

Review Article

Tailoring of chronic lymphatic leukemia therapy

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Abstract: Chronic lymphocytic leukemia (CLL) remains an incurable disease, with all patients who require therapy destined to relapse and understanding of the pathophysiology of chronic lymphocytic leukemia has advanced significantly. It is now clear that chronic lymphocytic leukemia is a relatively proliferative disorder that requires the help of its microenvironment to be maintained and to progress. The stimulation of the chronic lymphatic leukemia cell occurs in most, if not all, patients through antigen stimulation via the B cell receptors. In addition, there is now a appreciation of the role of the p53 pathway leading to chemoresistance and the elucidation of the molecular and intracellular signaling mechanisms of disease is just beginning to facilitate the development of several targeted small molecules that promise to revolutionize the treatment of Chronic lymphocytic leukemia.

Keywords: Chronic lymphocytic leukemia, pathophysiology, target therapy

Introduction

Chronic lymphatic leukemia (CLL) is a B-cell malignancy with significant variability in clinical presentation and it is the most common leukemia in western world with an incidence of 4.2/100000/year [1]. The incidence increases to more than 30/100000/year at an age of more than 80 year. The median age at diagnosis is 72 years. About 10% of chronic lymphatic patients are reported to be younger than 55 years. Chronic lymphocytic leukemia cells co-express the CD5 antigen and B-cell surface antigen CD19, CD20 and CD23 and the levels of surface immunoglobulin, CD20 and CD79b are characteristically low compared with those found on normal B-cells. Each clone of leukemia cells is restricted to expression of either κ or λ immunoglobulin light chains.

In contrast, the leukemia cells of mantle cell lymphoma, despite also expressing B-cell surface antigens and CD5, generally do not express CD23 and cases which express CD23, cyclin D1 staining or fluorescence in situ hybridization (FISH) for detecting a translocation (11;14) are useful to diagnose mantle cell lymphoma [2].

Cancer treatment strategies continue to evolve, with new drugs reaching the marketplace each year and patient survival data increasing steadily. Treatments are now based not only on the histopathological diagnosis of the lesion, but also on its underlying molecular basis. The use of non-specific radio- and chemotherapy that impacts on both healthy and cancerous cells is gradually being replaced by more targeted, and therefore less toxic, treatment strategies and the elucidation of the molecular and intracellular signaling mechanisms of disease is just beginning to facilitate the development of several targeted small molecules that promise to revolutionize the treatment of chronic lymphocytic leukemia.

Molecular pathophysiology of chronic lymphatic leukemia microenvironment

Molecular interactions between chronic lymphatic leukemia, stromal cells in the bone marrow and/or lymphoid tissue microenvironments were considered important for chronic lymphatic leukemia cell survival and proliferation, chronic lymphatic leukemia cell homing, and tissue retention [3]. Contact between chronic lymphatic leukemia cells and monocyte-derived nurse-like cells (NLCs) or bone marrow stromal

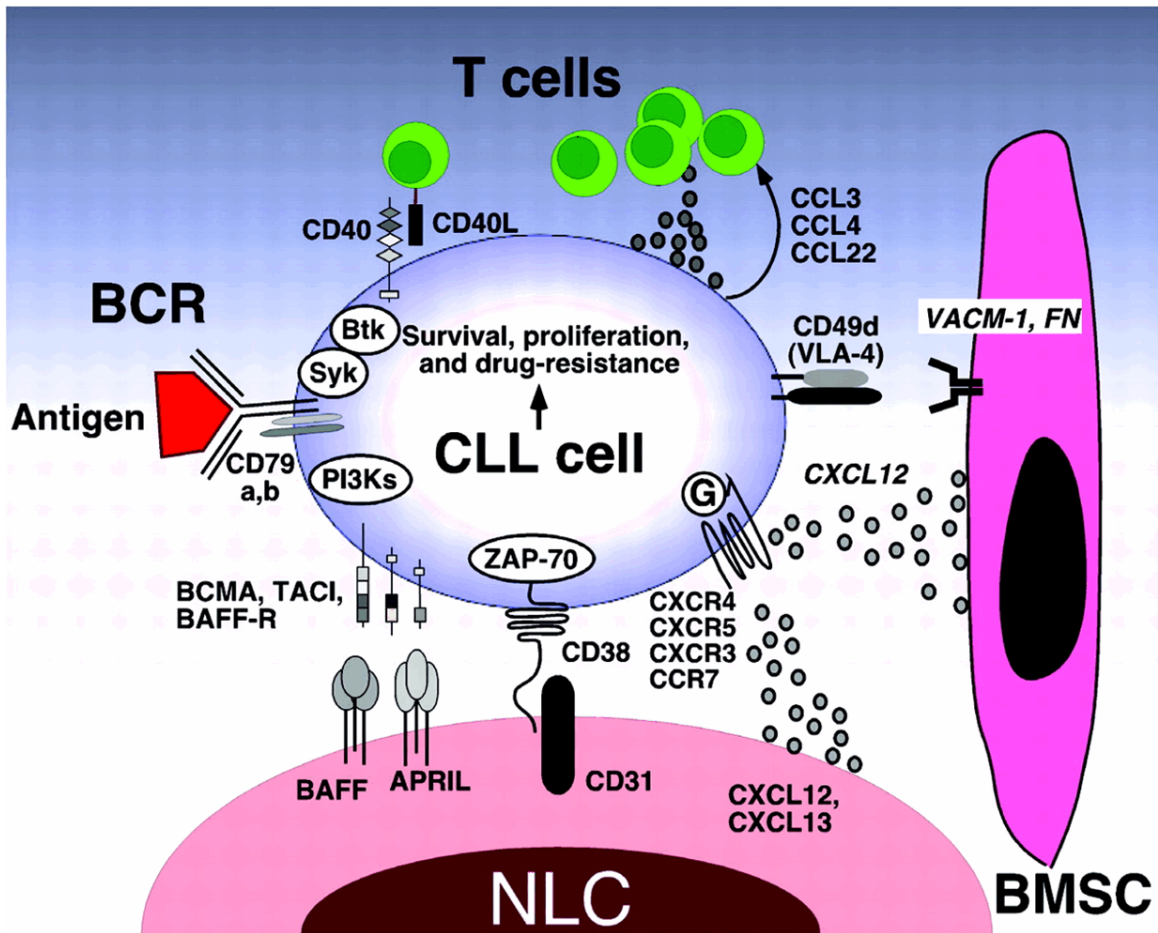


Figure 1. Main signaling pathways regulating the interactions between chronic lymphocytic leukemia cells and the microenvironment.

cells was established and maintained by chemokine receptors and adhesion molecules expressed on chronic lymphatic leukemia cells [4]. Monocyte-derived nurse-like cells (NLCs) expressed the chemokines CXCL12 and CXCL13, whereas bone marrow stromal cells predominantly expressed CXCL12 and the chemokine receptors CXCR3 and CCR7 were additional chemokine receptors on chronic lymphatic leukemia cells that were involved in lymphatic tissue homing [3]. Nurse-like cells and bone marrow stromal cells attract chronic lymphatic leukemia cells via the G protein-coupled chemokine receptors CXCR4 and CXCR5, which were expressed at high levels on chronic lymphatic leukemia cells. Integrins, particularly Very Late Adhesion molecule-4 integrins (CD49d), expressed on the surface of chronic lymphatic leukemia cells cooperate with chemokine receptors in establishing cell-cell adhesion through respective ligands on the stromal

cells (vascular cell adhesion molecule-1 and fibronectin) [10]. Monocyte-derived nurse-like cells (NLCs) also expressed the B cell-activating factor of the tumor necrosis factor (TNF), BAFF family and proliferation-inducing ligand (PRIL) and providing survival signals to chronic lymphatic leukemia cells via corresponding receptors (B-cell maturation antigen (BCMA), Transmembrane Activator and Calcium modulator and Cyclophilin ligand interactor (TACI), and BAFF receptors) [9]. CD38 expression allowed chronic lymphatic leukemia cells to interact with CD31, the ligand for CD38 that was expressed by stromal and monocyte-derived nurse-like cells (NLCs) activates Zeta chain Associated Protein-70 and downstream survival pathways [4]. Self- and/or environmental antigens were considered key factors in the activation and expansion of the chronic lymphatic leukemia clone by activation of the B cell receptor (BCR) and its downstream kinases

and stimulation of the BCR complex (BCR and CD79a,b) induces downstream signaling by recruitment and activation of spleen tyrosine kinase (Syk) and Bruton's tyrosine kinase (Btk) and Phosphatidylinositol-3-kinase (PI3K) [6]. B cell receptor (BCR) stimulation and coculture with monocyte-derived nurse-like cells induced chronic lymphatic leukemia cells to secrete chemokines (CCL3, CCL4, and CCL22) for the recruitment of immune cells (T cells and monocytes) and for cognate interactions. CD40L⁺ (CD154⁺) T cells were preferentially found in chronic lymphatic leukemia-proliferation centers and could interact with chronic lymphatic leukemia cells via CD40 [5].

Also chronic lymphatic leukemia cells almost universally had high expression of the anti-apoptotic molecule Bcl-2 with identification of many p53 pathway abnormalities, principally deletions of the p53 locus on the short arm of chromosome 17 [11].

The telomeres of chronic lymphatic leukemia cells, particularly in unmutated CLL, were short, indicating that they had undergone a large number of cell divisions suggesting the genomic instability and might contributed to the progression of chronic lymphatic leukemia [12, 13] (**Figure 1**).

Global and gene-specific aberrant DNA methylation had been detected in CLL and aberrant methylation had been described for genes that are specifically deregulated in CLL (for example, hypomethylation of BCL2 and T cell leukemia/lymphoma 1), the expression levels of ZAP70 correlate closely with the methylation of specific CpG islands in ZAP-70.

Epigenetic modifications were an attractive therapeutic target because they are reversible. Several compounds, including histone deacetylase inhibitors, had been tested on CLL cells in vitro and in clinical studies. Although these compounds had been shown to be biologically and clinically active, but the results in CLL had been less encouraging compared with other leukemias [39].

Diagnosis

The diagnosis of chronic lymphatic leukemia was established by presence in the peripheral blood of more than or equal 5000 monoclonal B lymphocytes/ μ l for the duration of at least 3

months. The clonality of the circulating B lymphocytes needed to be confirmed by flow cytometry. The leukemia cells found in the blood smear are characteristically small, mature lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin [7].

In the World Health Organization (WHO) classification, small lymphocytic lymphoma (SLL) and chronic lymphatic leukemia were considered to be the same entity and the diagnosis of small lymphocytic lymphoma requires the presence of lymphadenopathy and/or splenomegaly, with the number of B lymphocytes in the peripheral blood not exceeding 5×10^9 /l. small lymphocytic lymphoma cells show the same immunophenotype as chronic lymphatic leukemia cells. The diagnosis of small lymphocytic lymphoma should be confirmed by histopathological evaluation of a lymph node biopsy whenever possible [7]. In the absence of lymphadenopathy and organomegaly, cytopenias and clinical symptoms, the presence of less than 5000 monoclonal B lymphocytes/ μ l is defined as 'monoclonal B-lymphocytosis' (MBL) and progress to chronic lymphatic leukemia occurs in 1-2% of monoclonal B-lymphocytosis cases per year [8]. Although a bone marrow biopsy was not required for diagnosis, it was strongly recommended prior to initiating myelosuppressive therapies and for the diagnostic evaluation of unclear cytopenias with the detection of cytogenetic abnormalities, in particular of a deletion of chromosome 17 (del(17p)) and 11 (del(11q)) by FISH may had therapeutic consequences. Therefore, a FISH analysis is recommended prior to the start of therapy. Also the status of relevant infections (hepatitis B and C, cytomegalovirus (CMV), human immunodeficiency virus (HIV)) should be evaluated prior to chemoimmunotherapy, alemtuzumab or allogeneic stem cell transplantation to avoid virus reactivation [7].

Target therapy for chronic lymphatic leukemia

Understanding of the pathophysiology of chronic lymphocytic leukemia (CLL) had advanced significantly and it was clear that a relatively proliferative disorder that required the help of its microenvironment to be maintained and to progress, the stimulation of the chronic lymphocytic leukemia cell occurs in most, if not all,

patients through antigen stimulation via the B cell receptors. In addition, there was a clearer appreciation of the role of the p53 pathway leading to chemoresistance and these insights were allowing a more targeted approach with the use of p53-independent drugs such as monoclonal antibodies and high-dose steroids to overcome genetically poor-risk chronic lymphocytic leukemia [24].

P53 pathway abnormalities

At the time of initial treatment 7% of previously untreated patients with chronic lymphocytic leukemia requiring therapy had a deletion involving the short arm of chromosome 17 detected by FISH that covered the locus of the p53 gene and there was a small number of patients had mutations of the p53 gene without a 17p deletion [14]. Both 17p deletion and the p53 mutation rendered patients resistant to chemotherapy because the p53 pathway is critical in the cellular response to DNA damage, either by facilitating the repair of the damaged DNA or, if the damage is too great, leading to cell-cycle arrest and/or apoptosis [11].

Patients frequently did not respond to conventional chemotherapy with fludarabine or FC, even after FCR therapy, progression-free survival of these patients remains short [18]. Therefore, physically fit and young patients should be offered an effective initial regimen, of which alemtuzumab was currently the most widely explored, followed by an allogeneic stem cell transplantation within clinical trials [19]. Also combination of alemtuzumab with high-dose steroids had been reported to be even more effective in 17p-deleted chronic lymphocytic leukemia [24].

Patients with deleted chromosome 11q also had a poorer survival than those without this abnormality, although not as poor as patients with 17p deletion [15]. Chromosome 11q was the locus of the ataxia telangiectasia mutated (ATM) gene that lied on the p53 pathway but was not as pivotal in that pathway as the p53 gene itself [16]. The addition of rituximab to fludarabine plus cyclophosphamide appeared to overcome the poor prognostic impact of the chromosome 11q deletion [17].

B-cell receptors downstream signaling

There were multiple receptors and their ligands that participate in the proliferative signal gener-

ated by the interaction between the CLL cell and the microenvironment. It appeared that the role of the B cell receptor was central to this interaction. Therefore, targeting the receptor and its downstream signaling was an attractive therapeutic avenue. There were several molecules that were important in this pathway, including spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (Btk), Phosphatidylinositol-3-kinase (PI3K) and Zeta-chain Associated Protein-70 (ZAP-70).

PCI-32765

PCI-32765 was an irreversible covalent inhibitor of Bruton's tyrosine kinase (Btk) which was a B-cell receptor associated tyrosine kinase that was required for B-cell development and function, initial results of this drug specifically in CLL were reported at ASH 2010 and more mature results of the phase 1b/2 study are reported at the American Society of Clinical Oncology (ASCO) 2011 annual meeting in which patients are treated on a 28 days on/7 days off schedule as well as a continuous dosing schedule [20]. Toxicity had been generally minimal and primarily gastrointestinal, with nausea, vomiting, diarrhea, rash, and fatigue. Myelosuppression had not been seen. Therefore, PCI-32765 was a very promising novel therapy with excellent disease activity and minimal toxicity to date [21].

CAL-101

CAL-101 was a specific inhibitor of the delta isoform of Phosphatidylinositol-3-kinase (PI3K). The delta isoform had expression restricted to hematopoietic lineages, and the knockout mouse had a phenotype primarily affecting B-cell function. CAL-101 had been clinically beneficial and well-tolerated in refractory high-risk CLL patients for up to 2 years of therapy, raising the possibility that it can be continued for long-term maintenance therapy and the dose for phase II studies is 150 mg twice a day [22].

Fostamatinib

Fostamatinib was spleen tyrosine kinase (Syk) inhibitor which resulted in responses in over half of relapsed, refractory patients with CLL and there was a rapid and often dramatic reduction or even resolution of lymphadenopa-

thy within the first few months of therapy but coinciding with an increase in lymphocytosis. After continuous treatment for 6 or more months, the lymphocytosis was resolved and this pattern of response suggested that the proliferative compartment in the tissues had been disrupted by this agent [23].

Microenvironment cross-talk target agents

Plerixafor (Mozobil, AMD3100) and T140 analogs

These agents disrupt CLL-cell adhesion to bone marrow stromal cells and mobilize chronic lymphatic leukemia cells from protective tissue microenvironments to the blood, making them more accessible to conventional drugs and targeting the CXCR4-CXCL12 axis was currently being explored in a first clinical trial in CLL patients, combination of plerixafor with rituximab is a very promising novel combination with excellent disease activity and minimal toxicity and preliminary data show the safety of these drugs combination [25]. Studies in CLL would combine a CXCR4 antagonist with established cytotoxic agents or antibodies or in the setting of minimal residual disease, in which these agents would help to mobilize and eliminate residual CLL cells from tissue sanctuaries [26].

Cyclin-dependent kinase (CDK) inhibitors

Flavopiridol

Flavopiridol is a pan-inhibitor of cyclin-dependent kinases, including CDK9, that potently induces apoptosis in primary human CLL cells [27, 28]. Flavopiridol showed some promise as one of the cyclin-dependent kinase (CDK) inhibitors. However, there had been difficulties in identifying an acceptable schedule of administration and issues of toxicity that might ultimately limit the use of this agent and now there are second-generation CDK inhibitors in development [29].

Bcl-2 antagonists

Navitoclax (ABT-263)

Chronic lymphatic leukemia cells almost universally have high expression of the antiapoptotic molecule Bcl-2, although without the classical translocation seen in follicular lymphoma. Therefore, Bcl-2 is a sensible target in CLL, and

a variety of agents, such as antisense technology and small molecules, have been developed to inhibit this target. The latest showing some promise is navitoclax [30, 31].

Oblimersen

Oblimersen is a synthetic, bcl-2-directed antisense oligonucleotide which block Bcl-2 mRNA, resulting in decrement of bcl-2 protein levels thereby inducing apoptosis in treated CLL cells. It also makes treated cells more susceptible to apoptosis induced by fludarabine, dexamethasone, alemtuzumab, or rituximab and O'Brien et al study showed oblimersen had a higher response rate than fludarabine and cyclophosphamide [32]. Adverse effects of oblimersen included nausea, thrombocytopenia, tumor lysis syndrome (rare), and cytokine release reactions (rare).

Obatoclax

Obatoclax mesylate (GX15-070) is a synthetic small molecule pan-Bcl-2 antagonist with in vitro activity against CLL cells. In a phase I trial, obatoclax was administered to patients with advanced CLL at doses ranging from 3.5 mg/m² to 14 mg/m² as a 1-hour infusion and from 20 mg/m² to 40 mg/m² as a 3-hour infusion every 3 weeks. Although the clinical activity of this agent was limited (4% PR; 1 of 26 patients), patients with anemia (3 of 11 patients) or thrombocytopenia (4 of 14 patients) experienced improvements in hemoglobin and platelet counts. Dose-limiting reactions were neurologic (somnolence, euphoria, ataxia) and were associated with the infusion [33].

SPC2996

SPC2996 is a recently developed specific Bcl-2 mRNA antagonist. Patients with relapsed CLL were treated with a maximum of 6 doses of SPC2996 (0.2 mg/kg to 6 mg/kg) in a multicenter phase I trial-SPC2996 caused a 50% reduction of circulating lymphocytes in 5 of 18 patients (28%), which was found to be independent of its immunostimulatory and anti-Bcl-2 effects. Further studies with this agent are warranted [34].

Aptamers

Aptamers are nucleic acids (DNA or RNA) bind to their targets in a manner similar to antibody-

ies and can be generated completely in vitro and then rapidly produced by chemical synthesis in a form that is relatively stable and easy to modify [35]. Moreover, due to their low or non-immunogenic nature, small size and simple chemical structure, aptamers have advantages over monoclonal antibodies, which are inherently immunogenic due to their protein nature [36]. Aptamer binding can inhibit the biological activity of its target, including blocking either the catalytic site in the case of enzymes, or the ligand recognition site in the case of receptors, or induce loss-of-function conformational changes [37, 38]. The anti-nucleolin aptamer, AS1411, is the first nucleic acid based aptamer approved for Phase I clinical testing for the treatment of cancer in humans and nucleolin is an abundant cell surface receptor that has been associated with survival, growth and proliferation of cells, nuclear transport, transcription, packing and transport of rRNA, replication and recombination of DNA. It is also associated with a poor clinical prognosis for some cancer types [39]. Indeed, nucleolin overexpression has been shown to stabilize the expression of BCL2 in CLL cells and using an in vivo xenograft model, treatment with AS1411 resulted in initial cytostasis, followed by induction of apoptotic markers and cell death [40].

Heat shock protein 90 inhibitors (Hsp)

The zeta-associated protein of 70 kDa (ZAP70) was expressed in patients with aggressive CLL. Heat shock protein 90 (Hsp90) was a ubiquitous molecular chaperone that was necessary for the expression and activity of the tyrosine kinase ZAP70, which was expressed aberrantly in 45% of patients with CLL. ZAP70+ CLL cells expressed activated Hsp90 with high binding affinity for Hsp90 inhibitors. Treatment with Hsp90 inhibitors such as 17-AAG and 17-DMAG induced ZAP70 degradation and apoptosis in CLL cells but not in T cells, and it also impaired B-cell receptor signaling in leukemia cells. Hsp inhibitors were currently being investigated in a phase I clinical trial in relapsed CLL (NCT01126502, clinicaltrials.gov) [41].

Conclusion

During the coming years, increasing emphasis will be placed on targeting the microenvironment in chronic lymphocytic leukemia. CXCR4 and the B cell receptor-associated kinases Syk,

Btk, and PI3K δ represent the most advanced therapeutic targets in the complex cross-talk between chronic lymphatic leukemia cells and their microenvironment. Moreover, numerous other pathways of chronic lymphatic leukemia-microenvironment interactions, such as BAFF and APRIL, represent alternative therapeutic targets that are likely to be explored in the near future.

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