

Novel drug therapies in myeloid leukemia



Both acute myeloid leukemia and chronic myeloid leukemia are thought to arise from a subpopulation of primitive cells, termed leukemic stem cells that share properties with somatic stem cells. Leukemic stem cells are capable of continued self-renewal, and are resistant to conventional chemotherapy and are considered to be responsible for disease relapse. In recent years, improved understanding of the underlying mechanisms of myeloid leukemia biology has led to the development of novel and targeted therapies. This review focuses on clinically relevant patent applications and their relevance within the known literature in two areas of prevailing therapeutic interest, namely monoclonal antibody therapy and small molecule inhibitors in disease-relevant signaling pathways.

Myeloid leukemias are characterized by the accumulation of immature myeloid progenitors caused by aberrations in cellular function, where deregulation of differentiation, growth and apoptosis leads to progression of an oncogenic phenotype [1–3]. The two commonest types of myeloid leukemia represent distinct disease entities – **acute myeloid leukemia** (AML) and **chronic myeloid leukemia** (CML). Improved understanding of the underlying mechanisms in their leukemic biology has led to the development of novel and targeted therapies.

CML represents a prime example of the role that targeted therapies can play in improved disease outcome. CML is a clonal myeloproliferative disorder associated with a reciprocal translocation between the long arms of chromosomes 22 and 9 giving rise to the **Philadelphia chromosome** and the subsequent formation of the *BCR-ABL* fusion gene, encoding the constitutively active **tyrosine kinase** BCR-ABL [4–7]. Advances in targeted therapies in chronic phase CML, notably the use of tyrosine kinase inhibitors (TKIs), have led to a tenfold reduction in disease progression to an accelerated or blast phase [8]. However, if left untreated, or in patients where resistance to TKIs exists or develops, the dis-

ease eventually progresses to blast phase, with terminal outcome in most cases [9,10].

Imatinib (Glivec, Novartis), the first BCR-ABL-specific TKI to be used clinically, or the second generation TKIs, nilotinib (Tasigna, Novartis, Switzerland) and dasatinib (Sprycel, Bristol-Myers Squibb, USA), are used as first-line therapy for the management of patients with all phases of CML [11]. Second-generation TKIs demonstrate increased pharmacological potency compared with imatinib. Nilotinib, like imatinib, binds an inactive conformation of BCR-ABL1, with 30- to 50-fold increased binding affinity; whereas, dasatinib binds both active and inactive conformations of BCR-ABL1 and is 325-times more potent [12]. The aim of treatment is to achieve hematological, cytogenetic and molecular response at critical time points from treatment initiation (**Table 1**) [11]. This provides important prognostic information for disease progression [9,11,13].

With standard dose imatinib (400 mg daily), complete cytogenetic response (CCyR) is achieved in 49–77% of patients, with a major molecular response (MMR) in 18–58%, depending on the data studied [11,14–15]. However, despite this, 18% of patients do not achieve optimal response

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Key terms

Acute myeloid leukemia: Acute myeloid leukemia is a myeloproliferative neoplasm characterized by a rapid accumulation of abnormal white cells.

Chronic myeloid leukemia: Chronic myeloid leukemia is a myeloproliferative neoplasm that originates from an oncogenically active tyrosine kinase, *BCR-ABL*, generated by the Philadelphia translocation.

Philadelphia translocation: This is a chromosomal abnormality that is the result of a reciprocal translocation between chromosome 9 and chromosome 22.

Tyrosine kinase: A tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to the amino acid tyrosine in a protein in a cell, thereby functioning to alter cellular function.

T315I mutation: The T315I mutation is caused by a single cytosine to thymine base pair substitution at codon 315 of the Abl protein resulting in a threonine-isoleucine substitution

(defined as quantitative *BCR-ABL1* $\leq 0.1\%$ at 12 months and maintained thereafter [11]), 10% lose optimal response and 4–8% are unable to tolerate therapy [16,17]. Furthermore, 12–15% of patients will present in the advanced stages of disease and are resistant to therapy. Resistance to therapy can occur through *BCR-ABL*-dependent mechanisms that rely on abnor-

malities within the structure of the target oncoprotein, including point mutations, such as **T315I mutations**; the third-generation TKI ponatinib, has been shown to be active against this mutation [18]. Further to this, recent *in vitro* studies provide strong evidence that CML leukemic stem cells (LSCs) are responsible for disease persistence despite a *BCR-ABL*-targeted therapeutic approach, and suggest *BCR-ABL*-independent mechanisms are being exploited to sustain their survival and proliferation [2,19]. TKIs are unable to target the resistant population of quiescent LSCs [20,21] meaning that on cessation of TKI treatment the disease is likely to return [18,22–23].

AML, like CML, is a clonal disorder that arises following the gain of self-renewal potential of progenitor cells, giving rise to a LSC. It is the most common malignant myeloid disorder in adults, with an annual incidence of approximately 3.8 per 100,000. Untreated AML typically results in bone marrow failure, leading to fatal infection, bleeding or organ infiltration, within 1 year of diagnosis, but often within weeks to months. AML is categorized according to the WHO classification which is based on cytogenetic and molecular abnormalities in the leukemic clone with known prognostic significance (Table 2) [24]. Treatment and prognosis vary significantly between the subtypes; with

Table 1. European Leukemia Net criteria detailing minimum required response to first-line tyrosine kinase inhibitor therapy from initiation, with definitions of response^a.

Response	Criteria		
Complete hematologic response (CHR)	Normalization of peripheral blood and resolution of splenomegaly		
Minimal cytogenetic response	>65–95% Ph+ in bone marrow		
Minor cytogenetic response	>35–66% Ph+ in bone marrow		
Partial cytogenetic response	0–35% Ph+ in bone marrow		
Complete cytogenetic response (CCyR)	0% Ph+ in bone marrow		
Major molecular response (MMR)	3 log reduction in <i>BCR-ABL1</i> relative to control		
Complete molecular response (CMR)	Undetectable <i>BCR-ABL1</i> transcripts by qRT-PCR		
	Optimal	Warning	Failure
3 months	<i>BCR-ABL1</i> $\leq 10\%$ \pm Ph+ <35%	<i>BCR-ABL1</i> >10% \pm Ph+ 36–95%	No CHR, and/or Ph+ >95%
6 months	<i>BCR-ABL1</i> <1% \pm Ph+ 0	<i>BCR-ABL1</i> >10% \pm Ph+ 1–35%	<i>BCR-ABL1</i> >10% and/or Ph+ >35%
12 months	<i>BCR-ABL1</i> $\leq 0.1\%$	<i>BCR-ABL1</i> >0.1–1%	<i>BCR-ABL1</i> >1% and/or Ph+ >0
Thereafter	<i>BCR-ABL1</i> $\leq 0.1\%$	Confirmed clonal abnormalities, Ph	Loss of CHR; loss of CCyR; confirmed loss of MMR

^aData taken from [11].

BCR-ABL1: Quantitative PCR assessment of *BCR-ABL1:ABL1* (or other housekeeping gene) on the International Scale; CCyR: Complete cytogenetic response; CHR: Complete hematologic response; CMR: Complete molecular response; MMR: Major molecular response; Ph+: Philadelphia chromosome positive.

overall survival varying from 20 to 47%, depending on subtype, mutational status and age.

Treatment, in itself, is associated with considerable morbidity and mortality. Standard chemotherapy for AML includes cytosine arabinoside (Ara-c) in conjunction with an anthracycline, such as daunorubicin or the nucleoside analogue fludarabine. While chemotherapy effectively induces remission in the majority of patients with AML, the risk of relapse is high. The reasons for this are not completely understood, but conventional chemotherapeutics target proliferating cells and not the small number of quiescent LSCs postulated to initiate and maintain the disease [1,25–26]. Therefore, disease relapse is likely to be a consequence of failure to eradicate the residual LSC fraction with conventional chemotherapies. Advances in the understanding of different molecular mechanisms involved in the pathogenesis of AML, including mutational events, cell cycle aberrations and methylation status has led to the development of novel therapies. These have been reviewed extensively [26] and include the cell cycle inhibitors barasertib, volasertib and rigosertib and Flt3 receptor tyrosine kinases targeting mutational status, such as the inhibitors quizartinib, sorafenib, midostaurin and creolanib [26]. Changes in DNA methylation have been shown to be integral in the development of leukemogenesis through tumor suppressor gene silencing. In AML, hypermethylation has been shown to be a poor prognostic marker exerting a negative effect on chemotherapy induction outcome, via p53-independent apoptosis. Hypomethylating agents are already in clinical use in the treatment of AML, and are beyond the scope of this review [27]. In patients with acute promyelocytic leukemia, a non-

chemotherapy approach is increasingly being adopted with the use of all trans-retinoic acid in combination with arsenic trioxide [28].

The identification of novel therapies that target the LSC population is a growing area of research and is of vital importance for developing treatment strategies which maintain remission and lead to possible long-term cure of AML and CML. Targeted therapies for cancer aim to exploit molecular differences between normal and malignant cells to allow the specific removal of cancerous cells while sparing healthy cells. Many studies have examined the differences in gene expression between normal hematopoietic stem cells (HSC) and their leukemic counterparts to identify potential targets for therapy. This review focuses on clinically relevant patent applications of novel targeted therapies in myeloid leukemias, with particular attention focused on monoclonal antibody therapy and small molecule inhibitors of signal transduction within leukemia cells.

Antibody-mediated therapy

As a targeted form of cancer therapy, monoclonal antibodies have proven an attractive focus for recent research and have provided promising results for certain malignancies, usually in combination with conventional chemotherapeutics [29]. Antibodies as cancer-targeting therapies have been investigated since the early 1980s and immunotherapy with monoclonal antibodies has been used successfully in the clinic for the treatment of a number of cancers. Trastuzumab is used in cases of HER2-positive breast carcinomas [30]. Cetuximab targets EGFR in metastatic colorectal cancer [31], metastatic non-small-cell lung cancer [32] and head and neck squamous cell carcinomas [33].

Table 2. Prognostic risk in acute myeloid leukemia according to cytogenetic and molecular characteristics.		
Prognostic risks	Cytogenetic abnormalities	Molecular abnormalities
Favorable	Inv(16) or t(16;16) t(8;21) t(15;17)	Normal cytogenetics; with isolated NPM1 or CEBPA mutation in the absence of FLT3-ITD
Intermediate	Normal cytogenetics Exon 8 trisomy t(9;11)	t(8;21), inv(16), t(16;16): with c-KIT mutation
Unfavorable	Complex karyotype -5,5q-, -7,7q-11q23 t(6;9) t(9,22)	Normal cytogenetics: with FLT3-ITD inv(3), t(3;3)
Data taken from [24]		

Key term

Antibody-mediated therapy: Form of immunotherapy that typically uses a monoclonal antibody to specifically bind to a target cell, leading to the subsequent immune destruction of that cell.

Rituximab [34] and ofatumumab [35] target malignancies with characteristic expression of CD20 on B-cells such as B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia (CLL) and alemtuzumab is used in the treatment of CLL and cutaneous T-cell lymphoma, where it targets CD52 on the surface of lymphocytes [36].

Therapeutic monoclonal antibodies work by a number of different mechanisms to target tumor cells (Figure 1). Antibody-dependent cellular cytotoxicity (ADCC) occurs when effector cells of the immune system lyse target cells 'containing' a specific antibody following antibody binding to Fc receptors (FcR) on the surface of the effector cell. ADCC is classically mediated by activation of NK cells through binding of the FcR CD16a (FcγRIIIA), but neutrophils (through CD64 (FcγRI), CD32 (FcγRII) and CD16 (FcγRIIIB)), macrophages and eosinophils also participate in ADCC. Once binding of the FcR occurs, the natural killer (NK) cell releases cytokines, such as IFN-γ, that can signal to other immune mediators, and release cytotoxic factors such as perforin and granzyme to initiate apoptosis in the target cell.

Complement-dependent cytotoxicity (CDC) is not the dominant antibody mediated mechanism of tumor cell kill, but does play a significant role and can enhance ADCC. Activation of the complement cascade is most effectively carried out by IgM type antibodies, but these are rarely used in the clinical setting due to their difficulty in crossing the vascular wall [37]. However, IgG1 and IgG3 are able to activate complement via CDC. When antibody binds to antigen, C1q-binding sites are uncovered allowing higher avidity binding to IgG, triggering the complement cascade and proteolytic release of effector cell activating agents C3a and C5a. The membrane attack complex (MAC) containing C5b, C6, C7, C8 and C9, is then formed which causes osmotic lysis of target cells. Rituximab [38], alemtuzumab [39] and ofatumumab [40] have been shown to depend in part on CDC for their *in vivo* efficacy.

In addition to their effects on cellular cytotoxicity, the majority of clinically effective monoclonal antibodies work by perturbing signaling pathways essential for the promotion of proliferation and survival of tumor cells. For example, overexpression of growth factor receptors is common on tumor cells and can be targeted by antagonistic antibodies that modulate their

ability to influence mitogenic signaling pathways [41]. Cetuximab functions as a competitive antagonist of EGFR to inhibit ligand binding, resulting in inhibition of downstream signaling and cell proliferation [42]. IgG2 and IgG4 type antibodies are unable to activate either ADCC or CDC and work only by modulating signaling pathways, resulting in fewer immune related adverse events (irAE) [43,44].

Perhaps the most important factor associated with antibody-mediated therapy success is the induction of adaptive immunity. Following ADCC or CDC, fragments of tumor cells are released and taken up by antigen-presenting cells (APCs) such as dendritic cells (DCs) where they are presented on the surface by the major histocompatibility complex class II (MHCII) to prime CD4⁺ T-cells or by MHC I to generate tumor specific CD8⁺ T-cells [44,45]. These cytotoxic T-cells can then target and kill cells containing the tumor antigen or differentiate into tumor-specific memory T-cells [46]. Clinical data are emerging to suggest that the induction of adaptive immunity will likely play a substantial role in whether antibody-mediated therapies are successful [47,48].

As described, in AML the residual LSC fraction of cells following conventional chemotherapies can lead to disease relapse. CD123 [49], CD25 [50], CD32 [50], CD47 [51], CD44 [52], CD96 [53], CLL-1 [54] and CD33 [55] have all been demonstrated to be differentially expressed on AML LSC compared with normal HSC (Table 3). Of these, the most attractive antibodies currently being investigated for the treatment of AML are anti-CD33 and anti-CD123.

CD33 (Siglec-3) is a myeloid lineage-specific antigen expressed on early myeloid progenitors, most monocytic cells and approximately 90% of AML blasts, but absent on normal HSCs [66]. The humanized monoclonal anti-CD33 antibody lintuzumab appeared promising in early clinical trials [67], but was abandoned during Phase IIb trials after patient survival rates did not increase significantly [68]. Following this, the calicheamicin-conjugated anti-CD33 monoclonal antibody gemtuzumab ozogamicin (GO; Mylotarg, Pfizer, USA) was granted accelerated approval by the US FDA following good response rates in Phase II trials [69,70]. However, GO was voluntarily removed from the market in 2008 following increased incidence of death [71] and limited clinical benefits over conventional cancer therapies in Phase III clinical trials [71-73]. It is likely that the adverse effects of GO were due to dissociation of calicheamicin from the anti-CD33 antibody causing increased toxicity [74]. Further Phase III studies have demonstrated improved remission rates and survival in both younger and older patients receiving GO in combination with conven-

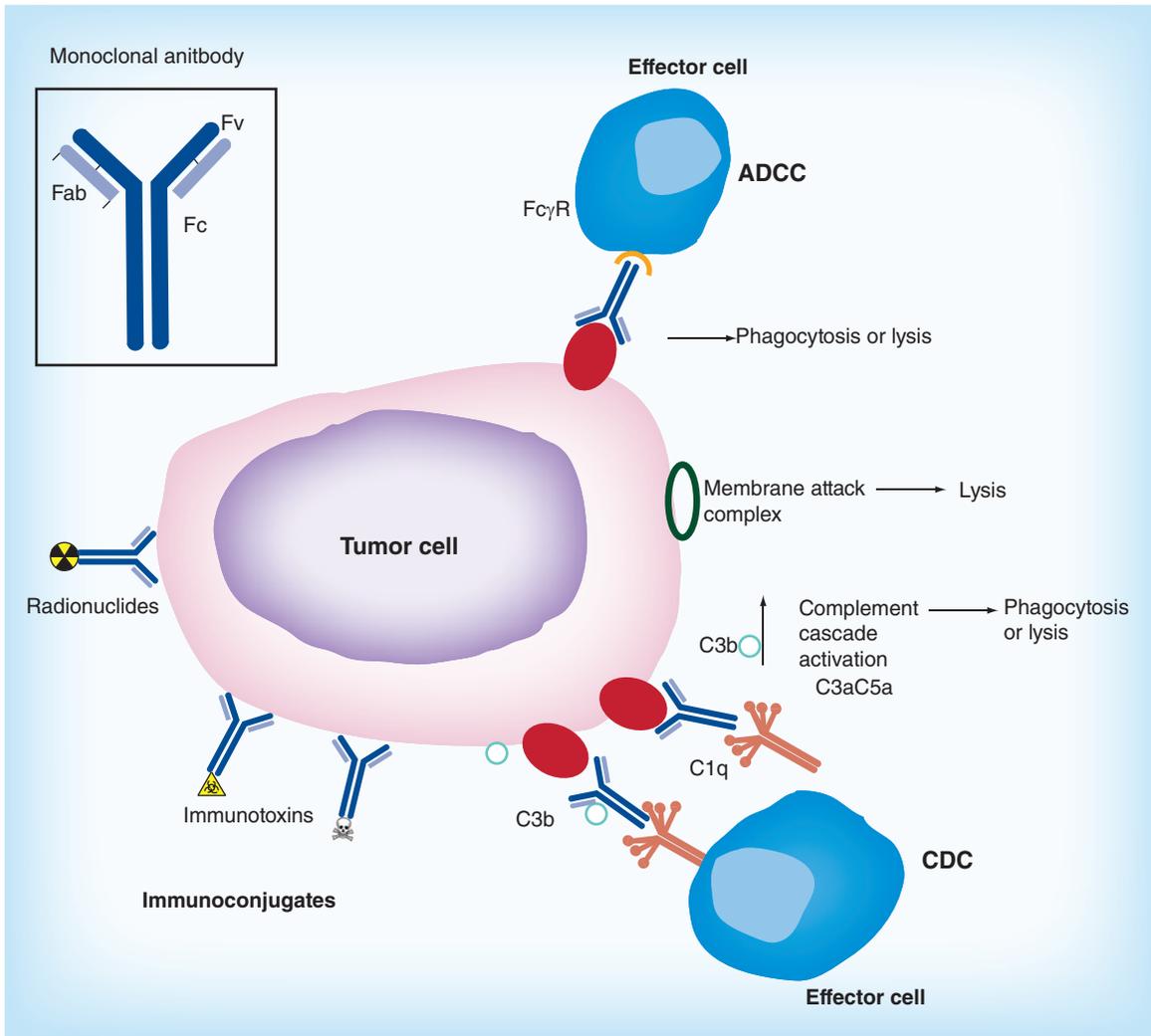


Figure 1. Mechanisms of action of monoclonal antibody-mediated therapy. Complement dependent cytotoxicity is an immune process that occurs following binding of an antibody to an antigen, where the complement cascade is activated. This leads to the formation of the terminal membrane attack complex in the cell membrane leading to lysis and death. Antibody-dependent cellular cytotoxicity occurs when the Fc receptor of effector cells (NK cells, macrophages) binds to the Fc portion of an antibody that has bound to a target cell. Following binding the effector cell can release cytokines for example that cause lysis of the target cell. Therapeutic monoclonal antibodies can also directly target cells by being conjugated to radionuclides, toxins, drugs and other substances that cause cells to die. ADCC: Antibody-dependent cellular cytotoxicity; CDC: Complement-dependent cytotoxicity.

tional chemotherapy as AML remission induction therapy [75,76]. Anti-CD33 therapy therefore remains a viable and potentially attractive option for the treatment of AML. A recent patent [56] has examined making these antibodies of higher affinity to the CD33 receptor enabling their use as recombinant fragments for immunotargeting. By varying the sequences of the antibody complementary determining regions (CDRs), it is proposed that more effective, higher affinity binding can be achieved with these modified CD33 antibodies. These antibodies remain bound to the cell surface for longer, improving binding affinity and can be further modified by addition of effector

groups such as pharmaceutically active substances and radioactive isotopes allowing tumor cells to be effectively targeted. In addition, antibodies can be conjugated to a further antibody to allow what is known as bispecificity or to two additional antibodies to allow trispecificity [77-79]. These are typically antibodies specific to effector cells such as NK cells, cytotoxic T-cells, monocytes, macrophages or DCs allowing more effective targeting for CDC or ADCC. In a recent patent, [57], a trispecific antibody for targeted AML therapy that included binding to CD33, CD123 and CD16 was described. Bispecific antibodies binding CD19 and CD16, with cytolytic activity directed

Table 3. Antibody-mediated therapies patent applications.

Target	Patent number		<i>In vitro</i> data in myeloid leukemia	<i>In vivo</i> data in myeloid leukemia	Clinical Trial data in myeloid leukemia	Ref.
CD33	US20120251554	Anti-CD33 antibodies and use thereof for immunotargeting in treating CD33-associated illnesses	+	-	-	[56]
CD123	US20120328619	Tri-specific therapeutics against acute myeloid leukemia	+	-	-	[57]
CD16 CD33						
CD123	EP2329847	Use of an antibody or an immunotoxin that selectively binds to CD123 to impair hematologic cancer progenitor cells	+	-	-	[58]
Siglec-15	CA2848074	Anti-Siglec-15 antibodies and uses thereof	+	-	-	[59]
CD44	EP2275444	Chimeric anti-CD44 antibodies and their use for treating acute myeloid leukemia	+	-	-	[60]
TIM-3	US20140134639	Method for treatment of blood tumor using anti-TIM-3 antibody	+	-	-	[61]
GM-CSF receptor α	US20140079708	Antibody molecule for human GM-CSF receptor α	+	-	-	[62]
CLL-1	US20140248633	Leukemia stem cell targeting ligands and methods of use	+	-	-	[63]
ITM2A	US20140193420	Diagnosis and treatment of cancer using anti-ITM2A antibody	+	-	-	[64]
CXCR4	WO2013071068	Treatment of hematologic malignancies with an anti-CXCR4 antibody	+	+	-	[65]

+: Evidence available; -: No evidence available.

against leukemic cells have been described [80,81]. A bispecific antibody (blinatumomab) to CD19 and CD3 has been evaluated in Phase I and II clinical trials with promising results in CD19-expressing lymphoid malignancies [82]. In the case of the invention in patent [57], the molecule consists of three scFvs covalently linked in tandem (single-chain Fv triplebody [sctb]), two that have specificity to the tumor cell (CD33 and CD123) and one to an effector cell (NK, monocytes or macrophage; CD16). The therapeutic effect of this trispecific antibody is direct elimination of the cancer cell by ADCC in addition to generating apoptotic fragments from the tumor cell that are taken up and presented by CD4⁺ and CD8⁺ T-cells [83].

CD123 (IL-3R α) is expressed on AML LSCs [49,84–87]. Initially described in the patent [58], CD123 monoclonal antibodies have been identified as potential therapies in the treatment of AML. Monoclonal antibodies to CD123 (CSL362) have proved effective *in vitro* and in animal models in enhancing ADCC through NK cells [88]; however, recent Phase I clinical trials of the anti-CD123 (CSL360) antibody for treat-

ment of relapsed, refractory or high-risk AML showed that while it was safe, there was limited efficacy [89].

Other siglecs (in addition to CD33/siglec-3) such as the recently described siglec-15 have also been identified as potential targets for antibody-mediated therapy [59]. The advantage of targeting siglec-15 in AML instead of CD33 is that theoretically the majority of the toxic side-effects associated with targeting CD33 such as myelosuppression and/or hepatotoxicity would be avoided. This is because there are much lower levels of siglec-15 on peripheral blood leukocytes and high levels on AML blasts, so nonspecific cell kill would be reduced. Other potential targets in AML such as CD44 [60], TIM-3 [61] and GM-CSF receptor α [62] have been the subject of recent patent applications. Anti-CD44 (H90/P245) showed some promise as an agent for targeting LSCs, with preclinical studies demonstrating eradication of LSCs and cure of leukemic NOD/SCID mice [52]. These antibodies would likely have limited effect *in vivo* as they were of murine origin, but chimeric antibodies (γ 1 construct and γ 4 construct) as described in the application [60] would in

theory remove some of the issues associated with the murine antibody, while retaining the ability to induce ADCC or CDC (γ 1). C-type lectin-like molecule-1 (CLL-1) is another good potential target for the eradication of AML stem cells [90]. The patent [63] describes the binding of specific peptides/polypeptides to the surface of AML LSC expressing CLL-1 and the subsequent delivery of toxic or drug payloads to the cell to allow an effective cell kill. The patent [91] describes the use of human monoclonal anti-CXCR4 antibodies to target AML cells by disrupting the interaction between CXCR4 and its ligand CXCL12. The CXCR4 antagonists AMD3100 and AMD3465 have previously been shown to increase chemosensitization of AML cells by blocking signaling through this pathway [92,93] and lower levels of CXCR4 on AML cells correlate with better prognosis and survival [94]. The anti-CXCR4 antibody described binds with low nanomolar affinity and is shown to decrease tumor proliferation and increase induction of apoptosis. The invention in patent [64] describes a monoclonal antibody against the type II membrane protein ITM2A for the potential treatment of AML by inducing ADCC.

Currently the most topical potential therapeutic monoclonal antibody in AML is CD47 (Table 4). CD47 binds the ligands thrombospondin-1 (TSP-1) and signal regulatory protein α (SIRP- α) and is involved in apoptosis, proliferation, adhesion and migration [95]. Increased expression of CD47 has been shown to be a poor prognostic factor in AML [51].

One of the major modes of clearance of apoptotic leukemic cells is by macrophage-mediated phagocytosis. To avoid immune surveillance by macrophages, AML cells upregulate CD47 [101]. The patents [96] and [97] describe methods to enhance phagocytosis of circulating CD47-expressing LSC by blocking cell surface CD47. Blocking CD47 with a monoclonal antibody prevents the interaction between CD47 and SIRP- α leading to increases in phagocytic clearance of LSCs. Additionally, the patents describe aspects of antibody bispecificity, where CD47 is used synergistically with other monoclonal antibodies directed against other cell surface markers unregulated in AML stem cells to enhance phagocytic clearance [96]. Additionally, by combining a monoclonal antibody specific to CD47 with a cytotoxic agent such as a chemotherapeutic agent, radio-isotope or toxin, LSCs expressing CD47 can be targeted directly on antibody binding. Reference [65] also describes therapeutic antibodies targeting CD47 that disrupt the interaction with SIRP- α leading to increased phagocytic uptake. Other methods targeting the interaction between CD47 and SIRP- α are described in [98]. By using polypeptides capable of binding specific sequences within CD47 or SIRP- α , the interaction between each can be modulated to enable macrophage activation, leading to clearance of leukemic cells. Patents describing humanized monoclonal antibodies that bind and neutralise CD47 and that have low immunogenicity in humans have been filed [99,100]. The benefits of using humanized antibody-

Table 4. CD47 antibody-related patent applications.

Patent number		<i>In vitro</i> data in myeloid leukemia	<i>In vivo</i> data in myeloid leukemia	Clinical trial data in myeloid leukemia	Ref.
WO2014093678	Therapeutic CD47 antibodies	-	+	-	[91]
US20140161805	Methods for manipulating phagocytosis mediated by CD47	+	+	-	[96]
US20140161825	Methods of treating acute myeloid leukemia by blocking CD47	+	+	-	[97]
US20120189625	Compositions and methods for treating hematological cancers targeting the SIRP- α CD47 interaction	+	+	-	[98]
US20120156724	Humanized anti-CD47 antibody	-	-	-	[99]
US20130142786	Humanized and chimeric monoclonal antibodies to CD47	+	-	-	[100]

+: Evidence available; -: No evidence available.

ies in immunotherapy are a significant reduction in host immune responses following administration.

There is limited evidence of the use of monoclonal antibody therapy in CML. There has been a limited number of reports indicating the use of GO in myeloid blast phase and rituximab in lymphoid blast phase CML, both in combination with conventional chemotherapy with some success, but there are no patents specific to these indications [102,103]. Additionally, one report identified CML stem cells based on their expression of the IL-1 receptor accessory protein (IL-1RAP) [104]. The authors demonstrated a reduction in CML stem cells by ADCC when a rabbit polyclonal antibody was administered to CML cell lines and to primary CD34⁺CD38⁻ cells. However, with the success of TKIs in CML, it is unlikely that antibody-mediated therapies will represent a major new therapeutic class in CML in the immediate future.

Small molecule inhibitors of signal transduction

Signal transduction is the primary method used by eukaryotic cells to respond to external cues within their microenvironment [105], and is fundamental to normal cellular processes. Deregulation within these signals is, unsurprisingly, critical in the pathogenesis of leukemia. Studies have shown that abnormal signal transduction enhances the self-renewal, proliferative and anti-apoptotic capacity in AML and CML LSC [106–111], and targeting these pathways offers an attractive alternative to conventional chemotherapy [112].

Large numbers of aberrant signaling proteins have been identified in both AML and CML – these reflect a more limited number of signaling pathways, such as mammalian target of rapamycin complex 1/phosphatidylinositol 3-kinases/AKT (mTORC1/PI3K/Akt) [113,114], Wingless-Int/ β -Catenin (Wnt/ β catenin) [115,116], Hedgehog (Hh) [117–119] and Notch [108–109,120]. These pathways are known regulators of cell survival, namely self-renewal and apoptosis, and are often differentially expressed following genetic events [111,114,121]. When deregulated, these signaling pathways have been implicated in neoplastic pathogenesis [107,109,113,116,119,122]. Targeting these unifying pathways may represent a more broadly applicable therapeutic strategy compared with conventional chemotherapy alone in myeloid leukemias. However, these pathways do not act in isolation, relying on intricately interwoven networks, leading to the development of myeloid leukemia, its progression [107] and LSC survival [123]. CML serves as a paradigm example into the complexities and orchestral approach between the pathways in leukemia pathogenesis.

In CML, the fundamental role of BCR-ABL is well documented [124,125]. The oncogenically active tyrosine

kinase, BCR-ABL, activates many signal transduction pathways essential for cell survival and proliferation. Activation of downstream pathways occurs, in part, from the autophosphorylation of the BCR-ABL domain with the subsequent suppression of forkhead O (FOXO) transcription [126], through activation of the PI3K/Akt pathway [127], and activation of RAS, promoting leukemic cell survival through MAPK. Furthermore, it has been demonstrated that BCR-ABL can lead to the direct phosphorylation and activation of STAT5 [128]. Thus, there are many potential small molecule inhibitors with targets downstream of BCR-ABL that have been assessed for their potential as LSC-eliminating agents. Furthermore, as self-renewal activation is characteristic of LSCs, further therapeutic targets could lie in the evolutionary conserved self-renewal pathways, Wnt, Hh and Notch [115,119–120]. Additional signaling abnormalities characterise CML progression to the more acute blast phase of disease, including the Numb-Musashi signaling network; although, whether this exerts its effects through the self-renewal pathways, Hh or Notch, has yet to be established [129].

The complexities in interaction between these pathways are well documented. In CML, the Hh pathway has been linked to LSC generation, disease progression and the modulation of the hematopoietic microenvironment [130]. Wnt has been shown to play a critical role in the survival of LSCs with acquired imatinib resistance [131], and it has been demonstrated that a recurrent misspliced, non-functional isoform of GSK3 β predominates in the granulocyte-macrophage progenitor population in myeloid blast phase, leading to enhanced self-renewal via the Wnt pathway [131,132]. In addition, interactions between the Hh and Wnt pathways have been shown to be deregulated in CML development and progression [106,115–116,133].

As with CML, the aberrations seen in AML can be related to similar signaling pathways [134]. However, the genetic variations seen in AML render the intricacy of signal transduction interaction more complicated. For example, Flt3 mutations (detected in approximately 30% of AML [135]) mediate their proliferative and anti-apoptotic oncogenic effects through varying signaling pathways, including STAT5, RAS/MAPK and PI3K/Akt. Therefore, activating mutations can lead to deregulation in more than one signaling pathway. The pathways can also be activated simultaneously, as demonstrated in patients with RAS mutations, where both RAS/MAPK and PI3k/Akt pathways can be activated simultaneously [136]. The diversity of molecular abnormalities in AML makes developing a therapeutic regimen that is efficacious in all activated pathways unachievable and, increasingly, we are moving to an individualized treatment approach in AML based on

molecular and cytogenetic abnormalities, prognostic risk group and age. In view of the complexities seen within the interactions of signal transduction pathways, each clinically relevant pathway and its associated patent applications will be discussed individually.

mTOR/PI3K/Akt signaling pathway

The PI3K/Akt signaling pathway plays a significant role in a number of cellular functions, including differentiation, apoptosis and cell cycle progression [137,138]. Aberrant signaling of the pathway has been linked to a number of oncogenic disease processes [139], including AML and CML [111,137], where it has been shown to influence survival and drug resistance of immature progenitors and LSCs [137]. The mTOR signaling pathway is critical to normal hematopoiesis and has been shown to be frequently mutated in hematological malignancies and TKI-resistant myeloid disease [137–138,140–141]. mTOR is a serine/threonine kinase with its activity within a cell being conducted by two distinct complexes (mTORC1 and mTORC2), with mTORC1 being strongly associated with neoplastic proliferation. One of its main substrates, p70-S6k, is known

to inhibit autophagy – an area of increasing interest in myeloid disease, particularly in CML [141,142]. Although patent applications have been generated for small molecule inhibitors toward autophagy in malignancy, their direct role in myeloid disease is not listed.

The interactions between mTOR and PI3K/Akt are complex, with negative feedback loops at its core; this has been previously reviewed in detail [138]. Briefly, the binding of a growth factor ligand (e.g., insulin, TGF- β or VEGF) to the receptor tyrosine kinase results in receptor activation, which in turn, activates PI3K, through phosphoinositide-dependent kinase 1 (PDK1). PI3K activity subsequently results in Akt activation that directly phosphorylates and activates mTOR, as well as inhibits the mTOR inhibitor proteins, proline-rich Akt/PKB substrate 40 kDa (PRAS40) and tuberlin. Combined, these actions promote cell growth, cell proliferation and inhibit autophagy through downstream targets (Figure 2). The pathways are frequently activated in both AML and CML, with aberrant PI3K/Akt activation being reported in 50–80% of AML cases [137]; simultaneous activation with other pathways conferring a poor prognosis. The PI3K/Akt

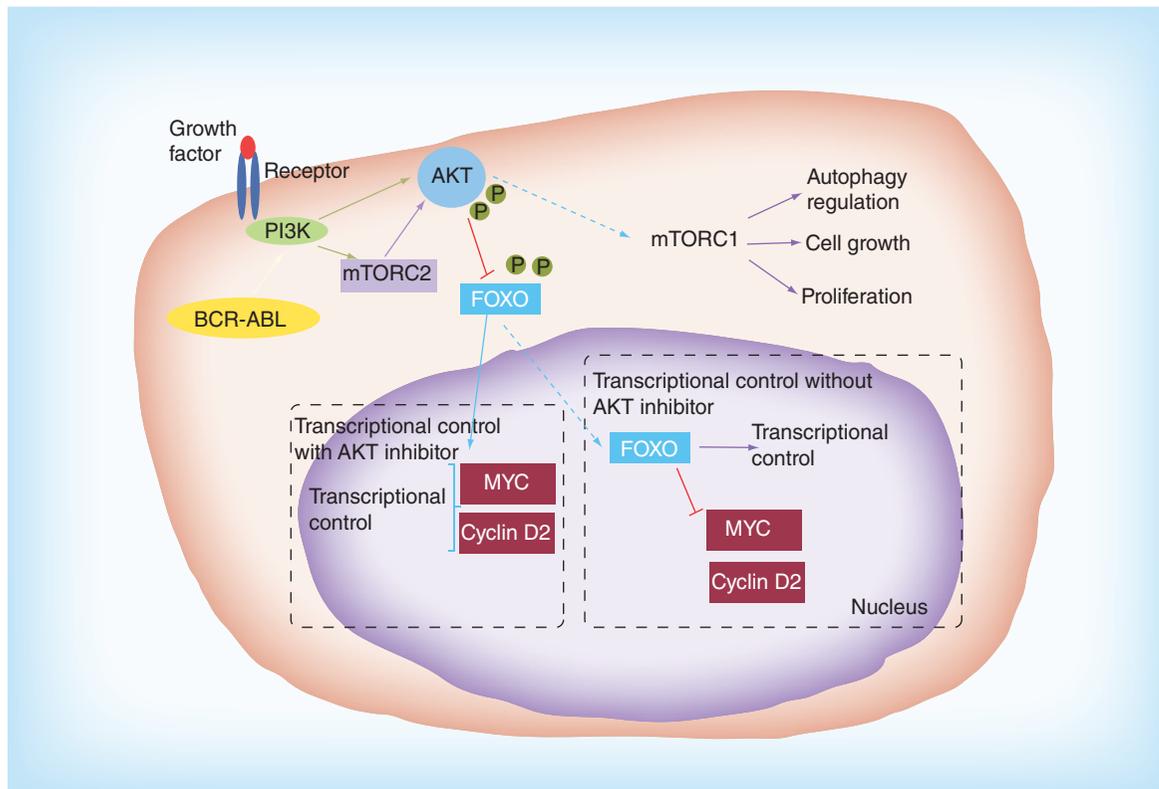


Figure 2. Regulation of cellular function through the PI3K/Akt-FOXO signaling network. The binding of a ligand (including insulin, TGF- β and VEGF) to the receptor tyrosine kinase results in receptor activation, which in turn, activates PI3K through PDK1. PI3K activity subsequently results in Akt activation that directly phosphorylates and activates mTORC1. BCR-ABL activates Akt. Akt negatively regulates the FOXO proteins and prevents translocation to the nucleus, and the FOXO proteins normal function, therefore leading to activation of cell cycle genes, including MYC and CYCLIN D2. Combined, these actions promote cell growth, cell proliferation and inhibit autophagy through downstream targets.

pathway is a key downstream target of BCR-ABL, with mTORC1 inhibition acting synergistically with TKIs to induce apoptosis [141,143]. Recent patent applications describe a selection of compounds available for the inhibition of mTOR/PI3K/Akt (Table 5), but their exact mechanism in myeloid disease or bioavailability for therapeutic use are not available via these patent applications. However, a recent publication describes their potential benefits in both *in vitro* and *in vivo* settings [143]. Furthermore, the synergistic effect *in vitro* of Akt-1 inhibitors and Flt3 TKIs has been documented extensively in the published literature, but without relevant associated patent applications [144]. Inhibition of PI3K or mTORC1 has previously demonstrated a growth disadvantage in AML. Inhibiting PI3K/Akt with LY294002, a potent PI3K inhibitor, can control blast cell proliferation [145]. First-generation mTORC1

inhibitors offered potentially promising initial results in terms of apoptotic ability, but long-term apoptotic advantages were disappointing [146]. Although, there are no patent applications specifically detailing the role of PI3K or mTORC1 inhibition in myeloid leukemias, early phase clinical trials are ongoing to assess the role of PI3K or mTOR inhibitors in both AML and CML. A Phase Ib study analyzing the combined effect of the mTORC1 inhibitor, RAD001 and conventional chemotherapy demonstrated the possibility of improved treatment outcome in AML [147]. The lack of specific patent application in this area, therefore, does not diminish the importance of this pathway as a clinically relevant therapeutic target in the future.

Dual inhibition of mTORC1 and mTORC2 may offer an interesting possibility for therapeutic advancement as simultaneous inhibition of mTORC1 and 2

Table 5. mTOR/PI3k/Akt signaling pathway-related patent applications.

Patent number		<i>In vitro</i> data in myeloid leukemia	<i>In vivo</i> data in myeloid leukemia	Clinical trial data in myeloid leukemia	Ref.
WO2009008992	Combination anticancer therapy comprising an inhibitor of bone mTORC1 and mTORC2	-	-	-	[148]
WO2012106702	Treatment of leukemia	+	+	-	[149]
US20130045188	Reduction of TGF- β signaling in myeloid cells in the treatment of cancer	+	+	-	[150]
US7928248	Benzoxepin PI3K inhibitor compounds and methods of use	-	-	-	[151]
CA2680853	Delta3-substituted quinoline or quinoxaline derivatives and their use as phosphatidylinositol 3-kinase (PI3K) inhibitors	-	-	-	[152]
WO2013141586	Novel pyridopyrimidine derivatives that inhibit PI3K	-	-	-	[153]
WO2013104611	Substituted pyrazolopyrimidines that are PI3K and Akt inhibitors	-	-	-	[154]
WO2013104610	Substituted imidazopyrazines that are PI3K and Akt inhibitors	-	-	-	[155]
WO2013049581	A PI3K inhibitor and a poly(ADP-Ribose) polymerase inhibitor; useful for treating cancer	-	-	-	[156]
WO2013052699	Novel Quinoxaline inhibitors or PI3K; useful for treating cancer and inflammatory diseases	-	-	-	[157]
WO2013095761	Imidazopyridine derivatives that are selective PI3KB inhibitors and can be used to treat cancer	-	-	-	[158]
WO2013066483	Synergistic combinations of PI3K and MEK inhibitors that can be used to treat proliferative disease	-	-	-	[159]

+: Evidence available; -: No evidence available.

induces apoptosis in T315I mutated BCR-ABL positive cells, indicating its potential relevance as a therapeutic target in TKI-resistant CML [127]. A patent application [148] described this dual inhibitory approach in mesenchymal and epithelial non-small-cell lung cancer and ovarian cancer cell lines, but did not specifically detail a myeloid phenotype.

The Akt network controls many different downstream targets including the FOXO family of transcription factors as described above (Figure 2) [160]. When unphosphorylated, the FOXOs localize in the nucleus, enabling transcription of a wide array of target genes involved in cell cycle and apoptosis. PI3K activation, downstream of growth factor receptors negatively regulates FOXO proteins. It is hypothesized that the FOXO transcription factors are activated via BCR-ABL-Akt signaling [126,161] and this maintains CML LSC quiescence. No patent applications are currently available in respect of FOXO signaling in CML.

Paradoxically, the patent application [149] suggests that inhibition of FOXO, rather than activation, could be protective in AML; in both *in vitro* CD34+ progenitor cells and *in vivo* in a MLL-AF9-induced AML mouse model. Moreover, TGF- β is a critical regulator of Akt activation in CML leukemia-initiating cells and also controls FOXO nuclear localization [161]. In a recent patent application, [148], Yang *et al.* provide evidence as to the importance of TGF- β in malignancy and metastasis, particularly in lung disease, by manipulating TGF- β receptor II expression in myeloid cells *in vitro*, *ex vivo* and *in vivo*, using RNA interference, and transgenic mouse models. Although not limited to myeloid disease, the invention suggests the importance of the TGF- β in the PI3K/Akt pathway in malignancies where TGF- β is upregulated.

Targeting self-renewal pathways

In view of the fact that committed progenitors gain self-renewal properties in AML and blast phase CML, a number of evolutionary conserved self-renewal pathways are generating increasing interest as novel therapeutic agents.

Hedgehog signaling pathway

The Hh signaling pathway is inappropriately activated in many human malignancies, including CML [162–165] and AML [117,118]. Recent evidence suggests that Hh signaling is critical for the maintenance and expansion of cancer stem cells, and is a key candidate for LSC-directed therapy.

The patent application [166] presents the invention of a dual-therapy treatment approach in CML, comprising a first agent that inhibits the Hh signaling pathway and a second that inhibits BCR-ABL.

The experimental data assessed primary patient samples obtained from newly diagnosed and untreated patients with CML in chronic phase. These cells were enriched for CD34+ progenitor cells prior to analysis. In addition, mouse bone marrow cells were infected with a bicistronic retroviral Bcr-Abl vector, cultured for 82 h in the presence of the Hh, compound A, then plated to assess clonogenic colony formation. In all cases, the Hh inhibitor reduced the clonogenic capacity of the BCR-ABL-expressing cells. These results were confirmed *in vivo* in the murine model. This suggests that a combinatorial approach may benefit patients with primary, relapsed, transformed or refractory forms of CML.

In a patent application, [167], the Smoothed inhibitor, PF-04449913, demonstrated reduction in leukemia progenitor survival in both *in vitro* and *in vivo* models of CML LSC. *In vivo*, blast phase CML LSC engrafted mice treated with and without combined PF-04449913 and dasatinib showed a significant reduction in disease and BCR-ABL expression if the Hh inhibitor was used in combination with TKI. It was demonstrated that inhibition of this pathway could push the LSC from G0 to G1 of the cell cycle, which increased their susceptibility to BCR-ABL inhibition. In a Phase Ia study presented at The American Society of Hematology (2013) it was demonstrated that PF-04449913 could synergize with BCR-ABL inhibition to reduce blast crisis LSC survival and self-renewal, but with increased expression of Hh pathway regulators, suggesting that it is selective Hh antagonism that induces cell cycle interference of dormant human blast crisis LSCs [168].

Other modulators of the pathway (Table 6) have been described via patent application, but the relevance in myeloid leukemia has yet to be determined.

Notch signaling pathway

The Notch pathway has been implicated in a number of malignancies with its role being cell and tissue-dependent. In cancer biology, Notch signaling has been demonstrated to play both an oncogenic and tumor suppressive role, depending on cell and cancer type [171–176]. In hematopoietic malignancies, accumulating evidence demonstrates its importance in growth, differentiation and apoptosis [177,178]. Reports about the role that 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 [179–182]. A recent patent application, [183], demonstrated that the Notch signaling pathway is silenced in human AML, as well as in AML-initiating cells in an *in vivo* model of the disease. Furthermore, *in vivo*

Table 6. Hedgehog pathway-related patent applications.

Patent number		<i>In vitro</i> data in myeloid leukemia	<i>In vivo</i> data in myeloid leukemia	Clinical trial data in myeloid leukemia	Ref.
CA2769300	Methods and compositions for treating leukemia	+	+	-	[166]
WO2013036867	Compositions and methods for cancer and cancer stem cell detection and elimination	+	+	-	[167]
WO2009074300	Benzimidazole derivatives as hedgeog pathway antagonists and therapeutic applications thereof	-	-	-	[169]
WO2008112913	Inhibitors of the Hedgehog pathway	-	-	-	[170]

+: Evidence available; -: No evidence available.

activation of Notch signaling using genetic Notch gain-of-function models or *in vitro* using synthetic Notch ligand induced rapid cell cycle arrest, differentiation and apoptosis of AML-initiating cells. These data demonstrated a potential novel tumor suppressor role for Notch signaling in AML and elucidated the potential therapeutic use of Notch receptor agonists in its treatment. There is currently no patent application focusing on the agonistic therapeutic implication of Notch signaling in CML.

Wnt/ β -catenin

The Wnt signaling pathway plays an essential role the proliferation, survival and differentiation of LSCs [184]. Constitutive activation of Wnt signaling has been reported in both AML and CML [115,184]. Zhao *et al.* revealed that β -catenin deletion reduces the ability of mice to develop Bcr-Abl positive leukemia, suggesting an integral role for β -catenin in the pathogenesis of CML [115]. Furthermore, increased activation of the pathway has been associated with poor response in blast phase CML [106]. The patent application, [185], discloses the use of an anthracene-9,10-dione dioxime compound: 2-((3R,5S)-3,5-dimethylpiperidin-1ylsulfonyl)-7-((3S,5R)-3,5-dimethylpiperidin-1-ylsulfonyl)anthracene-9,10-dione dioxime, in the disruption of transducin β -like protein 1 (TBL1)'s interaction with β -catenin in AML cells, as well as CML cell lines. Recent studies have shown that TBL1 is able to activate the Wnt/ β -catenin pathway by binding to both β -catenin and the Wnt promoter complex. Further, TBL1 appears to protect β -catenin from ubiquitination and degradation [186]. However, the mechanism of the interaction between TBL1 and β -catenin remain unknown.

Conclusion & future perspective

This review describes the clinically relevant novel therapeutic targets in myeloid leukemia that have been the subject of recent patent applications. The main areas of focus were monoclonal antibody therapies and clinically relevant small molecule inhibitors of signal transduction; however, this list is not exhaustive.

The treatment of myeloid leukemias represents an exciting area of research where novel therapeutic advances are being discovered; but it is an area of research with great complexity. A limiting factor in discussing the patent applications is the lack of consistency in describing cell line and primary cell *in vitro* models and *in vivo* murine models between the applications. Furthermore, the clinically important aspect of any novel therapeutic target is in its ability to be administered with manageable toxicity and still demonstrate efficacy. This relies on its biological effect on other systems and is not often described within the patent applications.

The novel therapeutic targets described offer exciting prospects for improving therapeutic outcomes in patients with myeloid leukemias with a potential reduction in treatment toxicity. Some of these novel therapeutic approaches may even target the elusive LSC. It remains to be seen if the inhibition of signaling pathways and monoclonal antibody therapy will, indeed, prove to be clinically effective in AML and CML, and few novel therapies (with the exception of BCR-ABL-specific TKIs) have been licensed for use in myeloid leukemias in recent years. It is likely that the identification of novel targets and novel therapeutic strategies in myeloid leukemias will remain a rich source of patent applications in the future.

Financial & competing interests disclosure

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Executive summary**Background**

- Both acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) are thought to arise from a subpopulation of primitive cells, termed leukemic stem cells (LSCs), that share properties with somatic stem cells.
- LSCs are capable of unlimited self-renewal, and are resistant to therapy, with conventional chemotherapy being unable to eradicate them.
- The identification of novel therapies that target the LSC population is a growing area of research of vital importance for maintaining remission and possible cure of AML and CML.

Antibody-mediated therapy

- Monoclonal antibodies have proven an attractive focus for recent research and have provided promising results for certain malignancies usually in combination with conventional chemotherapeutics.
- CD123, CD25, CD32, CD47, CD44, CD96, CLL-1 and CD33 have all been demonstrated to be differentially expressed on AML LSC compared with normal hematopoietic stem cells.
- Currently the most topical potential therapeutic monoclonal antibody in AML is CD47.
- Increased expression of CD47 has been shown to be a poor prognostic factor in AML.
- Examples of recent patent applications and the associated scientific literature is discussed within this section.

Small molecule inhibitors of signal transduction

- Studies have shown that abnormal signal transduction enhances the self-renewal, proliferative and anti-apoptotic capacity in both AML and CML LSC.
- Targeting these pathways offers an attractive alternative to conventional chemotherapy.
- Many signaling pathways have been shown to be deregulated, including mTOR/PI3K/Akt, Notch, Hedgehog and Wnt.
- Interactions within the pathways make developing a therapeutic regimen that is efficacious in all activated pathways difficult.
- Examples of recent patent applications and the associated scientific literature is discussed within this section.

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