

Signal transduction inhibitors in chronic lymphocytic leukemia

Shuo Ma and Steven T. Rosen

Robert H. Lurie Comprehensive Cancer Center and Division of Hematology and Oncology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Correspondence to Shuo Ma, MD, PhD, Robert H. Lurie Comprehensive Cancer Center and Division of Hematology and Oncology, Feinberg School of Medicine, Northwestern University, 303 E. Superior Street, Lurie 3-125, Chicago, IL 60611, USA
E-mail: shuo-ma@northwestern.edu

Current Opinion in Oncology 2011, 23:601–608

Purpose of review

Chronic lymphocytic leukemia (CLL) therapy has evolved over the past few decades as modern chemo-immunotherapy significantly improved the response and survival of CLL patients. However, treatment toxicity of the intensive chemo-immunotherapy often limits its use in the mostly elderly population of patients. Further, the disease eventually relapses and additional therapy options are required. Of particular interest are molecular targeted therapies that interfere with critical signal transduction pathways controlling cell growth and survival. This review will provide an update on the most recent preclinical and clinical development of signal transduction targeted therapy in CLL.

Recent findings

Small molecular kinase inhibitors have been developed to target the proximal B-cell receptor signaling pathway (e.g. spleen tyrosine kinase inhibitors and Bruton's tyrosine kinase inhibitors) or the downstream phosphatidylinositol-3 kinase/Akt/mammalian target of rapamycin pathway. These agents showed unique in-vitro activities by inducing apoptosis and blocking interaction of CLL cells and the protective microenvironment. They have also shown promising clinical activity in early-phase clinical studies and appear to alter lymphocyte trafficking. Inhibitors of the B-cell CLL/lymphoma 2 (BCL-2) family of antiapoptotic proteins and Cdk inhibitors are in active clinical development. Strategies modulating the CLL interaction with the microenvironment niche are emerging. Further understanding of novel signaling pathways helps to identify additional potential therapeutic targets.

Summary

Signal transduction inhibitors are promising new strategy for targeted CLL treatment. Identification of novel molecular targets and therapeutic agents will further expand our therapeutic options.

Keywords

chronic lymphocytic leukemia, signal transduction, small molecule inhibitors

Curr Opin Oncol 23:601–608
© 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins
1040-8746

Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western society with median age of diagnosis 64–72 years. The clinical course is highly heterogeneous among different CLL patients. Advanced clinical staging and high-risk molecular prognostic features (high-risk cytogenetic abnormalities, unmutated IgVH, ZAP70 and CD38 positivity) are associated with more rapid disease progression and shorter survival [1]. Whereas CLL is commonly diagnosed at an early asymptomatic stage, most patients will develop disease progression and ultimately require treatment. CLL therapy has evolved rapidly during the past decade. Most modern CLL treatments combine cytotoxic chemotherapy backbone with monoclonal antibodies, which have significantly improved the quality of response, duration of response, and survival of the patients [2]. However, treatment toxicity of the intensive chemo-immunotherapy often limits its use in the mostly

elderly population of CLL patients with comorbidities. In addition, subgroups of high-risk CLL carry inherent resistance to DNA-damaging chemotherapy agents and the clinical outcome of these patients remains dismal. Further, in most patients, the disease inevitably relapses and eventually develops acquired resistance to conventional treatment. Therefore, development of novel treatment strategies is highly desirable, especially those with targeted actions, less toxicities, and ability to circumvent the intrinsic cellular resistance mechanism. In recent years, our understanding of the biological basis of CLL pathogenesis has greatly improved which revealed new potential therapeutic targets and accelerated the development of novel agents [3^{••},4]. Of particular interest are molecular targeted therapies that directly interfere with central signal transduction pathways controlling cell growth and survival, and bypass resistance mechanisms to cytotoxic drugs [5[•],6]. This review will provide an update on the most recent preclinical and clinical development of signal transduction inhibitor therapy in CLL with

1040-8746 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins

DOI:10.1097/CCO.0b013e32834b8943

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Pharmacyclics LLC
Exhibit 2022
BeiGene v. Pharmacyclics
PGR2024-00009
EX2022.0001



emphasis on agents targeting the B-cell receptor (BCR) signaling pathway, the cell death machinery, and the leukemia's interaction with the microenvironment.

Targeting the B-cell receptor signaling pathway

BCR signaling is crucial for B-cell survival and growth. Following antigen engagement of BCR, intracellular recruitment and activation of BCR-associated protein tyrosine kinases (PTKs) occur [7]. The ordered activation of three different nonreceptor PTKs – prototypically represented by Lyn, spleen tyrosine kinase (Syk), and Bruton's tyrosine kinase (Btk) – is required for proper BCR signal transduction. These PTKs in turn will activate multiple signal transducers and pathways including the phosphatidylinositol-3 kinase (PI-3K)/Akt/mammalian target of rapamycin (mTOR) pathway, the Ras/Raf/mitogen-activated protein kinase and extracellular signal-regulated kinase/extracellular signal-regulated kinase cascade, phospholipase C γ 2 and protein kinase C (PKC), with subsequent activation of downstream transcription factors promoting cell survival (Fig. 1) [3^{**},7]. The aberrantly activated BCR signaling plays a key role in the pathogenesis of CLL by promoting survival of leukemia cells. In addition to ligand-induced BCR signaling, there are critical nonligand-dependent

Key points

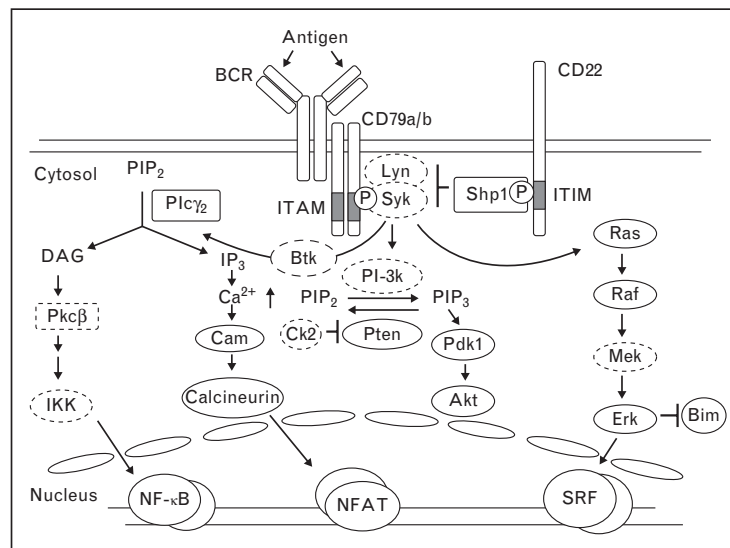
- Constitutively active B-cell receptor (BCR) signaling is crucial for chronic lymphocytic leukemia (CLL) cell survival and growth.
- Blocking the BCR signaling pathway with kinase inhibitors was shown *in vitro* to induce apoptosis in CLL cells and blocks the protective signals from the microenvironment.
- Clinical studies of BCR pathway inhibitors showed promising antileukemia response in CLL and interesting biological effect on altering CLL cell trafficking.
- The deregulated cell death machinery contributes to prolonged survival of CLL cells and provides important therapeutic targets.

BCR-mediated survival signals in malignant B cells [3^{**},4]. Blocking various components in the BCR signaling pathway holds great therapeutic potentials in CLL.

Blocking the proximal B-cell receptor-activated protein tyrosine kinases

The three proximal PTKs mediating BCR signaling, Lyn, Syk and Btk, are all found to be over-expressed and constitutively activated in CLL cells compared to

Figure 1 Signaling pathways activated consequent to engagement of the B-cell antigen receptor



Potential therapeutic drug targets are shown in dashed outline. BCR engagement rapidly activates the proximal Src family kinases Lyn, Btk, and Fyn as well as the Syk and Btk tyrosine kinases. This initiates the formation of a signaling complex composed of the BCR, the aforementioned tyrosine kinases, adaptor proteins such as CD19 and BLNK, and signaling enzymes such as PLC γ 2, PI3K, and Vav. Signals from this complex activate multiple downstream signaling cascades that involve kinases, GTPases, and transcription factors. This leads to changes in cell metabolism, gene expression and cytoskeletal organization [7]. Adapted with permission from Gitendra Wickremasinghe *et al.* [3^{**}].



normal B lymphocytes [4,8^{••}]. Inhibitors for these kinases are being studied in preclinical and clinical studies.

Lyn inhibitors

Two selective Lyn inhibitors, PP2 and SU6656 blocked Lyn activity and induced apoptosis in primary CLL cells *in vitro* [9]. No clinical study of these inhibitors has been reported in CLL.

Spleen tyrosine kinase inhibitors

Syk is activated by BCR and amplifies its survival signal [10[•]]. In-vitro siRNA knockdown studies of Syk suggest that constitutively active Syk maintains a high level of antiapoptotic Mcl-1 via PKC δ which blocks proteasome-dependent degradation of Mcl-1 [11]. Selective Syk inhibitors BAY-61–3606 and R406 induced apoptosis in primary CLL cells [12–14]. In addition, Syk inhibition by R406 also blocked the migration toward CXCL12 and CXCL13, as well as chemokine production by CLL cells, and blocks the chemokine and stromal protective effects of the microenvironment [10[•],14].

Fostamatinib disodium (R788, FosD, AstraZeneca/Rigel Pharmaceuticals), an orally bioavailable prodrug of R406, is the first clinically available selective Syk inhibitor. Preclinical study of fostamatinib in E μ –TCL1 transgenic mouse model of CLL showed reduced survival and proliferation of the malignant B cells [15[•]]. A multicenter phase 1/2 clinical trial of fostamatinib in patients with recurrent B-cell non-Hodgkin lymphoma (B-NHL) and CLL was presented initially at ASH 2009 annual meeting and subsequently published [16^{••}]. Dose-limiting toxicity in phase 1 was neutropenia, diarrhea and thrombocytopenia, and 200mg twice daily was chosen as the phase 2 dosing. Phase 2 enrolled 68 patients with B-NHL, including 11 patients with small lymphocytic lymphoma (SLL)/CLL. The objective response rate was the highest for SLL/CLL at 55% (6 of 11), as compared to 10–22% for the other B-NHLs. Despite these promising results, further clinical development of fostamatinib in B-NHL is on hold and its future is unclear.

Bruton's tyrosine kinase inhibitors

Btk plays a crucial role in development and function of normal B cells. Whereas Btk is expressed in multiple hematopoietic cells, the primary defect in Btk knockout mice is B-cell-specific, suggesting a more selective B-cell function [7]. Btk mutations in humans are associated with X-linked agammaglobulinemia which is characterized by severely decreased immunoglobulin production and absence of B cells [17]. Btk is found to be significantly overexpressed in CLL as compared to normal B cells [8^{••}], making it a promising therapeutic target for CLL.

PCI-32765 (Pharmacyclics) is an orally available, irreversible, small molecule inhibitor of Btk. Several preclinical

and clinical studies of this new agent were presented at ASH 2010 annual meeting (abstract #45, #1385, #57) with one recently published [8^{••}]. PCI-32765 directly induced modest apoptosis in CLL cells *in vitro*, and blocks external survival signals to CLL cells from environmental soluble factors and stromal contact [8^{••}]. In addition, it inhibits the ability of CLL cells to migrate to and adhere to lymph nodes (ASH2010, abstract #45). PCI-32765 is being studied in an ongoing phase 1a trial of patients with B-cell malignancies, with a subsequent phase 1b/2 study focusing on CLL/SLL. Pooled analysis of the 54 CLL/SLL patients from these studies was presented at ASH2010 (abstract #57, oral presentation). Each of the studies included patients with CLL/SLL relapsed or refractory after more than one prior treatment regimens (median three prior treatments), whereas the second phase 1b study also included a cohort of elderly patients (aged ≥ 65 years) with previously untreated CLL/SLL. The treatment is generally well tolerated with infrequent grade 3/4 adverse events, no hepatic or renal toxicity, and no accumulative hematologic toxicity. Thirty-four out of 39 evaluable patients had a lymph node response (over 50% reduction). Of the 45 patients evaluable for response, complete remission was achieved in one patient, partial response (PR) in 16 patients, and nodal response with lymphocytosis in 19 patients. The latter response describes a unique clinical response of this agent with reciprocal change of lymphocyte count and lymph node sizes – many patients experienced rapidly reduced lymphadenopathy with concurrently increased peripheral blood lymphocytosis during the initial few weeks of the treatment. The lymphocytosis will gradually decrease over the ensuing months, with improved quality of response with time. This phenomenon is likely due to the unique impact of Btk inhibition on promoting lymphocyte trafficking out of the protective lymph nodes and bone marrow microenvironment. Therefore, Btk inhibition could potentially make the CLL cells more vulnerable to spontaneous or treatment-induced apoptosis. The low-toxicity profile and the unique effect on lymphocyte trafficking indicate that Btk inhibitors could potentially be combined with other cytotoxic therapy. Combination therapies of PCI-32765 with ofatumumab (NCT01217749) or chemoimmunotherapy (fludarabine/cyclophosphamide/rituximab or bendamustine/rituximab) (NCT01292135) in CLL is currently recruiting.

A novel, orally available, covalent Btk inhibitor – AVL-292 (Avila Therapeutics) – will be tested in an upcoming multicenter phase 1 clinical trial in patients with previously treated B-NHL and CLL/SLL (NCT01351935).

Blocking the PI-3K/Akt/mTOR pathway

The PI-3K-mediated signaling pathway plays a central role in controlling CLL cell apoptosis and survival.



Selective inhibitors for various kinases along this pathway have been developed.

Phosphatidylinositol 3-kinase inhibitors

Constitutive PI-3K activity is significantly increased in CLL cells compared to normal B cells. Inhibition of PI-3K activity by broad PI-3K inhibitor (LY294002) induced apoptosis of CLL cells [18]. However, prior clinical development of broad PI-3K inhibitors has been hampered by toxicity, especially hyperglycemia, which is due to the ubiquitous expression of p110 α and β isoforms in many cell types.

CAL-101 (Calistoga Pharmaceuticals) is a first-in-class, orally available, selective small molecule inhibitor of the PI-3K p110 δ isoform, which is exclusively expressed in hematopoietic cells. CAL-101 exhibited promising pre-clinical activity by antagonizing cellular survival signals from both intrinsic and extrinsic sources [19^{••}]. A phase 1 study of CAL-101 was reported at ASH 2010 annual meeting (abstract #55) in 54 patients with relapsed/refractory CLL/SLL (median 5 prior therapies). A dose range of 50–350 mg q.d.–b.i.d. for up to 12 cycles of 28 days was tested. There was no dose-limiting toxicity (DLT) and the maximally tolerated dose (MTD) was not reached. Grade ≥ 3 pneumonias occurred in nine (24%) patients; grade ≥ 3 neutropenia, $n = 9$ (24%); thrombocytopenia, $n = 4$ (11%); and anemia, $n = 3$ (8%) that were not usually considered CAL-101-related. The overall response rate was 33% (all PR). Lymph node reduction was observed in 100% of patients, with 91% lymph node response ($>50\%$ reduction). Similar to PCI-32765, CAL-101 also caused rapid initial increase of lymphocytosis which then gradually decreased over time. This was thought to be a result of lymphocyte redistribution due to the effect of PI-3K δ inhibition on lymphocyte trafficking.

Akt inhibitors

It is controversial whether Akt directly mediates the pro-survival action of PI-3K. A recent study showed that Akt is activated in CLL and that inhibition of Akt activation by selective inhibitor Akti-1/2 induced apoptosis of CLL cells *in vitro* [20[•]]. Another study showed that Akt inhibitor AiX induced apoptosis preferentially in IgVH unmutated CLL cells, and that Tc11a directly interacts with Akt to modulate its signaling [21[•]]. Perifosine (Keryx Biopharmaceuticals), an Akt inhibitor, is currently being tested in a phase 2 trial in patients with relapsed/refractory CLL (NCT00873457).

Mammalian target of rapamycin inhibitors

mTOR is a kinase involved in cellular growth and proliferation that mediates signals from the PI-3k/Akt pathway. Rapamycin (sirolimus, Rapamune, Wyeth) is an immunosuppressive agent that also exhibits growth inhibitory and proapoptotic effect on lymphocytes [5[•]].

Everolimus (RAD001, Afinitor, Novartis) is a more readily orally available derivative of sirolimus. A pilot trial of oral everolimus in patients with advanced CLL induced one PR and three stable disease, but was terminated after the enrollment of only seven patients due to toxicity concerns [22]. A recent phase 2 study of everolimus (10 mg/day) in relapsed/refractory indolent B-NHL showed only modest effect with 18% PR in CLL patients [23[•]]. Interestingly, lymph node response with lymphocytosis was observed, similar to PCI-32765 and CAL-101, indicating alteration of lymphocyte trafficking.

Multikinase inhibitors

A recent study of gene expression profile of 505 protein kinases in CLL revealed a distinctive signature with homogenous up-regulation of several protein kinases in CLL [24[•]], suggesting a potential role of second-generation multikinase inhibitors.

Dasatinib (Sprycel, Bristol-Myers Squibb) is a tyrosine kinase inhibitor that targets the Abl kinase and Src family kinases. Dasatinib induces apoptosis of CLL cells *in vitro* with preferential effects in IgVH unmutated CLL [25]. A recent study showed that dasatinib inhibited BCR signaling. However, its proapoptotic effect is abrogated by stromal cell contact alone or in the presence of CD154 and IL-4 [26[•]]. Hsp90 inhibitor 17-DMAG exhibited synergy with dasatinib and induced apoptosis of CLL cells despite the presence of CD154/IL-4 [26[•]]. In a phase 2 study in relapsed/refractory CLL, dasatinib single agent resulted in 20% response rate but was difficult to administer due to significant myelosuppression (ASH2007 abstract #920). Dasatinib is being tested in combination with various other agents including rituximab (NCT00949988), fludarabine (NCT01051115) fludarabine/rituximab (NCT01173679), lenalidomide (NCT00829647), and bendamustine (NCT00872976).

Sorafenib (Nexavar, Bayer) is another multikinase inhibitor that targets Raf serine/threonine kinase, vascular endothelial growth factor receptors (VEGFRs), and platelet-derived growth factor receptor (PDGFR)- β . Sorafenib induces apoptosis in CLL cells, which appears to be independent of its effect on VEGFR or Lck signaling [27[•]]. Sorafenib has also been recently shown to disrupt the survival provided by the microenvironment (ASH2010 abstract 47). A phase 2 trial of sorafenib in CLL is ongoing (NCT00303966).

Targeting the cell death machinery

CLL is featured by prolonged survival of malignant B cells, which is thought to be due to deregulated cell death machinery with defective balance among proapoptotic



and antiapoptotic members of the B-cell CLL/lymphoma 2 (Bcl-2) family [28[•]]. Most CLLs overexpress of the Bcl-2 family of antiapoptotic proteins Bcl-2, Bcl-XL, and Mcl-1, making them rational targets for CLL therapy.

Inhibitors of Bcl-2 family of antiapoptotic proteins

Various inhibitors of the Bcl-2 family of antiapoptotic proteins have been developed using antisense approach or synthetic BH3-mimetics.

Oblimersen (Genasense, Genta) is an antisense DNA oligonucleotide that inhibits Bcl-2 transcription. An update of the phase III randomized study of fludarabine–cyclophosphamide with or without oblimersen in relapsed/refractory CLL was recently reported. The oblimersen arm showed superior complete remission/nodular complete remission rate (17 vs. 7%, $P=0.025$), significantly prolonged time to progression and increased median survival (56 vs. 38 months, $P=0.04$) [29,30]. In March 2009, the FDA stated that it would not support the approval of oblimersen until further confirmatory trials become available.

Obatoclax (GX15-070, Gemin X Pharmaceuticals) is a small molecular pan-Bcl-2 family inhibitor, active across all Bcl-2 proteins. A phase 1 trial of single agent obatoclax intravenously in relapsed CLL showed only modest activity with PR in one patient (4%), although improvement of hematopoietic function was observed. A single-center phase 1 trial combining obatoclax with fludarabine–rituximab in previously treated CLL is ongoing (NCT00612612).

ABT-263 (navitoclax, Abbott) is a second-generation, orally available, small molecule inhibitor of the Bcl-2 family proteins targeting Bcl-2, Bcl-XL, Bcl-W but not Mcl-1 [28[•]]. The preliminary results of a phase 1/2a study of ABT-263 in relapsed/refractory CLL were reported in ASH2009 (abstract 883). The most common adverse events are gastrointestinal reactions, fatigue, thrombocytopenia (20%), and neutropenia (12%). The DLT is thrombocytopenia occurring within 3–5 days following ABT-263 administration as a result of Bcl-XL inhibition in platelets. The recommended phase 2 dose of ABT-263 is 100 mg 7-day lead-in dose followed by 200 mg/day of continuous dosing. ABT-263 is currently being evaluated in combination studies with rituximab in previously untreated CLL (NCT01087151) or chemoimmunotherapies (FCR or bendamustine/rituximab) in relapsed/refractory CLL (NCT00868413).

AT-101 (Ascenta Therapeutics) is an orally available small molecule inhibitor of Bcl-2, Bcl-XL and Mcl-1. AT-101 induces apoptosis in CLL B cells and overcomes stromal cell-mediated Mcl-1 induction and drug resistance [31]. Phase 2 studies of single-agent AT-101

(NCT00275431) in B-cell malignancy or in combination with rituximab (NCT00286780) in relapsed/refractory CLL have recently completed. Another study of AT-101 and lenalidomide is ongoing (NCT01003769).

Targeting X-linked inhibitor of apoptosis

Because X-linked inhibitor of apoptosis (XIAP) blocks apoptosis at a central point via inhibition of caspases, there has been much interest exploiting it as a molecular target of cancer. This has led to the development of small-molecule inhibitors or antisense strategies directed against XIAP [32]. High levels of XIAP were detected in CLL cells, possibly as part of a nuclear factor-kappa B (NF- κ B)-regulated survival program [33]. XIAP-inhibiting compounds have been shown to sensitize primary CLL cells towards TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis [34,35[•]]. A multicenter phase 1/2 study of AEG35156 (Aegera Therapeutics), a second-generation antisense oligonucleotide targeting XIAP, in relapsed/refractory CLL/SLL and indolent B-NHL is currently ongoing (NCT00768339).

Cyclin-dependent kinase inhibitors

Antiapoptotic Bcl-2 family proteins such as Mcl-1 have short-lived transcripts and proteins [28[•]]. Therefore, transient inhibition of transcription by cyclin-dependent kinase (CDK) inhibitors has been explored as a strategy to induce apoptosis in malignant B cells.

Flavopiridol (Alvocidib, Sanofi-Aventis) is a broad CDK inhibitor that induces apoptosis in CLL cells *in vitro* independent of P53 [6]. DLT in phase 1 studies of flavopiridol in relapsed CLL was life-threatening tumor lysis syndrome, which was accompanied by cytokine-release syndrome due to IL-6 secretion. In a phase 2 study, flavopiridol treatment resulted in 53% response rate with 47% PRs, 5% nodular PRs and 1.6% complete remission in relapsed CLL [36]. A registration study in 165 relapsed CLL patients is ongoing (NCT00098371). In addition, combination of flavopiridol with lenalidomide (NCT00735930), fludarabine/rituximab (NCT00058227), and cyclophosphamide/rituximab (NCT01076556) is ongoing in CLL.

SNS-032 (Sunesis Pharmaceuticals) is a potent selective inhibitor of CDK2, 7 and 9. A phase 1 dose-escalation study of this agent in relapsed CLL and myeloma was recently published [37[•]]. In the CLL cohort, tumor lysis syndrome was the DLT, and the most frequent grade 3/4 toxicity was myelosuppression. Of the 19 CLL patients, one had 50% reduction of measurable disease without improvement of hematologic parameters, and another had stable disease.

SCH 727965 (dinaciclib, Merck), a novel inhibitor of CDK1, CDK2, CDK5 and CDK9, is currently being



tested in CLL and mantle cell lymphoma in a randomized phase 2 study (NCT00871546).

Targeting the microenvironment

CLL interaction with the bone marrow and lymph node microenvironment niche consisting of soluble factors (chemokines and cytokines), stromal cells, nurse-like cells, follicular dendritic cells and T cells is of critical importance in promoting CLL growth, survival and development of drug resistance [4]. Blocking such protective interaction could render CLL cells more susceptible to apoptosis and cytotoxic therapies. As described above, the BCR pathway kinase inhibitors, in addition to its pro-apoptotic effect, appeared to also impede the communication of CLL cells with the microenvironment. Other agents specifically targeting this aspect are being studied.

Immunomodulating agent: lenalidomide (Revlimid, Celgene)

Lenalidomide's antitumor effect *in vivo* appears to depend on microenvironmental factors. Although the exact mechanism of action is unclear, lenalidomide is thought to inhibit angiogenesis, change the cytokine microenvironment, and modulate the immune system by enhancing cytotoxic T cells and natural killer (NK) cell activity [38]. It is active clinically in relapsed/refractory CLL in two prior studies [39,40]. A recently published phase 1 study of single-agent lenalidomide in frontline treatment of CLL initially encountered severe toxicity (tumor lysis, septic shock) when using a starting dose of lenalidomide 10 mg/day for 21 days of a 28-day cycle and weekly 5-mg dose escalations to a target of 25 mg. The protocol was modified to use 2.5 mg/day starting dose with monthly escalation to target dose of 10 mg/day with extended tumor lysis prophylaxis and monitoring. There was no further tumor lysis event. Tumor flare was common (88%) but mild. Seventy-two percent of patients experienced grade 3 to 4 neutropenia, with only five episodes of febrile neutropenia. The overall response rate was 56% (all PR) [41**]. A phase 1 study of lenalidomide with fludarabine and rituximab combination was terminated early due to excessive toxicity even at the lowest dose of fludarabine and lenalidomide, with idiosyncratic febrile syndrome and persistent myelosuppression [42*]. Many ongoing studies are underway assessing single-agent lenalidomide or in combination with various agents (rituximab, ofatumumab, dexamethasone, azacitidine, AT101, bendamustine and rituximab, cyclophosphamide, rituximab, etc. www.clinicaltrials.gov).

Inhibitor of CXCR4 signaling

Blockade of CXCR4 signaling antagonizes stroma-mediated protection and restores CLL chemosensitivity [4]. Plerixafor (Mozobil, AMD3100, Genzyme) is a hema-

topoietic stem cell mobilizing agent that antagonizes SDF-1 binding to CXCR4. A phase I study of plerixafor and rituximab in patients with CLL is currently ongoing (NCT00694590).

Other potential targets

A number of other potential targets of CLL therapy have been recently described and/or extensively reviewed, including NF- κ B [43*], CD38 [44*], Pim kinase [45*], Hsp90 [46], CK2 [47*,48], Tcl-1A [49*], Wnt/ β -catenin/LEF-1 pathway [50], VEGFR [51,52*], and so on. Inhibitors of these signaling molecules or pathways are currently being tested in preclinical and clinical settings [3**,4]. Detailed discussion of these pathways is beyond the scope of this review.

Conclusion

Signal transduction inhibitors are promising new strategy for targeted CLL treatment. By selectively targeting different components of the critical signaling pathways, these agents may directly promote apoptosis and/or indirectly blocking CLL cells from their protective microenvironment, and thus make them more susceptible to cytotoxic therapy. Combining inhibitors of different signaling pathways or combining signaling inhibitor with conventional treatments may have synergistic antileukemic effect. Further understanding of the signaling pathways and cross-talks will help us identify novel molecular targets, expand our therapeutic options, and develop individualized therapies.

Acknowledgements

S.M. is supported by funding from the Leukemia and Lymphoma Society and the Zell Scholar Award from the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

Conflicts of interest

There are no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 702).

- 1 Hallek M, Cheson BD, Catovsky D, *et al*. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008; 111:5446–5456.
- 2 Hallek M. State-of-the-art treatment of chronic lymphocytic leukemia. *Hematology Am Soc Hematol Educ Program* 2009; 440–449.
- 3 Gitendra Wickremasinghe R, Prentice AG, Steele AJ. Aberrantly activated antiapoptotic signalling mechanisms in chronic lymphocytic leukaemia cells: clues to the identification of novel therapeutic targets. *Br J Haematol* 2011; 153:545–556.

This article provided an updated comprehensive review on the aberrant signal pathways in CLL.



- 4 Pleyer L, Egle A, Hartmann TN, Greil R. Molecular and cellular mechanisms of CLL: novel therapeutic approaches. *Nat Rev Clin Oncol* 2009; 6:405–418.
- 5 Abou-Nassar K, Brown JR. Novel agents for the treatment of chronic lymphocytic leukemia. *Clin Adv Hematol Oncol* 2011; 8:886–895.
This article reviewed novel agents in CLL treatment, with data table including dosage, response rates, response duration and toxicities of various new agents.
- 6 Lin TS. New agents in chronic lymphocytic leukemia. *Curr Hematol Malig Rep* 2010; 5:29–34.
- 7 Dal Porto JM, Gauld SB, Merrell KT, *et al.* B cell antigen receptor signaling 101. *Mol Immunol* 2004; 41:599–613.
- 8 Herman SE, Gordon AL, Hertlein E, *et al.* Bruton's tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood* 2011; 117:6287–6296.
This study dealt with the role of Btk in CLL and demonstrated the in-vitro activity of inhibitor PCI-32765.
- 9 Contri A, Brunati AM, Trentin L, *et al.* Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. *J Clin Invest* 2005; 115:369–378.
- 10 Buchner M, Baer C, Prinz G, *et al.* Spleen tyrosine kinase inhibition prevents chemokine- and integrin-mediated stromal protective effects in chronic lymphocytic leukemia. *Blood* 2010; 115:4497–4506.
This study demonstrated a role of Syk in mediating the CLL–microenvironment crosstalk and the effect of FosD in preventing stroma-contact mediated cell survival in CLL.
- 11 Baudot AD, Jeandel PY, Mouska X, *et al.* The tyrosine kinase Syk regulates the survival of chronic lymphocytic leukemia B cells through PKCdelta and proteasome-dependent regulation of Mcl-1 expression. *Oncogene* 2009; 28:3261–3273.
- 12 Buchner M, Fuchs S, Prinz G, *et al.* Spleen tyrosine kinase is overexpressed and represents a potential therapeutic target in chronic lymphocytic leukemia. *Cancer Res* 2009; 69:5424–5432.
- 13 Gobessi S, Laurenti L, Longo PG, *et al.* Inhibition of constitutive and BCR-induced Syk activation downregulates Mcl-1 and induces apoptosis in chronic lymphocytic leukemia B cells. *Leukemia* 2009; 23:686–697.
- 14 Quiroga MP, Balakrishnan K, Kurtova AV, *et al.* B-cell antigen receptor signaling enhances chronic lymphocytic leukemia cell migration and survival: specific targeting with a novel spleen tyrosine kinase inhibitor, R406. *Blood* 2009; 114:1029–1037.
- 15 Suljagic M, Longo PG, Bennardo S, *et al.* The Syk inhibitor fostamatinib disodium (R788) inhibits tumor growth in the Emu- TCL1 transgenic mouse model of CLL by blocking antigen-dependent B-cell receptor signaling. *Blood* 2010; 116:4894–4905.
This study tested the in-vivo effect of FosD using a CLL transgenic mouse model.
- 16 Friedberg JW, Sharman J, Sweetenham J, *et al.* Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Blood* 2010; 115:2578–2585.
This study reported the result of a phase 1/2 trial showing the promising clinical activity of fostamatinib in CLL.
- 17 Tsukada S, Saffran DC, Rawlings DJ, *et al.* Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993; 72:279–290.
- 18 Ringshausen I, Schneller F, Bogner C, *et al.* Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase Cdelta. *Blood* 2002; 100:3741–3748.
- 19 Herman SE, Gordon AL, Wagner AJ, *et al.* Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. *Blood* 2010; 116:2078–2088.
This study reports the important preclinical study of CAL-101 in CLL.
- 20 Zhuang J, Hawkins SF, Glenn MA, *et al.* Akt is activated in chronic lymphocytic leukemia cells and delivers a pro-survival signal: the therapeutic potential of Akt inhibition. *Haematologica* 2010; 95:110–118.
This study demonstrated that Akt is activated in CLL, and that a harsh method of cell extraction is needed for detection of the active enzymes.
- 21 Hofbauer SW, Pinon JD, Brachtl G, *et al.* Modifying akt signaling in B-cell chronic lymphocytic leukemia cells. *Cancer Res* 2010; 70:7336–7344.
This study investigated the role of Akt and its modulation by Tci 1a in the survival pathway of CLL.
- 22 Decker T, Sandherr M, Goetze K, *et al.* A pilot trial of the mTOR (mammalian target of rapamycin) inhibitor RAD001 in patients with advanced B-CLL. *Ann Hematol* 2009; 88:221–227.
- 23 Zent CS, LaPlant BR, Johnston PB, *et al.* The treatment of recurrent/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) with everolimus results in clinical responses and mobilization of CLL cells into the circulation. *Cancer* 2010; 116:2201–2207.
This is a phase 2 trial of single-agent everolimus in relapsed/refractory CLL, showing moderate activity.
- 24 Tavoraro S, Chiaretti S, Messina M, *et al.* Gene expression profile of protein kinases reveals a distinctive signature in chronic lymphocytic leukemia and in vitro experiments support a role of second generation protein kinase inhibitors. *Leuk Res* 2010; 34:733–741.
This study compared the gene expression profiles of 505 protein kinases between CLL and acute lymphoblastic leukemia.
- 25 Veldurthy A, Patz M, Hagist S, *et al.* The kinase inhibitor dasatinib induces apoptosis in chronic lymphocytic leukemia cells in vitro with preference for a subgroup of patients with unmutated IgVH genes. *Blood* 2008; 112:1443–1452.
- 26 McCaig AM, Cosimo E, Leach MT, Michie AM. Dasatinib inhibits B cell receptor signalling in chronic lymphocytic leukaemia but novel combination approaches are required to overcome additional pro-survival microenvironmental signals. *Br J Haematol* 2011; 153:199–211.
This study showed dasatinib reduced the viability of CLL cells *in vitro*, but this effect was abrogated by stromal cell co-culture.
- 27 Huber S, Oelsner M, Decker T, *et al.* Sorafenib induces cell death in chronic lymphocytic leukemia by translational downregulation of Mcl-1. *Leukemia* 2011; 25:838–847.
This study demonstrated the pro-apoptotic effect of sorafenib in CLL, which was mediated by rapid down-regulation of Mcl-1.
- 28 Capitanì N, Baldari CT. The Bcl-2 family as a rational target for the treatment of B-cell chronic lymphocytic leukaemia. *Curr Med Chem* 2010; 17:801–811.
This article reviewed the defective apoptosis in CLL and various Bcl-2 family inhibitors.
- 29 O'Brien S, Moore JO, Boyd TE, *et al.* Randomized phase III trial of fludarabine plus cyclophosphamide with or without oblimersen sodium (Bcl-2 antisense) in patients with relapsed or refractory chronic lymphocytic leukemia. *J Clin Oncol* 2007; 25:1114–1120.
- 30 O'Brien S, Moore JO, Boyd TE, *et al.* 5-year survival in patients with relapsed or refractory chronic lymphocytic leukemia in a randomized, phase III trial of fludarabine plus cyclophosphamide with or without oblimersen. *J Clin Oncol* 2009; 27:5208–5212.
- 31 Balakrishnan K, Burger JA, Wierda WG, Gandhi V. AT-101 induces apoptosis in CLL B cells and overcomes stromal cell-mediated Mcl-1 induction and drug resistance. *Blood* 2009; 113:149–153.
- 32 LaCasse EC, Mahoney DJ, Cheung HH, *et al.* IAP-targeted therapies for cancer. *Oncogene* 2008; 27:6252–6275.
- 33 Munzert G, Kirchner D, Stobbe H, *et al.* Tumor necrosis factor receptor-associated factor 1 gene overexpression in B-cell chronic lymphocytic leukemia: analysis of NF-kappa B/Rel-regulated inhibitors of apoptosis. *Blood* 2002; 100:3749–3756.
- 34 Fakler M, Loeder S, Vogler M, *et al.* Small molecule XIAP inhibitors cooperate with TRAIL to induce apoptosis in childhood acute leukemia cells and overcome Bcl-2-mediated resistance. *Blood* 2009; 113:1710–1722.
- 35 Frenzel LP, Patz M, Pallasch CP, *et al.* Novel X-linked inhibitor of apoptosis inhibiting compound as sensitizer for TRAIL-mediated apoptosis in chronic lymphocytic leukaemia with poor prognosis. *Br J Haematol* 2011; 152:191–200.
This study identified XIAP as the key factor rendering CLL cells' resistance to TRAIL.
- 36 Lin TS, Ruppert AS, Johnson AJ, *et al.* Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. *J Clin Oncol* 2009; 27:6012–6018.
- 37 Tong WG, Chen R, Plunkett W, *et al.* Phase I and pharmacologic study of SNS-032, a potent and selective Cdk2, 7, and 9 inhibitor, in patients with advanced chronic lymphocytic leukemia and multiple myeloma. *J Clin Oncol* 2010; 28:3015–3022.
This phase 1 study of selective Cdk inhibitor SNS-032 demonstrated mechanism-based pharmacodynamic activity but limited clinical efficacy in heavily pretreated patients with CLL and myeloma.
- 38 Molica S. Immunomodulatory drugs in chronic lymphocytic leukemia: a new treatment paradigm. *Leuk Lymphoma* 2007; 48:866–869.
- 39 Chanan-Khan A, Miller KC, Musial L, *et al.* Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J Clin Oncol* 2006; 24:5343–5349.
- 40 Ferrajoli A, Lee BN, Schlette EJ, *et al.* Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. *Blood* 2008; 111:5291–5297.
- 41 Chen CL, Bergsagel PL, Paul H, *et al.* Single-agent lenalidomide in the treatment of previously untreated chronic lymphocytic leukemia. *J Clin Oncol* 2011; 29:1175–1181.
This is the first study of using lenalidomide in first-line treatment of CLL.
- 42 Brown JR, Abramson J, Hochberg E, *et al.* A phase I study of lenalidomide in combination with fludarabine and rituximab in previously untreated CLL/SLL. *Leukemia* 2010; 24:1972–1975.
This is the first phase 1 study of lenalidomide as frontline treatment of CLL.



608 Therapeutic modalities

- 43** Lopez-Guerra M, Colomer D. NF-kappaB as a therapeutic target in chronic lymphocytic leukemia. *Expert Opin Ther Targets* 2010; 14:275–288.
- This article reviewed the role of NF-kB pathway in CLL and highlights different strategies inhibiting this pathway.

- 44** Deaglio S, Vaisitti T, Zucchetto A, *et al.* CD38 as a molecular compass guiding topographical decisions of chronic lymphocytic leukemia cells. *Semin Cancer Biol* 2010; 20:416–423.

This article discussed the potential crucial role CD38 played in tumor–host communication and that its signal was detrimental to clinical outcome.

- 45** Chen LS, Balakrishnan K, Gandhi V. Inflammation and survival pathways: chronic lymphocytic leukemia as a model system. *Biochem Pharmacol* 2010; 80:1936–1945.

This article reviewed the role of Bcl-2 family of protein and Pim kinase in CLL.

- 46** Best OG, Singh N, Forsyth C, Mulligan SP. The novel Hsp-90 inhibitor SNX7081 is significantly more potent than 17-AAG against primary CLL cells and a range of haematological cell lines, irrespective of lesions in the TP53 pathway. *Br J Haematol* 2010; 151:185–188.

- 47** Martins LR, Lucio P, Silva MC, *et al.* Targeting CK2 overexpression and hyperactivation as a novel therapeutic tool in chronic lymphocytic leukemia. *Blood* 2010; 116:2724–2731.

This study demonstrated the pro-apoptotic effect of CK2 inhibitor in CLL, which was mediated by inactivation of PKC and increased activity of PTEN.

- 48** Shehata M, Schnabl S, Demirtas D, *et al.* Reconstitution of PTEN activity by CK2 inhibitors and interference with the PI3-K/Akt cascade counteract the antiapoptotic effect of human stromal cells in chronic lymphocytic leukemia. *Blood* 2010; 116:2513–2521.

- 49** Popal W, Boucas J, Peer-Zada AA, Herling M. Pharmacologic interception in T-cell leukemia 1A associated pathways as a treatment rationale for chronic lymphocytic leukemia. *Leuk Lymphoma* 2010; 51:1375–1378.

This article discussed the role of Tcl 1A in CLL and the inhibitory strategies.

- 50** Gandhirajan RK, Poll-Wolbeck SJ, Gehrke I, Kreuzer KA. Wnt/beta-catenin/LEF-1 signaling in chronic lymphocytic leukemia (CLL): a target for current and potential therapeutic options. *Curr Cancer Drug Targets* 2010; 10:716–727.

- 51** Paesler J, Gehrke I, Gandhirajan RK, *et al.* The vascular endothelial growth factor receptor tyrosine kinase inhibitors vatalanib and pazopanib potentially induce apoptosis in chronic lymphocytic leukemia cells in vitro and in vivo. *Clin Cancer Res* 2010; 16:3390–3398.

- 52** Shanafelt T, Zent C, Byrd J, *et al.* Phase II trials of single-agent anti-VEGF therapy for patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2010; 51:2222–2229.

This article combined the three separate phase 2 trials of single agent bevacizumab as anti-VEGF therapy in relapsed/refractory CLL and demonstrated the lack of clinical activity of this strategy.