

Overview on Immunopathology of Chronic Lymphocytic Leukemia and Tumor-Associated Antigens with Therapeutic Applications

Mahdi Shabani ^{1*}, Davoud Rostamzadeh ², Mansoure Mansouri ¹ and Mahmood Jeddi-Tehrani ³

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

3. Monoclonal Antibody Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Abstract

Chronic Lymphocytic Leukemia (CLL) is a clinically and biologically heterogeneous disease with a variable clinical course. The induction of a generalized state of immunosuppression, leading to susceptibility to infections and the failure of anti-tumor immune responses, is a key feature of the clinical course of CLL. In addition to B-cell receptor (BCR) signaling in CLL, several receptor tyrosine kinases (RTKs) have been reported to be constitutively active in leukemic B cells, resulting in promoted survival and resistance to apoptosis induced by chemotherapy. Several treatment options are available for CLL, including a watch-and-wait strategy, chemotherapy, targeted therapies, immunotherapies such as adoptive cellular therapy (CAR T-Cell Therapy), stem cell transplantation (allogeneic transplantation), radiation therapy and surgery. The identification of Tumor-Associated Antigens (TAAs) is the bottleneck of tumor immunology and immunotherapy, serving as promising targets for precise diagnosis, monitoring, or therapeutic approaches. Numerous TAAs have been identified, and their application in immunotherapy holds promise for the treatment of CLL. Furthermore, extensive ongoing research aims to identify new cancer TAAs. In this review, our objective is to provide a comprehensive overview of CLL immunology and recent findings regarding advances in TAAs with therapeutic applications in CLL.

Keywords: Cell therapy, Chronic lymphocytic leukemia, Hematologic malignancies, Immunotherapy, Tumor antigens

To cite this article: Shabani M, Rostamzadeh D, Mansouri M, Jeddi-Tehrani M. Overview on Immunopathology of Chronic Lymphocytic Leukemia and Tumor-Associated Antigens with Therapeutic Applications. *Avicenna J Med Biotech* 2024;16(4):201-222.

* Corresponding author:

Mahdi Shabani, Ph.D.,
Department of Immunology,
School of Medicine
Shahid Beheshti University of
Medical Sciences, Tehran, Iran
Tel: +98 21 22439970
Fax: +98 21 22439970
E-mail:
msshabani@sbmu.ac.ir
Received: 2 Mar 2024
Accepted: 20 Jul 2024

Introduction

Chronic Lymphocytic Leukemia (CLL), a member of the Non-Hodgkin Lymphoma (NHL) family, is a common lymphoproliferative disorder with high heterogeneity in clinical behavior that accounts for approximately up to 30% of all adult leukemias ¹. The median age at CLL diagnosis is around 67-72 years ². Its diagnosis is based on absolute number (more than 5000 B-lymphocytes/ μ l for the duration of at least 3 months) and clonal proliferation of B cells, that represent a specific immune-phenotype (CD19⁺, CD20⁺, and CD23⁺) that is frequently associated with expression of CD5 antigen (95% of patients) within the peripheral blood, bone marrow as well as other lymphoid organs ^{3,4}. Clonal proliferation of circulating B cells must be confirmed by flow cytometry ². Marrow aspirate and biopsy is not mandatory for diagnosis of CLL, but it is rec-

ommended to differentiate between autoimmune cytopenias (anemia, and thrombocytopenia), which is not associated with leukemia-cell infiltration of the normal bone marrow ⁵. Marrow biopsy is highly recommended to start treatment in a clinical trial with potentially myelosuppressive agents ⁵. Lymph node biopsy is not necessary for diagnosis of CLL but is only recommended for diagnosis of transformation into a more aggressive type of lymphoma (suspected Richter's transformation) ^{5,6}.

Standard therapies in CLL have emerged from the use of alkylating agents and then switched toward more aggressive immunotherapy-based regimens in order to improve response rates and extended survival ⁷. For several decades, administration of chlorambucil has been considered as the "gold standard" first-line thera-

peutic approach in CLL⁷. Over the recent years, significant progress has been made in the treatment of CLL and several new drugs have been approved such as cytostatic agents (purine analogs and bendamustine), monoclonal Antibodies (mAb) such as rituximab, ofatumumab, obinutuzumab, alemtuzumab, agents targeting B-cell receptor signaling (idelalisib, ibrutinib and acalabrutinib), BCL-2 inhibitors (venetoclax), BTK inhibitor (zanubrutinib and ibrutinib) and immunomodulatory drugs^{2,8-10}. Constitutive activation of several tyrosine kinases in the form of receptors and non-receptors involved in B cell survival and resistance to apoptosis have been identified in CLL^{11,12}. Even with introduction of these drugs, identification of new ideal Tumor-Associated Antigens (TAAs) is the bottleneck of tumor immunotherapy approaches. Numerous TAAs have been identified and their application in immunotherapy is promising for treatment of CLL. Besides, research for identification of new cancer TAAs has been ongoing most extensively. In this review, aim is to give a comprehensive overview of CLL immunology and recent findings regarding the advances in TAAs and their therapeutic applications in CLL.

Immunological features of CLL patients

Alterations of B and T lymphocytes: A key feature of the clinical course of CLL is immunosuppression, causing augmented susceptibility to infections and failure of anti-tumor immune responses¹³. CLL patients show abnormal distribution pattern of circulating T cell subpopulations in peripheral blood. A surprising finding related to the immune cells in CLL was the increase in absolute number of circulating CD8⁺ T cells¹⁴. These CD8⁺ T cells secrete high levels of IL-4¹⁵. The IL-4 producing CD8⁺ T cells displayed increased expression of CD30. It has been revealed that ligation of CD30L on the surface of CLL B-cells stimulates their production of TNF- α and enhances their proliferation¹⁶. Furthermore, IL-4 is able to prevent apoptosis of B-CLL cells in a BCL-2 dependent manner and therefore plays an important role in pathogenesis of CLL disease¹⁷. Leukemic CLL cells induce CD30 and downregulate CD40L expression on T cells in OX40L and IL-4 dependent as well as in contact-dependent manners. Contrary to malignant B cells, upregulated CD30⁺ T cells inhibit CD40L mediated immunoglobulin class switching by engaging CD30L in non-malignant B cells¹⁸. Leukemic CLL cells are prominent in secreting several cytokines. In this context, CLL cells contribute to the T cell defects *via* secretion of IL-6. IL-9 secreted by leukemic cells negatively modulates the cytotoxic T cell-mediated killing by inducing PD-1 expression¹⁹. Stimulation of healthy T cells in the presence of tumor derived supernatant containing high levels of IL-6 increases their production of IL-4, and causes them to show impaired upregulation of CD40L expression²⁰. Furthermore, elevated level of IL-6 in CLL patients is correlated with poor survival

and diverse disease features²¹. Additionally, disrupted B cell function in CLL patients has been reported²².

Global gene expression profiles of peripheral blood T cells from CLL patients revealed altered gene expression profiles compared to healthy donors²³. Gene analysis demonstrated expression of genes mostly involved in cell differentiation, proliferation, survival, cytoskeleton formation, and vesicle trafficking of CD4⁺ T cells and cytoskeleton formation, intracellular transportation, vesicle trafficking, or cellular secretion as well as cytotoxicity pathways in CD8⁺ T cells. Alterations in cytoskeletal relevant gene expression resulting in functional defects in actin polymerization, and consequently CLL T cells exhibit defects in formation of immunological synapse with antigen presenting cells (APCs)²³.

Frequency of regulatory T (Treg) cells

Abnormally high regulatory T (Treg) cells absolute count observed in CLL patients is considered a critical mechanism of immunosuppression in these patients. CLL patients unusually show increased frequencies of CD4⁺CD25^{hi}FOXP3⁺ Tregs which may be correlated with the disease status such as tumor progression and expansion as well as clinically advanced disease^{24,25}. In addition, raised numbers of Tregs in CLL patients is correlated with decreased T cell responses against viral and tumor antigens. Treg cells from CLL patients displayed reduced amounts of CD25 expression intensity and may inhibit anti-tumor T cell responses by releasing soluble CD25 resulting in inhibition of Th1 differentiation²⁶. Besides immunosuppressive effects of Tregs, CD4⁺ T cells may be essential in controlling immune related diseases. Both CD4⁺ FoxP3⁺ and CD4⁺ FoxP3⁻ T cells have been demonstrated to act as cytotoxic populations of CD4⁺ T cells and express cytolytic markers like Fas ligand and CD107a, rendering them to kill autologous leukemic B cells *in vitro*²⁷. Increased frequency of Treg cells in CLL patients may be due to significantly elevated levels of anti-apoptotic BCL-2 resulting in decreased susceptibility to apoptosis and induction of Treg formation through CD27-CD70 co-stimulation in the Lymph Node (LN) follicular proliferation centers²⁷. TIGIT (T cell immunoreceptor with Ig and ITIM domains) expressing CD4⁺ T cells are enriched in CLL and these cells provide a supportive microenvironment for CLL cells, representing a potential therapeutic target for CLL treatment²⁸. In addition, it has been elucidated that CLL T cells show increased expression of CD57, CD71, CD69, and HLA-DR and decreased expression of CD28 and CD62L, which represent their systemic and chronically activated phenotypes²⁹.

Abnormality in NK cells

Impaired Natural Killer (NK) cell activity has also been reported in CLL patients. NK cells from CLL patients showed lack of cytoplasmic azurophilic granules resulting in reduced ability to lyse leukemia cell

lines³⁰. IL-2 is able to restore the impaired NK cell activity and increased granularity of the Large Granular Lymphocyte (LGL) subset³¹. However, based on downregulation of NK cell function by CLL cells, it has been suggested that malignant CLL cells may be capable of secreting immunosuppressive factors that down-regulate T cell and NK functions³². Similar to CLL T cells, NK cells from CLL patients show defective actin polymerization and impaired immunological synapse formation which affect the NK-cell mediated cytotoxic mechanisms³³. CD3⁺CD16⁺CD56⁺ NKT cell frequencies appear to be of clinical significance, as a decrease in number of these cells is related to disease progression and higher risk of death in CLL patients³⁴. Therefore, development of a chimeric antigen-receptor (CAR)-NK therapy strategy against the CLL cells such as CD19-specific CAR-NK cells have given great interest for treatment of hematological malignancies, particularly CLL^{35,36}.

In addition, dysregulation of monocyte and neutrophil functions has been reported in CLL. CLL monocytes and neutrophils have been shown to be deficient in myeloperoxidase and lysozyme activities. These events may affect CLL B-cell survival through changes in the secretion of TNF superfamily proteins³⁷.

Genomic alteration of CLL cells

Recently, comprehensive description of putative genomic landscape of CLL by Whole Exome Sequencing (WES) in large cohorts revealed that loss or addition of large chromosomal material (e.g., 13q, 11q, 17p deletions and trisomy 12) may conceivably be involved in disease initiation, aggressiveness and progression³⁸. The most frequent cytogenetic abnormality in the CLL leukemic cells is deletion on the long arm of chromosome 13q14 [del(13q14) that occurs in more than half of all cases (%55)]^{2,39}. Deletion at 13q14 is also associated with most mantle cell lymphomas (%50), multiple myeloma (%16-40) and prostate cancers (%60) and is related to their pathogenesis⁴⁰. *miR15* and *miR16* were recently identified to be exactly located on chromosome 13q14 and both miRNAs are deleted or down-regulated in most CLL sample cases (~68%)³⁹. Genetically modified mice carrying a targeted deletion of the *DLEU2/miR-15a/16-1* cluster recapitulates many features of CLL including development of CLL, monoclonal B cell lymphocytosis-like disorder, and lymphoma⁴¹. These data have suggested a crucial role for *DLEU2/miR-15a/16-1* locus in controlling the expansion for the mature B cell pool and may harbor unknown tumor suppressor genes in B cell lineage and indicate that *DLEU2/miR-15a/16-1* locus may play a critical and direct role in CLL leukemogenesis and pathogenesis⁴¹.

Chromosome region 11q22.3–q23.1 frequently harbors the *Ataxia Telangiectasia Mutated (ATM)* gene which activates p53 protein in response to DNA Double-Strand Breaks (DSBs)⁴². Prevalence of *ATM* mutations have been reported in 12% of CLL patients and

approximately in one-third of CLL patients harboring 11q23 deletion⁴². Deletions of *ATM* (11q22–q23) have been found in approximately 25% of patients with more advanced clinical stages of CLL and have been rarely found in early stage disease (10%)⁴³. Patients harboring mutations in 11q23 exhibit more rapid disease progression, extensive LN involvement and reduced overall survival⁴⁴. Trisomy 12 is one of the most frequent aberrations (10-20%) with unidentified susceptibility genes involved in the pathogenesis of CLL⁴³. However, in line with these observations, further studies remain to be performed to explore the probable correlation between the incidence of trisomy 12 and relevance of CLL prognosis.

Another chromosomal aberration is deletion in band 17p13 (involving the p53 locus) which is found in 5-8% of CLL patients at diagnosis or in chemotherapy-naïve CLL patients^{45,46}. The association between poor prognosis, drug resistance and poor survival of CLL patients with 17p deletion or p53 mutation has been reported in several studies⁴⁷⁻⁵¹. In patients diagnosed with 17p deletion, most cases have shown mutations in the remaining *TP53* allele (80%). Furthermore, in cases without deletion in 17p, *TP53* mutation is rare, but has a similar impact on chemotherapy response and overall survival⁵².

Using WES to characterize the genetic landscape of CLL patients, 44 recurrently mutated genes and 11 recurrent somatic copy number variations were identified⁵³. These mainly include the genes *NOTCH1*, *MYD88*, *KLHL6*, *TP53*, *ATM*, *SF3B1*, *FBXW7*, *POT1*, *CHD2*, *RPS15*, *IKZF3*, *ZNF292*, *PAX5*, *ZMYM3*, *ARID1A*, *XPO1*, and *PTPN11*⁵³⁻⁵⁵. These studies provide a comprehensive catalog of somatic mutations of the CLL genomic landscape which provides useful results in targeting several well-known genetic pathways and treatment of CLL and other malignancies.

Signaling pathways in CLL cells involved in pathogenesis and disease progression

Ligand-dependent B-cell receptor signaling pathways in B-CLL: Activation via B-Cell Receptor (BCR) signaling can trigger several cascades of signaling events that lead to multiple events in normal and malignant B cells including B cell selection, proliferation, differentiation, antibody production and isotype switching⁵⁶. Induction of BCR signaling pathway either via antigen (ligand-dependent) or "tonic signaling" (ligand-independent) seems to play an essential pro-survival role for the survival, growth and pathogenesis of CLL cells and in other NHLs⁵⁷. Analysis of Gene Expression Profiling (GEP) within the tissue microenvironment of lymphatic tissues explored BCR signaling as the most predominant signaling pathway engaged in CLL which promoted maintenance, proliferation and survival of CLL cells *in vivo*⁵⁸. Among genes upregulated in LN resident CLL cells, BCR signaling shows a highly over-represented profile in cooperation with upregulation of c-MYC and NF-κB, suggesting the LN as an important

site in CLL pathogenesis⁵⁸. Furthermore, genes involved in BCR signaling are stronger in clinically more aggressive CLL which supports the notion that inhibition of BCR signaling is an appropriate therapeutic strategy in CLL⁵⁸.

Growing evidence supports the idea that recognition of various autoantigens and other environmental or microbial antigens by polyreactive/autoreactive BCRs from U-CLL patients may lead CLL cells to programmed secretion of multispecific autoantibodies. It has been shown that CLL BCRs could bind to non-muscle Myosin Heavy chain IIA (MYHIIA) which probably stimulates CLL cells toward development, survival, and expansion⁵⁹. CLL cells have also been reported to be autoreactive against Fc domains of IgG, histones, cardiolipins, cytoskeletal components⁶⁰, ssDNA, dsDNA⁶¹, apoptotic cells, oxidized Low-Density Lipoprotein [oxLDL],⁶² and some pathogenic bacteria and fungi^{62,63}. CLL B cells, irrespective of their V gene mutational status, represent features of activated and of antigen-experienced B cells based on the overexpression of the activation markers CD23, CD25, CD69, and CD71⁶¹.

Ligand-independent B-cell receptor signaling pathways in CLL

It is well known that tonic signaling of BCR is essential for development, maintenance, and survival of normal B cells⁶⁴. It has been shown that unmanipulated primary or freshly isolated CLL cells display elevated intrinsic tonic signaling activity⁶⁵. Src kinase Lyn, plays an essential role in transducing survival or apoptosis signaling cascade which is triggered following BCR engagement⁶⁶. Analysis of protein tyrosine phosphorylation downstream of BCR in normal and CLL cells revealed overexpression and significant constitutive activation of Lyn in freshly isolated leukemic cells⁶⁷. Mice deficient in expression of Src kinases Lyn, Fyn, and Blk showed increased levels of apoptosis in pre-B cells⁶⁸. Syk as a key protein kinase downstream of BCR has been shown to be constitutively phosphorylated and active on the activating Y352 residue in CLL B cells and Syk inhibitor induced leukemic cell apoptosis⁶⁹. Constitutive activation of Syk has been shown in several common B-cell malignancies indicating a role for antigen-independent activation of Syk in the pathogenesis of these diseases^{70,71}. Therefore, Syk can be a potential target for inducing apoptosis in CLL leukemic cells and an appropriate therapeutic target by disrupting antigen-dependent and independent signaling pathways in CLL. Furthermore, CLL cells have been shown to exhibit constitutive phosphorylation of Erk, NFAT and subunits of NF- κ B^{72,73}. This constitutive activation of tonic BCR signaling suggests that antigen-independent BCR signaling might be associated with oncogenic mechanisms and clinical relevance in CLL and induces resistance of the leukemic B-cells to therapy. In particular, unmanipulated CLL cells show different N-glycosylation patterns in the μ -con-

stant region in IgM including glycoform similar to normal B cells and immature mannosylation. In contrast to IgVH mutated CLL (M-CLL) cells, unmutated CLL (U-CLL) cells display elevated levels of mannosylated surface μ chains which may increase the ongoing interaction with local lectin-bearing stromal cells⁷⁴. Immunophenotypic analysis of leukemic cells from CLL patients have shown characteristics of activated and antigen-experienced B cells irrespective of their V gene mutational status⁶¹.

Growing evidence supports the idea that recognition of various autoantigens and other environmental or microbial antigens by polyreactive/autoreactive BCRs from U-CLL patients may lead CLL cells to programmed secretion of multispecific autoantibodies. It has been shown that CLL BCRs could bind to non-muscle myosin heavy chain IIA (MYHIIA) which probably stimulates CLL cells toward development, survival, and expansion⁵⁹. CLL cells have also been reported to be autoreactive against Fc domains of IgG, histones, cardiolipins, cytoskeletal components⁶⁰, ssDNA, dsDNA⁶¹, apoptotic cells, oxLDL,⁶² and some pathogenic bacteria and fungi^{62,63}. As mentioned above, CLL B cells, irrespective of their V gene mutational status, represent features of activated and of antigen-experienced B cells based on the overexpression of the activation markers CD23, CD25, CD69, and CD71⁶¹.

Non-receptor tyrosine kinases signaling in CLL B cells

In addition to BCR signaling in CLL, several Receptor Tyrosine Kinases (RTKs) have been reported to be constitutively active in leukemic cells resulting in promoted survival and resistance to apoptosis induced by chemotherapeutic drugs. RTKs are cell surface glycoproteins and a subclass of tyrosine kinases involved in the regulation of various cellular processes, such as proliferation, carcinogenesis, growth, differentiation, survival, signaling and migration⁷⁵. For example, Insulin-like Growth Factor-I (IGF-I) released by stromal cell in bone marrow plays an important role in regulating B lymphopoiesis by enhancing the differentiation and development of normal pro-B to pre-B lymphocytes as well as stimulating μ -heavy chain gene rearrangement and protein expression⁷⁶. For the first time, IGF-I has been reported to be expressed by 44% of CLL patients and its expression has been positively correlated with BCL-2 anti-apoptotic protein expression and CLL cells survival⁷⁷, which supports the notion that the paracrine/autocrine control of CLL cells occurs through interaction of IGF-1 and IGF-1R. High level expression of Insulin Receptor (INSR) has been reported in the majority of CLL cases with 11q-del as compared to CLL cases with other genomic abnormalities and normal B cells in these patients⁷⁸, while normal B cells from peripheral blood express moderate levels of INSR⁷⁸. Interestingly, higher expression of INSR has been shown to be correlated with shorter time to first therapy and shorter overall survival⁷⁸.

These results highlighted the IGF-1 and INSR as a potential therapeutic target in CLL.

Receptor tyrosine kinase-like Orphan Receptor (ROR) protein is a member of RTKs that play an important role in developmental processes including skeletal and neuronal development, cell movement and cell polarity⁷⁹. ROR1 has been shown to be highly expressed in CLL and several other malignancies with functional activity and its involvement in tumor cells signaling⁸⁰⁻⁸⁵. Gene expression profiling of CLL B cells showed increased expression of ROR1 in leukemic cells^{77,86}. The majority of CLL cells (70% mean), but not normal B cells, and other normal blood cells, exhibited ROR1 surface expression and mRNA levels uniformly^{87,88}. No correlation between ROR1 protein expression and IgVH mutated and unmutated cases as well as progressive and non-progressive CLL patients has been reported⁸⁷⁻⁹⁰. Unique surface expression of ROR1 by CLL B cells, but not normal B cells and other tissues merits further studies of its role in the pathobiology of CLL and makes it a potential therapeutic target for CLL⁸⁴. Currently a mAb specific to ROR1 (cirmtuzumab) has been tested in phase I clinical trial (NCT02222688)⁹¹ and a few Small Molecule Inhibitors (SMI) are in preclinical stages for targeting ROR1^{80,92}.

In CLL, Vascular Endothelial Growth Factor A (VEGFA) promotes CLL B cell survival and progression which is most likely to be associated to activating the STAT1/STAT3 signaling pathway and upregulating the critical anti-apoptotic protein Myeloid cell leukemia-1 (Mcl-1) in leukemic B cells⁹³. Since overexpression of VEGF and Mcl-1 are associated with poor prognosis of B CLL, inhibition of VEGF and related signaling pathways can be therapeutically targeted for treatment of CLL. Furthermore, it has been proven that leukemic B cells spontaneously secrete both pro- and anti-angiogenic molecules, including basic Fibroblast Growth Factors (bFGF), VEGF and T thrombospondin-1 (TSP-1). Because of the co-expression of angiogenic molecules and receptors, CLL B cell biology is directly affected by autocrine pathways of stimulation⁹⁴. In addition, micro vessel density of bone marrow in CLL patients is correlated positively with the clinical stage of the disease⁹⁵. Some studies have also reported increased serum levels of VEGF and bFGF in CLL that can be considered useful targets for predicting the risk of disease progression⁹⁶.

Axl is another important RTK involved in pro-survival signaling pathway in CLL. Axl alongside with Tyro3 and Mer are members of the TAM receptor tyrosine kinase family⁹⁷. Axl was primarily identified in patients with Chronic Myelogenous Leukemia (CML)⁹⁸. Axl activation and signaling have been involved in several cellular responses, including cell survival, proliferation, migration, phagocytosis, adhesion and angiogenesis as well as implication in multiple types of human cancer, inflammatory, autoimmune, develop-

ment of atherosclerosis and kidney diseases⁹⁹. Analysis of freshly isolated CLL B cells has constitutively shown activation of Axl which is correlated with other constitutively phosphorylated kinases, including Lyn, phosphoinositide-3 kinase, SyK/ ζ -associated 70 kDa protein, phospholipase Cy2 in CLL cells¹⁰⁰. Inhibition of Src/Abl kinase or specific-inhibition of Axl may induce massive CLL B-cell apoptosis¹⁰⁰ which can be an attractive target for treatment of CLL.

c-MET is another member of RTKs that acts as a high affinity receptor for Hepatocyte Growth Factor (HGF)/scatter factor¹⁰¹. The HGF/c-MET signaling pathway has been reported to be dysregulated in a wide range of human malignancies with poor clinical outcomes and drug resistance¹⁰². Furthermore, it has been demonstrated that aberrant expression of c-MET and HGF/c-met pathways is in favor of survival and apoptotic resistance in CLL, but not on normal donor CD19⁺ cells. Increased expression of c-MET has also been associated with decreased expression of adhesion molecules¹⁰³.

These results suggest that RTKs inhibition can be potential pharmacological targets in CLL and recommend that combining anti-RTK agents with more traditional therapeutic drugs may emerge as new oncological targets for antibody-based therapy.

Targeted therapy drugs for chronic lymphocytic leukemia

There are several treatment options for CLL including chemotherapy, radiotherapy, surgery, stem cell transplantation (allogeneic transplantation), target based therapies such as mAbs¹⁰⁴, SMI (ibrutinib, acalabrutinib, and zanubrutinib)^{105,106} and immunotherapy methods like adoptive cellular therapy (CAR T-cell therapy)¹⁰⁷. There are a number of targets to specifically target malignant B cells, such as CD20, CD19, CD52, CD70, CD74, CD40, and CD37 (Figure 1, Table 1)^{107,108}. In addition, there are also numerous mAbs in clinical development for CLL therapy (Table 1)^{107,108}.

CD20; a pan B cell surface glycoprotein

CD20 is a potential cell surface target for elimination of circulating CD20-expressing B cells such as B cell malignancies. CD20 is a hydrophobic glycosylated transmembrane protein and its expression is relatively limited to the cell surface of normal mature B, CLL B cells and on >90% of B cell NHL, but not stem cells, pro-B cells and plasma cells¹⁰⁹. However, the precise function of CD20 remains poorly understood. CD20 knockout mice have been shown to exhibit normal B cell development but are deficient in CD19-induced calcium responses and BCR signaling¹¹⁰. Since internalization and shedding of specific targets may potentially determine the efficacy of targeted therapy, CD20 appears neither undergo cell-surface shedding nor internalization¹¹¹. In addition, its specific expression on B cells makes it a specific marker for targeted therapy in CLL. Rituximab is a chimeric anti-human CD20

Update on Tumor-Associated Antigens in Chronic Lymphocytic Leukemia

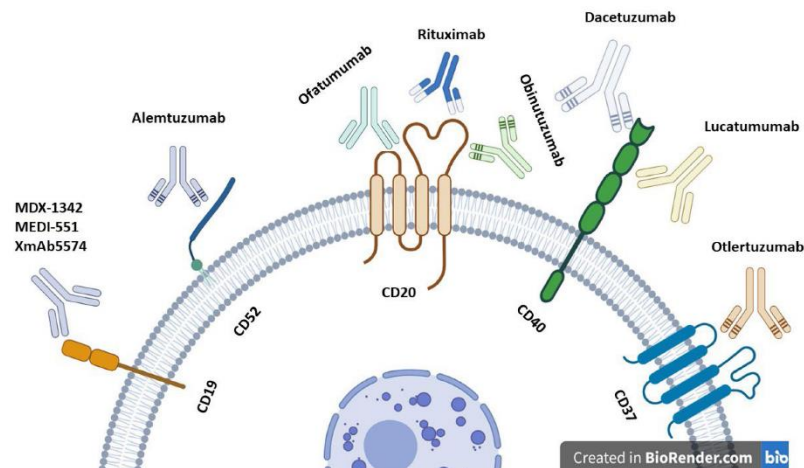


Figure 1. Tumor associated antigens targeted for monoclonal antibody-based immunotherapy of chronic lymphocytic leukemia.

Table 1. Approved monoclonal antibodies targeting tumor antigens in CLL patients

Potential target	Description	Function	Expression pattern	Intervention/treatment and therapeutic outcome	Ref
CD20	Specific marker for targeted therapy in CLL	Unknown	- Normal mature B cells - CLL B cells - >90% of B cell NHL	- Rituximab induction of ADCC, CDC, ADP, and direct apoptosis - Ofatumumab induction of ADCC, potent CDC and apoptosis - GA101/Obinutuzumab ADCC with higher capacity to deplete CLL B cells	(108, 109, 116, 117)
CD52	A 21-28 kDa glycosylphosphatidylinositol (GPI)-linked glycoprotein	May provide costimulatory signals for T-cell activation and proliferation	- High expression on all B and T cells - Overexpression on T-PLL and CLL cells - Soluble CD52 is an indicator of disease activity in CLL	- Alemtuzumab (Campath-1H) - Induction of CDC, ADCC effective therapy for CLL patients with high-risk cytogenetic markers including p53 mutations or 17p and 11q deletions	(123, 124, 126, 127, 234, 235)
CD19	Transmembrane glycoprotein of immunoglobulin superfamily	Acts as a co-receptor to augment signals by the pre-BCR/BCR	Highly expressed on most malignant B cells, particularly B-CLL	- MDX-1342 and MEDI-551 non-fucosylated fully human mAbs specifically directed against CD19 - ADCC and ADP - XmAb5574 - Fc-engineered anti CD19 mAb	(130, 236)
CD37	Heavily glycosylated 40 to 52 kDa glycoprotein		High expression on the surface of B cells and transformed mature B-cell leukemia and lymphoma cells	- Otlertuzumab (TRU-016) humanized anti-CD37 mAb - Induction of ADCC and caspase-independent cell death against CLL cells	(131)
CD40	A member of the tumour necrosis factor receptor (TNFR) superfamily	CD40 activation is associated with enhanced cytokine secretion, proliferation and survival of neoplastic B cells	Highly expressed by normal and neoplastic CLL cells	- Lucatumumab (HCD122) - Humanized anti-CD40 antagonist antibody inhibits CD40L-induced activation of signaling pathways, survival, cytokine secretion - Induction of ADCC - Dacetuzumab (SGN40) humanized IgG1 monoclonal antibody - Induction of ADCC and apoptosis	(133, 135, 136)

ADCC: Cytotoxic effect mainly by antibody dependent cellular cytotoxicity, CDC: Complement Dependent Cytotoxicity, ADP: Antibody Dependent Phagocytosis, CLL: Chronic Lymphocytic Leukemia, T-PLL: T Cell Prolymphocytic Leukemia.

IgG1 κ murine/human immunoglobulin containing both murine light- and heavy-chain variable region sequences with human constant region sequences (Figure 1) ¹⁰⁸. It has been demonstrated that rituximab ex-

erts its cytotoxic effect mainly by Antibody Dependent Cellular Cytotoxicity (ADCC), Complement Dependent Cytotoxicity (CDC), Antibody Dependent Phagocytosis (ADP and direct apoptosis with a cross-linking

antibody^{107,108}. Since CLL cells express relatively low levels of CD20, these cells show little susceptibility to CDC mediated by rituximab¹¹². However, CLL cells have been demonstrated to be susceptible to CD20-shedding after treatment and reduce the ability of rituximab to induce CDC¹¹³. Monocyte mediated ADP and NK cell mediated ADCC have also been reported in CLL cells exposed to rituximab *in vitro*¹¹⁴. It has been shown that Treg cells strikingly diminish NK cell mediated ADCC function toward rituximab-labeled tumor cells¹¹⁵.

A significant number of patients have been observed to relapse following treatment with rituximab. Therefore, development of novel anti-CD20 mAbs with improved therapeutic efficacy is necessary. A second-generation of fully humanized anti-CD20, ofatumumab recognizes a unique and different epitope on CD20 than rituximab, has been developed. This mAb exerts similar ADCC, potent CDC, slower off-rate, more stable CD20 binding and direct apoptosis after cross-linking to rituximab^{108,116}. Type II glycoengineered fully humanized high affinity mAb to CD20 epitope, GA101/Obinutuzumab showed greater effect to induce ADCC and higher capacity to deplete CLL B cells from peripheral blood compared to rituximab¹¹⁷⁻¹¹⁹. Obinutuzumab was approved by the Food and Drug Administration (FDA) in November 2013 for use in combination with chlorambucil for the treatment of patients with previously untreated CLL¹²⁰. Clinical trial of the triple combination of obinutuzumab, ibrutinib, and venetoclax (GIVE regimen) presented a promising outcome for this combination regimen and thus introduced it as a first-line treatment for patients with high-risk CLL¹²¹.

CD52; a small immunomodulator glycoprotein

Alemtuzumab (Campath-1H) is a fully humanized IgG1 κ mAb directed against cell-surface antigen CD52¹²². CD52 is a 21-28 kDa glycosylphosphatidylinositol (GPI)-linked glycoprotein expressed at high levels on all B and T cells. It is expressed at lower levels on monocytes and macrophages, eosinophils, NK cells, and monocyte-derived dendritic cells¹²³. Over-expression of CD52 antigen has also been demonstrated on T cell Prolymphocytic Leukemia (T-PLL) and CLL¹²⁴. Immunologically, alemtuzumab can mediate CDC, ADCC by virtue of its IgG Fc region and can induce direct caspase-independent cell death through a membrane raft-dependent mechanism (Figure 1)^{108,125}. Clinical studies have shown that alemtuzumab could be an effective therapy for CLL patients with high-risk cytogenetic markers including p53 mutations or 17p and 11q deletions as well as other genomic aberrations^{126,127}. Therefore, alemtuzumab might be a reasonable treatment for CLL patients with these poor prognostic features. Although alemtuzumab has been approved for the treatment of CLL, due to the strategic decision of Sanofi company, the license of this drug was withdrawn in August/September 2012 from the clinic and it

is only available through an international compassionate use program².

CD19; a B-cell surface glycoprotein

Co-stimulatory molecule CD19, is a transmembrane glycoprotein of immunoglobulin superfamily and is a B cell marker that is highly expressed on most malignant B cells, especially in leukemic CLL cells and is considered as a potential target for immunotherapies¹²⁸. Recent advances in engineered antibody technology have led to development of a series of modified antibodies for targeting CD19. MDX-1342 (NCT00593944) and MEDI-551 (NCT01466153) are non-fucosylated fully human mAbs specifically directed against CD19 that acts predominantly via ADCC and ADP (Figure 1). Data from a phase 2 clinical trial, has shown that MEDI-551 has demonstrated a 30% response rate in relapsed/refractory (RR) CLL as a monotherapy and further study showed clinical activity and comparable safety compared to rituximab and bendamustine (NCT-01466153)¹²⁹. XmAb5574 (MOR00208) is a new Fc-engineered anti CD19 mAb with an engineered Fc region to enhance Fc γ receptor binding affinity for improving ADCC and ADP¹³⁰. Phase 1 clinical trial as a monotherapy (NCT01161511) exhibited safety and preliminary efficacy in RR CLL patients¹³⁰. Phase 2 XmAb5574 in combination with lenalidomide for RR CLL patients is under investigation (NCT02005289).

CD37; a heavily glycosylated glycoprotein

Another lineage-specific B cell target, CD37, is a heavily glycosylated 40 to 52 kDa glycoprotein member of the transmembrane 4 superfamily (TM4SF) of tetraspanin family of proteins and is considered to be an attractive therapeutic target for CLL targeted therapy¹³¹. Otlertuzumab (TRU-016) is a novel humanized anti-CD37 mAb that shows potential capacity to induce ADCC and caspase-independent cell death against CLL cells (Figure 1)¹³¹. Otlertuzumab includes anti-CD37 antibody variable regions linked to immunoglobulin constant domains and is categorized as a small modular immunopharmaceutical (SMIP). Phase 1 escalation evaluation of otlertuzumab in RR CLL patients has demonstrated 23% response rate with acceptable toxicity¹³².

CD40; a TNF receptor superfamily member

A member of the Tumour Necrosis Factor Receptor (TNFR) superfamily, CD40, is highly expressed by normal and neoplastic CLL cells¹³³. CD40 activation has been found to be involved in enhanced cytokine secretion, proliferation and survival of neoplastic B cells, triggering phosphorylation of downstream signaling molecules associated to oncogenic mechanisms and clinical relevance in CLL. It induces resistance of the leukemic B cells to therapy such as ERK 1/2 and up-regulates Mcl-1 and Bcl-xl¹³⁴. Humanized anti-CD40 antagonist antibody, lucatumumab (HCD122), blocks the interaction of CD40 with CD40L and inhibits CD40L-induced activation of signaling pathways, sur-

vival, cytokine secretion as well as mediating ADCC (Figure 1) ¹³⁵. Data from a phase I study of lincatuzumab has shown that 17 out of 27 CLL patients have stable disease and acceptable toxicity ¹³⁶. Another anti-CD40 mAb, dacetuzumab (SGN40) mediates ADCC against CLL cells and its effect is further enhanced in combination with lenalidomide ¹³⁶. However, phase I dose escalation study as a single agent demonstrated minimal clinical activity (NCT00283101) ¹³⁷. Further studies are proposed to be performed on dacetuzumab in combination with other chronic lymphocytic leukemia therapies.

Tumor associated antigens: potential targets for CLL immunotherapy

Identification of human Tumor associated antigens (TAAs) is one of the main goals of targeted therapy and tumor immunology that serve as promising targets for diagnosis, disease monitoring and therapeutic approaches. Several investigations have been performed to detect new TAAs (Table 2). TAAs mostly are the mutational status of normal proteins expressed by normal tissues, aberrantly expressed normal genes, or genes encoding viral proteins which make them potential targets for immunotherapeutic vaccines ¹³⁸. These antigens should contain peptide sequences that are recognized by MHC molecules. Recently, several TAAs with the potential to be specifically recognized by T cells and induce a specific Cytotoxic T Cell (CTL) response and specific CTL-mediated antitumor immune response have been characterized in CLL ^{139,140}.

CD23; a low-affinity receptor for IgE (Fc ϵ RII)

CD23 that is expressed on the surface of B cells, belongs to type II integral membrane proteins (C-type lectin family) ¹⁴¹. CD23 is constitutively and strongly overexpressed by leukemic B cells with significant prognostic importance in CLL ¹⁴². Serum CD23 level has been shown to be selectively elevated in CLL patients and significantly contributes to significantly worsening the prognosis and overall survival ¹⁴³. Evaluation of serum level of CD23 in the early stage (stage A) of the disease can provide significant prognostic information, but during the course of the disease it is presumed to help to the early detection of patients who will rapidly progress to upper stages ¹⁴³. Moreover, analysis of GEF has revealed 5.9-fold increase in expression of CD23 in leukemic B cells compared to normal B cells ¹⁴⁴. Previous studies have demonstrated that CD23 could be naturally processed and presented in the context of MHC molecules. They showed that HLA-A2-restricted specific CTLs for CD23-derived peptides from CLL patients efficiently recognized native and CD40L-activated autologous malignant CLL cells ¹⁴⁵. These findings strongly support the notion that CD23 can potentially serve as a specific TAA in CLL and may also be employed as an attractive therapeutic target for the development of T cell-based immunotherapeutic approaches such as clinical vaccination

trials or adoptive T cell transfer of B-CLL and other CD23⁺ B-cell malignancies. phase 1, dose escalation and schedule optimization study of the macaque-human primatized mAb (lumiliximab), directed against CD23 in RR CLL patients has shown minimal activity, acceptable toxicity and well-tolerated profile (NCT-00046488) ¹⁴⁶. Although, phase 1/2 study of lumiliximab combined with fludarabine, cyclophosphamide, and rituximab (FCR) in patients with RR CLL shows potential benefit (NCT00103558) ¹⁴⁷, phase 3 did not confirm the ability of lumiliximab to improve response to treatment and Progression-Free Survival (PFS) when FCR plus lumiliximab was compared with FCR alone ¹⁰⁸.

MDM2; the human homolog of murine double-minute 2 oncoprotein

MDM2, also known as MDM2 E3 ubiquitin ligase, is typically expressed in the nucleus, which translocates to the nucleus following its activation and acts as an effective tumor suppressor protein p53 negative regulator ¹⁴⁸. Upon activation, MDM2 binds to the amino-terminus of P53 and targets it for ubiquitinylation and subsequent proteasomal degradation and therefore exerts its oncogenic affect by inhibiting its transcriptional activity ¹⁴⁸. MDM2 protein overexpression has been reported in multiple types of human cancers, especially in tumors with a wild-type p53, such as breast cancer, colorectal cancer, glioblastoma, cutaneous melanoma, differentiated liposarcoma, CLL, NHL, Hodgkin lymphomas, and osteosarcoma ¹⁴⁹⁻¹⁵². Northern blot analysis showed 10-fold higher expression of MDM2 RNA levels in neoplastic B cells from B-CLL or NHL patients than in normal B cells ¹⁵². In addition, MDM2 gene overexpression has been reported to be more frequent in patients at advanced clinical stages (stage IV) than in those at lower clinical stages (stage II or III) ¹⁵². These results may suggest an important role for MDM2 in tumorigenicity and/or disease progression of CLL. Mayr *et al.* defined MDM2 as a novel TAA recognized by CD8⁺ autologous T cells in B-CLL and also showed that MDM2 expression was detectable in 85% of CLL patients. They identified MDM2 HLA-A2-restricted specific CTLs that recognized T2 target cells loaded with MDM2 peptides and autologous MDM2 overexpressing CLL cells which naturally processed and presented it in the context of MHC-I molecules ¹⁵³. Inhibition of the MDM2-p53 protein-protein interaction or MDM2 E3 ligase function promotes steady state p53 levels, inhibits its p53-degradation activity and in some cancer cells stimulates apoptosis ^{154,155}. Investigation of apoptosis induction after treatment of highly pure CLL tumor cells *ex vivo* with 2 MDM2 inhibitors, Nutlin-3 and MI-63 showed that p53 status potentially determines the CLL cells response to MDM2 inhibitors ¹⁵⁵. These results indicated that MDM2 could be an interesting TAA target in therapeutic approaches in CLL patients. Furthermore, in the setting of CLL, utilizing MDM2 inhibitors in combination with drugs less sensi-

Table 2. Tumor antigens with potential treatment utility in CLL patients

TAA	Treatment approach	Clinical significance	Comments	Ref
CD23 (Fc γ RII)	Primatized	Phase I clinical trial with minimal activity, acceptable toxicity and well-tolerated profile (NCT00046488)	Strongly overexpressed by leukemic B-cell with significant prognostic importance in CLL	(142, 146)
	Lumiliximab	Phase 1/2 study of lumiliximab combined with fludarabine, cyclophosphamide, and rituximab (FCR) showed potential benefit (NCT00103558)	Elevated level of serum CD23 has shown to be selectively elevated in CLL patients and contributed to significantly worse prognosis and overall survival	(108, 143, 147)
		Phase 3 didn't confirmed the ability of lumiliximab to improve response to treatment and PFS	Evaluation of serum level of CD23 in the early stage (stage A) of disease can provides significant prognostic information	
MDM2	SPC2996	Molecular variability in p53 might influence strong quantitative influence on MDM2 inhibitor-mediated cell kill in CLL	Targets P53 for ubiquitylation and subsequent proteasomal degradation	(148-152, 155, 189)
		Acquired p53 mutations is of significant concern	MDM2 protein overexpression has been reported in multiple types of human cancers	
		Great importance to monitor p53 mutation status serially in patients treated with MDM2 inhibitor	-	
		Using MDM2 inhibitors in combination with drugs less sensitive to p53 mutation status, like alemtuzumab or flavipiridol might be more effective immunotherapeutic strategies in treatment of CLL	-	-
Survivin		YM155 treatment suppressed proliferation and effectively induce apoptosis in the proliferative subset of CLL might pave the way for novel therapeutic applications of TM155in treatment of CLL	A critical protein associated with apoptosis and regulation of cell division	(157, 159, 167, 237)
		Phase I/II clinical trial of terameprocol (NCT00664677) in different hematological malignancies showed that the drug was safe, the maximum tolerated dose was established.	Its upregulation is related to poor prognosis and drug/radiation resistance	
Bax and Bcl-2		Selective pharmacological inhibition of BCL-2 by ABT-199 highlighted the potential role of bcl-2 family proteins in the context of target therapies	Proteasomal-mediated degradation of Bax has been reported in advanced CLL and was associated with poor prognosis and chemoresistance	(181, 188, 238, 239)
		Administration of SPC2996, a novel Bcl-2 mRNA antagonist, resulted in rapid leukemic cell clearance and immune activation in CLL patients	Increase in the BCL2/BAX ratio was associated with resistant to apoptosis, and the clinical significance of CLL	
LY9 (CD229)		CD229-specific T cells represented specific and strong killing activity against primary unmodified HLA-A0201+/CD229+ B-CLL	Overexpression of CD229 has been clarified in all naïve C-CLL patients with stable expression during the course of disease	(192)
hTERT		DCs pulsed with hTERT derived peptide were able to stimulate autologous T cells in hTERT-negative B-CLL patients	High telomerase activity that is correlate with expression of hTERT has been demonstrated in more than 90% of all human tumors, particularly in CLL	(201, 202)

TAA: Tumor associated antigen, PFS: Progression-Free Survival, MDM2: Mouse Double Minute 2 homolog, hTERT: human Telomerase Reverse Transcriptase, PFS: Progression-free survival, CLL: Chronic Lymphocytic Leukemia.

tive to p53 mutation status, like alemtuzumab or flavopiridol (a potent CDK4-blocking activity), might be more effective immunotherapeutic strategies in treatment of CLL. The results of phase I clinical study of RG7112 (NCT00623870), a small-molecule MDM2 antagonist showed its clinical activity in RR AML and CLL patients ¹⁵⁶.

Survivin

Survivin, a member of inhibitor of apoptosis protein family (IAPs) seems to play a key role in suppression of apoptosis and regulation of cell division ¹⁵⁷. Overex-

pression of survivin has been reported in several cancer types, including bladder, lung, breast, stomach, oesophagus, liver, and ovarian cancers, ALL and AML ¹⁵⁸. The overexpression of survivin in most human cancers suggests its important role in tumor progression. Aberrant expression of survivin is a biomarker of poor prognosis and in drug/radiation resistance ¹⁵⁹. Survivin overexpression has been found to happen in Bone Marrow (BM) and LNs, especially in pseudofollicles from patients with B-CLL, as well as CD40 stimulated B-CLL cells ¹⁶⁰. Survivin expressing cells actively proliferate

erated and uniformly and intensely expressed BCL-2, indicating a remarkable resistance to apoptosis ¹⁶⁰. A vaccine targeting survivin could potentially target a proliferation compartment of B-CLL. The immune reaction takes place in pseudofollicles of LNs and provides a new way of eliminating B-CLL by promoting the eradication of the proliferating tumor cell pool ¹⁶⁰. Nonetheless, survivin-reactive T cells have been detected in the peripheral blood of patients with CLL. Specific CTL responses against two survivin-derived peptide epitopes were also identified in CLL patients, but not in healthy controls ¹⁶¹. Effective and specific *in-vitro* CTL responses against survivin have also been induced by autologous Dendritic Cells (DCs) pulsed with soluble recombinant survivin protein. These survivin-specific CTLs were capable of recognizing Epstein-Barr virus (EBV) B lymphocytes transfected with survivin cDNA or allogeneic lung tumor cells ¹⁶². In other studies, induced survivin-specific CTLs from Peripheral Blood Mononuclear Cells (PBMCs) efficiently recognized and lysed autologous mature DCs pulsed with the antigenic peptide or transfected with whole tumor RNA purified from a survivin-expressing cell line derived from primary autologous malignant CLL cells. In addition, survivin-specific CTLs were not able to lyse mature DCs or activate B and T cells ¹⁶³. Specific T-cell reactivity against survivin-derived HLA-B35 restricted epitopes was also detected in the peripheral blood from patients with different malignancies, especially B-CLL, multiple myeloma and melanoma. Spontaneous T cell responses against survivin-derived peptides were found in 6 of 10 B-CLL patients ¹⁶⁴. These findings raise the possibility that targeting survivin can be an innovative and efficient approach for designing potential protein- and peptide-based anti-cancer vaccines. Moreover, S12, a small molecule inhibitor of survivin, remarkably inhibited the growth of representative B lymphoma lines *in vitro* ¹⁶⁵. Another small molecule inhibitor of survivin YM155 potentially suppressed proliferation and effectively induced apoptosis in the proliferative subset of CLL cells. Interestingly, YM155 treatment diminished anti-apoptotic proteins Mcl-1 and BCL-2 expression levels independently of the level of survivin expression which might pave the way for novel therapeutic applications of YM155 in treatment of CLL ¹⁶⁶. A phase I/II clinical trial of terameprocol (NCT00664677), a novel survivin and cdc2/CDK1 Inhibitor, in patients with various advanced leukemias and hematological malignancies, including CLL showed that the drug was safe with a potential clinical activity ¹⁶⁷.

FCRL; Fc receptor-like molecules

Fc Receptor-Like (FCRL) molecules, as novel members of the Immunoglobulin Superfamily (IgSF), are preferentially expressed by B-cells with potential activating and inhibitory roles ¹⁶⁸. FCRL1-5 are dominantly expressed on developing B cells at different stages of development. But, FCRL3 and FCRL6 are

especially expressed in different subsets of T and NK cells ¹⁶⁸. Expression profiles of different FCRL family members have been investigated in autoimmune diseases, infectious diseases and B-cell malignancies ^{168,169}. Interestingly, FCRL2 has shown 94% concordance with IgHV mutational status suggesting the importance of FCRL2 as a novel and potential prognostic biomarker in CLL. In addition, FCRL2 expression was also inversely associated with clinical progression in B-CLL ¹⁷⁰. We showed elevated expression levels of CD305/LAIR-1 (leukocyte associated Ig like receptor 1) and FCRL2 in mutated compared to unmutated B-CLLs ¹⁷¹. High and exclusive expression levels of FCRL2 in CLL hold enormous potential to bring it as a novel diagnostic marker and therapeutic target in CLL patients.

Fibromodulin

Fibromodulin (FMOD) belongs to small leucine rich repeat (LRR) protein family, first identified as a 59 kDa collagen-binding protein with a broad range of tissue distribution ¹⁷². FMOD involved in regulation of collagen organization and assembly of matrix components by interaction with type I and type II collagen fibrils ¹⁷³. Several independent GEF analyses have revealed aberrant expression of FMOD (287-fold) in leukemic B cells compared to normal B cells ^{144, 174, 175}. Overexpression of FMOD in CLL B cells samples has also been confirmed at mRNA and protein levels ^{176,177}. Analysis of FMOD revealed widespread cell surface expression of GPI-anchored FMOD in CLL patients compared to healthy individuals ¹⁷⁸. Specific CTL responses have been identified against four different HLA-A2 binding FMOD derived peptides ¹⁷⁶. A recent study demonstrated that T cells from CLL patients were able to precisely recognize FMOD overexpressing leukemic B cells and responded to naturally processed and presented HLA-A0201 binding peptides derived from FMOD. They also showed that FMOD specific CD8⁺ T cells derived from CLL patients could be expanded ¹⁷⁶. Besides the possible role of FMOD in the pathophysiology of CLL, it could be a beneficial TAA candidate for immunotherapeutic intervention of CLL.

Bax and BCL-2

The ability of tumor cells to evade apoptosis is one of the hallmarks of human cancers. Evasion of apoptosis is often mediated by pro- and anti-apoptotic BCL-2 family proteins that are frequently highly expressed in cancers ¹⁷⁹. Expression of Bax or Bak plays a critical role in suppressing cancer development and their reduced expression has been reported in several malignancies ¹⁸⁰. Furthermore, proteasome-mediated degradation of Bax has been reported in advanced CLL that is associated with poor prognosis and chemoresistance of the disease ¹⁸¹. Generation of immunogenic peptides following proteasomal degradation of Bax might be related to immunologic consequences via presentation by MHC class I molecules. To address this hypothesis,

Nunes *et al* showed that Bax peptide-specific T cells had been able to recognize and kill primary malignant cells from CLL patients¹⁸². This finding suggests that generating Bax specific T cells for adoptive cell therapy protocols can be a promising therapeutic approach for treatment of CLL and other malignancies. It has been demonstrated that BCL-2 is an important protein in predicting survival in CLL. Increase in the BCL-2/BAX ratio in CD38 and CD49d positive patients was associated with resistance to apoptosis, and the clinical significance of this change was contributed to pathogenesis, chemorefractoriness and clinical outcome of CLL¹⁸³. Selective inhibition of BCL-2 by ABT-199 highlighted the potential role of BCL-2 family proteins in the context of targeted therapies¹⁸⁴. CD38 and CD49d overexpression are well known to be potential adverse prognostic markers in CLL¹⁸⁵⁻¹⁸⁷. Administration of SPC2996, a novel BCL-2 mRNA antagonist, resulted in rapid leukemic cell clearance and immune activation in CLL patients¹⁸⁸. Phase I/II dose-escalating study of SPC2996 in 25 patients with relapsed CLL showed all six CLL patients in the treatment group with maximum drug concentration (4 mg/kg/dose) had a significant reduction in lymphocyte count with shrinkage of nodes at the higher tolerated doses¹⁸⁹. Currently, a large number of clinical trials assessing monotherapy and combination therapies, which are typically based on BCL-2 targeting, are underway. Venetoclax is first and only BCL-2 inhibitor approved for CLL as monotherapy or in combination with obinutuzumab or rituximab. A 4-year follow-up from a multicentre, open-label, randomised, phase 3 trial demonstrated that CLL patients treated with venetoclax-obinutuzumab or venetoclax-obinutuzumab-ibrutinib versus the chemoimmunotherapy, improved undetectable Measurable Residual Disease (MRD) rates and progression free survival¹⁹⁰.

CD229 (Ly9); a homophilic receptor

subset of the immunoglobulin superfamily¹⁸⁰. CD229 is expressed on thymocytes and mature T and B lymphocytes¹⁹¹. Overexpression of CD229 has been clarified in all naïve CLL patients with stable expressions during the course of the disease¹⁸¹. CD229-specific T cells presented a specific and strong killing activity against primary unmodified HLA-A0201⁺/CD229⁺ B-CLL cells and T2 cells pulsed with synthetic peptides derived from CD229 protein *in vitro*¹⁹². In addition, CD40L-stimulated B-CLL cells and native unmodified B-CLL cells as APCs were able to specifically expand antigen-specific autologous T cells from B-CLL patients¹⁹². These results showed that CD229 is a naturally processed and presented antigen in B-CLL which can be considered a TAA in this disease. Thus, these findings provide strong evidence that CD229 can be an attractive target for the design and implementation of T-cell-based adoptive immunotherapeutic approaches for CD229-expressing malignancies including B-CLL.

FMNL1; a formin like protein 1

Formins are cytoskeleton-organizing proteins that play essential roles in cytokinesis and driving alterations in cell polarity, vesicular trafficking, signaling to the nucleus and embryonic development¹⁹³. Recently, a novel human gene, related to the formin family has been described. mRNA expression analysis demonstrated restricted expression of a formin like protein (FMNL1) in peripheral blood leukocytes, spleen, and thymus. However, at the protein level, low expression of FMNL1 was found to be restricted to lymph nodes and peripheral blood leukocytes. Interestingly, overexpression of FMNL1 was observed in Jurkat and Molt-4 cell lines and CLL tumor cells¹⁹⁴. This study also showed interaction between FMNL1 and Akt, suggesting a possible role for this protein in Akt signaling pathway¹⁹⁴. Another human leukocyte formin gene, termed KW-13, has been defined as a novel TAA overexpressed in CLL¹⁹⁵. Further research should clarify the exact role of formin protein family in CLL and also in immunotherapeutic based strategies.

RHAMM/CD168; the receptor for hyaluronan-mediated motility

Differential mRNA expression of TAAs in B-CLL patients has identified tumor-restricted antigen expression of RHAMM/CD168, fibromodulin, PRAME and MPP11 in CLL samples, but not in healthy donors. Higher expression levels of HSP27 (100%), MAZ (93%) and OFAI1 (100%) have also been detected in CLL patients. Analysis by conventional RT-PCR showed a more frequent expression of RHAMM/CD168 in advanced stages of CLL¹⁹⁶. RHAMM/CD168 expression was detected in both ZAP-70-positive and negative B-CLL patients. In addition, IFN- γ and granzyme-B secreting CD8⁺ T cells showed specific response against T2 cells pulsed with RHAMM/CD168 derived peptides¹⁹⁶. In another study, significantly increased specific CTLs against RHAMM/CD168 derived R3 peptide was detected after vaccination with DCs pulsed with CLL cell lysates¹⁹⁷. CD8⁺ T-cell responses against RHAMM/CD168 have also been described in AML patients¹⁹⁸. These results showed that targeting RHAMM/CD168 as a TAA may serve as an effective cell-based immunotherapeutic strategy for RHAMM/CD168 overexpressing CLL patients.

hTERT; human telomerase reverse transcriptase

A functional catalytic protein subunit of a RNA-dependent DNA-polymerase, named human telomerase reverse transcriptase (hTERT), adds repeat sequences of DNA (TTAGGG) to the 3' end of chromosomes¹⁹⁹. Telomere length maintained by telomerase and ectopic telomerase expression leads to survival and unlimited proliferative capacity of malignant cells²⁰⁰. High telomerase activity that is correlated with expression of hTERT has been demonstrated in more than 90% of all human tumors²⁰⁰. High telomerase activity has also been reported in the majority of acute and chronic leukemias. Significantly higher levels of telomerase activ-

ity in various B-cell malignancies including CLL, Hairy Cell Leukemia (HCL) and Mantle Cell Lymphoma (MCL) have been detected in late stages than in early stages ²⁰¹. In addition, the activity and therefore length of telomere might be associated with disease prognosis in B-CLL ²⁰¹. hTERT overexpression was found in about 75% of CLL patients ²⁰². DCs pulsed with a hTERT derived peptide (hTERT 611–626 peptide) were able to stimulate autologous T cells in hTERT expressing but not in hTERT-negative B-CLL patients and healthy control donors. Expanded autologous hTERT-specific cytotoxic T cells showed cytotoxic activities against hTERT overexpressing B-CLL cells in MHC class I-restricted manner ²⁰². These findings suggest targeting hTERT as a suitable vaccine in B-CLL patients.

OFA-iLRP; oncofetal antigen immature laminin receptor protein

As a highly conserved protein, oncofetal antigen immature laminin receptor protein (OFA-iLRP) is preferentially expressed in fetal tissues and has been identified in many types of tumors ^{203, 204}. Siegel *et al* demonstrated that OFA-iLRP could act as a potential target for T-cell-based immunotherapeutic approaches against hematologic malignancies such as lymphomas, AML and CLL ²⁰⁵. They primarily showed significant overexpression of OFA-iLRP in all hematologic tumor cell lines and all B-CLL and AML samples. OFA-iLRP was neither detectable in early stages of cancer nor in healthy donors. OFA-iLRP-specific CTLs were able to kill primary AML blasts, malignant B-CLL cells and OFA-iLRP-loaded T cells ²⁰⁵. Furthermore, it was also shown that mice treated with DCs transfected with OFA-iLRP-coding RNA, fully rejected the tumor and improved overall survival ²⁰⁵. Since OFA-iLRP mRNA was strongly expressed in PBMCs from healthy donors and B-CLL patients ¹⁹⁶ as well as renal patients ²⁰⁶, it could not serve as a suitable target for B-CLL immunotherapy.

SLLP1; sperm lysozyme-like protein 1

SLLP1 is a unique c-lysozyme-like protein predominantly expressed in the acrosome of human sperm ²⁰⁷. A study by Wang *et al.* showed that SLLP1 could serve as a novel cancer–testis antigen in some hematologic malignancies including AML, CLL, CML and multiple myeloma ²⁰⁸. In contrast to healthy donors, tumor cells of hematologic malignancies including AML, CML, CLL and multiple myeloma, showed aberrant expression of SLLP1. High titer IgG antibodies against SLLP1 were thus detected in the sera of malignant patients ²⁰⁸.

TCL1; T-cell leukemia/lymphoma 1

TCL1 onco-protein overexpression has been detected in many B-cell malignancies, including follicular lymphoma, CLL, MCL, diffuse large B-cell lymphoma, and splenic marginal zone B-cell lymphoma ²⁰⁹⁻²¹². TCL1-specific T cells were detectable in the peripheral

blood and tumor-infiltrating lymphocytes of lymphoma patients. TCL1-specific T cells could also be expanded in an autologous setting and were able to recognize and lyse TCL1₆₅₋₇₉ peptide-pulsed T2 cells in MHC-I restricted manner ²¹¹. Therefore, TCL1 was demonstrated to be naturally processed and presented on the surface of primary human lymphoma cells to be recognized by CTLs and seems to be an ideal TAA that can act as a therapeutic target for development of new immunotherapeutic strategies against B-cell lymphomas especially for CLL patients ²¹¹.

Adipophilin

Adipophilin as a marker of lipid accumulation initially is involved in lipid storage and is considered to be expressed only in adipocytes ²¹³, but it has been found to be expressed by a variety of cells including macrophages and tumor cells ^{214,215}. Adipophilin derived peptides are capable of inducing effective adipophilin-specific CTLs in a wide variety of malignancies including renal cell carcinoma, breast cancer, melanoma, multiple myeloma, and primary autologous CLL cells or cells from plasma cell leukemia ²¹⁶. Generated adipophilin-specific CTLs from PBMCs of CLL patients are able to recognize and lyse autologous RNA transfected DCs purified from adipophilin-positive tumor cell lines and autologous primary CLL cells, while nonmalignant B cells, T cells, monocytes, and DCs were unharmed ²¹⁶. It may thus be suggested that adipophilin can act as an ideal candidate TAA candidate for development of CLL specific T-cell-based immunotherapy.

APRIL; a proliferation-inducing ligand and BAFF; B-cell activating factor

A proliferation-inducing ligand (APRIL) and B-cell Activating Factor (BAFF) belong to the Tumor Necrosis Factor (TNF) ligand family and are critical for the maturation, survival, and differentiation of normal and malignant B cells ²¹⁷⁻²¹⁹. BAFF and APRIL have been shown to play a crucial role in the pathogenesis and maintenance of B CLL tumor cells ²²⁰. Additionally, APRIL enhances tumor growth in human and murine tumor cell lines *in vitro* and *in vivo* ²²¹. Interestingly, B CLL malignant cells express BAFF and APRIL and stimulation of these receptors promote CLL cell proliferation and survival *in vitro* ^{222,223}. Additionally, BAFF and APRIL protected B-CLL cells against spontaneous and drug-induced apoptosis ²²⁰. Elevated BAFF levels are observed in patients with CLL, particularly those with unmutated IgHV and Higher BAFF expression is linked to unfavorable outcomes in these patients ²²⁴. Increased serum levels of BAFF (sBAFF) and APRIL (sAPRIL) are considered as potential predictive factors in B-CLL patients ²²⁵. Enhanced intracellular APRIL and BAFF levels within CLL cells correlate with elevated expression of unfavorable prognostic markers CD38 and ZAP70, and poorer clinical outcomes ²²⁴. BAFF, APRIL, and their receptors have therefore at-

tracted great attention as potential targets for B-CLL therapy. BAFF-neutralizing antibody belimumab remarkably increased the sensitivity of the leukemic cells to all three small molecule inhibitors including B cell receptor inhibitors ibrutinib and idelalisib as well as the BCL-2 antagonist venetoclax ²²⁶. These findings show that BAFF neutralization using belimumab in combination with small molecule inhibitors can serve as a promising therapeutic strategy for patients with CLL. Belimumab is currently undergoing phase II clinical trials (NCT05069051) for the treatment of relapsed and/or refractory CLL ²¹⁹. Patients who undergo treatment with CD19-targeting chimeric antigen receptor (CAR)-T cells for B-cell lymphoid leukemias and lymphomas experience relapsed and/or refractory (R/R) disease. CAR)-T cells targeting BAFF-R (BAFF-R CAR, also known as MC10029 CAR) showed significant *in vitro* and *in vivo* antigen-specific cytotoxicity against CLL cell lines and against CLL patients' tumors, respectively ²²⁷.

Pim-1; provirus integration site for Moloney murine leukemia virus

Pim-1 is a highly conserved serine/threonine kinase that fine-tunes several cellular functions such as cell cycle, cell survival, drug resistance ²²⁸. Upregulation of Pin-1 expression has been reported in CLL compared with normal lymphocytes and PIM kinase inhibitors showed an effective therapeutic efficacy for CLL patients ²²⁹. HLA Ligandome Analysis showed the highly expression of SET nuclear proto-oncogene (SET), Pim-1 oncogene and Mucin 1 as new TAA in CLL cells ²³⁰. The SET oncoprotein is overexpressed in CLL cells, and SET levels are predictive of Overall Survival (OS) and the Time To Treatment (TTT) ²³¹. Inhibition of SET protein leads to the enhance apoptosis, decrease Mcl-1 levels, and also is highly cytotoxic to malignant B cells *in vitro* and *in vivo* ²³¹. These data show that targeting SET and Pim-1 could be a promising strategy to the treatment of CLL.

IGLV3-21; immunoglobulin lambda variable 3-21

The CLL B CLL subsets bearing IGLV3-21^{R110} BCR light chain represents an aggressive clinical course and serves as a poor prognostic factor in CLL patients ²³². Surface expression IGLV3-21^{R110} neoantigen with oncogenic activity may be considered a potential target for CAR T cell therapy in CLL patients. New types of CAR-T cells with CAR construct of BCR light chain neoepitope composed of point mutation IGLV3-21^{R110} are able to selectively eradicate poor-risk subset of IGLV3-21^{R110} expressing cell lines and primary CLL cells ²³³.

Conclusion

Tumor cells express a variety of poorly immunogenic antigens at different stages of cancer. However, identification of novel immunogenic CLL associated antigens that are generally expressed is essential to

overcome the barriers of patient-specific idotype vaccines. Furthermore, identification of human TAAs in cancer is developing as a critical part of clinical trials at this time (Table 2). mAbs exhibit an exciting addition to the growing list of agents that are used to treat CLL. Molecules that are tumor-specific or overexpressed in cancers may play fundamental roles to contribute to tumor cell development, cellular transformation, and migration. Today, new promising molecules for CLL are being tested in clinical trials. Targeting of such molecules can promote the anti-tumor effect and therefore might be valuable approaches for cancer therapy. Application of specific mAbs to target certain antigens have shown great potentials as valuable approaches in preclinical and clinical investigations and will thereby continue to revolutionize the treatment of CLL as we know it today. Malignant CLL cells exhibit features of activated and antigen-experienced B cells. Constitutive and stable expression of specific targets on malignant B cells and natural processing and presentation of these molecules on the surface of lymphoma cells for recognition by cytotoxic T cells as TAA in primary B-CLL, enabling the expansion of autologous tumor-specific T cells, can serve as novel targets for development of immunotherapeutic strategies against common B-cell malignancies. Recently there has been significant interest in multiple TAA as therapeutic targets in CLL patients as evidenced by several studies that have demonstrated the existence of autologous T cells against TAAs which functionally respond with IFN- γ secretion after recognition of these antigens. These findings are promising in the identification of specific target-derived peptides as TAAs in B-CLL that open numerous pathways to consider these molecules for therapeutic interventions in this disease. However, preclinical studies are essential to achieve the optimal vaccine formulation and identify the safety and efficacy of TAA-derived vaccines before evaluation in clinical trials.

Conflict of Interest

The authors have no financial conflicts of interest.

References

1. Hus I, Rolinski J. Current concepts in diagnosis and treatment of chronic lymphocytic leukemia. *Contemp Oncol (Pozn)* 2015;19(5):361-7.
2. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. *Am J Hematol* 2017;92(9):946-65.
3. Rozman C, Montserrat E. Chronic lymphocytic leukemia. *N Engl J Med* 1995;333(16):1052-7.
4. Jovanovic D, Djurdjevic P, Andjelkovic N, Zivic L. Possible role of CD22, CD79b and CD20 expression in distinguishing small lymphocytic lymphoma from chronic lymphocytic leukemia. *Contemp Oncol (Pozn)* 2014; 18(1):29-33.

5. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008;111(12):5446-56.
6. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood* 2018;131(25):2745-60.
7. Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group. *J Natl Cancer Inst* 1999;91(10):861-8.
8. Brown J, Hillmen P, O'Brien S, Barrientos J, Reddy N, Coutre S, et al. Extended follow-up and impact of high-risk prognostic factors from the phase 3 RESONATE study in patients with previously treated CLL/SLL. *Leukemia* 2018;32(1):83-91.
9. Molica S, Tam C, Allsup D, Polliack A. Advancements in the treatment of CLL: The rise of zanubrutinib as a preferred therapeutic option. *Cancers* 2023;15(14):3737.
10. Andreescu M, Andreescu B. Immune Evasion Through Human Leukocyte Antigen Implications and Its Impact on Targeted Therapy. *Cureus* 2024;16(1):e52737.
11. Ghosh AK, Kay NE. Critical signal transduction pathways in CLL. *Adv Exp Med Biol* 2013;792:215-39.
12. Mouawad N, Ruggeri E, Capasso G, Martinello L, Visentin A, Frezzato F, et al. How receptor tyrosine kinase-like orphan receptor 1 meets its partners in chronic lymphocytic leukemia. *Hematol Oncol* 2024;42(2):e3250.
13. Riches JC, Ramsay AG, Gribben JG. Immune dysfunction in chronic lymphocytic leukemia: the role for immunotherapy. *Curr Pharm Des* 2012;18(23):3389-98.
14. Platsoucas CD, Galinski M, Kempin S, Reich L, Clarkson B, Good RA. Abnormal T lymphocyte subpopulations in patients with B cell chronic lymphocytic leukemia: an analysis by monoclonal antibodies. *J Immunol* 1982;129(5):2305-12.
15. Mu X, Kay NE, Gosland MP, Jennings CD. Analysis of blood T-cell cytokine expression in B-chronic lymphocytic leukaemia: evidence for increased levels of cytoplasmic IL-4 in resting and activated CD8 T cells. *Br J Haematol* 1997;96(4):733-5.
16. de Toter D, Reato G, Mauro F, Cignetti A, Ferrini S, Guarini A, et al. IL4 production and increased CD30 expression by a unique CD8+ T-cell subset in B-cell chronic lymphocytic leukaemia. *Br J Haematol* 1999;104(3):589-99.
17. 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):1319-26.
18. Cerutti A, Kim EC, Shah S, Schattner EJ, Zan H, Schaffer A, et al. Dysregulation of CD30+ T cells by leukemia impairs isotype switching in normal B cells. *Nat Immunol* 2001;2(2):150-6.
19. Boncompagni G, Tatangelo V, Lopresti L, Olivieri C, Capitani N, Tangredi C, et al. Leukemic cell-secreted interleukin-9 suppresses cytotoxic T cell-mediated killing in chronic lymphocytic leukemia. *Cell Death Dis* 2024;15(2):144.
20. Buggins AG, Patten PE, Richards J, Thomas NS, Mufti GJ, Devereux S. Tumor-derived IL-6 may contribute to the immunological defect in CLL. *Leukemia* 2008;22(5):1084-7.
21. Fayad L, Keating MJ, Reuben JM, O'Brien S, Lee BN, Lerner S, et al. Interleukin-6 and interleukin-10 levels in chronic lymphocytic leukemia: correlation with phenotypic characteristics and outcome. *Blood* 2001;97(1):256-63.
22. Gadi D, Griffith A, Tyekucheva S, Wang Z, Rai V, Vartanov A, et al. AT cell inflammatory phenotype is associated with autoimmune toxicity of the PI3K inhibitor duvelisib in chronic lymphocytic leukemia. *Leukemia* 2022;36(3):723-32.
23. Gorgun G, Holderried TA, Zahrieh D, Neuberg D, Gribben JG. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. *J Clin Invest* 2005;115(7):1797-805.
24. D'Arena G, Laurenti L, Minervini MM, Deaglio S, Bonello L, De Martino L, et al. Regulatory T-cell number is increased in chronic lymphocytic leukemia patients and correlates with progressive disease. *Leuk Res* 2011;35(3):363-8.
25. Giannopoulos K, Schmitt M, Kowal M, Wlasiuk P, Bojarska-Junak A, Chen J, et al. Characterization of regulatory T cells in patients with B-cell chronic lymphocytic leukemia. *Oncol Rep* 2008;20(3):677-82.
26. Lindqvist CA, Christiansson LH, Simonsson B, Enblad G, Olsson-Stromberg U, Loskog AS. T regulatory cells control T-cell proliferation partly by the release of soluble CD25 in patients with B-cell malignancies. *Immunology* 2010;131(3):371-6.
27. Lindqvist CA, Christiansson LH, Thorn I, Mangsbo S, Paul-Wetterberg G, Sundstrom C, et al. Both CD4+ FoxP3+ and CD4+ FoxP3- T cells from patients with B-cell malignancy express cytolytic markers and kill autologous leukaemic B cells in vitro. *Immunology* 2011;133(3):296-306.
28. Catakovic K, Gassner FJ, Ratswohl C, Zaborsky N, Rebhandl S, Schubert M, et al. TIGIT expressing CD4+ T cells represent a tumor-supportive T cell subset in chronic lymphocytic leukemia. *Oncoimmunology* 2018;7(1):e1371399.
29. Van den Hove LE, Vandenbergh P, Van Gool SW, Ceuppens JL, Demuyneck H, Verhoef GE, et al. Peripheral blood lymphocyte subset shifts in patients with untreated hematological tumors: evidence for systemic activation of the T cell compartment. *Leuk Res* 1998;22(2):175-84.
30. Kay NE, Zarlign JM. Impaired natural killer activity in patients with chronic lymphocytic leukemia is associated with a deficiency of azurophilic cytoplasmic granules in putative NK cells. *Blood* 1984;63(2):305-9.

31. Kay NE, Zarling J. Restoration of impaired natural killer cell activity of B-chronic lymphocytic leukemia patients by recombinant interleukin-2. *Am J Hematol* 1987;24 (2):161-7.
32. Burton JD, Weitz CH, Kay NE. Malignant chronic lymphocytic leukemia B cells elaborate soluble factors that down-regulate T cell and NK function. *Am J Hematol* 1989;30(2):61-7.
33. Acebes-Huerta A, Huergo-Zapico L, Gonzalez-Rodriguez AP, Fernandez-Guizan A, Payer AR, Lopez-Soto A, et al. Lenalidomide induces immunomodulation in chronic lymphocytic leukemia and enhances antitumor immune responses mediated by NK and CD4 T cells. *Biomed Res Int* 2014;2014:265840.
34. Bojarska-Junak A, Hus I, Sieklucka M, Wasik-Szczepanek E, Mazurkiewicz T, Polak P, et al. Natural killer-like T CD3+/CD16+CD56+ cells in chronic lymphocytic leukemia: intracellular cytokine expression and relationship with clinical outcome. *Oncol Rep* 2010;24(3):803-10.
35. Marin D, Li Y, Basar R, Rafei H, Daher M, Dou J, et al. Safety, efficacy and determinants of response of allogeneic CD19-specific CAR-NK cells in CD19+ B cell tumors: a phase 1/2 trial. *Nat Med* 2024;772-84.
36. Huang M, Liu Y, Yan Q, Peng M, Ge J, Mo Y, et al. NK cells as powerful therapeutic tool in cancer immunotherapy. *Cellular Oncology*. 2024;1-25.
37. Zeya HI, Keku E, Richards F, 2nd, Spurr CL. Monocyte and granulocyte defect in chronic lymphocytic leukemia. *Am J Pathol* 1979;95(1):43-54.
38. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature* 2015; 526(7574):525-30.
39. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002;99(24):15524-9.
40. Dong JT, Boyd JC, Frierson HF, Jr. Loss of heterozygosity at 13q14 and 13q21 in high grade, high stage prostate cancer. *Prostate* 2001;49(3):166-71.
41. Klein U, Lia M, Crespo M, Siegel R, Shen Q, Mo T, et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell* 2010;17(1):28-40.
42. Austen B, Powell JE, Alvi A, Edwards I, Hooper L, Starczynski J, et al. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. *Blood* 2005;106(9):3175-82.
43. Zenz T, Mertens D, Kuppers R, Dohner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia. *Nat Rev Cancer* 2010;10(1):37-50.
44. Dohner H, Stilgenbauer S, James MR, Benner A, Weillguni T, Bentz M, et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood* 1997;89(7):2516-22.
45. Catovsky D, Richards S, Matutes E, Oscier D, Dyer M, Bezares RF, et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. *Lancet* 2007;370(9583):230-9.
46. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med* 2000; 343(26):1910-6.
47. Dohner H, Fischer K, Bentz M, Hansen K, Benner A, Cabot G, et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood*. 1995;85(6):1580-9.
48. Geisler CH, Philip P, Christensen BE, Hou-Jensen K, Pedersen NT, Jensen OM, et al. In B-cell chronic lymphocytic leukaemia chromosome 17 abnormalities and not trisomy 12 are the single most important cytogenetic abnormalities for the prognosis: a cytogenetic and immunophenotypic study of 480 unselected newly diagnosed patients. *Leuk Res* 1997;21 (11-12):1011-23.
49. el Rouby S, Thomas A, Costin D, Rosenberg CR, Potmesil M, Silber R, et al. p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. *Blood* 1993;82(11):3452-9.
50. Zenz T, Vollmer D, Trbusek M, Smardova J, Benner A, Soussi T, et al. TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. *Leukemia* 2010;24(12):2072-9.
51. Trbusek M, Smardova J, Malcikova J, Sebejova L, Dobes P, Svitakova M, et al. Missense mutations located in structural p53 DNA-binding motifs are associated with extremely poor survival in chronic lymphocytic leukemia. *Journal of clinical oncology : official journal of the American Society of Clin Oncol* 2011;29(19):2703-8.
52. Seiffert M, Dietrich S, Jethwa A, Glimm H, Lichter P, Zenz T. Exploiting biological diversity and genomic aberrations in chronic lymphocytic leukemia. *Leuk Lymphoma* 2012;53(6):1023-31.
53. Ljungstrom V, Cortese D, Young E, Pandzic T, Mansouri L, Plevova K, et al. Whole-exome sequencing in relapsing chronic lymphocytic leukemia: clinical impact of recurrent RPS15 mutations. *Blood* 2016;127(8):1007-16.
54. Puente XS, Pinyol M, Quesada V, Conde L, Ordenez GR, Villamor N, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature* 2011;475(7354):101-5.
55. Puente XS, Bea S, Valdes-Mas R, Villamor N, Gutierrez-Abril J, Martin-Subero JI, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* 2015;526(7574):519-24.
56. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood* 2008;112(5):1570-80.
57. Burger JA, Chiorazzi N. B cell receptor signaling in chronic lymphocytic leukemia. *Trends Immunol* 2013;34 (12):592-601.

58. Herishanu Y, Perez-Galan P, Liu D, Biancotto A, Pitaluga S, Vire B, et al. The lymph node micro-environment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood* 2011;117(2):563-74.
59. Chu CC, CATERA R, HATZI K, YAN XJ, ZHANG L, WANG XB, et al. Chronic lymphocytic leukemia antibodies with a common stereotypic rearrangement recognize non-muscle myosin heavy chain IIA. *Blood* 2008;112(13):5122-9.
60. Broker BM, Klajman A, Youinou P, Jouquan J, Worman CP, Murphy J, et al. Chronic lymphocytic leukemic (CLL) cells secrete multispecific autoantibodies. *J Autoimmun* 1988;1(5):469-81.
61. Damle RN, Ghiotto F, Valetto A, Albesiano E, Fais F, Yan XJ, et al. B-cell chronic lymphocytic leukemia cells express a surface membrane phenotype of activated, antigen-experienced B lymphocytes. *Blood* 2002;99(11):4087-93.
62. Lanemo Myhrinder A, Hellqvist E, Sidorova E, Soderberg A, Baxendale H, Dahle C, et al. A new perspective: molecular motifs on oxidized LDL, apoptotic cells, and bacteria are targets for chronic lymphocytic leukemia antibodies. *Blood* 2008;111(7):3838-48.
63. Hoogeboom R, van Kessel KP, Hochstenbach F, Wormhoudt TA, Reinten RJ, Wagner K, et al. A mutated B cell chronic lymphocytic leukemia subset that recognizes and responds to fungi. *J Exp Med* 2013;210(1):59-70.
64. Stadanlick JE, Kaileh M, Karnell FG, Scholz JL, Miller JP, Quinn WJ, 3rd, et al. Tonic B cell antigen receptor signals supply an NF-kappaB substrate for prosurvival BLyS signaling. *Nat Immunol* 2008;9(12):1379-87.
65. Scupoli MT, Pizzolo G. Signaling pathways activated by the B-cell receptor in chronic lymphocytic leukemia. *Expert Rev Hematol* 2012;5(3):341-8.
66. Healy JJ, Goodnow CC. Positive versus negative signaling by lymphocyte antigen receptors. *Annu Rev Immunol* 1998;16:645-70.
67. Contri A, Brunati AM, Trentin L, Cabrelle A, Miorin M, Cesaro L, et al. Chronic lymphocytic leukemia B cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. *J Clin Invest* 2005;115(2):369-78.
68. Saijo K, Schmedt C, Su IH, Karasuyama H, Lowell CA, Reth M, et al. Essential role of Src-family protein tyrosine kinases in NF-kappaB activation during B cell development. *Nat Immunol* 2003;4(3):274-9.
69. Gobessi S, Laurenti L, Longo PG, Carsetti L, Berno V, Sica S, et al. Inhibition of constitutive and BCR-induced Syk activation downregulates Mcl-1 and induces apoptosis in chronic lymphocytic leukemia B cells. *Leukemia* 2009;23(4):686-97.
70. Rinaldi A, Kwee I, Taborelli M, Largo C, Uccella S, Martin V, et al. Genomic and expression profiling identifies the B-cell associated tyrosine kinase Syk as a possible therapeutic target in mantle cell lymphoma. *Br J Haematol* 2006;132(3):303-16.
71. Chen L, Monti S, Juszczynski P, Daley J, Chen W, Witzig TE, et al. SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. *Blood* 2008;111(4):2230-7.
72. Muzio M, Apollonio B, Scielzo C, Frenquelli M, Vandoni I, Boussiotis V, et al. Constitutive activation of distinct BCR-signaling pathways in a subset of CLL patients: a molecular signature of anergy. *Blood* 2008;112(1):188-95.
73. Hewamana S, Alghazal S, Lin TT, Clement M, Jenkins C, Guzman ML, et al. The NF-kappaB subunit Rel A is associated with in vitro survival and clinical disease progression in chronic lymphocytic leukemia and represents a promising therapeutic target. *Blood* 2008;111(9):4681-9.
74. Krysov S, Potter KN, Mockridge CI, Coelho V, Wheatley I, Packham G, et al. Surface IgM of CLL cells displays unusual glycans indicative of engagement of antigen in vivo. *Blood* 2010;115(21):4198-205.
75. Zhang XY, Zhang PY. Receptor tyrosine kinases in carcinogenesis. *Oncol Lett* 2020;20(5):195.
76. Landreth KS, Narayanan R, Dorshkind K. Insulin-like growth factor-I regulates pro-B cell differentiation. *Blood* 1992;80(5):1207-12.
77. Schillaci R, Galeano A, Becu-Villalobos D, Spinelli O, Sapia S, Bezares RF. Autocrine/paracrine involvement of insulin-like growth factor-I and its receptor in chronic lymphocytic leukaemia. *Br J Haematol* 2005;130(1):58-66.
78. Saiya-Cork K, Collins R, Parkin B, Ouillet P, Kuizon E, Kujawski L, et al. A pathobiological role of the insulin receptor in chronic lymphocytic leukemia. *Clin Cancer Res* 2011;17(9):2679-92.
79. Green JL, Kuntz SG, Sternberg PW. Ror receptor tyrosine kinases: orphans no more. *Trends Cell Biol* 2008;18(11):536-44.
80. Daneshmanesh AH, Hojjat-Farsangi M, Ghaderi A, Moshfegh A, Hansson L, Schultz J, et al. A receptor tyrosine kinase ROR1 inhibitor (KAN0439834) induced significant apoptosis of pancreatic cells which was enhanced by erlotinib and ibrutinib. *PLoS One* 2018;13(6):e0198038.
81. Hojjat-Farsangi M, Moshfegh A, Daneshmanesh AH, Khan AS, Mikaelsson E, Osterborg A, et al. The receptor tyrosine kinase ROR1--an oncofetal antigen for targeted cancer therapy. *Semin Cancer Biol*. 2014;29:21-31.
82. Hojjat-Farsangi M, Khan AS, Daneshmanesh AH, Moshfegh A, Sandin A, Mansouri L, et al. The tyrosine kinase receptor ROR1 is constitutively phosphorylated in chronic lymphocytic leukemia (CLL) cells. *PLoS One* 2013;8(10):e78339.
83. Hojjat-Farsangi M, Ghaemimanesh F, Daneshmanesh AH, Bayat AA, Mahmoudian J, Jeddi-Tehrani M, et al. Inhibition of the receptor tyrosine kinase ROR1 by anti-ROR1 monoclonal antibodies and siRNA induced apoptosis of melanoma cells. *PLoS One* 2013;8(4):e61167. Retraction.
84. Daneshmanesh AH, Hojjat-Farsangi M, Khan AS, Jeddi-

- Tehrani M, Akhondi MM, Bayat AA, et al. Monoclonal antibodies against ROR1 induce apoptosis of chronic lymphocytic leukemia (CLL) cells. *Leukemia* 2012;26(6):1348-55.
85. Daneshmanesh AH, Porwit A, Hojjat-Farsangi M, Jeddi-Tehrani M, Tamm KP, Grander D, et al. Orphan receptor tyrosine kinases ROR1 and ROR2 in hematological malignancies. *Leuk Lymphoma* 2013;54(4):843-50.
 86. Shabani M, Asgarian Omran H, Farsangi MH, Vossough P, Sharifian RA, Touge GR, et al. Comparative expression profile of orphan receptor tyrosine kinase ROR1 in Iranian patients with lymphoid and myeloid leukemias. *Avicenna J Med Biotechnol* 2011;3(3):119-25.
 87. Baskar S, Kwong KY, Hofer T, Levy JM, Kennedy MG, Lee E, et al. Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia. *Clin Cancer Res* 2008;14(2):396-404.
 88. Daneshmanesh AH, Mikaelsson E, Jeddi-Tehrani M, Bayat AA, Ghods R, Ostadkarampour M, et al. Ror1, a cell surface receptor tyrosine kinase is expressed in chronic lymphocytic leukemia and may serve as a putative target for therapy. *Int J Cancer* 2008;123(5):1190-5.
 89. Hojjat-Farsangi M, Jeddi-Tehrani M, Razavi SM, Sharifian RA, Mellstedt H, Shokri F, et al. Immunoglobulin heavy chain variable region gene usage and mutational status of the leukemic B cells in Iranian patients with chronic lymphocytic leukemia. *Cancer Sci* 2009;100(12):2346-53.
 90. Farsangi MH, Jeddi-Tehrani M, Sharifian RA, Razavi SM, Khoshnoodi J, Rabbani H, et al. Analysis of the immunoglobulin heavy chain variable region gene expression in Iranian patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2007;48(1):109-16.
 91. Choi MY, Widhopf GF, 2nd, Ghia EM, Kidwell RL, Hasan MK, Yu J, et al. Phase I Trial: Cirmtuzumab Inhibits ROR1 Signaling and Stemness Signatures in Patients with Chronic Lymphocytic Leukemia. *Cell Stem Cell* 2018;22(6):951-9 e3.
 92. Hojjat-Farsangi M, Daneshmanesh AH, Khan AS, Shetye J, Mozaffari F, Kharaziha P, et al. First-in-class oral small molecule inhibitor of the tyrosine kinase ROR1 (KAN0439834) induced significant apoptosis of chronic lymphocytic leukemia cells. *Leukemia* 2018;32(10):2291-5.
 93. Veronese L, Tournilhac O, Verrelle P, Davi F, Dighiero G, Chautard E, et al. Strong correlation between VEGF and MCL-1 mRNA expression levels in B-cell chronic lymphocytic leukemia. *Leuk Res* 2009;33(12):1623-6.
 94. Kay NE, Bone ND, Tschumper RC, Howell KH, Geyer SM, Dewald GW, et al. B-CLL cells are capable of synthesis and secretion of both pro- and anti-angiogenic molecules. *Leukemia* 2002;16(5):911-9.
 95. Kini AR, Kay NE, Peterson LC. Increased bone marrow angiogenesis in B cell chronic lymphocytic leukemia. *Leukemia* 2000;14(8):1414-8.
 96. Molica S, Vitelli G, Levato D, Ricciotti A, Digiesi G. Clinicoprognostic implications of increased serum levels of vascular endothelial growth factor and basic fibroblastic growth factor in early B-cell chronic lymphocytic leukaemia. *Br J Cancer* 2002;86(1):31-5.
 97. Lai C, Lemke G. An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 1991;6(5):691-704.
 98. O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, et al. axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol* 1991;11(10):5016-31.
 99. Hafizi S, Dahlback B. Signalling and functional diversity within the Axl subfamily of receptor tyrosine kinases. *Cytokine Growth Factor Rev* 2006;17(4):295-304.
 100. Ghosh AK, Secreto C, Boysen J, Sassoon T, Shanafelt TD, Mukhopadhyay D, et al. The novel receptor tyrosine kinase Axl is constitutively active in B-cell chronic lymphocytic leukemia and acts as a docking site of nonreceptor kinases: implications for therapy. *Blood* 2011;117(6):1928-37.
 101. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, et al. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature* 1984;311(5981):29-33.
 102. Liu X, Yao W, Newton RC, Scherle PA. Targeting the c-MET signaling pathway for cancer therapy. *Expert Opin Investig Drugs* 2008;17(7):997-1011.
 103. Eksioglu-Demiralp E, Akdeniz T, Bayik M. Aberrant expression of c-met and HGF/c-met pathway provides survival advantage in B-chronic lymphocytic leukemia. *Cytometry B Clin Cytom* 2011;80(1):1-7.
 104. Shabani M, Hojjat-Farsangi M. Targeting Receptor Tyrosine Kinases Using Monoclonal Antibodies: The Most Specific Tools for Targeted-Based Cancer Therapy. *Curr Drug Targets* 2016;17(14):1687-703.
 105. Hojjat-Farsangi M. Small-molecule inhibitors of the receptor tyrosine kinases: promising tools for targeted cancer therapies. *Int J Mol Sci* 2014;15(8):13768-801.
 106. Alsouqi A, Woyach JA. Covalent Bruton's Tyrosine Kinase Inhibitors in Chronic Lymphocytic Leukemia. *Clin Lymphoma Myeloma Leuk* 2024 May 31:S2152-2650(24)00210-6.
 107. Freeman CL, Gribben JG. Immunotherapy in Chronic Lymphocytic Leukaemia (CLL). *Curr Hematol Malig Rep* 2016;11(1):29-36.
 108. Jaglowski SM, Alinari L, Lapalombella R, Muthusamy N, Byrd JC. The clinical application of monoclonal antibodies in chronic lymphocytic leukemia. *Blood* 2010;116(19):3705-14.
 109. Plosker GL, Figgitt DP. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. *Drugs* 2003;63(8):803-43.
 110. Uchida J, Lee Y, Hasegawa M, Liang Y, Bradney A, Oliver JA, et al. Mouse CD20 expression and function. *Int Immunol* 2004;16(1):119-29.
 111. Press OW, Howell-Clark J, Anderson S, Bernstein I. Retention of B-cell-specific monoclonal antibodies by human lymphoma cells. *Blood* 1994;83(5):1390-7.

112. Golay J, Lazzari M, Facchinetti V, Bernasconi S, Borleri G, Barbui T, et al. CD20 levels determine the in vitro susceptibility to rituximab and complement of B-cell chronic lymphocytic leukemia: further regulation by CD55 and CD59. *Blood* 2001;98(12):3383-9.
113. Aue G, Lindorfer MA, Beum PV, Pawluczukowycz AW, Vire B, Hughes T, et al. Fractionated subcutaneous rituximab is well-tolerated and preserves CD20 expression on tumor cells in patients with chronic lymphocytic leukemia. *Haematologica* 2010;95(2):329-32.
114. Gowda A, Roda J, Hussain SR, Ramanunni A, Joshi T, Schmidt S, et al. IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells in vitro. *Blood* 2008;111(9):4723-30.
115. Gowda A, Ramanunni A, Cheney C, Rozewski D, Kindsvogel W, Lehman A, et al. Differential effects of IL-2 and IL-21 on expansion of the CD4⁺ CD25⁺ Foxp3⁺ T regulatory cells with redundant roles in natural killer cell mediated antibody dependent cellular cytotoxicity in chronic lymphocytic leukemia. *MAbs* 2010;2(1):35-41.
116. Teeling JL, French RR, Cragg MS, van den Brakel J, Pluyter M, Huang H, et al. Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. *Blood* 2004;104(6):1793-800.
117. Patz M, Isaeva P, Forcob N, Muller B, Frenzel LP, Wendtner CM, et al. Comparison of the in vitro effects of the anti-CD20 antibodies rituximab and GA101 on chronic lymphocytic leukaemia cells. *Br J Haematol*. 2011;152(3):295-306.
118. Bologna L, Gotti E, Manganini M, Rambaldi A, Interimesoli T, Introna M, et al. Mechanism of action of type II, glycoengineered, anti-CD20 monoclonal antibody GA101 in B-chronic lymphocytic leukemia whole blood assays in comparison with rituximab and alemtuzumab. *J Immunol*. 2011;186(6):3762-9.
119. Lei MM, Sorial MN, Lou U, Yu M, Medrano A, Ford J, et al. Real-world evidence of obinutuzumab and venetoclax in previously treated patients with chronic lymphocytic leukemia or small lymphocytic lymphoma. *Leuk Lymphoma* 2024;65(5):653-9.
120. Sachdeva M, Dhingra S. Obinutuzumab: A FDA approved monoclonal antibody in the treatment of untreated chronic lymphocytic leukemia. *Int J Appl Basic Med Res* 2015;5(1):54-7.
121. Huber H, Tausch E, Schneider C, Edenhofer S, von Tresckow J, Robrecht S, et al. Final analysis of the CLL2-GIVE trial: obinutuzumab, ibrutinib, and venetoclax for untreated CLL with del(17p)/TP53mut. *Blood* 2023;142(11):961-72.
122. Golay J, Manganini M, Rambaldi A, Introna M. Effect of alemtuzumab on neoplastic B cells. *Haematologica*. 2004;89(12):1476-83.
123. Rossmann ED, Lundin J, Lenkei R, Mellstedt H, Osterborg A. Variability in B-cell antigen expression: implications for the treatment of B-cell lymphomas and leukemias with monoclonal antibodies. *Hematol J* 2001; 2(5):300-6.
124. Ginaldi L, De Martinis M, Matutes E, Farahat N, Morilla R, Dyer MJ, et al. Levels of expression of CD52 in normal and leukemic B and T cells: correlation with in vivo therapeutic responses to Campath-1H. *Leuk Res* 1998;22(2):185-91.
125. Mone AP, Cheney C, Banks AL, Tridandapani S, Meh-ter N, Guster S, et al. Alemtuzumab induces caspase-independent cell death in human chronic lymphocytic leukemia cells through a lipid raft-dependent mechanism. *Leukemia* 2006;20(2):272-9.
126. Stilgenbauer S, Zenz T, Winkler D, Buhler A, Schlenk RF, Groner S, et al. Subcutaneous alemtuzumab in fludarabine-refractory chronic lymphocytic leukemia: clinical results and prognostic marker analyses from the CLL2H study of the German Chronic Lymphocytic Leukemia Study Group. *J Clin Oncol* 2009;27(24):3994-4001.
127. Lozanski G, Heerema NA, Flinn IW, Smith L, Harbison J, Webb J, et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. *Blood*. 2004;103(9):3278-81.
128. Tedder TF, Inaoki M, Sato S. The CD19-CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. *Immunity* 1997;6 (2):107-18.
129. Gladstone D, Andre M, Zaucha J, Assouline S, Bellam N, Cascavilla N, et al. Results of a phase 2 study of MEDI-551 and bendamustine vs rituximab and bendamustine in relapsed or refractory chronic lymphocytic leukemia. *Am Soc Hematology* 2014.
130. Woyach JA, Awan F, Flinn IW, Berdeja JG, Wiley E, Mansoor S, et al. A phase 1 trial of the Fc-engineered CD19 antibody XmAb5574 (MOR00208) demonstrates safety and preliminary efficacy in relapsed CLL. *Blood* 2014;124(24):3553-60.
131. Zhao X, Lapalombella R, Joshi T, Cheney C, Gowda A, Hayden-Ledbetter MS, et al. Targeting CD37-positive lymphoid malignancies with a novel engineered small modular immunopharmaceutical. *Blood* 2007;110(7): 2569-77.
132. Byrd JC, Pagel JM, Awan FT, Forero A, Flinn IW, Deauna-Limayo DP, et al. A phase 1 study evaluating the safety and tolerability of oltretuzumab, an anti-CD37 mono-specific ADAPTIR therapeutic protein in chronic lymphocytic leukemia. *Blood* 2014;123(9):1302-8.
133. Hulkkonen J, Vilpo L, Hurme M, Vilpo J. Surface antigen expression in chronic lymphocytic leukemia: clustering analysis, interrelationships and effects of chromosomal abnormalities. *Leukemia* 2002;16(2):178-85.
134. Luqman M, Klabunde S, Lin K, Georgakis GV, Cherukuri A, Holash J, et al. The antileukemia activity of a human anti-CD40 antagonist antibody, HCD122, on human chronic lymphocytic leukemia cells. *Blood* 2008; 112(3):711-20.
135. Byrd JC, Kipps TJ, Flinn IW, Cooper M, Odenike O, Bendiske J, et al. Phase I study of the anti-CD40

- humanized monoclonal antibody lcatumumab (HCD-122) in relapsed chronic lymphocytic leukemia. *Leuk Lymphoma* 2012;53(11):2136-42.
136. Lapalombella R, Gowda A, Joshi T, Mehter N, Cheney C, Lehman A, et al. The humanized CD40 antibody SGN-40 demonstrates pre-clinical activity that is enhanced by lenalidomide in chronic lymphocytic leukaemia. *Br J Haematol* 2009;144(6):848-55.
 137. Furman RR, Forero-Torres A, Shustov A, Drachman JG. A phase I study of dacetuzumab (SGN-40, a humanized anti-CD40 monoclonal antibody) in patients with chronic lymphocytic leukemia. *Leuk Lymphoma* 2010; 51(2):228-35.
 138. Lewis JD, Reilly BD, Bright RK. Tumor-associated antigens: from discovery to immunity. *Int Rev Immunol* 2003;22(2):81-112.
 139. Nagorsen D, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U. Natural T cell immunity against cancer. *Clin Cancer Res* 2003;9(12):4296-303.
 140. Babiak A, Steinhauser M, Gotz M, Herbst C, Dohner H, Greiner J. Frequent T cell responses against immunogenic targets in lung cancer patients for targeted immunotherapy. *Oncol Rep* 2014;31(1):384-90.
 141. Liu C, Richard K, Wiggins M, Zhu X, Conrad DH, Song W. CD23 can negatively regulate B-cell receptor signaling. *Sci Rep* 2016;6:25629.
 142. Fournier S, Delespesse G, Rubio M, Biron G, Sarfati M. CD23 antigen regulation and signaling in chronic lymphocytic leukemia. *J Clin Invest* 1992;89(4):1312-21.
 143. Sarfati M, Chevret S, Chastang C, Biron G, Stryckmans P, Delespesse G, et al. Prognostic importance of serum soluble CD23 level in chronic lymphocytic leukemia. *Blood* 1996;88(11):4259-64.
 144. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J Exp Med* 2001;194(11):1625-38.
 145. Bund D, Mayr C, Kofler DM, Hallek M, Wendtner CM. CD23 is recognized as tumor-associated antigen (TAA) in B-CLL by CD8+ autologous T lymphocytes. *Exp Hematol* 2007;35(6):920-30.
 146. Byrd JC, O'Brien S, Flinn IW, Kipps TJ, Weiss M, Rai K, et al. Phase 1 study of lumiliximab with detailed pharmacokinetic and pharmacodynamic measurements in patients with relapsed or refractory chronic lymphocytic leukemia. *Clin Cancer Res* 2007;13(15 Pt 1):4448-55.
 147. Byrd JC, Kipps TJ, Flinn IW, Castro J, Lin TS, Wierda W, et al. Phase 1/2 study of lumiliximab combined with fludarabine, cyclophosphamide, and rituximab in patients with relapsed or refractory chronic lymphocytic leukemia. *Blood* 2010;115(3):489-95.
 148. Urso L, Calabrese F, Favaretto A, Conte P, Pasello G. Critical review about MDM2 in cancer: Possible role in malignant mesothelioma and implications for treatment. *Crit Rev Oncol Hematol* 2016;97:220-30.
 149. Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer* 2013; 13(2):83-96.
 150. Seliger B, Papadilleris S, Vogel D, Hess G, Brendel C, Storkel S, et al. Analysis of the p53 and MDM-2 gene in acute myeloid leukemia. *Eur J Haematol* 1996;57(3): 230-40.
 151. Finnegan MC, Goepel JR, Royds J, Hancock BW, Goyns MH. Elevated levels of MDM-2 and p53 expression are associated with high grade non-Hodgkin's lymphomas. *Cancer Lett* 1994;86(2):215-21.
 152. Watanabe T, Hotta T, Ichikawa A, Kinoshita T, Nagai H, Uchida T, et al. The MDM2 oncogene over-expression in chronic lymphocytic leukemia and low-grade lymphoma of B-cell origin. *Blood* 1994;84(9): 3158-65.
 153. Mayr C, Bund D, Schlee M, Bamberger M, Kofler DM, Hallek M, et al. MDM2 is recognized as a tumor-associated antigen in chronic lymphocytic leukemia by CD8+ autologous T lymphocytes. *Exp Hematol* 2006;34 (1):44-53.
 154. Wang H, Yu D, Agrawal S, Zhang R. Experimental therapy of human prostate cancer by inhibiting MDM2 expression with novel mixed-backbone antisense oligonucleotides: in vitro and in vivo activities and mechanisms. *Prostate* 2003;54(3):194-205.
 155. Bixby D, Kujawski L, Wang S, Malek SN. The pre-clinical development of MDM2 inhibitors in chronic lymphocytic leukemia uncovers a central role for p53 status in sensitivity to MDM2 inhibitor-mediated apoptosis. *Cell Cycle* 2008;7(8):971-9.
 156. Andreeff M, Kelly KR, Yee K, Assouline S, Strair R, Popplewell L, et al. Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. *Clin Cancer Res* 2016;22(4):868-76.
 157. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997;3(8):917-21.
 158. Jaiswal PK, Goel A, Mittal R. Survivin: A molecular biomarker in cancer. *Indian J Med Res* 2015;141(4):389.
 159. Lv Y-G, Yu F, Yao Q, Chen J-H, Wang L. The role of survivin in diagnosis, prognosis and treatment of breast cancer. *J Thorac Dis* 2010;2(2):100.
 160. Granziero L, Ghia P, Circosta P, Gottardi D, Strola G, Geuna M, et al. Survivin is expressed on CD40 stimulation and interfaces proliferation and apoptosis in B-cell chronic lymphocytic leukemia. *Blood* 2001;97(9):2777-83.
 161. Andersen MH, Pedersen LØ, Becker JC, thor Straten P. Identification of a cytotoxic T lymphocyte response to the apoptosis inhibitor protein survivin in cancer patients. *Cancer Res* 2001;61(3):869-72.
 162. Schmitz M, Diestelkoetter P, Weigle B, Schmachtenberg F, Stevanovic S, Ockert D, et al. Generation of survivin-specific CD8+ T effector cells by dendritic cells pulsed with protein or selected peptides. *Cancer Res* 2000;60(17):4845-9.

163. Schmidt SM, Schag K, Müller MR, Weck MM, Appel S, Kanz L, et al. Survivin is a shared tumor-associated antigen expressed in a broad variety of malignancies and recognized by specific cytotoxic T cells. *Blood* 2003; 102(2):571-6.
164. Reker S, Becker JC, Svane IM, Ralfkiaer E, Straten Pt, Andersen MH. HLA-B35-restricted immune responses against survivin in cancer patients. *Int J Cancer* 2004; 108(6):937-41.
165. Miletic AV, Jellusova J, Cato MH, Lee CR, Baracho GV, Conway EM, et al. Essential role for Survivin in the proliferative expansion of progenitor and mature B cells. *J Immunol* 2016;196(5):2195-204.
166. Purroy N, Abrisqueta P, Carabia J, Carpio C, Calpe E, Palacio C, et al. Targeting the proliferative and chemoresistant compartment in chronic lymphocytic leukemia by inhibiting survivin protein. *Leukemia* 2014;28(10):1993-2004.
167. Tibes R, McDonagh K, Lekakis L, Bogenberger J, Kim S, Frazer N, et al. Phase I study of the novel Cdc2/CDK1 and AKT inhibitor terameprocol in patients with advanced leukemias. *Invest New Drugs* 2015;33(2):389-96.
168. Rostamzadeh D, Kazemi T, Amirghofran Z, Shabani M. Update on Fc receptor-like (FCRL) family: new immunoregulatory players in health and diseases. *Expert Opin Ther Targets* 2018;22(6):487-502.
169. Rostamzadeh D, Dabbaghmanesh M, Shabani M, Hosseini A, Amirghofran Z. Expression profile of human Fc receptor-like 1, 2, and 4 molecules in peripheral blood mononuclear cells of patients with Hashimoto's thyroiditis and Graves' disease. *Horm Metab Res* 2015;47(09):693-8.
170. Li FJ, Ding S, Pan J, Shakhmatov MA, Kashentseva E, Wu J, et al. FCRL2 expression predicts IGHV mutation status and clinical progression in chronic lymphocytic leukemia. *Blood* 2008;112(1):179-87.
171. Benedetti D, Perini C, Tissino E, Dal Bo M, Bulian P, Bomben R, et al. The B-cell receptor signaling inhibitor molecules CD305 and CD307b are markers of favorable prognosis in chronic lymphocytic leukemia with both mutated and unmutated IGHV gene status. *Blood* 2016 Dec 2;128(22):4358.
172. Oldberg A, Antonsson P, Lindblom K, Heinegård D. A collagen-binding 59-kD protein (fibromodulin) is structurally related to the small interstitial proteoglycans PG-S1 and PG-S2 (decorin). *EMBO J* 1989;8(9):2601-4.
173. Hedbom E, Heinegård D. Interaction of a 59-kDa connective tissue matrix protein with collagen I and collagen II. *J Biol Chem* 1989;264(12):6898-905.
174. Jelinek DF, Tschumper RC, Stolovitzky GA, Iturria SJ, Tu Y, Lepre J, et al. Identification of a Global Gene Expression Signature of B-Chronic Lymphocytic Leukemia1 1 Mayo Comprehensive Cancer Center, National Cancer Institute CA91542 (awarded to NE Kay), and generous philanthropic support provided by Edson Spencer. *Mol Cancer Res* 2003;1(5):346-61.
175. Vallat L, Magdelénat H, Merle-Béral Hln, Masdehors P, Potocki de Montalk G, Davi F, et al. The resistance of B-CLL cells to DNA damage-induced apoptosis defined by DNA microarrays. *Blood* 2003;101(11):4598-606.
176. Mayr C, Bund D, Schlee M, Moosmann A, Kofler DM, Hallek M, et al. Fibromodulin as a novel tumor-associated antigen (TAA) in chronic lymphocytic leukemia (CLL), which allows expansion of specific CD8+ autologous T lymphocytes. *Blood* 2005;105(4):1566-73.
177. Mikaelsson E, Jeddi-Tehrani M, Osterborg A, Shokri F, Mellstedt H, Rabbani H. Fibromodulin—a novel tumor associated antigen exclusively expressed in tumor B-cells from patients with chronic lymphocytic leukemia and mantle cell lymphoma. *Leuk Lymphoma* 2003; 44(suppl 2):21.
178. Farahi L, Ghaemimanesh F, Milani S, Razavi SM, Hadavi R, Bayat AA, et al. GPI-anchored fibromodulin as a novel target in chronic lymphocytic leukemia: diagnostic and therapeutic implications. *Iran J Immunol* 2019;16(2):127-41.
179. Yip K, Reed J. Bcl-2 family proteins and cancer. *Oncogene* 2008;27(50):6398-406.
180. Fecker LF, Geilen CC, Tchernev G, Trefzer U, Assaf C, Kurbanov BM, et al. Loss of proapoptotic Bcl-2-related multidomain proteins in primary melanomas is associated with poor prognosis. *J Invest Dermatol* 2006;126(6):1366-71.
181. Agrawal SG, Liu F-T, Wiseman C, Shirali S, Liu H, Lillington D, et al. Increased proteasomal degradation of Bax is a common feature of poor prognosis chronic lymphocytic leukemia. *Blood* 2008;111(5):2790-6.
182. Nunes CT, Miners KL, Dolton G, Pepper C, Fegan C, Mason MD, et al. A novel tumor antigen derived from enhanced degradation of bax protein in human cancers. *Cancer research*. 2011;71(16):5435-44.
183. Del Principe MI, Dal Bo M, Bittolo T, Buccisano F, Rossi FM, Zucchetto A, et al. Clinical significance of bax/bcl-2 ratio in chronic lymphocytic leukemia. *Haematologica* 2016;101(1):77.
184. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013;19(2):202-8.
185. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V Gene Mutation Status and CD38 Expression As Novel Prognostic Indicators in Chronic Lymphocytic Leukemia: Presented in part at the 40th Annual Meeting of The American Society of Hematology, held in Miami Beach, FL, December 4-8, 1998. *Blood* 1999;94(6):1840-7.
186. Del Poeta G, Del Principe MI, Maurillo L, Rossi FM, Buccisano F, Ammatuna E, et al. Spontaneous apoptosis and proliferation detected by BCL-2 and CD71 proteins are important progression indicators within ZAP-70 negative chronic lymphocytic leukemia. *Leuk Lymphoma* 2010;51(1):95-106.
187. Gattei V, Bulian P, Del Principe MI, Zucchetto A, Maurillo L, Buccisano F, et al. Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. *Blood* 2008;111(2):865-73.

188. Dürig J, Dührsen U, Klein-Hitpass L, Worm J, Hansen JR, Ørum H, et al. The novel antisense Bcl-2 inhibitor SPC2996 causes rapid leukemic cell clearance and immune activation in chronic lymphocytic leukemia. *Leukemia* 2011;25(4):638-47.
189. Tilly H, Coiffier B, Michallet A, Radford J, Geisler C, Gadeberg O, et al. Phase I/II study of SPC2996, an RNA antagonist of Bcl-2, in patients with advanced chronic lymphocytic leukemia (CLL). *Journal of Clinical Oncology*. 2007;25(18_suppl):7036.
190. Furstenau M, Kater AP, Robrecht S, von Tresckow J, Zhang C, Gregor M, et al. First-line venetoclax combinations versus chemoimmunotherapy in fit patients with chronic lymphocytic leukaemia (GAIA/CLL13): 4-year follow-up from a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol* 2024;25(6):744-59.
191. de la Fuente MA, Tovar V, Villamor N, Zapater N, Pizcueta P, Campo E, et al. Molecular characterization and expression of a novel human leukocyte cell-surface marker homologous to mouse Ly-9. *Blood* 2001;97(11):3513-20.
192. Bund D, Mayr C, Kofler DM, Hallek M, Wendtner C-M. Human Ly9 (CD229) as novel tumor-associated antigen (TAA) in chronic lymphocytic leukemia (B-CLL) recognized by autologous CD8⁺ T cells. *Exp Hematol* 2006;34(7):860-9.
193. Wallar BJ, Alberts AS. The formins: active scaffolds that remodel the cytoskeleton. *Trends Cell Biol* 2003; 13(8):435-46.
194. Favaro PMB, de Souza Medina S, Traina Fo, Bassères DS, Costa FF, Saad STO. Human leukocyte formin: a novel protein expressed in lymphoid malignancies and associated with Akt. *Biochem Biophys Res Commun* 2003;311(2):365-71.
195. Krackhardt AM, Witzens M, Harig S, Hodi FS, Zauls AJ, Chessia M, et al. Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. *Blood* 2002;100(6):2123-31.
196. Giannopoulos K, Li L, Bojarska-Junak A, Rolinski J, Dmoszynska A, Hus I, et al. Expression of RHAMM/CD168 and other tumor-associated antigens in patients with B-cell chronic lymphocytic leukemia. *Int J Oncol* 2006;29(1):95-103.
197. Hus I, Roliński J, Tabarkiewicz J, Wojas K, Bojarska-Junak A, Greiner J, et al. Allogeneic dendritic cells pulsed with tumor lysates or apoptotic bodies as immunotherapy for patients with early-stage B-cell chronic lymphocytic leukemia. *Leukemia* 2005;19(9):1621-7.
198. Greiner J, Li L, Ringhoffer M, Barth TF, Giannopoulos K, Guillaume P, et al. Identification and characterization of epitopes of the receptor for hyaluronic acid-mediated motility (RHAMM/CD168) recognized by CD8⁺ T cells of HLA-A2-positive patients with acute myeloid leukemia. *Blood* 2005;106(3):938-45.
199. Nugent CI, Lundblad V. The telomerase reverse transcriptase: components and regulation. *Genes Dev* 1998; 12(8):1073-85.
200. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. *Genome Med* 2016;8(1): 69.
201. Trentin L, Ballon G, Ometto L, Perin A, Basso U, Chieco-Bianchi L, et al. Telomerase activity in chronic lymphoproliferative disorders of B-cell lineage. *Br J Haematol* 1999;106(3):662-8.
202. Kokhaei P, Palma M, Hansson L, Österborg A, Melstedt H, Choudhury A. Telomerase (hTERT 611–626) serves as a tumor antigen in B-cell chronic lymphocytic leukemia and generates spontaneously antileukemic, cytotoxic T cells. *Exp Hematol* 2007;35(2):297-304.
203. Rohrer J, Barsoum A, Coggin Jr J. The development of a new universal tumor rejection antigen expressed on human and rodent cancers for vaccination, prevention of cancer, and anti-tumor therapy. *Mod Asp Immunobiol* 2001;5:191-5.
204. Barsoum A, Rohrer J, Coggin J. 37kDa oncofetal antigen is an autoimmunogenic homologue of the 37kDa laminin receptor precursor. *Cellular and Molecular Biology Letters* 2000;2(05).
205. Siegel S, Wagner A, Kabelitz D, Marget M, Coggin Jr J, Barsoum A, et al. Induction of cytotoxic T-cell responses against the oncofetal antigen-immature laminin receptor for the treatment of hematologic malignancies. *Blood* 2003;102(13):4416-23.
206. Su Z, Dannull J, Heiser A, Yancey D, Pruitt S, Madden J, et al. Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res* 2003;63(9): 2127-33.
207. Mandal A, Klotz KL, Shetty J, Jayes FL, Wolkowicz MJ, Bolling LC, et al. SLLP1, a unique, intra-acrosomal, non-bacteriolytic, c lysozyme-like protein of human spermatozoa. *Biol Reprod* 2003;68(5):1525-37.
208. Wang Z, Zhang Y, Mandal A, Zhang J, Giles FJ, Herr JC, et al. The spermatozoa protein, SLLP1, is a novel cancer-testis antigen in hematologic malignancies. *Clin Cancer Res* 2004;10(19):6544-50.
209. Said JW, Hoyer KK, French SW, Rosenfelt L, Garcia-Lloret M, Koh PJ, et al. TCL1 oncogene expression in B cell subsets from lymphoid hyperplasia and distinct classes of B cell lymphoma. *Lab Invest* 2001;81(4):555-64.
210. Narducci MG, Pescarmona E, Lazzeri C, Signoretti S, Lavinia AM, Remotti D, et al. Regulation of TCL1 expression in B-and T-cell lymphomas and reactive lymphoid tissues. *Cancer Res* 2000;60(8):2095-100.
211. Weng J, Rawal S, Chu F, Park HJ, Sharma R, Delgado DA, et al. TCL1: a shared tumor-associated antigen for immunotherapy against B-cell lymphomas. *Blood* 2012; 120(8):1613-23.
212. Sahasrabuddhe AA, Elenitoba-Johnson KS. TCL1A expression promotes aggressive biology in CLL. *Blood* 2023;141(12):1371-3.
213. Heid HW, Moll R, Schwetlick I, Rackwitz H-R, Keenan TW. Adipophilin is a specific marker of lipid accumulation in diverse cell types and diseases. *Cell Tissue Res* 1998;294(2):309-21.

214. Buechler C, Ritter M, Duong CQ, Orso E, Kapinsky M, Schmitz G. Adipophilin is a sensitive marker for lipid loading in human blood monocytes. *Biochim Biophys Acta* 2001;1532(1-2):97-104.
215. Rae FK, Stephenson SA, Nicol DL, Clements JA. Novel association of a diverse range of genes with renal cell carcinoma as identified by differential display. *Int J Cancer* 2000;88(5):726-32.
216. Schmidt SM, Schag K, Müller MR, Weinschenk T, Appel S, Schoor O, et al. Induction of adipophilin-specific cytotoxic T lymphocytes using a novel HLA-A2-binding peptide that mediates tumor cell lysis. *Cancer Res* 2004;64(3):1164-70.
217. Bolkun L, Tynecka M, Wasiluk T, Piszcz J, Starosz A, Grubczak K, et al. A Proliferation-Inducing Ligand and B-Cell Activating Factor Are Upregulated in Patients with Essential Thrombocythemia. *J Clin Med* 2022;11(16):4663.
218. O'Donnell A, Pepper C, Mitchell S, Pepper A. NF- κ B and the CLL microenvironment. *Front Oncol* 2023;13:1169397.
219. Ullah MA, Mackay F. The BAFF-APRIL System in Cancer. *Cancers (Basel)* 2023;15(6):1791.
220. Kern C, Cornuel J-F, Billard C, Tang R, Rouillard D, Stenou V, et al. Involvement of BAFF and APRIL in the resistance to apoptosis of B-CLL through an autocrine pathway. *Blood* 2004;103(2):679-88.
221. Hahne M, Kataoka T, Schröter M, Hofmann K, Irmeler M, Bodmer J-L, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. *J Exp Med* 1998;188(6):1185-90.
222. He B, Chadburn A, Jou E, Schattner EJ, Knowles DM, Cerutti A. Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. *J Immunol* 2004;172(5):3268-79.
223. Endo T, Nishio M, Enzler T, Cottam HB, Fukuda T, James DF, et al. BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF- κ B pathway. *Blood* 2007;109(2):703-10.
224. Bojarska-Junak A, Hus I, Chocholska S, Wąsik-Szczepek E, Sieklucka M, Dmoszyńska A, et al. BAFF and APRIL expression in B-cell chronic lymphocytic leukemia: correlation with biological and clinical features. *Leuk Res* 2009;33(10):1319-27.
225. Haiat S, Billard C, Quiney C, Ajchenbaum-Cymbalista F, Kolb JP. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. *Immunology* 2006;118(3):281-92.
226. Tandler C, Schmidt M, Heitmann JS, Hierold J, Schmidt J, Schneider P, et al. Neutralization of B-cell activating factor (BAFF) by belimumab reinforces small molecule inhibitor treatment in chronic lymphocytic leukemia. *Cancers (Basel)* 2020;12(10):2725.
227. Luo Y, Qie Y, Gadd ME, Manna A, To T, Li S, et al. Translational development of BAFF-R-specific chimeric antigen receptor T-cell therapy targeting B-cell lymphoid malignancies. *American Society of Clinical Oncology* 2023.
228. Chen L, Mao W, Ren C, Li J, Zhang J. Comprehensive Insights that Targeting PIM for Cancer Therapy: Prospects and Obstacles. *J Med Chem* 2024;67(1):38-64.
229. Decker S, Finter J, Forde AJ, Kissel S, Schwaller J, Mack TS, et al. PIM kinases are essential for chronic lymphocytic leukemia cell survival (PIM2/3) and CXCR4-mediated microenvironmental interactions (PIM1). *Mol Cancer Ther* 2014;13(5):1231-45.
230. Kowalewski DJ, Schuster H, Berlin C, Kanz L, Salih HR, Rammensee H-G, et al. Identification of Novel Tumor-Associated Antigens for Chronic Lymphocytic Leukemia (CLL) Based On HLA Ligandome Analysis—New Targets for Peptide Based Immunotherapy. *Blood* 2012;120(21):4119.
231. Christensen DJ, Chen Y, Oddo J, Matta KM, Neil J, Davis ED, et al. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin lymphoma: a predictor of aggressive disease and a new treatment target. *Blood* 2011;118(15):4150-8.
232. Nadeu F, Royo R, Clot G, Duran-Ferrer M, Navarro A, Martín S, et al. IGLV3-21R110 identifies an aggressive biological subtype of chronic lymphocytic leukemia with intermediate epigenetics. *Blood* 2021;137(21):2935-46.
233. Märkl F, Schultheiß C, Ali M, Chen S-S, Zintchenko M, Egli L, et al. Mutation-specific CAR T cells as precision therapy for IGLV3-21R110 expressing high-risk chronic lymphocytic leukemia. *Nat Commun* 2024;15(1):993.
234. Zhao Y, Su H, Shen X, Du J, Zhang X, Zhao Y. The immunological function of CD52 and its targeting in organ transplantation. *Inflamm Res* 2017;66(7):571-8.
235. Vojdeman FJ, Herman SEM, Kirkby N, Wiestner A, van T' Veer MB, Tjonnfjord GE, et al. Soluble CD52 is an indicator of disease activity in chronic lymphocytic leukemia. *Leuk Lymphoma* 2017;58(10):2356-62.
236. Czuczman MS, Kahanic S, Forero A, Davis G, Munteanu M, Van Den Neste E, et al. Results of a phase II study of bendamustine and ofatumumab in untreated indolent B cell non-Hodgkin's lymphoma. *Ann Hematol* 2015;94(4):633-41.
237. Purroy N, Abrisqueta P, Carabia J, Carpio C, Calpe E, Palacio C, et al. Targeting the proliferative and chemoresistant compartment in chronic lymphocytic leukemia by inhibiting survivin protein. *Leukemia* 2014;28(10):1993-2004.
238. Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013;19(2):202-8.
239. Del Principe MI, Dal Bo M, Bittolo T, Buccisano F, Rossi FM, Zucchetto A, et al. Clinical significance of bax/bcl-2 ratio in chronic lymphocytic leukemia. *Haematologica* 2016;101(1):77-85.