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Role of chemokines and their receptors in chronic lymphocytic leukemia

Function in microenvironment and targeted therapy

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Chemokines produced in distinct tissue microenvironments sustain migration of mature lymphocytes in lymphoglandula. Chemokine receptors expressed on chronic lymphocytic leukemia (CLL) cells regulate the migration of the leukemia cells within the bone marrow (BM), lymphoid organs in collaboration with chemokines. Chemokines form a pro-survival circuitry by regulating leukocyte trafficking, maintaining extended lymphocyte survival. Therefore, chemokines in tumor cell–microenvironment interactions represent a target for treatment of CLL. AMD3100 disrupts the CLL/microenvironment interactions and influences CXCL12/CXCR4 survival signaling. Fostamatinib, ibrutinib, and GS-1101 as B-cell receptor (BCR)-related kinase inhibitors inhibit BCR- and chemokine-receptor-signal-regulated kinase and have a good clinical response in CLL. Lenalidomide, sorafenib, and dasatinib are other additional drugs associated with chemokine in microenvironment. Inhibiting signaling through chemokine and microenvironment associated signaling are emerging as innovative therapeutic targets in CLL. In this article, we reviewed the role of chemokines in CLL microenvironment and novel therapeutics targeting CLL microenvironment.

Introduction

Chronic lymphocytic leukemia (CLL) is considered as the accumulation of mature monoclonal B cells rather than proliferation indolent B cell characterized by defective apoptosis.[1] Single gene mutations are rapidly being uncovered by sequencing the coding genome of CLL cases, including NOTCH1, splicing factor 3b subunit 1 (SF3B1), and myeloid differentiation primary response gene 88 (MYD88).[2] There is significant heterogeneity in the disease progression between CLL patients. Coding unmutated immunoglobulin variable heavy-chain (IGHV) genes and expressing the protein tyrosine kinase ZAP-70 and the type II transmembrane glycoprotein CD38 predict poor prognosis among leukemia patients who develop aggressive disease and need immediate therapy.[1,3]

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Compared with normal lymphocytes, CLL cells are accumulated in the bone marrow (BM), lymphoid tissues, and are flowed into peripheral blood and prolong survival time in vivo. CLL cells are spontaneous apoptosis in vitro but can be rescued by microenvironment of BM and lymphoid tissues.[4,5] CLL cells home to the BM by chemotaxis, increasing cell survival and probably the extent of marrow infiltration.[6] In vitro, adding stromal cells promotes survival of CLL cells through the secretion of several soluble growth factors and proteins.[7,8] CLL-accessory cell direct cross-talk in microenvironment appears to be meaningful in CLL cells survival and disease progression.[9] The microenvironment in the BM and lymph nodes (LNs) provides drug-resistance signals for CLL cells and drug resistance mechanism can interpret minimal residual disease (MRD) after conventional treatments.[10,11] Stromal cells protect CLL cells from conventional drug-induced apoptosis through cell adhesion-mediated drug resistance.

We will review the relationship of chemokines/chemokines receptors and CLL in microenvironment and then discuss therapeutic approaches of targeting the microenvironment or microenvironment associated signaling, as showed in **Figure 1**.

Role of Chemokines in CLL Microenvironment

Chemokines as a family of approximately 50 peptides are first proposed as "chemotactic cytokines" in 1992 which play a role in regulating homing of immune cells, leukocyte trafficking and maturation.[12,13] Physical interactions between CLL cells and bone marrow mesenchymal stem cells (BMSC), nurse-like cells (NLCs) are mediated through the molecular interaction of vascular cell adhesion molecule (VCAM-1), CD11a (leukocyte function associated antigen-1), and CD49d (very late antigen-4) and so on. Contact between the neoplastic cells and stroma-derived cells supports CLL cells growth and survival in vitro and in vivo.[14] Topical study show long-term survival demands direct interaction between CLL cells and the stroma cell co-cultures, whereas short-term survival of CLL cells in vitro can be sustained by soluble factors produced by stromal cells.[15] Stromal cell-derived factor-1 (SDF-1) as a homeostatic chemokine, binding to chemoreceptor CXCR4 not only plays a role in homing of CLL cells into the BM but also prolonging CLL cells survival by cell-to-cell interaction with BMSCs and NLCs.[16] In CLL cells, homeostatic chemokine receptors CXCR5 and CCR7 lead in resistance-mediated apoptosis.[17] The CX3CR1/CX3CL1 system

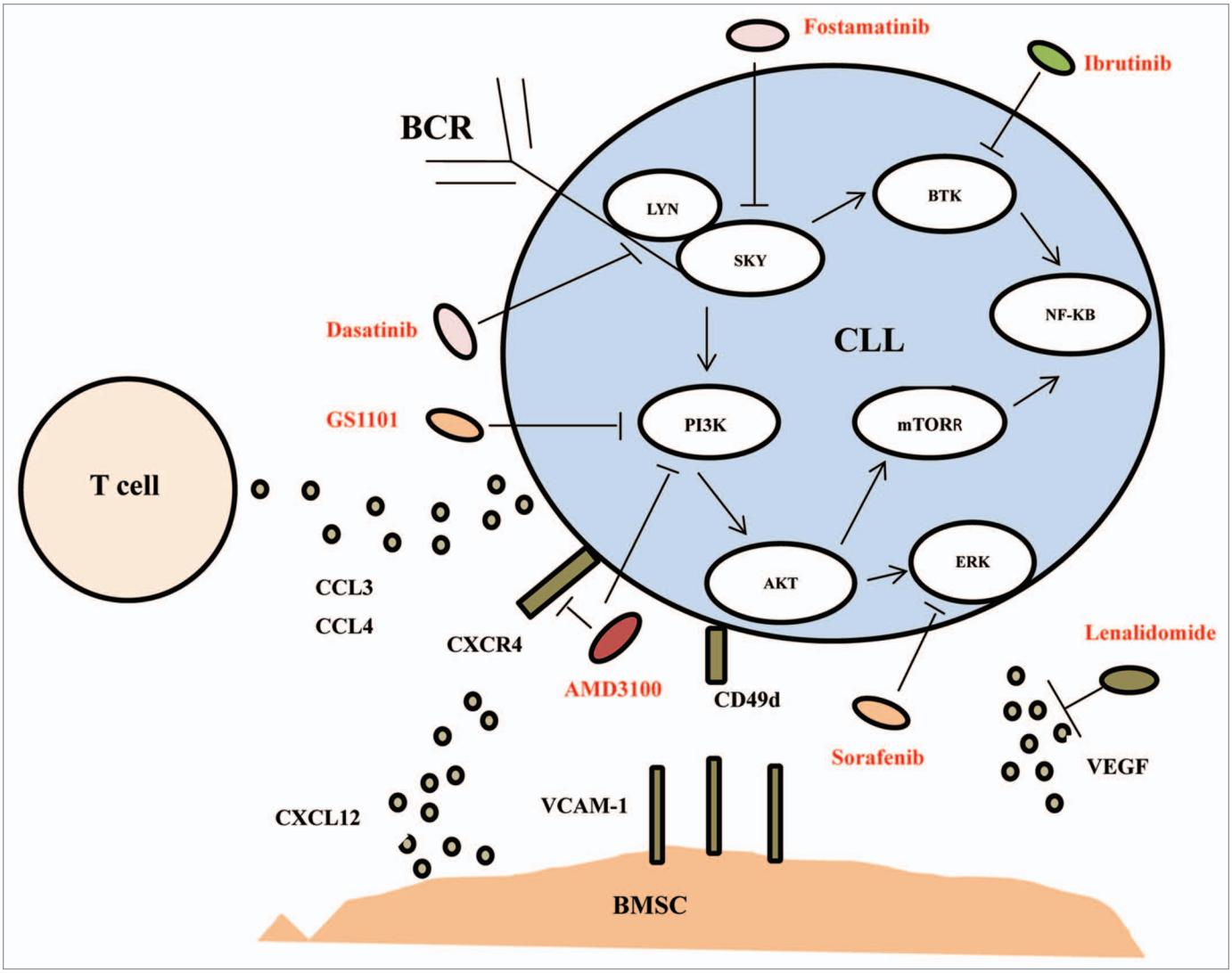

Figure 1. A schematic drawing of the microenvironmental interactions among CLL cells, T cells, and stromal cells described in the current review with targeting drugs.

may play a role in interactions between CLL cells and microenvironment by studying CXCL12- mediated adherence of leukemic cells to NLCs.[18] Other chemokines like CLL-generated CCL3 and CCL4 significantly lead to the recruitment of cells from the monocyte/macrophage lineage to BM microenvironmental sites.[19] Chemokines appear to form a pro-survival circuitry by regulating leukocyte trafficking, maintaining extended lymphocyte survival.[20]

Novel Therapeutics Targeting CLL Microenvironment

CXCR4 antagonists

CXCR4 (CD184), as a receptor for SDF-1(CXCL12) is highly expressed on the membrane of peripheral blood CLL cells which take advantage of CXCR4/CXCL12 axis to remain in a favorable environment.[21] CXCL12 binding to CXCR4 can regulate leukemia cells adhesion to actin polymerization and vascular endothelium, and accommodate migration beneath and underneath BMSCs.[22] In CLL cells, higher levels of CD49d in fact conduct migration beneath BMSCs in assistance with CXCR4.[23] $CD38^+$ CLL cells show higher levels of chemotaxis compared with $CD38^-$ CLL cells and activation of $CD38^+$ CLL cells with a monoclonal antibody (mAb) enhanced CXCR4 chemotaxis toward CXCL12, and a blocking anti-CD38 mAb can inhibit this chemotaxis.[24] Migration and survival in response to CXCL12 are associated with ZAP-70 expression, which is stimulated by B-cell receptor (BCR) signaling.[25] When engaging in adhesion to stromal cells, CLL cells are resistant to the cytotoxic effects of common drugs in CLL patients, like corticosteroids and fludarabine.[26] This adhesion-mediated drug resistance mechanism may explain MRD in the marrow and relapse found in CLL patients.[27]

CXCR4 antagonists were initially identified for HIV treatment and then found to induce leukocytosis, and recently are applied clinically for hematopoietic progenitors mobilization in lymphoma patients.[28] CXCR4 antagonists, such as AMD3100,

T140, and ALX40-4C, can block CLL–stroma interactions and then mobilize CLL cells from their protective microenvironments to the blood, becoming accessible to conventional drugs.[29,30] Namely, AMD3100 not only inhibits CXCL12-mediated guanosine diphosphate (GDP) binding, free of calcium, chemotaxis but also disrupts the cell/MSCs or NLCs-based microenvironment interactions, blocks survival stimuli, and influences the survival signal provided by CXCL12.[30,31] In CLL, mobilization and sensitization of leukemia cells could be achieved via combining a CXCR4 antagonist with conventional cytotoxic agents like fludarabine, cyclophosphamide, or established CLL drugs, like antibodies (rituximab or alemtuzumab), or combined immunochemotherapy.[31-33]

Andritsos et al.[34] went on a study of the maximum tolerated dose of AMD3100 in combination with rituximab in relapsed CLL patients and the results showed that of 14 estimable patients, 5 (36%) had partial response (PR), 3 (21%) had stable disease for ≥ 2 mo and 6 (43%) had progressive disease (PD). On day 8, there was a median 3.8-fold increase in peripheral blood CLL cells, demonstrating CLL cells mobilization. On day 26 fewer peripheral blood CLL cells were found with a median fold increase of 1.5-fold. Under certain circumstance, maximum responses were detected in several months after completion treatment of rituximab. From above data, we can find AMD3100 in combination with rituximab is a new therapeutic avenue for relapsed CLL patients.

The underway CLL trial combines plerixafor with rituximab, and original data demonstate a plerixafor dose-dependent CLL-cell mobilization to the peripheral blood from tissue sanctuaries and indicate the safety of combination of plerixafor with rituximab. Future studies in CLL may combine a CXCR4 antagonist with established convention agents or antibodies so as to help to mobilize and eliminate residual CLL cells.

BCR-related kinase inhibitors

BCR stimulation signal plays a significant role in the occurrence and prognosis of CLL.[35] First of all, disease progress of CLL patients are closely related with BCR variable area mutations; second, CLL cells restrictively express IgVH sequence BCR; third, no mutation of Ig and/or ZAP-70$^+$ has priority response to stimulation of BCR.[36,37] BCR launches a signaling cascade leading in expansion of CLL clone in company with other signals, like CD40 ligand, B-cell activating factors (BAFF), a proliferation-inducing ligand (APRIL), and so on.[38] In CLL, targeting different components of the BCR pathway can accomplish through kinds of constitutively active pathways including spleen tyrosine kinase (Syk), bruton tyrosine kinase (Btk), and phosphatidylinositol 3-kinases (PI3K).[39] Inhibition of both Syk and the PI3K pathway block the cross-talk between CLL cells with the microenvironment and what is more, inhibition of Btk, Syk, and PI3K would promote pro-apoptotic signals.[40]

Syk inhibitors (fostamatinib disodium), Btk inhibitors (ibrutinib), and PI3K inhibitors (GS-1101), which are BCR-related kinase inhibitors, have common characteristics in CLL treatment that these drugs can make LNs shrinkage and lymphocytes transitional increase in the first weeks of treatment because of CLL cells mobilization to peripheral blood from tissues.[41-43] Interference of BCR signal not only affects related survival pathways, but also influences tissue homing and CLL cells residual. BCR-related kinase inhibitors inhibit CLL cells chemokine-mediated adhesion in response to CXCL12 or CXCL13 and migration beneath stromal cells. These drugs also can downregulate secretion of BCR-dependent chemokines (CCL3, CCL4) produced by CLL cells. They inhibit BCR- and chemokine-receptor-induced Akt and extracellular signal-regulated kinase (ERK 1/2) activation and then markedly inhibit CLL cells survival and migration.[44-46]

PI3Ks inhibitor

PI3Ks as mediating signals of cell surface receptors enzymes has four class I PI3K isozymes (PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ) accommodating different cellular functions by the production of phosphatidylinositol-3,4,5-triphosphate.[47] Generation of phosphatidylinositol-3,4,5-triphosphate activates the downstream Akt, and the mammalian target of rapamycin (mTOR), which both have positive effects on cell survival, proliferation, and growth.[48] Of the all PI3K isoforms, PI3Kδ has been shown to play a significant role in homeostasis and function in response to chemokines. GS-110 1 is an up-to-date PI3Kδ-specific inhibitor that promotes CLL apoptosis, migration, homing.[43] Akt activation is suppressed by GS-1101 by means of CD40-, TNFα-, fibronectin-, and BCR-derived PI3K signaling.[49]

Overall response rate (ORR) of single agent GS-1101 treatment in relapsed or refractory indolent CLL patients is 33%. Ninety-one percent of CLL patients treated with GS-1101 lead to a greater than 50% decrease in their lymph node disease.[50] Recently, GS-1101-based combination therapies are in clinical trial and Coutre et al.[51] launched a phase I study that showed overall ORR for the GS-1101/rituximab (R), GS-1101/bendamustine (B), and GS-1101/BR respectively were 78%, 82%, and 87%, and 1-y progression-free survival (PFS) rates were 74%, 88%, and 87% respectively. Base on GS-1101 data on the American Society of Hematology Congress, we find that GS-1101 and GS-1101-based combination therapies are becoming a valid therapeutic target in relapsed or refractory CLL patients.

BTK inhibitor

BTK as a member of the Tec family kinases is activated upstream by Src-family kinases and leads to downstream activation of essential cell survival pathways such as nuclear factor-κB (NFκB) and mitogen activated protein-kinase (MAPK).[52] Mouse genetic ablation studies show that other BCR-pathway kinases rather than Btk have pleiotropic effects on kinds of cells; moreover BTK mutations in humans result in X-linked agammaglobulinemia (XLA), which is designated as a severe B cell-specific defects inherited disorder.[53] Based on these evidences, we suppose Btk is a distinctively attractive kinase target for selective B-cell inhibition.

Research finds that the mouse B cells deficient in function of cytoplasmic tyrosine kinase Btk are also lack of CXCL12-CXCR4, CXCL13-CXCR5, VCAM-1, or α4 integrin.[54] Combining this notion with the recent finding, we presume that Btk may be involved in the signaling mechanism underlying chemokine-controlled integrin-mediated migration. It is becoming clear that Btk signaling downstream other receptors including CXCR4 and CXCR5 greatly influence the CLL hazard rank and

disease progression.[55] Ibrutinib is the first human Btk inhibitor binding particularly and irreversibly to Btk protein through a cysteine residue and then inhibiting Btk phosphorylation. Hoellenreiger et al.[56] evaluate the effect of ibrutinib on CLL cell viability after anti-IgM stimulation and find ibrutinib blocks BCR-triggered CLL cells survival but CLL cells from ibrutinib-treated patients still are anti-IgM. Btk-independent pro-survival effects could not be inhibited by ibrutinib in vitro. Shortly after ibrutinib treatment, most circulating CLL cells display low CXCR4 expression which is characteristic of LN- and BM-derived CLL cells.

Brown et al.[57] conduct the major clinical trials of BTK inhibitor. The result shows the ORR of ibrutinib in relapsed refractory CLL is 67% and PFS 88% at 15 mo. In a cohort of untreated patients 65 y and over, the estimated 15 mo PFS is 96%. ORR of combination of ibrutinib with BR is 93%, PFS 90% at 11 mo, compared with ibrutinib with ofatumumab, ORR 100%, PFS 89% at 10 mo. Burger and his colleagues conduct a phase II single-center clinical trials of ibrutinib and rituximab. Early evaluable response is 50% at the inital 3 mo; ORR is 85%, CR 40%, and PR 45%. On this combination trial, in contrast with single-agent ibrutinib, re-distribution lymphocytosis peaks earlier and the duration is shorter, presumably because of the addition of rituximab.[58] On the basis of above data, ibrutinib and ibrutinib-based combination therapies is a safe, well-tolerated treatment for high-risk CLL patients and induce very high initial response rates.

Syk inhibitor

Syk as a member of the Syk/ZAP-70 family of non-receptor kinases activates BCR downstream signaling pathways, like Btk and activated B-cell linker protein (BLNK), which then activate the downstream signaling molecules NFκB, Raf, MEK, and ERK.[59] Syk signaling is required for B-cell development, proliferation, and survival. Syk-deficient mice show an interdict at the pro-B to pre-B transition.[60] R406 as an ATP competitive kinase inhibitor has limited specificity toward Syk, because of its activity against other kinases including FMS-related tyrosine kinases 3 (Flt3), Janus kinase 1, and Janus kinase 3. R406 is effective in CLL and other B-cell malignancies through disrupting BCR signals and micro-environmental interactions.[61]

Herman et al.[62] research shows that NFκB signature genes and MYC signature genes are downregulated due to fostamatinib. Expression of CD69, CD86, and the percentage of CLL cells expressing Ki67 are also remarkably reduced by fostamatinib. Cytotoxic effects of Syk inhibitor is associated with Syk protein expression and is stronger in unmutated IGVH and ZAP70$^+$ CLL cases. Compared with fludarabine therapy alone, combination of fludarabine with R406 increase cytotoxicity that provides potential mechanistic for a novel treatment option for the poor prognosis of CLL patients. Friedberg et al.[63] launch a clinical trial, 68 patients are enrolled in three cohorts: diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), and other non-Hodgkin lymphoma (NHL). CLL/small lymphoma leukemia (SLL) has the highest RR that is 55% (6/11) to fostamatinib. Collectively the data provide a blueprint to further study fostamatinib-targeted therapeutics.

In these trials, we find BCR-related kinase inhibitors both can make LNs shrinkage and lymphocytes transitional increase in the initial weeks of treatment owing to CLL cells mobilization to blood. Data regard to the frequency of relapses and progression of BCR-related kinase inhibitors are at the present stage very juvenile, but preliminary results are satisfactory and myelosuppression is scarce. Now novel insights regarding BCR inhibitor drugs not only provide support for their further application as monotherapy but also for their use as equitable combination therapy like coalescence with rituximab, ofatumumab, and bendamustine, utilizing the microenvironment dependence in CLL. Potential mechanism of resistance to BCR inhibitor drugs as yet are unknown and probably will become an innovative avenue in future, especially when these drugs are widely application.

Lenalidomide

Lenalidomide as an immune modulatory agent are recently approved for application in multiple myeloma, lymphoma, acute myeloid leukemia and CLL.[64] Application of lenalidomide in CLL has been combined with development of antitumor antibodies and induce a disease-specific side effect of tumor flare and cytokine release, such as serum basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).[65,66] bFGF and VEGF both have been associated with promoting CLL survival and circulating levels of bFGF is associated with response to therapy for CLL.[65] Recent study shows that activation of CLL cells induced by lenalidomide depends on the PI3Kδ pathway. Inhibition of PI3Kδ signaling by the PI3Kδ inhibitor can block up CLL cell activation, VEGF and bFGF gene expression induced by lenalidomide.[67]

Now, the encouraging results with single-agent lenalidomide as first-line treatment option for CLL shows ORR is 56% (no CR) and tumor flare is common.[68] Combination lenalidomide with rituximab of phase II clinical trials are ongoing in untreated CLL patients. The data present ORR is 66% and low toxicity have been observed.[69] In vitro, combination of PI3Kδ inhibitor is effective for preventing the tumor flare produced by lenalidomide treatment. The evidence provides support for the combination of lenalidomide with PI3K inhibitors, and potentially other BCR signaling inhibitors as a novel chemoimmunotherapy in future.

Sorafenib

The multikinase inhibitor sorafenib as a promising agent for treating tumors is a small molecule inhibitor of RAF.[70] Sorafenib could be particularly relevant in CLL cells by blocking CXCL12-induced phosphorylation of ERK and MEK in ZAP-70$^+$ CLL cells. What is more, ZAP-70$^+$ CLL cells represent more sensitive to the cytotoxic effects of sorafenib in vitro compared with ZAP-70$^-$ CLL cells.[71] This agent could overcome the protective effect of the CLL microenvironment at different ranks, like pro-survival signaling, chemokine signaling. Thus, further discussion of these factors and their effects on CLL provide vast ground for the development of additional strategies to improve the effectiveness of treatment with high risk CLL patients.

Dasatinib

Dasatinib as a tyrosine kinase inhibitor is a "second-generation" ATP-competitive inhibitor of the oncogenic BCR-ABL

Table 1. Clinical efficacy of targeted environmental drugs in chronic lymphocytic leukemia

Agent	Current status	Target	Nodal decrease > 50%	Response rate by 2008 IW-CLL criteria
AMD3100[34]	Under clinic phase II	Inhibiting CXCL12/CXCR4 axis	-	36%
GS-1101[50]	Under clinic phase III	Inhibiting PI3K signaling	91%	33%
Ibrutinib[57]	Under clinic phase III	Inhibiting BTK signaling	87%	79%
Fostamatinib[63]	Under clinic phase II	Inhibiting SYN signaling	63%	55%
Lenalidomide[68]	Complete clinic phase II	Increasing bFGF and VEGF secretion; inhibiting PI3K signaling	50%	56%
Sorafenib[71]	In malignant B cell lines	Blocking phosphorylation of ERK and MEK	-	-
Dasatinib[75]	Complete clinic phase II	Inhibit BCR signaling	44.4%	20%

IW-CLL, International Workshop on Chronic Lymphocytic Leukemia; -, not provided

kinase.[72] Given dasatinib inhibiting all Src-family tyrosine kinases, research shows dasatinib can inhibit BCR signal transduction and furthermore block BCR-mediated survival of CLL cells.[73] Dasatinib also significantly interferes migration of CLL cells toward CXCL12 through inhibiting CXCR4 signaling.[74] There is only one published phase II trial of dasatinib in CLL. In this study, Amrein et al. reported PR is 20%; moreover, notable nodal response is achieved more frequently than a reduction in peripheral blood leukocytosis.[75] Dasatinib exhibits novel chemotherapeutic agents without a protective microenvironment.

From data of the phase II trial of dasatinib in relapsed and refractory CLL, we find dasatinib shows intense activity in high-risk del(11q) patients; at the same time, the toxicity of myelosuppression is frequently encountered. To date, dasatinib in combination with other drugs in CLL has not been performed in clinical trial except in vitro. Given studies providing support for efficacy in combination with other drugs in vitro, we believe combination therapy of dasatinib in CLL will become a fresh area in future studies.

Conclusion

Therapeutic approaches of targeting the microenvironment or microenvironment associated signaling in CLL is becoming as the most outstanding therapeutic strategy in B-cell malignancies. By targeting selected microenvironmental interactions mediated by the immune system in CLL, it could possibly disrupt the protective of malignant cells derived from cross-talk microenvironment, and as well create enhanced features with established therapeutics and overcome resistance mechanisms in high-risk and relapse CLL. A vast quantity of targeted microenvironmental interactions of clinical trials are ongoing and preliminary results are favorable. Clinical trial outcome of these targeted drugs are presented in **Table 1**. We should closely monitor durability time of responses, MRD, risk for disease progression, and long-term side effects. The development of these related targeted treatment, whether single agent or in combination with conventional therapeutics is supposed to improve the quality of life of CLL patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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