REVIEW ARTICLE

Chronic lymphocytic leukemia cells are active participants in microenvironmental cross-talk

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ABSTRACT

The importance of the tumor microenvironment in chronic lymphocytic leukemia is widely accepted. Nevertheless, the understanding of the complex interplay between the various types of bystander cells and chronic lymphocytic leukemia cells is incomplete. Numerous studies have indicated that bystander cells provide chronic lymphocytic leukemia-supportive functions, but it has also become clear that chronic lymphocytic leukemia cells actively engage in the formation of a supportive tumor microenvironment through several cross-talk mechanisms. In this review, we describe how chronic lymphocytic leukemia cells participate in this interplay by inducing migration and tumor-supportive differentiation of bystander cells. Furthermore, chronic lymphocytic leukemia-mediated alterations in the interactions between bystander cells are discussed. Upon bystander cell interaction, chronic lymphocytic leukemia cells secrete cytokines and chemokines such as migratory factors [chemokine (C-C motif) ligand 22 and chemokine (C-C motif) ligand 2], which result in further recruitment of T cells but also of monocyte-derived cells. Within the tumor microenvironment, chronic lymphocytic leukemia cells induce differentiation towards a tumor-supportive M2 phenotype of monocyte-derived cells and suppress phagocytosis, but also induce increased numbers of supportive regulatory T cells. Like other tumor types, the differentiation of stromal cells towards supportive cancer-associated fibroblasts is critically dependent on chronic lymphocytic leukemia-derived factors such as exosomes and platelet-derived growth factor. Lastly, both chronic lymphocytic leukemia and bystander cells induce a tolerogenic tumor microenvironment; chronic lymphocytic leukemia-secreted cytokines, such as interleukin-10, suppress cytotoxic T-cell functions, while chronic lymphocytic leukemia-associated monocyte-derived cells contribute to suppression of T-cell function by producing the immune checkpoint factor, programmed cell death-ligand 1. Deeper understanding of the active involvement and cross-talk of chronic lymphocytic leukemia cells in shaping the tumor microenvironment may offer novel clues for designing therapeutic strategies.

Introduction

Chronic lymphocytic leukemia (CLL) is a prototypic malignancy that not only depends on intrinsic genetic defects, but is maintained by interactions with bystander cells in microenvironmental niches such as the lymph node. Bystander cells involved include T cells, monocyte-derived cells (MDC), and stromal cells (such as endothelial cells, fibroblastic reticular cells, and pericytes). Signals emanating from these cells critically affect several key features of malignancy of CLL cells,

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such as cell survival, chemo-resistance, cell proliferation, and migration.[1] Moreover, these signals result in an immunotolerant milieu in the CLL lymph node, in which the response to both pathogens[2] and neo-antigen-expressing malignant cells[3] is dampened.

Multiple types of regulators are involved in these communication processes: first, interleukins, such as interleukin (IL)-4 and IL-21, are involved in cell survival and proliferation[4,5] and IL-10 in immunosuppression.[6] Second, chemokines, including C-C motif chemokine (CCL)2, 3, 4, and 22, have an important role in chemo-attraction of cells towards the tumor microenvironment (TME).[7,8] In addition, CCL2 might play a role in tumor cell survival by indirect support via the microenvironment.[9] Third, growth factors, such as insulin-like growth factor 1, can promote survival.[10] Fourth, membrane-bound factors from bystander cells, such as CD40L and integrins, can induce cell survival.[11] Fifth, small vesicles, such as microvesicles and exosomes containing RNA, proteins, lipids or metabolites that are produced by either bystander cells[12] or CLL cells,[13,14] could transmit signals. Sixth, nucleoside adenosine is involved in dampening the local immune response and causing chemoresistance in CLL cells.[15]

Although it is by now well established that the factors secreted by bystander cells are essential for sustaining CLL (summarized in a recent review by Ten Hacken & Burger[1]), it has also become clear that these interactions are reciprocal in nature. As shown in other tumor types, upon contact with tumor cells, bystander cells can undergo changes that drive tumor progression.[7] Considering that CLL bystander cells include immune cells normally involved in highly adaptable immune responses, they are highly susceptible to (malignant) B-cell-derived signals. Alongside local changes leading to tumor progression, bystander cell alterations lead to systemic changes that can orchestrate recruitment of peripheral cells towards the TME.[7] Although various studies have suggested that bystander cell changes can take place at the genetic level,[7] recent evidence has shown unaltered stromal genomes, suggesting that microenvironmental signals are not mediated via genetic events.[7] These findings indicate that the stromal alterations are reversible, and that identification of the factors driving stromal cell changes may yield new therapeutic options.

In this review we analyze contemporary literature and our own recent findings to provide an overview of current evidence that signals emanating from CLL cells are crucial in creating a tumor-supportive TME. Second, as several reports show interdependency of bystander cells, we address how communication among bystander cells can contribute, in the context of CLL, to supportive TME interactions. We focus on T cells, MDC and stromal cells which together with CLL cells can form a tetrad exchanging reciprocal signals. For each of these, the functional effects of CLL cells towards the bystander cells are discussed followed by the relevant mechanisms. Lastly, we discuss effects between bystander cells.

T-cell interactions

Although it has been described that $CD4^+$ T helper type 1 (Th1) cells recognize CLL antigens,[3] activated Th1 cells also induce CLL-cell proliferation and survival.[16] Furthermore, T cells activate mitochondrial metabolism in CLL cells, which renders CLL cells more resistant to chemotherapy and contributes to cell proliferation.[17] Pro-tumor signals from T cells include both antigen-independent proliferation factors (CD40L in combination with IL-21[5]) as well as survival inducing factors [interferon (IFN)-γ,[18] IL-4,[4] and CD40L[19]] (Figure 1). These pro-survival signals result in a nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent upregulation of B-cell lymphoma 2 (BCL2) family members BCL2-related protein A1 (BFL-1) and B-cell lymphoma-extra large (BCL-X_L),[20] and protein kinase B (AKT)-dependent upregulation of induced myeloid leukemia cell differentiation protein (MCL-1).[21] With respect to CLL proliferation, mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT)3 pathways play additional roles.[22]

The interaction of CLL cells with T cells sensitizes CLL cells to additional TME-derived pro-tumor signals; first, B-cell receptor (BCR) signaling is enhanced by a microRNA-155-dependent mechanism after CD40L stimulation.[23] Second, CLL cells upregulate adhesion protein CD44 after CD40L stimulation, leading to hyaluronic acid binding, which increases retention in the lymph node.[24] Third, alongside a direct survival-inducing effect of T-cell-secreted IFN-γ on CLL cells, CD38 is upregulated on CLL cells after IFN-γ stimulation. CD38 can subsequently relay MDC-derived CD31 survival signals,[25] although this has been difficult to confirm *in vitro*.[26] These findings indicate that the pro-tumor effects of T cells might be partially mediated via other TME elements.

Various groups have described aberrations in the T-cell population in CLL patients. The total number of both $CD4^+$ and $CD8^+$ T cells is increased[27] and a skewing of their ratio towards $CD8^+$ cells occurs in both mice[28] and humans.[29] This skewing does not precede the occurrence of CLL, as it is not present during monoclonal B-cell lymphocytosis,[30] but even at an early stage of CLL, expansion of the $CD8^+$ T-cell population is correlated with an adverse outcome.[29] These findings indicate that CLL cells are the causative agent in this correlation. Furthermore, with respect to T-cell developmental stages, an increase in effector cells at the expense of naïve cells is observed.[31] The functional consequences of this skewing are currently unknown, but it could be speculated that a decreased naïve T-cell pool reduces the number of potential cytotoxic T cells directed towards CLL neo-antigens. Alongside the effects of CLL cells on T-cell skewing, CLL cells induce an exhausted T-cell phenotype.[32] This phenotype is characterized by increased expression of exhaustion markers CD160, CD244, BLIMP-1, and programmed cell death protein (PD)-1[32] and an inability to produce adequate levels of immune-activating cytokines upon stimulation,[33] similar to the phenotype of T cells directed towards chronic virus infections. Concurrently, effective synapse formation of T cells is suppressed by causing non-polarized release of lytic granules.[34] These mechanisms likely contribute to T-cell dysfunction. Lastly, CLL cells are involved in the induction of migration of T cells towards the lymph node.[8]

Several mechanisms have been linked to the observed suppression of T-cell function by CLL cells; it has been observed that CLL cells overexpress immune inhibitory factors such as programmed death ligand (PD-L)1 and PD-L2[35] and T cells from CLL patients have increased levels of the PD-1 receptor.[29] As PD-1 expression also increases with age, these observations should be interpreted with caution. To study causality, the Eμ-TCL1 mouse model, in

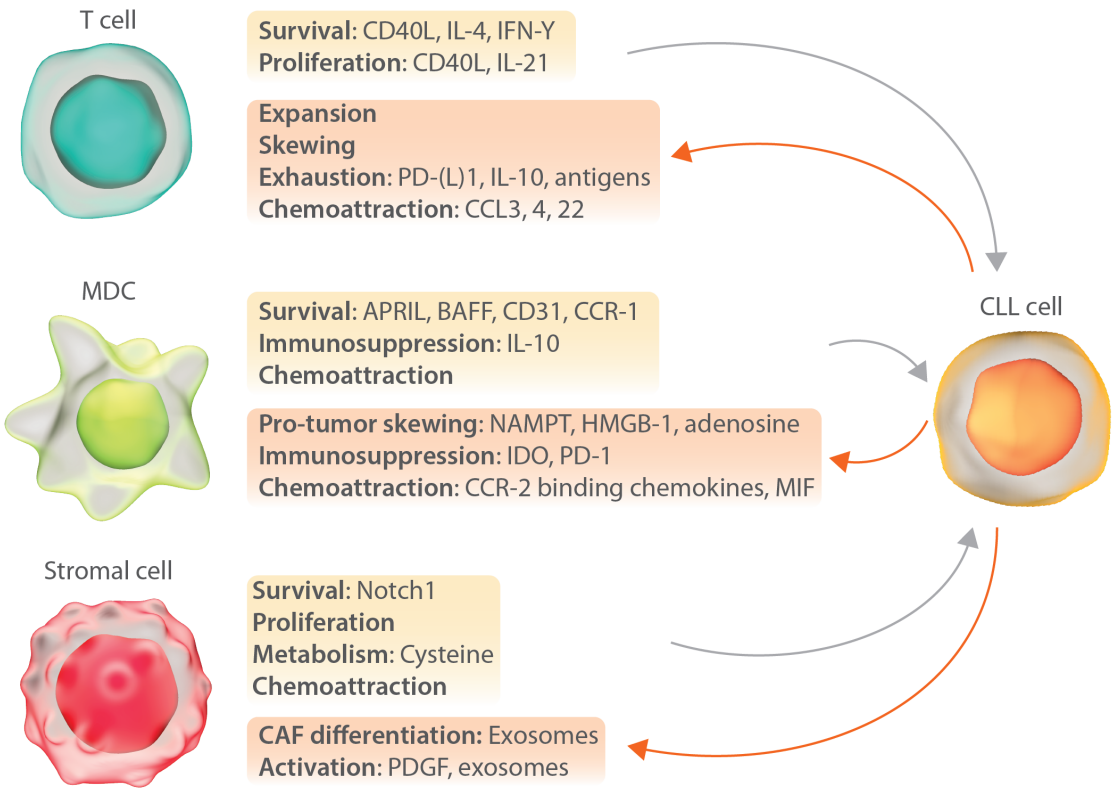

Figure 1. Interactions between chronic lymphocytic leukemia cells and bystander cells that contribute to the formation of a tumor-supportive microenvironment. Within the tetrad of CLL cells, T cells, MDC, and stromal cell, relevant effects (in bold) and signaling molecules involved in the interaction of CLL cells with T cells, MDC, and stromal cells are indicated. IL: interleukin; CCL: chemokine (C-C motif) ligand; IFN: interferon; APRIL: a proliferation inducing ligand; BAFF: B-cell activating factor; NAMPT: nicotinamide phosphoribosyltransferase; HMGB-1: high mobility group box 1; IDO: indoleamine 2,3-dioxygenase; MIF: migration inhibitory factor; CAF: cancer associated fibroblasts; PDGF: platelet derived growth factor.

which oncogene T-cell leukemia/lymphoma protein 1 (TCL1) is overexpressed under control of the B-cell-specific immunoglobulin heavy enhancer, has been used.[36] Although CLL in this mouse model is mainly driven by TCL1 in contrast to heterogeneous drivers in human disease, findings in this model have been valuable in explaining at least some of the observed immune disturbances in human CLL.[36] Using this model, aging bias was excluded by showing that adoptive transfer of $CD19^+$ cells of either wild-type or TCL-1 donor mice towards young wild-type recipients also induces PD-1 on T cells.[35] Alongside PD-1-mediated signaling, CLL cells produce the immune inhibitory cytokine IL-10.[6] Also, unknown contact-dependent factors produced by CLL cells actively impair T-cell synapse formation.[37] In addition, adenosine, which is produced in the hypoxic CLL TME, can also contribute to decreased T-cell proliferation.[15]

Very recently, a link between CLL-mediated T-cell dysfunction and altered immune metabolism was made by showing CLL-mediated suppression of T-cell glucose metabolism.[38] Whether impaired metabolism is a direct consequence of competition for fuels between the tumor cells and T cells, as has been shown in experimental models in other tumors,[39] or is solely due to CLL-mediated decreased AKT/mTOR signaling[38] has still to be resolved. It is important to note that the mechanistic causes of T-cell expansion and skewing remain largely obscure, but the defects in T-cell function might underlie the compensatory expansion seen in CLL patients.[29]

Several factors secreted by CLL cells can induce migration of T cells towards the CLL lymph node. CCL22 for instance, is secreted by CLL cells in the lymph node, which results in the recruitment of T cells.[8] Interestingly, as CCL22 preferentially induces migration of T helper type 2 (Th2) and T regulatory (T_{reg}) $CD4^+$ cells,[40] secretion of this chemokine could lead to skewing in the lymph node towards CLL-supporting and immunosuppressive T cells at the expense of cytotoxic T cells. Together with T-cell recruitment via CCL22, CLL cells secrete CCL3 and CCL4 upon interaction with MDC[41] and levels of CCL3 correlate with increased T-cell numbers in CLL lymph nodes.[42] Finally, the fact that T cells show reduced motility upon direct contact with CLL cells[43] could indicate that T cells are retained in the lymph node once recruited.

It has recently become evident that CLL cells also affect the phenotype of non-conventional T cells. A small population (1-10%) of the total T-cell pool carries the highly conserved γδ T-cell receptor (TCR) instead of the more prevalent αβ TCR.[44] Within this population, Vγ9Vδ2 T cells are the predominant subset present in the peripheral blood. In contrast to the recognition of peptide antigens by αβ T cells, Vγ9Vδ2 T cells respond to stress molecules

in malignant cells, in a TCR-dependent yet major histocompatibility complex (MHC)-independent process. As a consequence, these γδ T cells could suppress CLL cells acting independently of MHC antigen presentation.[44] Compared to cells from healthy donors, however, these γδ cells show a dysfunctional phenotype in CLL.[45] Interestingly, we found that these defects are spontaneously reverted when patient-derived γδ T cells are cultured in the absence of CLL cells,[46] in support of continuous, active subversion by CLL cells.

In their role as immunosuppressive cells, T_{reg} cells, on the other hand, secrete several immunosuppressive cytokines such as IL-10 and their number correlates with worse prognosis in several tumors.[47] In CLL, the frequency of forkhead box protein (FOXP)3$^+$ T_{reg} cells is increased in advanced disease.[48] IL-10 production by T_{reg} cells is higher in the CLL lymph node than in peripheral blood,[49] in accordance with microenvironmental signals engaging in immunosuppressive skewing.

Monocyte-derived cell interactions

MDC include monocytes, macrophages, and dendritic cells. These cells can, on the one hand, secrete essential survival factors for CLL cells, while, on the other hand, they can potentially mount an immune response against malignant cells as co-stimulators of B- or T-cell-mediated responses.[50] According to the dichotomized view of macrophage differentiation proposed in normal biology, M1 differentiated immunogenic macrophages mainly convey anti-tumor signals, while M2 wound healing macrophages are pro-tumorigenic overall.[51] The delayed disease development associated with MDC depletion in the TCL1 mouse model[52,53] suggests that MDC have a crucial, tumor-supportive function in CLL. Their supportive role is further indicated by the observation that a higher number of MDC correlates with worse prognosis in CLL patients.[54] Whereas MDC play important roles in inducing CLL-cell survival[55] and have migratory effects on CLL cells[55] (Figure 1), their role in inducing proliferation is subordinate; stimulation of CLL cells by macrophages does not induce proliferation (*unpublished observation*) and furthermore no spatial correlation between the MDC marker CD68 and the proliferation marker Ki67 exists in lymph nodes from CLL patients.[56] We recently found that MDC-mediated survival depended on chemokine signaling via CCR1.[21] Nurse-like cells are monocyte-derived cells which develop following prolonged *in vitro* culture with CLL cells[55] and have been identified in both the spleen and lymph nodes of CLL patients.[57] Nurse-like cells are thought to induce CLL survival effects via factors such as A proliferation inducing ligand (APRIL), B-cell activating factor (BAFF) or C-X-C motif chemokine (CXCL)12 (reviewed by Ten Hacken & Burger[1]). In line with this, transgenic APRIL overexpression in the TCL1 mouse led to faster disease progression.[58] By contrast, using a novel APRIL-overexpression system and an APRIL decoy receptor, we recently found *in vitro* that direct effects of APRIL produced by macrophages on CLL cells are negligible.[56] This discrepancy could be reconciled by postulating that *in vivo* effects of APRIL may be indirect, as exemplified by the recent finding that immunosuppressive IL-10 is produced by non-malignant B cells upon stimulation of APRIL receptor TACI.[59-61]

In line with the overall pro-tumor effect of CLL-associated MDC, pro-tumor M2 differentiation of macrophages in the presence of CLL cells has been found *ex vivo* and *in vitro*.[62-64] Functionally, these cells show impaired immunocompetence, as antigen presentation and immune response initiation are decreased.[65] In addition, CLL-associated monocytes are defective in their phagocytic function.[66] Moreover, dendritic cells in mice that have undergone adoptive transfer of TCL1 CLL cells show a decrease of MHC class II expression and an increase of the immunosuppressive molecule PD-L1,[52] and in CLL patients they have a suppressed immature phenotype showing decreased proliferation and cytokine production after stimulation.[67]

Several groups,[53,68] including our group,[56] have found that the CLL lymph node is interspersed with macrophages. As recruitment of these supportive macrophages depends on chemokine gradients emanating from the lymph node, it is postulated that CLL cells can provide these migratory signals. Indeed, it has recently been shown that in the TCL1 mouse model, CLL-infiltrated tissues harbor an increased number of monocytes compared to non-transgenic mice.[52]

Several CLL-secreted factors have been suggested to contribute to the pro-tumorigenic M2 differentiation of monocytes, which include nicotinamide phosphoribosyltransferase (NAMPT)[63] and high mobility group box 1 (HMGB1).[68] As NAMPT is also secreted by CLL-differentiated MDC, it could form a positive feedback loop keeping MDC in a CLL-supportive state.[63] Furthermore, by generating a hypoxic TME, CLL cells indirectly induce M2 differentiation as hypoxia increases adenosine production by MDC, which is known for its M2 differentiating capacity.[15] Besides the direct effects of these factors in inducing M2 differentiation, CLL-associated monocytes are primed for M2 differentiation as they show increased phosphorylation of downstream STAT molecules in response to M2-differentiating cytokines IL-4 and IL-10.[52] The persistent M2-differentiating signals emanating from CLL cells residing in the lymph node, in combination with PD-1 stimulation,[69] could explain the immune dysfunction of MDC. These effects are further enhanced by autocrine stimulation via PD-L1 or IL-10 expressed by the MDC themselves.[52] Furthermore, IL-10 is responsible for immune dysfunction seen in dendritic cells,[67] as here it leads to STAT6 suppression via suppressor of cytokine signaling 5.[70]

Interestingly, the tumor supportive phenotype in MDC is reversible, as IFN-γ stimulation results in transdifferentiation of pro-tumorigenic (M2) CLL-associated monocytes towards M1 macrophages.[71] Similarly, inhibiting PD1 signals could restore macrophage function,[69] suggesting there is potential for therapeutic intervention in these pathways.

Although several chemokines could account for the recruitment of monocytes towards the CLL lymph node, a critical role for CCR2 has recently been proposed. Adoptive transfer of CLL cells from TCL1 mice to CCR2 knockout mice led to a decrease in monocyte numbers in the spleen.[52] We recently found that primary CLL cells are able to secrete several monocyte-attracting chemokines such as CCL2, 3, 4, 5, 7, and 24, and CXCL5 and 10 after stimulation with the T-cell factor CD40L, resulting in monocyte migration. In line with data from Hanna *et al.*,[52] specific inhibition of CCR2 by small molecules could completely abrogate the migration towards CLL cells.[62] Others have found that knockout of macrophage migra-

tion inhibitory factor (MIF) reduced the number of macrophages in the spleen of TCL1 mice, suggesting an additional role for this chemokine in MDC migration.[72]

Stromal-cell interactions

Stromal cells constitute the connective tissue of organs and supply them with structure, anchoring and supportive signals. By definition, they are of non-hematopoietic origin. Different types of stromal cells include fibroblasts, reticular cells, and endothelial cells. Stromal cells can play a supportive role in various tumor environments, including the CLL lymph node[73] and stromal cell numbers generally correlate with tumor progression and worse prognosis.[74] Via several mechanisms, stromal cells can directly support CLL cells, for example, by inducing chemoresistance, promoting migration, and increasing cell survival via factors such as NOTCH1 (reviewed by Ten Hacken & Burger[1]). In addition, they induce CLL-cell proliferation[75] and change CLL-cell metabolism[76] (Figure 1). At the level of metabolism, stromal cells can supplement the defective CLL cystine transport by secreting large amounts of cystein into the TME.[77]

Alongside these direct effects, stromal cells can govern changes in CLL cells that make them more receptive to other microenvironmental signals. Upon co-culture with stromal cells, CLL cells upregulate transcription factor hypoxia-inducible factor-1α, which can induce changes in chemokine receptor expression in CLL cells that consequently retain them in the TME.[78]

As is the case for T cells and MDC, the stromal-cell secretome depends on the extracellular signals it receives. In order to provide tumor support, different stromal cell types have been shown to transdifferentiate into so-called cancer-associated fibroblasts in different malignancies.[74] In CLL, it has been suggested that this differentiation takes place via specific microRNA delivered through exosomes.[14] To support CLL cells, cancer-associated fibroblasts require AKT signaling.[79] A bidirectional cross-talk in which CLL cells induce AKT and extracellular signal-regulated kinase (ERK) signaling has been described[80] and platelet-derived growth factor is one secreted factor that can cause this activation.[81] In summary, these mechanisms underpin the dependence on CLL-secreted factors for tumor-supportive differentiation of stromal cells.

Interactions between bystander cells: monocyte-derived cells and T cells

We have so far discussed several direct reciprocal interactions between CLL cells and bystander cells. Considering that all cells within an ecosystem partake in reciprocal signaling, interactions between bystander cells can likewise contribute to the formation of a supportive TME in CLL.

Based on their role in the normal immune response, it is to be expected that MDC can also affect the phenotype of T cells in the context of CLL. Indeed, MDC contribute to T-cell skewing in CLL as skewing was reverted after depletion of MDC via clodronate treatment in the TCL1 mouse model.[52]

Furthermore, MDC are involved at several levels of T-cell suppression; first, in the context of CLL, MDC induce expression of PD-1 on T cells,[63] while PD-L1 is upregulated on CLL-differentiated monocytes,[52] both contributing to T-cell suppression. Second, CLL-differentiated monocytes inhibit T-cell proliferation[63] and third, they can inhibit T-cell activation and promote the differentiation towards T_{reg} cells via secretion of immunosuppressive indoleamine 2,3-dioxygenase (IDO).[64]

Like CLL cells, CLL-differentiated MDC can secrete chemokines that can attract T cells towards the lymph node, such as CXCL12. Furthermore, this chemokine enhances the expression of CLL-cell survival stimuli such as IFN-γ by T cells.[82] Similarly, in mouse studies, splenic monocytes show increased levels of T-cell-attracting chemokines such as CXCL9 and 10 after adoptive transfer of TCL1 CLL cells.[52] Concurrently, expression of the receptor for these chemokines (CXCR3), increases on T cells.[52] This indicates that supporting cells are not only recruited to the TME via induction of attracting chemokines in the lymph node, but also by an increased susceptibility to recruitment via chemokine receptor upregulation.

A subset of MDC, myeloid-derived suppressor cells (MDSC; expressing CD11b and CD33 and low levels of human leukocyte antigen-DR), provides important tumor-supportive factors in several other malignancies due to its immunosuppressive nature.[83] In CLL, it has been shown that the MDSC population is expanded[64,84] and that T cells are suppressed by MDSC.[64] Furthermore, the number of MDSC correlated with the number of CLL cells in patients.[84] These data indicate that MDSC might also suppress the T-cell response in the context of CLL.

Conclusion and therapeutic consequences

Cellular cross-talk is the driving force in establishing supportive interactions between elements within the TME. In this review, we have described several CLL-supportive mechanisms by bystander cells and the contribution of CLL cells. It is, however, important to keep in mind that CLL is an intra- and inter-tumoral genetically heterogeneous disease and that several of the described supportive TME mechanisms might depend on a specific genetic background. As an example, CLL cells harboring a NOTCH1 mutation might be more sensitive to NOTCH1 ligands present in the TME.[85] Likewise, a subset of CLL, specifically cases that harbor the trisomy 12 aberration, overexpresses CD49d, which might make them more sensitive to lymph node homing.[86] In the same vein, it has been shown that CD38-overexpressing CLL cells are more reactive to (microenvironmental) CXCL12 signaling and BCR signals.[87] Lastly, *IGHV* mutation status and expression levels and mutations of intracellular BCR signaling proteins such as zeta-chain-associated protein kinase 70, spleen tyrosine kinase, and Bruton tyrosine kinase (BTK) can dictate CLL-cell responses to TME BCR signals.[1] This shows that the receptiveness of CLL cells to TME support and subsequent disease outcome might depend on genetic alterations specific to CLL patients or to particular clones.

With these caveats in mind, we here discuss potential consequences for CLL therapy. With the advent of new treatment modalities for CLL, the potential side-effects that novel therapies have on bystander cells should be considered. For instance, because MDC-mediated antibody responses depend on BTK,[88] ibrutinib treatment reduces FcγR-mediated cytokine production,[88] inhibits activation,[69] and changes metabolism[69] in monocytes, which can inhibit their immune function, as has been shown for antibody-dependent cell-mediated cytotoxicity.[89] The outgrowth of adoptively transferred CLL cells

was, however, impaired in Btk knockout recipient mice, and macrophages deficient for its upstream tyrosine-protein kinase, Lyn, showed diminished CLL-supportive capacity *ex vivo*.[90] This suggests that the effects of ibrutinib on macrophages would be clinically beneficial. The depletion of immunosuppressive MDSC by ibrutinib[91] could furthermore support its beneficial clinical effects. Lastly, ibrutinib targets the T-cell-expressed BTK homolog, interleukin-2-inducible kinase (ITK), which is an important modulator of T-cell signaling and function.[92] Interestingly, as ITK inhibition preferentially affects Th2 cells because Th1 cells express a compensatory kinase, a potentially beneficial Th1 anti-tumor skewing occurs,[92] as was recently observed *in vivo* in pancreas carcinoma-engrafted mice.[93] In the context of chimeric antigen receptor T-cell therapy, T-cell expansion and increased tumor clearance were found when this therapy was used concurrently with ibrutinib,[94] indicating that ibrutinib treatment can overcome the suppressive effects of CLL cells on T cells. The effects of the kinase inhibitor idelalisib on bystander cells are generally CLL-supportive, as idelalisib reduces cytotoxic cytokine production of T cells[95] and in macrophages it reduces antibody-dependent cell-mediated cytotoxicity[89] and migration,[96] although specific inhibition of phosphoinositide 3-kinase γ leads to an immunostimulatory macrophage differentiation.[97] Given the critical pro-tumor effects of bystander cells, these findings suggest that complete tumor eradication after debulking treatment with chemotherapeutics can only be achieved after restoration of T-cell function by ibrutinib[94] or lenalidomide,[98] which can be complemented with CLL-directed chimeric antigen receptor T cells and PD-L1 inhibition.[99] In addition, as ibrutinib treatment results in migration of CLL cells out of the lymph node, subsequent CLL-attracting chemokine inhibition could avoid (re)formation of a tumor-supportive microenvironment and increase the effectiveness of cytotoxic therapies. The effectiveness of this strategy of migration inhibition has been shown, for instance, *in vivo* in prostate cancer, in which metastases were reduced after CXCR4 inhibition.[100] In conclusion, future insights into the dynamics of cellular interactions and the effects of (existing) therapies on these dynamics would substantially aid in designing optimal treatment strategies.

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References

1. Ten Hacken E, Burger JA. Microenvironment interactions and B-cell receptor signaling in chronic lymphocytic leukemia:implications for disease pathogenesis and treatment. Biochim Biophys Acta. 2016;1863(3):401-413.
2. Jurado-Camino T, Cordoba R, Esteban-Burgos L, et al. Chronic lymphocytic leukemia: a paradigm of innate immune cross-tolerance. J Immunol. 2015;194(2):719-727.
3. Rajasagi M, Shukla SA, Fritsch EF, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. Blood. 2014;124(3):453-462.
4. Dancescu M, Rubio-Trujillo M, Biron G, Bron D, Delespesse G, Sarfati M. Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and upregulates Bcl-2 expression. J Exp Med. 1992;176(5):1319-1326.
5. Pascutti MF, Jak M, Tromp JM, et al. IL-21 and CD40L signals from autologous T cells can induce antigen-independent proliferation of CLL cells. Blood. 2013;122(17):3010-3019.
6. DiLillo DJ, Weinberg JB, Yoshizaki A, et al. Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function. Leukemia. 2013;27(1):170-182.
7. Polyak K, Haviv I, Campbell IG. Co-evolution of tumor cells and their microenvironment. Trends Genet. 2009;25(1):30-38.
8. Ghia P, Strola G, Granziero L, et al. Chronic lymphocytic leukemia B cells are endowed with the capacity to attract CD4+, CD40L+ T cells by producing CCL22. Eur J Immunol. 2002;32(5):1403-1413.
9. Burgess M, Cheung C, Chambers L, et al. CCL2 and CXCL2 enhance survival of primary chronic lymphocytic leukemia cells in vitro. Leuk Lymphoma. 2012;53(10):1988-1998.
10. Yaktapour N, Ubelhart R, Schuler J, et al. Insulin-like growth factor-1 receptor (IGF1R) as a novel target in chronic lymphocytic leukemia. Blood. 2013;122(9):1621-1633.
11. de la Fuente MT, Casanova B, Garcia-Gila M, Silva A, Garcia-Pardo A. Fibronectin interaction with alpha4beta1 integrin prevents apoptosis in B cell chronic lymphocytic leukemia: correlation with Bcl-2 and Bax. Leukemia. 1999;13(2):266-274.
12. Webber J, Yeung V, Clayton A. Extracellular vesicles as modulators of the cancer microenvironment. Semin Cell Dev Biol. 2015;40:27-34.
13. Yeh YY, Ozer HG, Lehman AM, et al. Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling. Blood. 2015;125(21):3297-3305.
14. Paggetti J, Haderk F, Seiffert M, et al. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood. 2015;126(9):1106-1117.
15. Serra S, Vaisitti T, Audrito V, et al. Adenosine signaling mediates hypoxic responses in the chronic lymphocytic leukemia microenvironment. Blood advances. 2016;1(1):47-61.
16. Os A, Burgler S, Ribes AP, et al. Chronic lymphocytic leukemia cells are activated and proliferate in response to specific T helper cells. Cell Rep. 2013;4(3):566-577.
17. van der Windt GJ, van Bruggen JA, Gessel S, Terpstra S, Eldering E, Kater AP, Impact of signals from the tumor microenvironment on chronic lymphocytic leukemia metabolism. Keystone Cancer Metabolism; 2016.
18. Buschle M, Campana D, Carding SR, Richard C, Hoffbrand AV, Brenner MK. Interferon gamma inhibits apoptotic cell death in B cell chronic lymphocytic leukemia. J Exp Med. 1993;177(1):213-218.
19. Kitada S, Zapata JM, Andreeff M, Reed JC. Bryostatin and CD40-ligand enhance apoptosis resistance and induce expression of cell survival genes in B-cell chronic lymphocytic leukaemia. Br J Haematol. 1999;106(4):995-1004.
20. Tromp JM, Tonino SH, Elias JA, et al. Dichotomy in NF-kappaB signaling and chemoresistance in immunoglobulin variable heavy-chain-mutated versus unmutated CLL cells upon CD40/TLR9 triggering. Oncogene. 2010;29(36):5071-5082.
21. van Attekum MH, Terpstra S, Slinger E, et al. Macrophages confer survival signals via CCR1-dependent translational MCL-1 induction in chronic lymphocytic leukemia. Oncogene. 2017 Feb 13. [Epub ahead of print]
22. Slinger E, Kater AP, Thijssen R, Eldering E. Targeting BCR-independent proliferation of CLL cells. Blood. 2015;126(23):2916.
23. Cui B, Chen L, Zhang S, et al. MicroRNA-155 influences B-cell receptor signaling and associates with aggressive disease in chronic lymphocytic leukemia. Blood. 2014;124(4):546-554.
24. Girbl T, Hinterseer E, Grossinger EM, et al. CD40-mediated activation of chronic lymphocytic leukemia cells promotes their CD44-dependent adhesion to hyaluronan and restricts CCL21-induced motility. Cancer Res. 2013;73(2):561-570.
25. Burgler S, Gimeno A, Parente-Ribes A, et al. Chronic lymphocytic leukemia cells express CD38 in response to Th1 cell-derived IFN-gamma by a T-bet-dependent mechanism. J Immunol. 2015;194(2):827-835.
26. Tonino SH, Spijker R, Luijks DM, van Oers MH, Kater AP. No convincing evidence for a role of CD31-CD38 interactions in the pathogenesis of chronic lymphocytic leukemia. Blood. 2008;112(3):840-843.
27. Mackus WJ, Frakking FN, Grummels A, et al. Expansion of CMV-specific CD8+CD45RA+CD27- T cells in B-cell chronic lymphocytic leukemia. Blood. 2003;102(3):1057-1063.
28. Hofbauer JP, Heyder C, Denk U, et al. Development of CLL in the TCL1 transgenic

mouse model is associated with severe skewing of the T-cell compartment homologous to human CLL. Leukemia. 2011;25(9):1452-1458.
29. Nunes C, Wong R, Mason M, Fegan C, Man S, Pepper C. Expansion of a CD8(+)PD-1(+) replicative senescence phenotype in early stage CLL patients is associated with inverted CD4:CD8 ratios and disease progression. Clin Cancer Res. 2012;18(3):678-687.
30. te Raa GD, Tonino SH, Remmerswaal EB, et al. Chronic lymphocytic leukemia specific T-cell subset alterations are clone-size dependent and not present in monoclonal B lymphocytosis. Leuk Lymphoma. 2012;53(11):2321-2325.
31. Tonino SH, van de Berg PJ, Yong SL, et al. Expansion of effector T cells associated with decreased PD-1 expression in patients with indolent B cell lymphomas and chronic lymphocytic leukemia. Leuk Lymphoma. 2012;53(9):1785-1794.
32. Riches JC, Davies JK, McClanahan F, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013;121(9):1612-1621.
33. Christopoulos P, Pfeifer D, Bartholome K, et al. Definition and characterization of the systemic T-cell dysregulation in untreated indolent B-cell lymphoma and very early CLL. Blood. 2011;117(14):3836-3846.
34. Kabanova A, Sanseviero F, Veronica C, et al. Human cytotoxic T lymphocytes form dysfunctional immune synapses with B cells characterized by non-polarized lytic granule release. Cell Rep. 2016;15(1):9-18.
35. McClanahan F, Riches JC, Miller S, et al. Mechanisms of PD-L1/PD-1-mediated CD8 T-cell dysfunction in the context of aging-related immune defects in the Emicro-TCL1 CLL mouse model. Blood. 2015;126(2):212-221.
36. Simonetti G, Bertilaccio MT, Ghia P, Klein U. Mouse models in the study of chronic lymphocytic leukemia pathogenesis and therapy. Blood. 2014;124(7):1010-1019.
37. Ramsay AG, Johnson AJ, Lee AM, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. J Clin Invest. 2008;118(7):2427-2437.
38. Siska PJ, van der Windt GJ, Kishton RJ, et al. Suppression of glut1 and glucose metabolism by decreased Akt/mTORC1 signaling drives T cell impairment in B cell leukemia. J Immunol. 2016;197(6):2532-2540.
39. Chang CH, Qiu J, O'Sullivan D, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell. 2015;162(6):1229-1241.
40. White GE, Iqbal AJ, Greaves DR. CC chemokine receptors and chronic inflammation--therapeutic opportunities and pharmacological challenges. Pharmacol Rev. 2013;65(1):47-89.
41. Burger JA, Quiroga MP, Hartmann E, et al. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurselike cell cocultures and after BCR stimulation. Blood. 2009;113(13):3050-3058.
42. Hartmann EM, Rudelius M, Burger JA, Rosenwald A. CCL3 chemokine expression by chronic lymphocytic leukemia cells orchestrates the composition of the microenvironment in lymph node infiltrates. Leuk Lymphoma. 2016;57(3):563-571.
43. Ramsay AG, Evans R, Kiaii S, Svensson L, Hogg N, Gribben JG. Chronic lymphocytic leukemia cells induce defective LFA-1-directed T-cell motility by altering Rho GTPase signaling that is reversible with lenalidomide. Blood. 2013;121(14):2704-2714.
44. Born WK, Reardon CL, O'Brien RL. The function of gammadelta T cells in innate immunity. Curr Opin Immunol. 2006;18(1):31-38.
45. Coscia M, Vitale C, Peola S, et al. Dysfunctional Vgamma9Vdelta2 T cells are negative prognosticators and markers of dysregulated mevalonate pathway activity in chronic lymphocytic leukemia cells. Blood. 2012;120(16):3271-3279.
46. de Weerdt I, Terpstra S, Hofland T, et al. Chronic lymphocytic leukemia (CLL) cells are susceptible to γδ-T cell mediated killing, provided CLL-derived γδ-T cell dysfunction Can be Reversed. Blood. 2015;126(23):2914.
47. Halvorsen EC, Mahmoud SM, Bennewith KL. Emerging roles of regulatory T cells in tumour progression and metastasis. Cancer Metastasis Rev. 2014;33(4):1025-1041.
48. Piper KP, Karanth M, McLarnon A, et al. Chronic lymphocytic leukaemia cells drive the global CD4+ T cell repertoire towards a regulatory phenotype and leads to the accumulation of CD4+ forkhead box P3+ T cells. Clin Exp Immunol. 2011;166(2):154-163.
49. Lad DP, Varma S, Varma N, Sachdeva MU, Bose P, Malhotra P. Regulatory T-cell and T-helper 17 balance in chronic lymphocytic leukemia progression and autoimmune cytopenias. Leuk Lymphoma. 2015;56(8):2424-2428.
50. Erb P, Feldmann M. Role of macrophages in in vitro induction of T-helper cells. Nature. 1975;254(5498):352-354.
51. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res. 2006;66(2):605-612.
52. Hanna BS, McClanahan F, Yazdanparast H, et al. Depletion of CLL-associated patrolling monocytes and macrophages controls disease development and repairs immune dysfunction in vivo. Leukemia. 2016;30(3):570-579.
53. Galletti G, Scielzo C, Barbaglio F, et al. Targeting macrophages sensitizes chronic lymphocytic leukemia to apoptosis and inhibits disease progression. Cell Rep. 2016;14(7):1748-1760.
54. Gustafson MP, Abraham RS, Lin Y, et al. Association of an increased frequency of CD14+ HLA-DR lo/neg monocytes with decreased time to progression in chronic lymphocytic leukaemia (CLL). Br J Haematol. 2012;156(5):674-676.
55. Burger JA, Tsukada N, Burger M, Zvaifler NJ, Dell'Aquila M, Kipps TJ. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. Blood. 2000;96(8):2655-2663.
56. van Attekum MHA, Terpstra S, Reinen E, Kater AP, Eldering E. Macrophage-mediated chronic lymphocytic leukemia cell survival is independent of APRIL signaling. Cell Death Discov. 2016;2:16020.
57. Tsukada N, Burger JA, Zvaifler NJ, Kipps TJ. Distinctive features of "nurselike" cells that differentiate in the context of chronic lymphocytic leukemia. Blood. 2002;99(3):1030-1037.
58. Lascano V, Guadagnoli M, Schot JG, et al. Chronic lymphocytic leukemia disease progression is accelerated by APRIL-TACI interaction in the TCL1 transgenic mouse model. Blood. 2013;122(24):3960-3963.
59. Saulep-Easton D, Vincent FB, Quah PS, et al. The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B cells. Leukemia. 2016;30(1):163-172.
60. Hua C, Audo R, Yeremenko N, et al. A proliferation inducing ligand (APRIL) promotes IL-10 production and regulatory functions of human B cells. J Autoimmun. 2016;73:64-72.
61. van Attekum MH, Kater AP, Eldering E. The APRIL paradox in normal versus malignant B cell biology. Cell Death Dis. 2016;7(6):e2276.
62. van Attekum MHA, Terpstra S, Reinen E, et al. The T-cell/CLL/macrophage triad shapes a supportive tumor microenvironment in CLL. Blood. 2015;126(23):1715.
63. Audrito V, Serra S, Brusa D, et al. Extracellular nicotinamide phosphoribosyltransferase (NAMPT) promotes M2 macrophage polarization in chronic lymphocytic leukemia. Blood. 2015;125(1):111-123.
64. Jitschin R, Braun M, Buttner M, et al. CLL-cells induce IDOhi CD14+HLA-DRlo myeloid-derived suppressor cells that inhibit T-cell responses and promote TRegs. Blood. 2014;124(5):750-760.
65. Bhattacharya N, Diener S, Idler IS, et al. Nurse-like cells show deregulated expression of genes involved in immunocompetence. Br J Haematol. 2011;154(3):349-356.
66. Maffei R, Bulgarelli J, Fiorcari S, et al. The monocytic population in chronic lymphocytic leukemia shows altered composition and deregulation of genes involved in phagocytosis and inflammation. Haematologica. 2013;98(7):1115-1123.
67. Orsini E, Guarini A, Chiaretti S, Mauro FR, Foa R. The circulating dendritic cell compartment in patients with chronic lymphocytic leukemia is severely defective and unable to stimulate an effective T-cell response. Cancer Res. 2003;63(15):4497-4506.
68. Jia L, Clear A, Liu FT, et al. Extracellular HMGB1 promotes differentiation of nurse-like cells in chronic lymphocytic leukemia. Blood. 2014;123(11):1709-1719.
69. Qorraj M, Bruns H, Bottcher M, et al. The PD-1/PD-L1 axis contributes to immune metabolic dysfunctions of monocytes in chronic lymphocytic leukemia. Leukemia. 2017;31(2):470-478.
70. Toniolo PA, Liu S, Yeh JE, Ye DQ, Barbuto JA, Frank DA. Deregulation of SOCS5 suppresses dendritic cell function in chronic lymphocytic leukemia. Oncotarget. 2016;7(29):46301-46314.
71. Gautam S, Fatehchand K, Elavazhagan S, et al. Reprogramming nurse-like cells with Interferon-gamma to interrupt chronic lymphocytic leukemia cell survival. J Biol Chem. 2016;291(27):14356-14362.
72. Reinart N, Nguyen PH, Boucas J, et al. Delayed development of chronic lymphocytic leukemia in the absence of macrophage migration inhibitory factor. Blood. 2013;121(5):812-821.
73. Malhotra D, Fletcher AL, Turley SJ. Stromal and hematopoietic cells in secondary lymphoid organs: partners in immunity. Immunol Rev. 2013;251(1):160-176.
74. Raffaghello L, Vacca A, Pistoia V, Ribatti D. Cancer associated fibroblasts in hematological malignancies. Oncotarget. 2015;6(5):2589-2603.
75. Mittal AK, Chaturvedi NK, Rai KJ, et al. Chronic lymphocytic leukemia cells in a lymph node microenvironment depict molecular signature associated with an

aggressive disease. Mol Med. 2014;20:290-301.
76. Jitschin R, Braun M, Qorraj M, et al. Stromal cell-mediated glycolytic switch in CLL cells involves Notch-c-Myc signaling. Blood. 2015;125(22):3432-3436.
77. Zhang W, Trachootham D, Liu J, et al. Stromal control of cystine metabolism promotes cancer cell survival in chronic lymphocytic leukaemia. Nat Cell Biol. 2012;14(3):276-286.
78. Valsecchi R, Coltella N, Belloni D, et al. HIF-1alpha regulates the interaction of chronic lymphocytic leukemia cells with the tumor microenvironment. Blood. 2016;127(16):1987-1997.
79. Lutzny G, Kocher T, Schmidt-Supprian M, et al. Protein kinase c-beta-dependent activation of NF-kappaB in stromal cells is indispensable for the survival of chronic lymphocytic leukemia B cells in vivo. Cancer Cell. 2013;23(1):77-92.
80. Ding W, Nowakowski GS, Knox TR, et al. Bi-directional activation between mesenchymal stem cells and CLL B-cells: implication for CLL disease progression. Br J Haematol. 2009;147(4):471-483.
81. Ding W, Knox TR, Tschumper RC, et al. Platelet-derived growth factor (PDGF)-PDGF receptor interaction activates bone marrow-derived mesenchymal stromal cells derived from chronic lymphocytic leukemia: implications for an angiogenic switch. Blood. 2010;116(16):2984-2993.
82. Borge M, Nannini PR, Morande PE, et al. CXCL12 is a costimulator for CD4+ T cell activation and proliferation in chronic lymphocytic leukemia patients. Cancer Immunol Immunother. 2013;62(1):113-124.
83. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12(4):253-268.
84. Ferrer G, Yan X-J, Franca B, et al. Chronic Lymphocytic leukemia patients and Eμ-TCL1 mice share a phenotype of functional granulocyte-like and dysfunctional monocyte-like myeloid derived suppressor cells. Blood. 2015;126(23):614.
85. Seke Etet PF, Vecchio L, Nwabo Kamdje AH. Interactions between bone marrow stromal microenvironment and B-chronic lymphocytic leukemia cells: any role for Notch, Wnt and Hh signaling pathways? Cell Signal. 2012;24(7):1433-1443.
86. Zucchetto A, Caldana C, Benedetti D, et al. CD49d is overexpressed by trisomy 12 chronic lymphocytic leukemia cells: evidence for a methylation-dependent regulation mechanism. Blood. 2013;122(19):3317-3321.
87. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N. CD38 and chronic lymphocytic leukemia: a decade later. Blood. 2011;118(13):3470-3478.
88. Ren L, Campbell A, Fang H, et al. Analysis of the effects of the Bruton's tyrosine kinase (Btk) inhibitor ibrutinib on monocyte Fcgamma receptor (FcgammaR) function. J Biol Chem. 2016;291(6):3043-3052.
89. Da Roit F, Engelberts PJ, Taylor RP, et al. Ibrutinib interferes with the cell-mediated anti-tumor activities of therapeutic CD20 antibodies: implications for combination therapy. Haematologica. 2015;100(1):77-86.
90. Nguyen PH, Fedorchenko O, Rosen N, et al. LYN Kinase in the tumor microenvironment is essential for the progression of chronic lymphocytic leukemia. Cancer Cell. 2016;30(4):610-622.
91. Stiff A, Trikha P, Wesolowski R, et al. Myeloid-derived suppressor cells express Bruton's tyrosine kinase and can be depleted in tumor-bearing hosts by ibrutinib treatment. Cancer Res. 2016;76(8):2125-2136.
92. Dubovsky JA, Beckwith KA, Natarajan G, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. Blood. 2013;122(15):2539-2549.
93. Gunderson AJ, Kaneda MM, Tsujikawa T, et al. Bruton tyrosine kinase-dependent immune cell cross-talk drives pancreas cancer. Cancer Discov. 2016;6(3):270-285.
94. Fraietta JA, Beckwith KA, Patel PR, et al. Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. Blood. 2016;127(9):1117-1127.
95. 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(12):2078-2088.
96. Mouchemore KA, Sampaio NG, Murrey MW, Stanley ER, Lannutti BJ, Pixley FJ. Specific inhibition of PI3K p110delta inhibits CSF-1-induced macrophage spreading and invasive capacity. FEBS J. 2013;280(21):5228-5236.
97. Kaneda MM, Messer KS, Ralainirina N, et al. PI3Kgamma is a molecular switch that controls immune suppression. Nature. 2016;539(7629):437-442.
98. Gandhi AK, Kang J, Havens CG, et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN.). Br J Haematol. 2014;164(6):811-821.
99. McClanahan F, Hanna B, Miller S, et al. PD-L1 checkpoint blockade prevents immune dysfunction and leukemia development in a mouse model of chronic lymphocytic leukemia. Blood. 2015;126(2):203-211.
100. Wong D, Kandagatla P, Korz W, Chinni SR. Targeting CXCR4 with CTCE-9908 inhibits prostate tumor metastasis. BMC Urol. 2014;14:12.