al. Genome-wide comparison of the transcriptomes of highly enriched normal and chronic myeloid leukemia stem and progenitor cell populations. Oncotarget. 2013;4(5):715-28.
131. Toofan P, Irvine D, Hopcroft L, et al. The role of the bone morphogenetic proteins in leukaemic stem cell persistence. Biochemical Society Transactions. 2014;42:809-15.
132. Krause DS, Fulzele K, Catic A, et al. Differential regulation of myeloid leukemias by the bone marrow microenvironment. Nature Medicine. 2013;19(11):1513-+.
133. Zhang JW, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003;425(6960):836-41.
134. Zhang B, Li L, Ho YW, et al. Heterogeneity of leukemia-initiating capacity of chronic myelogenous leukemia stem cells. Journal of Clinical Investigation. 2016;126(3):975-91.
135. Bowers M, Zhang B, Ho YW, et al. Osteoblast ablation reduces normal long-term hematopoietic stem cell self-renewal but accelerates leukemia development. Blood. 2015;125(17):2678-88.
136. Bower H, Bjorkholm M, Dickman PW, et al. Life Expectancy of Patients With Chronic Myeloid Leukemia Approaches the Life Expectancy of the General Population. J Clin Oncol. 2016;34(24):2851-7.
137. Herrmann H, Sadovnik I, Cerny-Reiterer S, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. Blood. 2014;123(25):3951-62.
138. Landberg N, Hansen N, Askmyr M, et al. IL1RAP expression as a measure of leukemic stem cell burden at diagnosis of chronic myeloid leukemia predicts therapy outcome. Leukemia. 2016;30(1):253-7.
139. Kinstrie R, Horne GA, Morrison H, et al. CD93 Is a Novel Biomarker of Leukemia Stem Cells in Chronic Myeloid Leukemia. Blood. 2015;126(23).

140. Petersdorf SH, Kopecky KJ, Slovak M, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. Blood. 2013;121(24):4854-60.
141. Rowe JM, Lowenberg B. Gemtuzumab ozogamicin in acute myeloid leukemia: a remarkable saga about an active drug. Blood. 2013;121(24):4838-41.


Canonical Wnt signalling
Notch signalling
Hh signalling
JAG1
SMO
DVL
JAG2
GSK3
B-catenin
HES1
GLI1
Downstream target genes