Chronic lymphocytic leukaemia

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Abstract | Chronic lymphocytic leukaemia (CLL) is a malignancy of CD5$^+$ B cells that is characterized by the accumulation of small, mature-appearing lymphocytes in the blood, marrow and lymphoid tissues. Signalling via surface immunoglobulin, which constitutes the major part of the B cell receptor, and several genetic alterations play a part in CLL pathogenesis, in addition to interactions between CLL cells and other cell types, such as stromal cells, T cells and nurse-like cells in the lymph nodes. The clinical progression of CLL is heterogeneous and ranges from patients who require treatment soon after diagnosis to others who do not require therapy for many years, if at all. Several factors, including the immunoglobulin heavy-chain variable region gene (*IGHV*) mutational status, genomic changes, patient age and the presence of comorbidities, should be considered when defining the optimal management strategies, which include chemotherapy, chemoimmunotherapy and/or drugs targeting B cell receptor signalling or inhibitors of apoptosis, such as BCL-2. Research on the biology of CLL has profoundly enhanced our ability to identify patients who are at higher risk for disease progression and our capacity to treat patients with drugs that selectively target distinctive phenotypic or physiological features of CLL. How these and other advances have shaped our current understanding and treatment of patients with CLL is the subject of this Primer.

Chronic lymphocytic leukaemia (CLL) is a malignancy of CD5$^+$ B cells that is characterized by the accumulation of small, mature-appearing neoplastic lymphocytes in the blood, marrow and secondary lymphoid tissues, resulting in lymphocytosis, leukaemia cell infiltration of the marrow, lymphadenopathy and splenomegaly. Genetic factors contribute to the development of CLL; although CLL is the most common adult leukaemia in western countries, it is less common in Asia and relatively rare in Japan and Korea, even among Japanese people who immigrate to western counties.

CLL can be divided into two main subsets, which differ in their clinical behaviour. These subsets are distinguished by whether CLL cells express an unmutated or mutated immunoglobulin heavy-chain variable region gene (*IGHV*), reflecting the stage of normal B cell differentiation from which they originate[1,2]. CLL cells that express an unmutated *IGHV* originate from a B cell that has not undergone differentiation in germinal centres, which are the sites in the lymph nodes where B cells experience somatic hypermutation in their immunoglobulin variable region genes and selection during an immune response. Patients with CLL cells that express an unmutated *IGHV* typically have more-aggressive disease than patients with CLL cells that express a mutated *IGHV*. CLL cells with mutated *IGHV* arise from a post-germinal centre B cell that expresses immunoglobulin that has undergone somatic hypermutation and, in some cases, also immunoglobulin isotype switching (FIG. 1), similar to what occurs in normal B cells during an immune response to antigen. It should be emphasized that the high level of somatic mutations that arise in *IGHV* in the germinal centre are a natural part of affinity maturation of antibodies and, unlike mutations in other genes, are not pathological. The tumours are simply reflecting the stage of maturation of the parental B cell. In addition, some CLL cells have been described that are similar to unmutated *IGHV* CLL, but originate from B cells with limited somatic mutation, such as CLL with immunoglobulin heavy chains encoded by mutated *IGHV3-21* and immunoglobulin light chains encoded by unmutated *IGLV3-21* (REFS 3,4).

The repertoire of immunoglobin molecules produced by the CLL cells of all patients is considerably more limited than the repertoire of immunoglobulin molecules that can be made by the B cells of any one person[5,6], reflecting the biased use in CLL of certain *IGHV* genes that have restricted somatic mutation and limited junctional and heavy–light chain combinatorial diversity. In as many as one-third of patients, the CLL cells express immunoglobulin 'stereotypes', which are stretches of primary structure in the variable region that can also be identified in the immunoglobulins produced by the CLL cells of other patients[7]. The restricted immunoglobulin repertoire in CLL is underscored by the

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finding that ~1 in 75 patients have CLL cells that express immunoglobulin molecules that are virtually identical[8]. The limited immunoglobulin diversity provides compelling evidence that CLL B cells are selected based on the binding activity of their expressed surface immunoglobulin, suggesting that B cell receptor (BCR) signalling plays a crucial part in CLL pathogenesis.

Several large genetic studies have revealed numerous genetic alterations in CLL, including single-nucleotide polymorphisms (SNPs), chromosomal alterations and alterations in non-coding RNA, such as microRNA (miRNA), some of which can be used to determine prognosis and to guide management strategies. Interactions between CLL cells and their microenvironment, including interactions with other cell types, such as T cells, nurse-like cells and stromal cells, can induce B cell proliferation and contribute to disease.

The distinctive cytogenesis of CLL contrasts with most other B cell malignancies, such as follicular lymphoma, which is a germinal centre neoplasm, or myeloma (a post-germinal centre neoplasm)[9,10]. However, diffuse large B cell lymphoma (DLBCL) resembles CLL in consisting of two main subtypes: a germinal centre B-type DLBCL, which is derived from germinal centre light zone B cells, and an activated B cell (or non-germinal centre) DLBCL, which is derived from a later stage of germinal centre differentiation (before plasmablastic differentiation)[10]. As in CLL, these two subtypes of DLBCL generally have distinctive responses to therapy and clinical outcomes.

In this Primer, we describe the molecular pathogenesis of CLL and discuss the current advances that are shaping our understanding and treatment of patients with this disease.

Epidemiology

CLL is estimated to account for ~19,000 of all newly detected cancers in the United States in 2016 (REF. 11). The average incidence of CLL varies between individuals in different geographical regions and ranges from <0.01% of individuals in eastern Asia to ~0.06% of individuals in Europe and the United States. The risk of developing CLL is about two-times higher for men than for women and increases with age; the median age at diagnosis ranges from 70 to 72 years[11–14].

The US National Cancer Institute Surveillance, Epidemiology, and End Results programme has estimated the number of new cases of CLL to be 6.3 per 100,000 men and 3.3 per 100,000 women. The incidence in white populations is estimated to be 6.8 per 100,000 men and 3.5 per 100,000 women, 4.9 per 100,000 men and 2.4 per 100,000 women in African Americans, 2.7 per 100,000 men and 1.6 per 100,000 women in Hispanic Americans, 1.7 per 100,000 men and 1.3 per 100,000 women in Indigenous Americans, and 1.7 per 100,000 men and 0.3 per 100,000 women in people of Asian or Pacific Island descent in the United States[13].

Hereditary factors

Genetic factors contribute to disease susceptibility; among patients who are registered in the CLL Research Consortium, 9% of patients have a relative with CLL. In addition, first-degree relatives of patients with CLL have an 8.5-fold increased risk of developing this disease[15], and the concordance of CLL is higher among monozygotic twins than among dizygotic twins[16]. Genome-wide association studies have identified SNPs in nearly 30 loci that are associated with familial CLL, demonstrating that common genetic variation contributes to heritable risk[17–22] (see Supplementary information S1 (table)).

The altered expression of genes that are located in or near CLL-associated SNPs might contribute to disease development. For example, a SNP in *IRF4* is associated with low expression of interferon regulatory factor 4; mice that are deficient in this protein can develop CLL[23], partly owing to hyperactivation of Notch signalling[24]. SNPs in *LEF1* might be associated with high expression of lymphoid enhancer-binding factor 1, which is a downstream effector of WNT signalling; normally, *LEF1* is expressed at high levels in CLL and, among other functions, can enhance resistance to cell death[25]. In addition, CLL-associated SNPs have been found in *BCL2*, which encodes an anti-apoptotic protein that is expressed at high levels in CLL, and in *PMAIP1*, which encodes a pro-apoptotic protein. A SNP that is associated with reduced expression of *mir-15a* and *mir-16-1* is associated with familial CLL[26,27]. Because *mir-15a* and *mir-16-1* repress the expression of *BCL2* and *ZAP70* (REFS 26,27), reduced expression of these miRNAs allows for the increased expression of these genes, which encode proteins that respectively confer increased resistance to cell death[28] or enhanced BCR signalling[29]. Similarly, New Zealand black mice have an allele at the *mir-16-1* locus with shared synteny to human 13q14, which is associated with low expression of *mir-16-1*; this allele is linked to the genetic propensity of these mice to develop a B cell lymphoproliferative disease that resembles CLL[30]. Finally, a CLL-linked SNP in *TERT* is associated with a long leukocyte telomere length[31], conceivably contributing to the high rates of telomeric sister chromatid exchange observed in CLL cells that could slow telomere erosion leading to cellular senescence[32].

Environmental factors

The US Department of Veterans Affairs has accepted that exposure to Agent Orange is a risk factor for CLL, which has enabled veterans with CLL to claim benefits if they were previously exposed to Agent Orange while in military service[33]. In addition, evidence suggests that exposure to insecticides might be a risk factor for CLL[34]. By contrast, little evidence indicates that ionizing radiation can increase the risk of CLL[35,36]. Furthermore, there is scant evidence that viral infections are risk factors, and epidemiological studies have not found evidence that blood transfusions can transmit CLL[37]. No evidence suggests that dietary factors or lifestyle factors increase the risk of CLL.

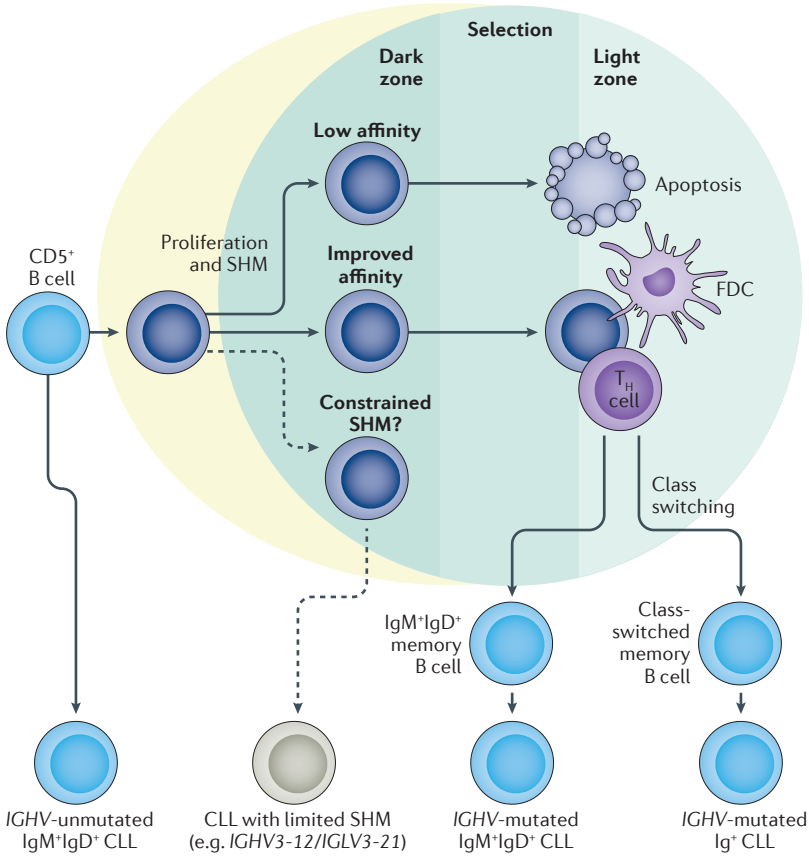


Figure 1 | **Cellular origins of CLL cells.** Normal naive B cells that have undergone successful V(D)J recombination and express functional B cell receptors that are capable of binding to antigen interact with CD4⁺ T cells and accessory cells, which aggregate to form follicles that become germinal centres. Germinal cells each have a dark zone, comprising rapidly dividing B cells, and a light zone, comprising B cells mixed with follicular dendritic cells (FDCs), macrophages and helper T cells (T_H cells). The B cells enter the dark zone of the germinal centre where they experience rapid proliferation and somatic hypermutation (SHM) in the genes encoding the immunoglobulin variable regions of the heavy chain (*IGHV*) and the light chain (*IGVL*). As they pass through to the light zone, the B cells that express the fittest B cell receptors for binding antigen are selected and may undergo immunoglobulin class-switch recombination. Chronic lymphocytic leukaemia (CLL) cells that use unmutated *IGHV* apparently originate from CD5⁺ B cells prior to experiencing SHM, whereas CLL cells that use mutated *IGHV* most likely originate from CD5⁺ B cells that have passed through and differentiated in the germinal centre. Some CLL cells might be derived from B cells that also have undergone immunoglobulin class-switch recombination and express immunoglobulin isotypes other than IgM and IgD, for example, IgG or IgA. Another subset is one with CLL cells that express immunoglobulin with only modest somatic mutations, such as CLL cells that use *IGHV3-21* with ~97% homology to the inherited *IGHV3-21* gene and an immunoglobulin light chain encoded by an unmutated *IGLV3-21*; these cells might derive from a B cell that has had constrained SHM, possibly owing to a limited need for immunoglobulin somatic diveresification and selection. Dashed arrows indicate speculated pathways.

Mechanisms/pathophysiology

Genetics

Genetic alterations in CLL can include chromosomal alterations, mutations, alterations in the expression of miRNAs and epigenetic modifications.

Chromosomal alterations. Approximately 80% of patients with CLL carry at least one of four common chromosomal alterations: a deletion in chromosome 13q14.3 (del(13q)), del(11q), del(17p) and trisomy 12 (REF. 38). Del(13q) is the most common chromosomal alteration, evident in >50% of patients, and is associated with favourable prognosis. Within this deleted region is the *DLEU2–mir-15-16* cluster, which regulates the expression of proteins that can inhibit apoptosis or that are involved in cell cycle progression[39] (see Supplementary information S2 (table)). Del(17p) is found in 7% of patients and is associated with loss of the tumour suppressor gene *TP53* (REF. 40), whereas del(11q) is found in 18% of patients and is often associated with alterations in *ATM* (which encodes a protein involved in DNA repair); each of these chromosomal alterations is associated with adverse clinical outcome[38], although this has improved in recent years[41]. Trisomy 12 is found in 16% of patients with CLL and is associated with an intermediate prognosis. Unlike the neoplastic B cells in mantle cell lymphoma, CLL cells do not have the translocation t(11;14)(q13;q32) or other genetic alterations that enhance the expression of *CCND1*, which encodes the cell cycle regulator cyclin D1.

Somatic mutations. The advent of massively parallel sequencing and the application of whole-exome sequencing to CLL have transformed our understanding of the genetic heterogeneity of CLL and have established that CLL harbours a high degree of genetic variability[42–45] (FIG. 2). From these studies, recurrent somatic mutations have been consistently observed in genes that have a role in DNA damage (for example, *TP53* and *ATM*), mRNA processing (for example, *SF3B1* and *XPO1*), chromatin modification (for example, *HIST1H1E, CHD2* and *ZMYM3*), WNT signalling, Notch signalling (for example, *NOTCH1*) and inflammatory pathways (for example, *MYD88*). Other mutations, such as those found in *EGR2* or *BRAF*, can affect B cell-related signalling and transcription[46] (FIG. 2).

The functional role of several putative driver mutations has been confirmed; for example, silencing mutated WNT pathway genes in primary CLL cells resulted in decreased cell viability[47]. Mutations in *POT1*, which has a role in the protection of telomeres, prevented the binding of protection of telomeres protein 1 to telomeric DNA, resulting in numerous chromosomal abnormalities, in addition to the development of abnormal telomeres. Mutations in *SF3B1* have been found to be associated with aberrant RNA splicing[45,48,49] and

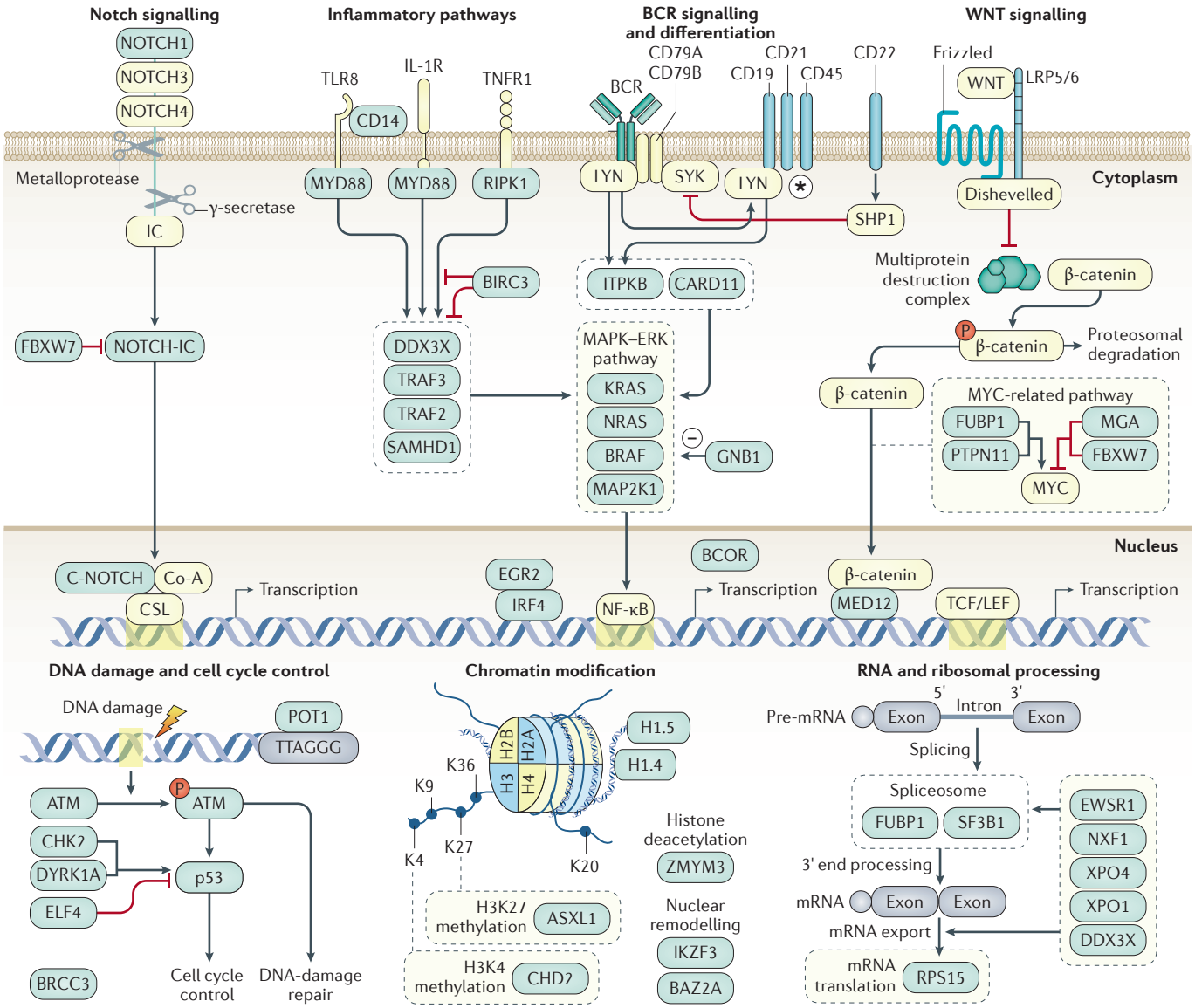


Figure 2 | **Range of somatic mutations in CLL.** Genes that are mutated in chronic lymphocytic leukaemia (CLL) are involved in several cellular pathways (blue boxes). As such, mutations in these genes can lead to a range of cellular consequences, such as aberrant DNA repair and B cell receptor (BCR) signalling, among others[51,213]. The minus sign from GBN1 to the MAPK–ERK pathway indicates negative regulation. *For more detail of the BCR and its associated signalling, see FIG. 3. ASXL1, additional sex combs-like protein 1; ATM, ataxia telangiectasia mutated; BAZ2A, bromodomain adjacent to zinc-finger domain protein 2A; BCOR, BCL-6 co-repressor; BIRC3, baculoviral IAP repeat-containing protein 3; BRCC3, BRCA1/BRCA2-containing complex subunit 3; C-NOTCH, carboxy-terminal domain of NOTCH; CARD11, caspase recruitment domain-containing protein 11; CHD2, chromodomain-helicase-DNA-binding protein 2; CHK2, checkpoint kinase 2; Co-A, co-activator; CSL, CBF1–Suppressor of Hairless–LAG1 (also known as RBPJ); DDX3X, ATP-dependent RNA helicase DDX3X; DYRK1A, dual-specificity tyrosine-phosphorylation-regulated kinase 1A; EGR2, early growth response 2; ELF4, ETS-related transcription factor Elf-4; ERK, extracellular signal-regulated kinase; EWSR1, Ewing sarcoma breakpoint region 1 protein; FBXW7, F-box/WD repeat-containing protein 7; FUBP1, far upstream element-binding protein 1; GNB1, guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit β1; H3K4, histone H3 lysine 4; IC, intracellular domain; IKZF3, Ikaros family zinc-finger protein 3; IL-1R, IL-1 receptor; IRF4, interferon regulatory factor 4; ITPKB, inositol-trisphosphate 3-kinase B; LRP, low-density lipoprotein receptor-related protein; MAP2K1, dual-specificity mitogen-activated protein kinase kinase 1; MAPK, mitogen-activated protein kinase; MED12, Mediator of RNA polymerase II transcription subunit 12; MGA, MAX gene-associated protein; MYD88, myeloid differentiation primary response protein MyD88; NF-κB, nuclear factor-κB; NXF1, nuclear RNA export factor 1; P, phosphate; POT1, protection of telomeres protein 1; PTPN11, tyrosine-protein phosphatase non-receptor type 11; RIPK1, receptor-interacting serine/threonine-protein kinase 1; RPS15, 40S ribosomal protein S15; SAMHD1, SAM domain and HD domain-containing protein 1; SF3B1, splicing factor 3B subunit 1; SHP1, Src homology region 2 domain-containing phosphatase 1 (also known as PTPN6); SYK, spleen tyrosine kinase; TCF/LEF, T cell factor/lymphoid enhancer factor; TLR8, Toll-like receptor 8; TNFR1, tumour necrosis factor receptor 1 (also known as TNFRSF1A); TRAF, TNFR-associated factor; XPO, exportin; ZMYM3, zinc-finger MYM-type protein 3. Adapted with permission from REF. 51, Macmillan Publishers Limited.

an altered DNA-damage response[50]. *SAMHD1* encodes a protein involved in the regulation of intracellular deoxynucleotide pools, which are recruited to the site of DNA damage and are probably involved in the response to DNA double-strand breaks[50].

The detection of somatic mutations and their relative frequencies is variable, which possibly reflects differences in the composition of the cohorts studied worldwide. Two seminal studies have provided the largest sequenced collections to date[51,52], in which >500 CLL samples were characterized by whole-exome sequencing or whole-genome sequencing. The clinical and/or biological features of the patients examined in each study were notably distinct; one study analysed matched pretreatment samples from patients who required initial treatment and noted mutations in *SF3B1* (21% of patients), *ATM* (15% of patients), *TP53* (7% of patients), *NOTCH1* (6% of patients) or *BIRC3* (4% of patients). The other study assessed patients with early-stage CLL and patients with monoclonal B cell lymphocytosis and identified *NOTCH1* (12.6% of patients), *ATM* (11% of patients), *BIRC3* (8.8% of patients) and *SF3B1* (8.6% of patients) as the most frequently mutated genes.

These large sample cohorts have provided the sensitivity to discover novel candidate cancer genes that are altered in CLL. Both studies also identified somatic mutations in *MGA* and *PTPN11*, which encode modulators of *MYC*, *IKZF3*, which encodes a key transcription factor, and *RPS15*, which encodes 40S ribosomal protein S15 and is recurrently mutated in ~20% of patients who relapse after combination chemotherapy[53]. Other recurrent somatic mutations include those in the 3′ untranslated region of *NOTCH1* and a *PAX5* enhancer, which increases the expression of these B cell-associated transcription factors[54,55]. Patients with mutations in the 3′ untranslated region of *NOTCH1* have a shorter time from diagnosis to treatment and poorer overall survival, similar to that of patients with non-synonymous mutations, which alter the amino acid sequence of NOTCH1.

Next-generation sequencing has revealed intratumoural heterogeneity in CLL. Some somatic mutations, such as those in *MYD88*, or chromosomal abnormalities, such trisomy 12 or del(13q), are most often found in all the CLL cells of any one patient, indicating that these genetic alterations occurred early in the evolution of the leukaemia. Other mutations, such as those found in *SF3B1* or *NOTCH1,* or chromosomal alterations, such as del(17p), are typically found in only a fraction of the leukaemia cells and thus represent subclonal events, which occur after the development of CLL. Across studies, subclonal driver mutations are associated with more-aggressive disease, particularly when two or more are found concurrent in a leukaemia cell population[51,56,57]. In addition, studies have demonstrated that large clonal shifts can occur following chemotherapy, owing to increases in the proportions of CLL cells that have a *TP53* mutation or del(17p), indicating that such genetic changes provide a strong fitness advantage in the setting of therapy[51]. By contrast, one study of CLL cells from patients treated with ibrutinib (an inhibitor of Bruton tyrosine kinase (BTK)), revealed mutations associated with drug resistance that were distinct from those observed in CLL cells of patients treated with standard chemotherapy[58].

miRNA alterations. CLL was the first human disease that was found to be associated with alterations in miRNA. Specifically, *mir-15a* and *mir-16-1* (REF. 59) are deleted, altered or downregulated in ~60% of patients with CLL and are dysfunctional in a few cases of familial CLL[26]. *mir-15a* and *mir-16-1* both target *BCL2* and *MCL1* (REF. 28), which encode anti-apoptotic proteins of the BCL-2 family[60]; reduced expression or loss of these miRNAs can enhance the expression of these target genes. Attention has also focused on miRNAs that are dysregulated or that are differentially expressed in subgroups with distinctive clinical and/or biological features[61] (see Supplementary information S2 (table)). For example, miR-29a/b, miR-29c, miR-34b, miR-181b and miR-3676 target the 3′ untranslated region of *TCL1A*[62]; loss or reduced expression of all or some of these miRNAs can lead to enhanced expression of *TCL1A*, which, when constitutively expressed in mature B cells, promotes the development of CLL in transgenic mice[63]. By contrast, increased expression of *mir-155* is associated with enhanced BCR signalling, B cell proliferation and lymphomagenesis[64,65].

Epigenetic changes. The CLL epigenome shows global hypomethylation combined with local hypermethylation, as has been observed in other cancers[66–68]. Indeed, comprehensive methylation profiling has demonstrated substantial intra-tumoural methylation heterogeneity[52,69–73]. Increasing methylation heterogeneity has consistently been associated with increased genetic complexity owing to the acquisition of subclonal mutations, thus linking genomic and methylomic evolution in CLL[52,70,71]. Indeed, locally disordered methylation in CLL might enhance the evolutionary adaptive capacity of CLL cells by increasing the background 'noise' of the genome, thereby providing increased opportunities for somatic mutations within the leukaemia clone. In support of this notion is the observed association between methylation evolution and adverse clinical outcome[52,70,71].

Methylation signatures can classify distinct clinical CLL subgroups[69,74]. As these methylation patterns are a heritable trait, they have been used to 'trace' back to the type of normal B cell from which the CLL cells were derived[75]. These studies revealed that the CLL cells of different patients derive from a continuum of B cell maturation states, which are not restricted to discrete maturation stages. Nevertheless, CLL cells that use unmutated *IGHV* versus mutated *IGHV* generally have distinctive methylation patterns, which respectively approximate to those of pre-germinal centre versus post-germinal centre memory B cells, as depicted in FIG. 1. The diversity in the likely cells of origin of CLL cells highlights the biological and phenotypic heterogeneity of this disease. These findings also suggest that epigenetic programming that is dependent of transcription factors has a potentially important role in the development of CLL.

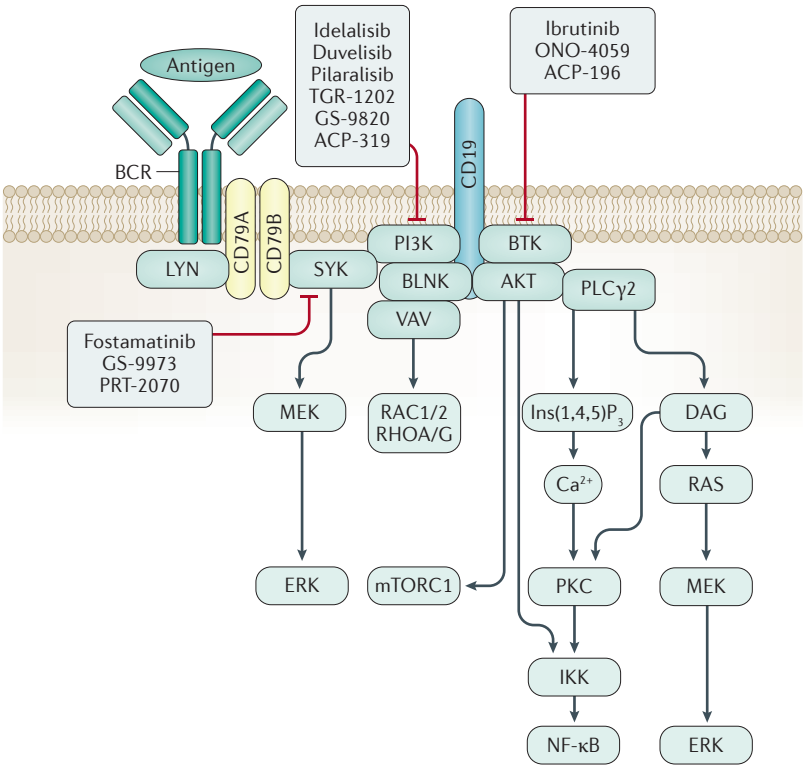


Figure 3 | **B cell receptor signalling response.** B cell receptor (BCR) signalling is initiated by SRC-family kinase-dependent phosphorylation (mainly LYN) of CD79A and CD79B that creates a docking site for the binding and activation of spleen tyrosine kinase (SYK). SYK then triggers the formation of a multi-component 'signalosome', comprising Bruton tyrosine kinase (BTK), AKT, phosphoinositide 3-kinase (PI3K), phospholipase Cγ2 (PLCγ2) and B cell-linker protein (BLNK), among others. CD19 is a co-receptor for BCR and is important for PI3K activation, which recruits and activates PLCγ2, BTK and AKT. PLCγ2 generates diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), which triggers Ca^{2+} release from the endoplasmic reticulum, leading to the activation of the MEK–extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB) signalling pathways. Other effects of BCR signalling include activation of mechanistic target of rapamycin complex 1 (mTORC1) and of Rho-family GTPases, RAC1 and RHOA, which can affect the cytoskeleton. Inhibitors of SYK, PI3K and BTK are shown. Note that this figure describes the main molecules and interactions that are involved in positive BCR signalling, but is not an exhaustive description of all signalling pathways or molecules activated. IKK, IκB kinase; PKC, protein kinase C.

BCR and B cell signalling

The BCR is composed of a ligand-binding transmembrane immunoglobulin molecule (either IgA, IgD, IgE, IgG or IgM) and the signalling Igα (also known as CD79A)–Igβ (also known as CD79B) heterodimer. CLL cells typically co-express IgD and IgM, although at low levels compared with normal B cells; less than a few per cent of CLL cases express class-switched isotypes, most commonly IgG. The CD79A and CD79B molecules contain immunoreceptor tyrosine-based activation motifs, which can be phosphorylated following the crosslinking of surface immunoglobulin, thereby triggering BCR signalling. A functional BCR is required for the survival of mature B cells[76] and is maintained in most mature B cell malignancies, including CLL. In CLL, evidence suggests that the surface immunoglobulin of CLL B cells is engaged by autoantigen, which leads to constitutive BCR signalling *in vivo*[77–79]. The importance of this interaction is underscored by the clinical success of kinase inhibitors that block BCR signalling (see Management), although effects on other receptors might also have a role[80].

Like most cancers, CLL is heterogeneous and the outcome of BCR signalling ranges from enhanced B cell activation to B cell anergy[81,82]. The main pathways that lead to cell survival and proliferation downstream of the BCR are shown in FIG. 3, along with drugs targeted against key signalling intermediates. BCR signalling that leads to anergy is less well defined, but seems to involve biased activation of inhibitory molecules with only partial activation of the pathways that are typically associated with B cell activation[81]. One important molecule that may be involved is the inositol lipid phosphatase SHIP1. SHIP1 is activated by the tyrosine-protein kinase LYN and may limit B cell activation by counteracting phosphoinositide 3-kinase (PI3K) activity at both chronically engaged receptors and distant non-ligated BCRs, rendering them insensitive to stimulation[82,83].

Enhanced B cell activation is more commonly observed in CLL that expresses unmutated *IGHV*, whereas anergy predominates in most cases of CLL that express mutated *IGHV*[84]. Anergy is a state of cellular lethargy induced by chronic engagement of the surface antigen receptors in the absence of adequate T cell help. Although capable of reversing their phenotype, anergic cells are less likely to proliferate in response to BCR signalling than more activated cells, which might, in part, account for the observation that patients with CLL cells that express mutated *IGHV* generally have more indolent disease than patients with CLL cells with unmutated *IGHV*[85]. The fate of the cell (activation versus anergy) might be influenced by the CLL cell of origin (FIG. 1), as the cell types that can form CLL differ in their patterns of DNA methylation[73], and are likely to respond differently to autoantigens. An unresolved question is whether anergy can be reversed *in vivo*, mirroring what occurs *in vitro*[78].

The BCR also coordinates the activity of other cell surface receptors, including integrins, such as α4β1 integrin. BCR stimulation can result in increased adhesion of CLL cells to α4β1 integrin substrates, for example, fibronectin and vascular cell adhesion protein 1 (REF. 86). By contrast, CXC-chemokine receptor 4 (CXCR4) is downmodulated by BCR engagement and both can trigger 'inside-out' signalling, resulting in the activation of α4β1 integrin[87,88]. Thus, recognized antigen encountered in lymphoid tissue is likely to affect adhesion and migration of CLL cells. Modulation of these pathways, coupled with the role of BTK and PI3K in chemokine receptor signalling[89], contribute to the increased lymphocytosis observed in patients upon initiation of treatment with inhibitors of BTK or PI3K (see Management).

Cancer microenvironment

CLL cells depend on survival signals that they receive in lymphoid tissues from neighbouring non-neoplastic cells within the so-called cancer microenvironment. CLL cells follow chemokine gradients into lymph nodes, where they form 'proliferation centres' (REF. 77), as opposed to normal germinal centres. In these proliferation centres,

the CLL cells contact non-malignant stromal cells, nurse-like cells (also known as lymphoma-associated macrophages), T cells and mesenchymal-derived stromal cells (FIG. 4). Engagement with autoantigen may occur during this transit, thereby stimulating CLL cell activation and proliferation if sufficient T cell help is available. Only a few per cent of the CLL cells undergo proliferation at any one time; the remainder of the cells are either unstimulated or driven into anergy[84]. However, within such proliferation centres, all CLL cells are exposed to chemokines, integrins, cytokines and survival factors (such as tumour necrosis factor (TNF) ligand superfamily member 13B (also known as BAFF) or TNF ligand superfamily member 13 (also known as APRIL)), which activate canonical nuclear factor-κB (NF-κB)[90], before they exit to the blood. Activation of NF-κB can induce the expression of *mir-155*, which enhances BCR signalling and activation by reducing the expression of *INPP5D*, which encodes SHIP1 (REF. 65). Cytokines that are secreted by T cells, such as IL-4, can upregulate surface IgM, which potentially facilitates the interaction of the CLL cell with autoantigen[91]. In addition, the elaboration of various WNT proteins by cells in the microenvironment can activate canonical and non-canonical WNT signalling pathways[92,93]. Activation of the tyrosine-kinase-like transmembrane receptor ROR1 by WNT5A can induce the activation of RAC1 and RHOA, and thereby enhance CLL cell proliferation and promote

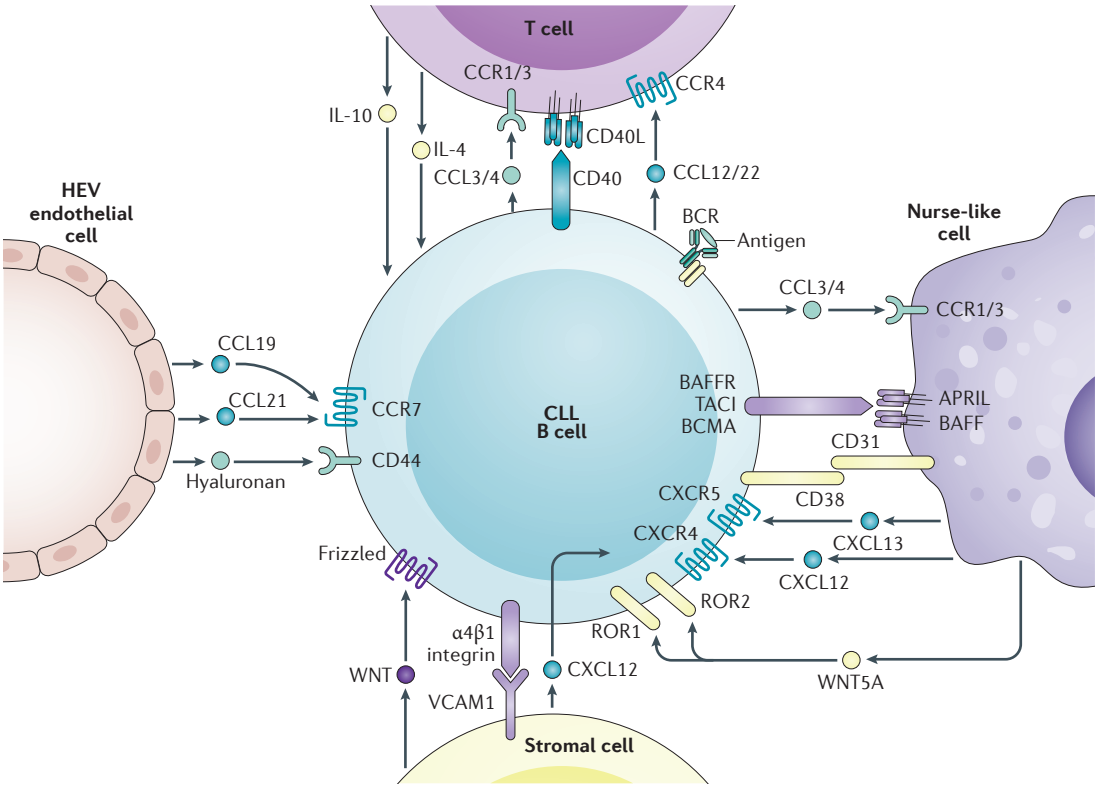


Figure 4 | **CLL microenvironment.** Migration of chronic lymphocytic leukaemia (CLL) cells into the lymphoid tissue is primarily mediated through CXC-chemokine receptor 4 (CXCR4) in response to CXC-chemokine ligand 12 (CXCL12), which is secreted mainly by nurse-like cells (NLCs) and mesenchymal-derived stromal cells. Migration of CLL cells into lymph nodes also occurs via CC-chemokine receptor 7 (CCR7) in response to CC-chemokine ligand 19 (CCL19) and CCL21, which are produced by the endothelial cells of high endothelial venules (HEVs). HEV endothelial cells also express hyaluronan, which can interact with CD44, to facilitate B cell signalling and might enhance the production of active matrix metalloproteinase 9 (MMP9). Once in tissues, several chemokines promote B cell survival, including CXCL12, B cell-activating factor (BAFF; also known as TNFSF13B) and a proliferation-inducing ligand (APRIL; also known as TNFSF13). In addition, CLL cell survival can be promoted through cognate interactions between CD31 and CD38, and the production by stromal cells of WNT factors, which can interact with ROR1, ROR2 and/or various Frizzled receptors. CLL cell contact with mesenchymal stromal cells can also be established through vascular cell adhesion protein 1 (VCAM1)–α4β1 integrin interactions that contribute to CLL cell survival. In turn, CLL cells can secrete chemokines, such as CCL3 and CCL4, which can recruit T cells and NLC-precursor cells (monocytes) to the CLL microenvironment. Activated T cells can provide CLL cells with proliferative signals through CD40 ligand (CD40L)–CD40 interactions and the secretion of several cytokines, such as IL-2, IL-4 and IL-10. In turn, activated CLL cells secrete CCL12 and CCL22, which attract more T cells into the CLL microenvironment. In tissues, CLL cells can be exposed to environmental and/or self-antigens that might trigger B cell activation through interactions with the surface immunoglobulin; this could amplify the responsiveness of CLL cells to the signals and factors that are provided by the CLL microenvironment. BAFFR, BAFF receptor (also known as TNFRSF13C); BCMA, B cell maturation protein (also known as TNFRSF17); BCR, B cell receptor; TACI, transmembrane activator and CAML interactor (also known as TNFRSF13B).

migration in response to chemokines[93]; in part, for this reason, high-level CLL cell expression of ROR1 is associated with accelerated disease progression[94]. Finally, Notch signalling in response to Jagged, or Hedgehog signalling in response to Sonic Hedgehog or Desert Hedgehog, can provide pro-survival stimulation for at least a subset of patients with CLL, particularly those with trisomy 12 (REFS 95–97).

As CLL cells leave the tissue site, engagement with antigen will be transient and its effects are likely to reverse in the blood, leading to variable increases in the expression of surface IgM and CXCR4 (REF. 98). CLL cell expression of CXCR4 is downmodulated upon exposure to CXC-chemokine ligand 12 (CXCL12)[99], which is produced by nurse-like cells in the microenvironment[100]. Consequently, CLL cells in the blood that have just exited lymphoid tissue express low levels of CXCR4 (known as $CXCR4^{dim}$ cells) and higher levels of CD5 (known as $CD5^{bright}$ cells) relative to the CLL cells that are poised to re-enter lymphoid compartments[101]. For unexplained reasons, a high level of expression of CXCR4 by circulating CLL cells is associated with poorer prognosis in patients with CLL that use mutated *IGHV*[102], possibly by influencing tissue re-entry. In terms of treatment effects, kinase inhibitors, such as ibrutinib, inhibit BCR-associated pathways, which remain important for cancer cells that are retained in lymphoid tissues, but can also directly inhibit integrin-mediated and chemokine-mediated pathways, thereby contributing to the increased lymphocytosis that occurs following the initiation of kinase inhibitor therapy[103].

Box 1 | **Differential diagnosis of CLL**

Small lymphocytic lymphoma

Diagnosis of small lymphocytic lymphoma is generally made following biopsy of an enlarged lymph node, which typically has a disrupted architecture owing to the infiltration of well-differentiated, clonal B cells with the same phenotype as chronic lymphocytic leukaemia (CLL) cells. Patients with small lymphocytic lymphoma have <5,000 clonal B cells per μl in the blood, but over time, patients can develop lymphocyte counts of >5,000 cells per μl, which allows them to be reclassified as having CLL.

Monoclonal B lymphocytosis

Monoclonal B lymphocytosis is defined as <5,000 clonal B cells per μl in the blood without other signs of lymphoma, such as enlarged lymph nodes (>1.5 cm), which would suggest the diagnosis of small lymphocytic lymphoma[204]. In most, but not all, cases, the clonal B cells in monoclonal B lymphocytosis express CD5 and have the same immune phenotype as CLL[205]. Although biopsies are not generally performed, the incidental finding of CLL-like cells in the marrow or in normal-sized lymph nodes does not exclude the diagnosis of monoclonal B lymphocytosis.

Cases of monoclonal B lymphocytosis are classified as being low count (<500 monoclonal B cells per μl) or high count (>500 monoclonal B cells per μl). Approximately 5% of adults of European ancestry >40 years of age have low-count monoclonal B lymphocytosis, as assessed via flow cytometry on blood mononuclear cells. Although subjects with low-count monoclonal B lymphocytosis rarely progress to CLL, 1–2% of patients with high-count monoclonal B lymphocytosis will develop CLL per year[206].

Other lymphoproliferative diseases

Other chronic B cell lymphoproliferative diseases can present like CLL, including B cell prolymphocytic leukaemia, follicular lymphoma, hairy cell leukaemia, mantle cell lymphoma or marginal zone lymphoma. In addition to clinical features and pathology, which are characteristic of these other conditions, the immune phenotype of neoplastic lymphocytes helps to differentiate these conditions from CLL.

Immune deficiency

One clinically important aspect of CLL is the development of hypogammaglobulinaemia with consequent risk of infection. The mechanism involved is unclear, but IL-10, a known T cell-derived immunosuppressive factor, might have a role[104]. For CLL, emerging evidence suggests that the cancer cells themselves can produce IL-10 (REF. 105). Apparently, more IL-10 is produced by CLL cells that express mutated *IGHV* than by CLL cells that express unmutated *IGHV*. However, systemic levels of IL-10 and other suppressive factors can also be influenced by the cumulative total-body numbers of cancer cells, which are often higher in patients with CLL cells that express unmutated *IGHV*. This might account in part for the finding of immune deficiency in patients with CLL cells that express either mutated *IGHV* or unmutated *IGHV*. Furthermore, CLL cells express high levels of programmed cell death 1 ligand 1 (PD-L1) and PD-L2, which suppress the effector responses of T cells that express programmed cell death protein 1 (PD-1), leading to an 'exhausted' T cell phenotype and impaired cellular immune function[106].

Diagnosis, screening and prevention

Diagnostic work-up

Most often, patients with CLL are asymptomatic at the time of diagnosis and become aware of the disease following the detection of lymphocytosis in a routine blood count. However, CLL can have a range of clinical presentations; some patients feel well and are fully active, but a minority have disease-related symptoms. The usual symptoms of CLL include fatigue, involuntary weight loss, excessive night sweats, abdominal fullness with early satiety and increased frequency of infections, which might be associated with hypogammaglobulinaemia. Some patients can present with symptoms of an autoimmune cytopenia (for example, autoimmune haemolytic anaemia or immune thrombocytopenic purpura). Patients can also have or develop enlarged lymph nodes, hepatomegaly and splenomegaly, which are palpable on physical examination. Enlarged lymph nodes can be easily palpable at three sites: the cervical, axillary and inguino-femoral regions.

Laboratory features. Laboratory assessment for CLL includes a full blood cell count and flow cytometry. The most consistent laboratory abnormality observed is an increase in the absolute number of blood lymphocytes above the normal adult upper limit of ~3,500 cells per μl, detected by a blood count. Most patients present with ≥10,000 cells per μl, but some might have fewer numbers of blood lymphocytes upon relapse after therapy. The initial diagnosis requires detection of ≥5,000 cells per μl of clonal CLL B cells[107], which typically express low levels of surface immunoglobulin with either κ-immunoglobulin or λ-immunoglobulin light chains.

Flow cytometric or immunohistochemical analyses of the mononuclear cells in the blood, marrow or lymph nodes can help to distinguish CLL from other types of lymphoma (BOX 1). CLL B cells typically express CD5, CD19 and CD23 (also known as low-affinity

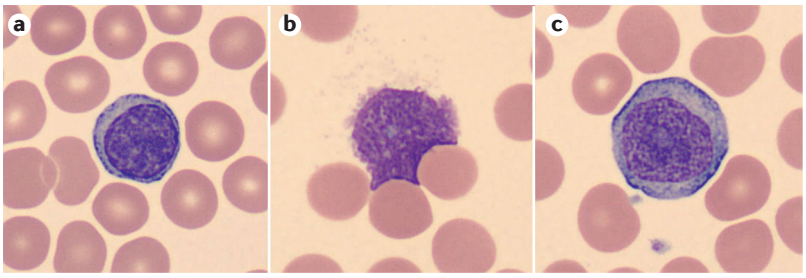


Figure 5 | **Blood smears from patients with CLL.** Wright–Giemsa-stained blood smears showing the typical chronic lymphocytic leukaemia (CLL) B lymphocyte (part **a**), smudge cell (part **b**) and a prolymphocyte with a prominent nucleolus (part **c**). Magnification ×500. Images courtesy of H. E. Broome, University of California, San Diego, La Jolla, California, USA.

immunoglobulin-ε Fc receptor), and have low levels of CD20, but lack expression of CD10 and stain poorly, if at all, with the FMC7 monoclonal antibody, which recognizes an epitope of CD20 (REF. 108). CLL cells also express CD200 (also known as OX-2 membrane glycoprotein), which can help to distinguish CLL from mantle cell lymphoma[109]. In addition, the CLL cells of >95% of patients express the onco-embryonic surface antigen ROR1 (REFS 94,110).

Morphologically, CLL cells are small mature-appearing lymphocytes with dense chromatin, a nucleus that virtually fills the cell with only a rim of visible cytoplasm and no (or occasionally small) nucleoli (FIG. 5a). In CLL, the presence of smudged cells on the blood smear is common and represents lymphocytes that were crushed in the process of making the slide (FIG. 5b). CLL cells also can appear as prolymphocytes, which are larger than typical CLL cells, have less-condensed nuclei and a single prominent nucleolus (FIG. 5c). However, if >55% of cells on the blood smear are prolymphocytes, a diagnosis of prolymphocytic leukaemia should be considered[111].

No abnormalities are considered specific for CLL in the blood chemistry panel. Quantitative levels of serum immunoglobulins (for example, IgA, IgG and IgM) are usually normal at diagnosis, but generally decline with disease progression. A direct Coombs test (which is used to detect erythrocytes that are coated with anti-red blood cell autoantibodies) might be positive in the absence of overt autoimmune haemolytic anaemia in a large minority of patients; however, patients with a positive direct Coombs test might be at increased risk of developing this autoimmune disease.

Although not required for establishing a diagnosis of CLL, a marrow biopsy is often performed; this usually shows hypercellularity owing to an increased percentage of mature-appearing lymphocytes. Four patterns of lymphocytic infiltration in the marrow have been described: nodular, interstitial, mixed (nodular and interstitial) or diffuse; the diffuse pattern is typically associated with advanced disease[112] (FIG. 6). In addition, the marrow usually shows reduced numbers of myeloid and erythroid cells, which otherwise have normal maturation.

A lymph node biopsy might be performed in a patient with an enlarged lymph node as part of a diagnostic evaluation for suspected lymphoma. Excised lymph nodes typically have a diffuse infiltration of well-differentiated small lymphocytes, often obliterating the normal nodal architecture, and scattered, vaguely nodular, pale haematoxylin and eosin-staining areas, appearing as pseudofollicles (FIG. 7a), which are enriched with prolymphocytes and paraimmunoblasts (FIG. 7b); these areas comprise the proliferation centres[113]. The pseudofollicles or proliferation centres are hallmark features in the lymph nodes of patients with CLL or small lymphocytic lymphoma, as they are not observed in other types of lymphomas.

Staging

Two clinical staging systems are widely used to divide patients with CLL into three broad prognostic groups[114,115]. The Rai staging system (TABLE 1) is more commonly used in the United States, whereas the Binet classification (TABLE 2) is more commonly used in Europe. The staging systems each recognize the importance of marrow function and define late-stage, or high-risk, disease by the presence of pronounced anaemia or thrombocytopenia.

Prognostic factors and nomograms

The clinical course of newly diagnosed CLL is extremely variable; some patients remain free of symptoms and are fully active for decades, whereas others rapidly become symptomatic or develop high-risk disease, which requires treatment soon after diagnosis and might result in death due to therapy-related and/or disease-related complications. However, most patients have a clinical course that is in between these two extremes.

Prognostic factors that can help to identify patients who require therapy relatively soon after diagnosis include certain clinical features and genetic, molecular and biochemical characteristics of the CLL cell. Multivariable models, prognostic indexes[116–118] and nomograms[119] have been developed to consolidate such prognostic factors so that they can more robustly predict clinical outcome. Commonly used parameters that are associated with poorer outcome are male sex, ≥65 years of age, poor performance status due to medical comorbidities, certain CLL cell characteristics, such as the expression of unmutated *IGHV*[1,2], ZAP70 (REFS 120,121), CD49d (also known as integrin α4)[122] or CD38 (REF. 2), the presence of del(17p)[38] or del(11q)[123], high serum levels of β2-microglobulin (>3.5 mg per l)[124], complex karyotype (that is, the presence of three or more chromosomal aberrations observed on a karyotype test)[125,126], or a high absolute lymphocyte count (>50,000 cells per μl) and/or late-stage disease at initial presentation. Del(17p) is often associated with inactivating mutations in *TP53* and is a predictor of poor outcome to treatment with regimens that involve conventional chemotherapy[127].

Currently, the most reliable prognostic models are those developed for treatment-free survival, as evolving treatments have yet to change the indications for therapy. Predictive models to define overall survival with a given type of therapy are challenged by the chronicity of CLL and the fact that patients often receive serial treatments, each of which can affect outcome; moreover, death might be due to an indirect or unrelated cause. Furthermore, treatment options are changing, with newly identified,

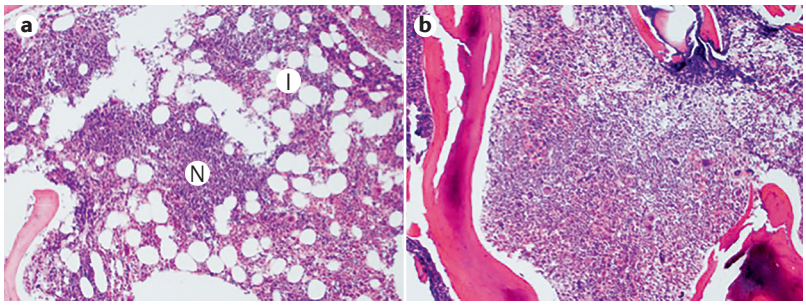


Figure 6 | **Marrow biopsies from patients with CLL.** Tissue sections of a marrow biopsy specimen stained with haemotoxylin and eosin showing interstitial (I) or nodular (N) chronic lymphocytic leukaemia (CLL) cell involvement (part **a**) or diffuse CLL cell marrow involvement (part **b**), which is typically associated with advanced-stage disease (original magnification ×100). Images courtesy of H. E. Broome, University of California, San Diego, La Jolla, California, USA.

highly effective agents that are clearly prolonging survival and have activity among patients who would have been considered high risk when the only option was conventional chemotherapy.

Management

Generally, indications to initiate therapy include pronounced disease-related anaemia or thrombocytopenia (patients with Rai stage III or stage IV disease, or Binet stage C disease), symptomatic lymphadenopathy and/or symptoms that are associated with active disease, such as night sweats, fatigue, unintentional weight loss or fever without evidence of infection[107]. However, when basing a treatment decision on constitutional symptoms alone, the physician should consider other medical conditions, such as hypothyroidism, hyperthyroidism, hypoglycaemia, chronic inflammation, uncommon opportunistic infections or sleep disorders, including sleep apnoea.

No established absolute lymphocyte count or lymph node size alone should form the basis for the initiation of therapy. Instead, patients who are asymptomatic with early-stage or intermediate-stage disease (such as Rai stage I or stage II, or Binet stage A or stage B) are not recommended for therapy unless they have symptomatic disease or evidence for disease progression. Evidence for disease progression can include a lymphocyte doubling time of <1 year, progressive palpable lymphadenopathy and/or progressive palpable splenomegaly in serial examinations. In the absence of indications for treatment, patients are examined for palpable lymphadenopathy and splenomegaly and have complete blood counts at 3–12-month intervals, the frequency of which depends on the presence of signs of disease progression. Clinical or laboratory features of anaemia or thrombocytopenia should prompt evaluation for autoimmune haemolytic anaemia or immune thrombocytopenic purpura, respectively; such autoimmune cytopenias might require treatment that is independent of the consideration for therapy directed against the underlying CLL. Finally, patients should be cautioned to seek prompt medical attention for signs or symptoms of infection; because of the acquired immune deficiency associated with CLL, the threshold for considering the use of antimicrobial therapy should be low. Nonetheless, development of frequent or serious infections is not an indication for CLL-directed therapy.

For patients who need treatment, the presence of del(17p) or mutated *TP53* are the most important features that are currently directing the choice of therapy (FIG. 8). Next, advanced age of >65 years, the presence of medical comorbidities and the objectives of treatment have substantial bearing on the choice of therapy. Increasingly, *IGHV* mutational status is considered as a parameter when determining the type of therapy; for example, chemotherapy-based regimens are reserved for patients with CLL and mutated *IGHV*. Conversely, the specific Rai or Binet stage of the patient who requires treatment does not necessarily influence the choice of therapy.

Systemic treatments

The treatment of patients with CLL can include chemotherapy, a combination of chemotherapy and immunotherapy, or drugs that target the signalling pathways that promote the growth and/or survival of CLL cells (for example, BCR signalling and BCL-2)[128,129].

Chemotherapy. Chemotherapy has been the mainstay of therapy for the past 50 years. Purine analogues (most commonly fludarabine, but also pentostatin or cladribine) and alkylating agents (including chlorambucil, cyclophosphamide or bendamustine) are used in the treatment of CLL[130–132]. Chemotherapy-based regimens can cause myelosuppression, an increased risk of infections and, in a small subset of patients, post-therapy myelodysplasia or secondary cancers, such as acute myeloid leukaemia (see Secondary cancers).

Chemoimmunotherapy. Phase III clinical trials have validated the benefit of anti-CD20 monoclonal antibodies, such as rituximab, obinutuzumab or ofatumumab, in combination with chemotherapy for the treatment of patients with CLL. In one trial (the CLL8 trial of the German CLL Study Group), patients who received fludarabine and cyclophosphamide with rituximab had higher response rates and a longer median progression-free survival (PFS) than patients who were treated with fludarabine and cyclophosphamide[133]. In a separate study (the CLL11 trial), patients >65 years of age with medical comorbidities who were treated with chlorambucil and either obinutuzumab or rituximab had improved response rates and longer median PFS than patients who were treated with chlorambucil alone[134]. However, the median PFS was significantly longer for patients who received obinutuzumab (26.7 months) than in those who received rituximab (11.1 months). In a third phase III trial, median PFS significantly improved from 13.1 months for patients treated with chlorambucil to only 22.4 months for patients treated with chlorambucil and ofatumumab[135]. As a consequence of these three trials, the US FDA approved the use of rituximab, obinutuzumab or ofatumumab in combination with chemotherapy for the first-line treatment of patients with CLL. The FDA also approved the use of ofatumumab as a single agent for the treatment of patients with relapsed or refractory disease based on data from a phase II study[136].

Bendamustine is commonly used with rituximab and has good response rates in treatment-naive patients without del(17p)[137], although no randomized trials comparing bendamustine and rituximab versus bendamustine alone have been conducted. Bendamustine has also been used in combination with obinutuzumab, which showed highly encouraging results[138] and is being evaluated in larger clinical trials.

In a randomized trial, the rates of complete response and complete response without evidence for minimal residual disease (MRD) were higher in patients treated with fludarabine, cyclophosphamide and rituximab than in those treated with bendamustine and rituximab, and the median PFS was ~1 year longer[139]. However, patients in the bendamustine and rituximab treatment subgroup were older and had a higher proportion of patients who had CLL cells expressing unmutated *IGHV*, making this cohort at higher risk for a poorer outcome than the cohort of patients treated with fludarabine, cyclophosphamide and rituximab. It also should be noted that patients treated with fludarabine, cyclophosphamide and rituximab had higher rates of neutropenia and infections than patients treated with bendamustine and rituximab. Because of this, many physicians currently provide patients with growth factors (for example, filgrastim or pegfilgrastim) and prophylactic antimicrobial therapy when they are treated with the fludarabine, cyclophosphamide and rituximab regimen, but such measures were not recommended for patients treated in this trial[139]. In any case, there has not been significant difference observed in overall survival between the two treatment arms, but events are limited.

Some patients can experience a prolonged PFS following treatment with fludarabine, cyclophosphamide and rituximab, particularly those with CLL with mutated *IGHV* that lack del(17p) or del(11q), which are associated with chemotherapy resistance or relatively short PFS, respectively. Long-term follow-up data on patient outcomes following therapy with this regimen indicate that patients with mutated *IGHV* might achieve a long-term survival benefit (and possible 'cure') with chemoimmunotherapy[140–142].

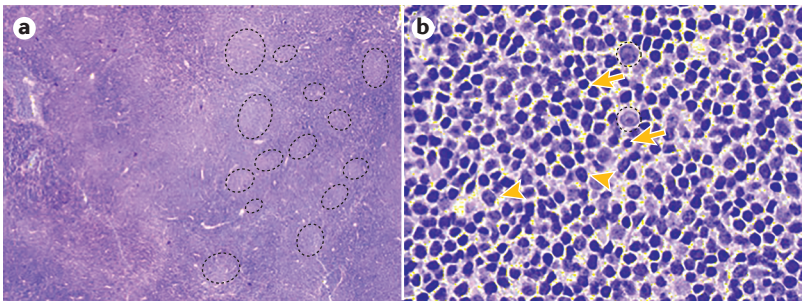


Figure 7 | **Lymph node of patients with CLL. a** | Tissue sections of a lymph node stained with haemotoxylin and eosin showing numerous pale-staining pseudofollicles, which are circled (original magnification ×20). **b** | Higher (×400) magnification of a proliferation centre. Representative lymphocytes (arrows), prolymphocytes (arrowheads) or paraimmunoblasts (circles) in a proliferation centre are shown. Images courtesy of H.-Y. Wang, University of California, San Diego, La Jolla, California, USA.

Inhibitors of BCR signalling. Three main classes of drugs that each can inhibit BCR signalling have been evaluated in patients with CLL: BTK inhibitors, PI3K inhibitors and spleen tyrosine kinase (SYK) inhibitors[86,143]. CLL cells with unmutated *IGHV* seem to be more sensitive to inhibitors of BCR signalling than CLL cells with mutated *IGHV*[144], but whether inhibitors, such as ibrutinib, are more effective in patients with CLL and unmutated *IGHV*, remains to be validated in clinical trials.

Ibrutinib has been approved in the United States and Europe for use as initial therapy, as well as in patients with relapsed disease, which followed results from a randomized trial that showed a significantly higher response rate to therapy with ibrutinib than with ofatumumab[145]. In addition, with continuous therapy, patients treated with ibrutinib had a significantly longer median PFS and overall survival than patients treated for 8 months with ofatumumab. Approval of ibrutinib as initial therapy was based on the results of a randomized trial that showed a significant improvement in median PFS and overall survival in patients ≥65 years of age with del(17p) who were treated indefinitely with ibrutinib than in patients treated for up to 48 weeks with chlorambucil[146].

Upon initiation of treatment with ibrutinib, lymphadenopathy is rapidly reduced, which is associated with a concomitant increase in absolute lymphocyte count[147]. The rise in absolute lymphocyte count is related to the inhibition of chemokine receptor signalling, which inhibits the migration of CLL cells from the blood into the lymphoid tissues. This resulting lymphocytosis should not be considered a sign of progression; over time, the lymphocytosis subsides as the overall tumour burden decreases with continued therapy.

Adverse effects of ibrutinib include fatigue, diarrhoea, bleeding, ecchymoses, rash, arthralgia, myalgia, increased blood pressure and atrial fibrillation. Clinical trials are currently evaluating second-generation BTK inhibitors (for example, acalabrutinib[148], ONO/GS-4059 (REF. 149) or BGB-3111) to determine whether any one of these drugs has a superior therapeutic index than that of ibrutinib[150].

PI3K inhibitors include idelalisib, duvelisib (also known as IPI-145), TGR-1022 and ACP-319 (also known as AMG-319)[151]; the latter three drugs are being evaluated in clinical trials, whereas idelalisib was approved in the United States and Europe for the treatment of patients with relapsed CLL; this approval was based on the outcome of a clinical trial that showed that patients treated with rituximab and idelalisib had significantly higher response rates and a significantly longer median PFS and overall survival than patients treated with rituximab and placebo[152]. As with ibrutinib, patients who initiate therapy with idelalisib can experience a rapid reduction in lymphadenopathy that is associated with lymphocytosis. Similarly, this event should not be considered as a sign of disease progression.

Adverse effects of idelalisib include transaminitis (usually in the first few months of therapy), pneumonitis and colitis; the latter usually occurs >6 months after the initiation of therapy with this drug and is often severe enough to require cessation of therapy[153]. Transaminitis

seemed to be more severe in patients who received idelalisib as their initial therapy for CLL than in patients with relapsed disease[153], suggesting that transaminitis is not directly caused by idelalisib. This is also suggested by the observations that mild increases in the levels of serum transaminase can subside over time with continued drug administration; furthermore, patients who have had idelalisib withheld because of transaminitis can be restarted on this drug without experiencing apparent hepatic toxicity. The decision to halt therapy or to re-administer the drug following resolution of transaminitis should consider the severity and duration of hepatic function test abnormalities, which often do not recur upon re-institution of idelalisib therapy[154].

In 2016, the FDA recommended the closure of clinical trials investigating idelalisib and rituximab combination therapy for first-line treatment of patients with CLL, owing to a higher number of infections and deaths in the experimental arm. As such, patients undergoing therapy with idelalisib and rituximab should be considered for concomitant treatment with prophylactic low-dose acyclovir to protect against reactivation of varicella zoster virus, which causes chicken pox and shingles. Patients also should be treated with prophylactic antibiotics to mitigate the risk for opportunistic infection, such as that caused by *Pneumocystis jiroveci*. Finally, as with any patient receiving therapy with anti-CD20 monoclonal antibodies, patients should be screened for active infection with hepatitis B virus before the initiation of therapy[155], and periodically monitored for reactivation of cytomegalovirus, especially if they should develop unexplained symptoms of infection.

Phase I/II clinical trials of fostamatinib, an oral SYK inhibitor, caused reduction in lymphadenopathy with concomitant lymphocytosis, an improvement in disease-related cytopenias and relief of disease-related symptoms in most of the treated patients with CLL[156]. However, dose-limiting toxicities of fostamatinib treatment include neutropenia, thrombocytopenia and diarrhoea. Other inhibitors of SYK, such as entospletinib, are being evaluated in preclinical and clinical studies.

BCL-2 inhibitors. Venetoclax is a small molecule that functions as a BH3 mimetic to inhibit BCL-2 (REF. 157). This drug is highly potent in inducing apoptosis in CLL cells, possibly by diminishing the capacity of BCL-2 to sequester the pro-apoptotic molecule BCL-2-interacting mediator of cell death (BIM; also known as BCL2L11)[158]. As such, venetoclax is effective in patients with relapsed and/or refractory disease[159] or in patients with relapsed disease and del(17p)[160]. Indeed, the overall response rate for patients with relapsed disease and del(17p) was 79%, with 8% achieving a complete response. In addition, the estimated 12-month PFS was 72% and overall survival was 87%. On the basis of these results, the FDA approved the use of venetoclax for patients with relapsed disease and del(17p). Ongoing studies have shown that venetoclax can be safely combined with rituximab or obinutuzumab. Moreover, studies are examining the use of venetoclax with or without an anti-CD20 monoclonal antibody, and with or without ibrutinib[161,162], which might provide higher response rates to therapy than that with venetoclax alone.

Toxicities of venetoclax include gastrointestinal disturbances, neutropenia and tumour lysis syndrome[159], which is characterized by hyperkalaemia, hyperuricaemia and/or azotaemia. Tumour lysis syndrome results from the rapid destruction of cancer cells and the release of their cellular contents into the blood. Tumour lysis syndrome typically occurs when initiating venetoclax therapy or when dosing is increased. Thus, patients start venetoclax with a low daily dose, which is escalated each week over 5 weeks to mitigate the risk of developing tumour lysis syndrome. Even with this strategy, patients who are at high risk for tumour lysis syndrome because of bulky lymphadenopathy and/or lymphocytosis of >25,000 cells per μl must be hydrated and closely monitored during therapy initiation and during dose escalation.

Table 1 | **Rai staging system**

Risk group	Clinical features	Median life expectancy*
Low risk (Rai stage 0/I)	Lymphocytosis without cytopenia, lymphadenopathy or splenomegaly	13 years
Intermediate risk (Rai stage II)	Lymphocytosis, lymphadenopathy and/or splenomegaly, but without cytopenia	8 years
High risk (Rai stage III/IV)	Lymphocytosis and cytopenia (a haemoglobin level of ≤11 g per dl and/or a platelet count of ≤100,000 cells per μl)	2 years

*These life-expectancy estimates are increasing with the advent of newer therapies.

Assessment of response

Historically, a favourable response to therapy has been defined as a partial remission or complete remission. Partial remission requires a 50% reduction in tumour bulk (for example, lymphadenopathy and splenomegaly), a 50% reduction in lymphocytosis, and platelet counts of >100,000 cells per μl (or 50% improvement over baseline) or a haemoglobin level of >11 g per dl (or 50% improvement over baseline) without requiring transfusions or exogenous growth factors[107]. Complete remission requires the normalization of blood counts, resolution in lymphadenopathy and splenomegaly, and normal marrow function. The use of CT to assess response in CLL is becoming more common, particularly in clinical trials. However, the benefit of using repeated CT scans to monitor disease is uncertain, and seems unlikely to change patient outcome. Because of the distinct pattern of response observed with BCR inhibitors, a new response category, namely, partial response with lymphocytosis, has been described. Partial response with lymphocytosis is defined as a >50% reduction in lymphadenopathy and splenomegaly, with persistent lymphocytosis; often the blood lymphocyte counts are equal to or greater than those observed prior to therapy.

In clinical trials, it is becoming more common to evaluate for MRD with ≥0.01% of CLL cells among the total population of mononuclear cells in the blood or marrow. MRD can be measured by flow cytometry or PCR with next-generation sequencing of the clonal

Table 2 | **Binet staging system**

Risk group	Clinical features	Median life expectancy*
Low risk (Binet stage A)	Less than three palpably enlarged sites‡ without cytopenia	13 years
Intermediate risk (Binet stage B)	Three or more palpably enlarged sites‡ without cytopenia	8 years
High risk (Binet stage C)	Cytopenia (a haemoglobin level of ≤10 g per dl and/or a platelet count of ≤100,000 cells per µl)	2 years

*These life-expectancy estimates are increasing with the advent of newer therapies. ‡There are five sites of lymphoid organs: cervical, axillary and inguinal nodes, the spleen and the liver.

immunoglobulin variable region gene rearrangements[163]. In most clinical trials for patients with CLL, particularly those conducted in Europe, evaluation of MRD has been performed by flow cytometry of mononuclear cells from the marrow aspirate (the preferred method) or from the peripheral blood. In the 6 months following anti-CD20 monoclonal antibody treatment, the assessment of MRD is more sensitive on the mononuclear cells of the marrow aspirate than on cells that are isolated from the blood, which will often lack detectable CLL even when they are readily found in the marrow. Beyond a complete response, the best predictor of long-term PFS and overall survival is the achievement of a complete remission without evidence for MRD.

Relapsed disease

The treatment landscape for relapsed and refractory CLL will be changing owing to the first-line approval of ibrutinib. Currently, most patients with relapsed or refractory disease receive chemoimmunotherapy. Standard salvage regimens include BCR inhibitors or BCL-2 inhibitors, particularly for patients with CLL and del(17p). For patients who received first-line BTK inhibitor therapy, salvage options include chemoimmunotherapy (fludarabine, cyclophosphamide and rituximab (or bendamustine with an anti-CD20 monoclonal antibody), PI3K inhibitor and an anti-CD20 monoclonal antibody[152], high-dose methylprednisolone and an anti-CD20 monoclonal antibody[164], or lenalidomide alone or with an anti-CD20 monoclonal antibody, although lenalidomide has not been approved for the treatment of patients with CLL by the FDA[165,166]. Treatment choice depends on individual patient characteristics and the intent of treatment. Scant data are available regarding the activity of small-molecule inhibitors in patients who are refractory to another small-molecule inhibitor; better efficacy is expected for patients who discontinued use of another small-molecule inhibitor due to intolerance[167]. Ibrutinib resistance is an adverse predictor of clinical outcome, particularly for patients who were previously exposed to chemoimmunotherapy. If a previously treated patient develops del(17p), or mutated *TP53*, treatment options include ibrutinib, venetoclax, or idelalisib and an anti-CD20 monoclonal antibody. The patient could also participate in a clinical trial. The preference for non-chemotherapy-based treatment should be driven by prior exposure to a small-molecule inhibitor and a review of the safety profile of the drug.

Maintenance therapy with an anti-CD20 monoclonal antibody after chemoimmunotherapy has been shown to prolong PFS, but not overall survival, and was associated with a significantly higher incidence of neutropenia and risk for infections[168]. This regimen is currently not considered the standard of care, but might be useful in patients with medical comorbidities that limit other treatment options.

Quality of life

Comorbidities

As CLL is a disease of the elderly population, assessing the effect of CLL on the patient's quality of life (QOL) and the coexisting comorbidities that occur in this patient population is important[169]. Awareness regarding the importance of a patient's QOL, not only during and after treatment but also during the watch-and-wait period, is increasing.

Until recently, few clinical trials included elderly or frail patients, who account for most patients with CLL. As such, the recommendations for therapy were largely based on results from clinical trials that were conducted with younger patients who could better tolerate combination drug therapies. Trials have moved away from using eligibility criteria based on age or creatinine clearance, to using more objective measures of fitness, such as the cumulative illness rating score, which can stratify patients for appropriate first-line or subsequent therapy. This has led to an increased number of published clinical trials targeting patients who would not be deemed fit for aggressive chemoimmunotherapy approaches. Importantly, this has also demonstrated that 'unfit' patients with CLL can be recruited to clinical trials in a timely manner.

Risk of other diseases

Patients with CLL have an increased risk of other medical conditions, such as infections, autoimmune disorders or secondary cancers, any one of which can result in substantial morbidity and mortality.

Infections. CLL is characterized by progressive defects in both cell-mediated and antibody-mediated immunity, including hypogammaglobulinaemia and B cell and T cell quantitative and functional defects[170]. The risk of infections increases with worsening hypogammaglobulinaemia. The degree of immune impairment worsens with disease progression and can be exacerbated by the immunosuppressive effects of purine analogue chemotherapy, anti-CD20 monoclonal antibodies or drugs that inhibit kinases involved in immune receptor signalling. Consequently, infectious complications represent a frequent cause of morbidity and mortality in patients with CLL.

Infections are typically bacterial and frequently involve the respiratory tract. Intravenous immunoglobulin replacement therapy can mitigate the risk of infection, particularly in patients with hypogammaglobulinaemia who have frequent infections or a severe life-threatening infection[171]. Immunoglobulin formulations that are administered subcutaneously seem to be

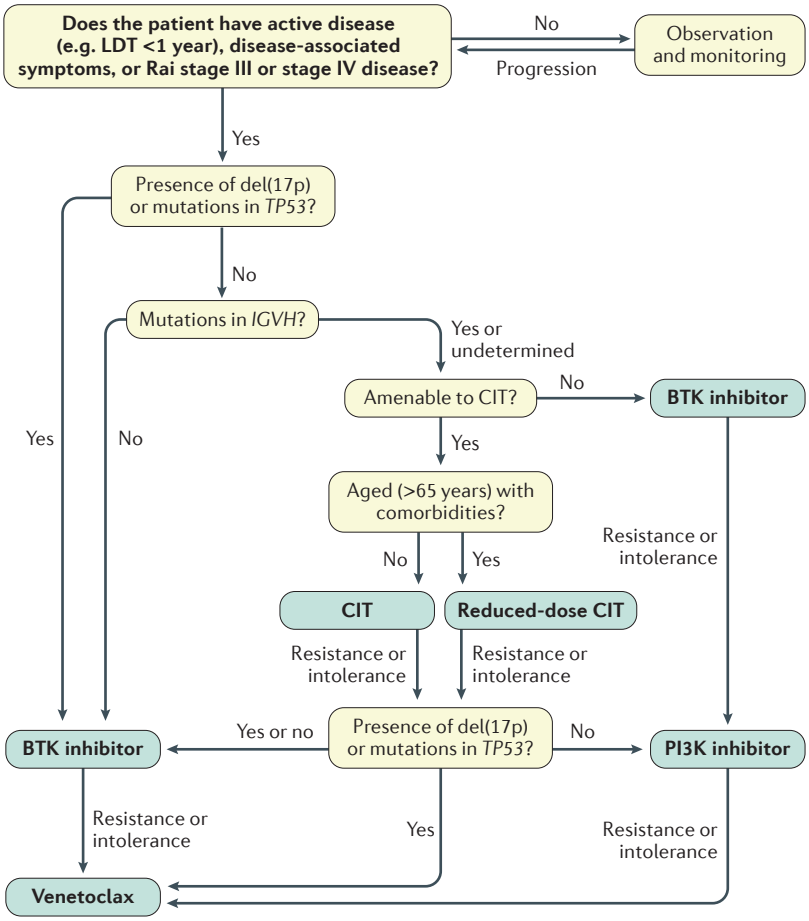


Figure 8 | **Management algorithm for patients with CLL.** Indications for therapy of patients with chronic lymphocytic leukaemia (CLL) include late-stage disease, evidence for rapid disease progression or disease-related symptoms. Patients with del(17p) or mutated *TP53* should be treated with therapy that does not require functional *TP53*, such as ibrutinib (a Bruton tyrosine kinase (BTK) inhibitor), given the relatively poor outcome for such patients with chemotherapy. For patients without del(17p) or known mutations in *TP53*, immunoglobulin heavy-chain variable region (*IGHV*) mutational status can help to define the treatment strategy; patients with unmutated *IGHV* could be considered for therapy with a BTK inhibitor (such as ibrutinib) and patients with mutated *IGHV* might be good candidates for chemoimmunotherapy (CIT), if amenable. Indeed, patients with mutated *IGHV* can have excellent outcomes with CIT regimens, such as fludarabine, cyclophosphamide and rituximab, with >50% of patients having a median progression-free survival of >10 years, including the potential for cure. If the patient is amenable to CIT, age, medical comorbidities and myeloid reserve should be taken into consideration. Patients >65 years of age commonly have medical comorbidities and are less able to tolerate myelosuppressive regimens, such as fludarabine, cyclophosphamide and rituximab. Thus, considerations should be given to using reduced dose or less myelosuppressive chemotherapy regimens, such as chlorambucil or reduced-dose bendamustine and an anti-CD20 monoclonal antibody for patients with limited myeloid reserve. Patients who either do not respond, have a poor tolerance to CIT or relapse following CIT, should be re-evaluated for del(17p) or *TP53* mutations. Patients who develop *de novo* del(17p) or *TP53* mutations, or have known del(17p) and/or *TP53* mutations, or who develop resistance or intolerance to ibrutinib, could be considered for therapy with idelalisib and rituximab or the BCL-2 inhibitor venetoclax. Patients treated with CIT who do not have del(17p) or *TP53* mutations could be considered for repeat CIT if their progression-free survival after CIT is >2 years and the patient has sufficient myeloid reserve. Such patients also might be treated with a BTK inhibitor or a phosphoinositide 3-kinase (PI3K) inhibitor, which also could be considered for patients who develop intolerance or resistance to therapy with ibrutinib. Patients who develop resistance or intolerance to inhibitors of BTK, PI3K and/or BCL-2 should be considered for clinical trials or alternative agents. LDT, lymphocyte doubling time.

as effective and might be less costly[172]. Unfortunately, randomized studies assessing the relative benefit of intravenous immunoglobulin replacement therapy versus prophylactic antibiotics in patients with CLL, hypogammaglobulinaemia and recurrent or serious infections have not been conducted. However, the development of another infection soon after completing a course of antibiotics, requiring repeated antibiotic therapy, is not uncommon in patients with CLL. These patients should be considered for immunoglobulin replacement therapy. The use of prophylactic antimicrobial agents to prevent opportunistic infections should be considered, particularly in patients undergoing therapy with drugs that might worsen immune function[173]. Finally, because of their suppressed immune function, patients should avoid having live vaccines, such as those used to vaccinate against shingles.

Autoimmune complications. Autoimmune complications are common in patients with CLL and occur in up to 25% of patients. Autoimmunity in CLL primarily targets the haematological lineages, resulting in autoimmune haemolytic anaemia, immune thrombocytopenic purpura, pure red cell aplasia or autoimmune granulocytopenia[174]. Spontaneous or drug-related autoimmune haemolytic anaemia is the most common autoimmune complication of CLL, the prevalence of which is related to disease stage and progression. For example, the prevalence of autoimmune haemolytic anaemia is 2.9% in patients with stable Binet stage A disease and >10% in patients with Binet stage B or stage C disease. Approximately 1–5% of patients with CLL develop clinically apparent immune thrombocytopenic purpura, which makes CLL the most common disease associated with this disorder in adults. Pure red cell aplasia, in which the marrow ceases to produce erythrocytes resulting in reticulocytopenia, occurs in <1% of patients[174]; diagnostic evaluation requires a marrow biopsy showing virtual absence of erythroid precursor cells without myelodysplasia, as well as the exclusion of viral infections that can impair erythropoiesis, such Parvovirus B19, Epstein–Barr virus, viral hepatitis B or hepatitis C and HIV infections. Even rarer is secondary autoimmune granulocytopenia, which occurs in <0.2% of patients[174]; the diagnosis requires a marrow biopsy showing maturation arrest at a late stage in granulocyte differentiation and exclusion of other causes of isolated acquired neutropenia, such as myelodysplasia, concomitant granular lymphocyte leukaemia or diseases that might cause secondary immune neutropenia, such as rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and related systemic autoimmune diseases.

No systematic controlled trials of treatment for autoimmune cytopenias in patients with CLL have been conducted. Corticosteroids remain the mainstay of initial treatment, but mycophenolate and thrombopoietin-like agents might be helpful for patients with immune thrombocytopenic purpura[175,176]. Second-line treatments include cyclosporine or rituximab. Splenectomy can be helpful in patients with severe or recurrent immune cytopenias who are good-risk surgical candidates,

Box 2 | **Molecular biology of Richter syndrome**

The lymphoma cells in Richter syndrome are malignant B cells that most often resemble those of non-germinal centre diffuse large B cell lymphoma (DLBCL), differing morphologically from the original chronic lymphocytic leukaemia (CLL) population. In addition, the lymphoma cells of over half the patients with Richter syndrome might not express CD5 or CD23, which are almost invariably expressed by CLL cells. Nevertheless, the DLBCL-like lymphoma in Richter syndrome often shares the same *IGHV-DJ* rearrangement as the original CLL clone[207]. As such, the lymphoma cells in Richter syndrome can express unmutated immunoglobulin heavy-chain variable region gene (*IGHV*), unlike *de novo* DLBCL, which virtually always expresses *IGHV* with somatic mutations. However, ~20% of the DLBCL-type Richter syndrome and ~50% of Hodgkin lymphoma-type Richter syndrome have *IGHV-DJ* rearrangements that differ from that of the original CLL clone, suggesting that these lymphomas might represent a *de novo* secondary malignancy; some of these seem to be associated with Epstein–Barr virus infection and may resemble post-transplant lymphomas, particularly in patients with severe disease-related immune dysfunction and/or treatment-related immune suppression[208].

Although the lymphoma cells of DLBCL-type Richter syndrome resemble those of *de novo* DLBCL, they have distinctive genetic differences[209]. Of DLBCL-type Richter syndrome lymphomas, ~60% have inactivating mutations and/or deletions in *TP53*, often with deregulation of *MYC*, which is observed in ~40% of cases; such deregulation is caused by translocations juxtaposing *MYC* to immunoglobulin loci, gene amplification of *MYC* at 8q24 or somatic mutations affecting *MYC trans*-regulatory factors, such as *NOTCH1*, which is mutated in ~30% of cases[210,211]. *CDKN2A*, which encodes p16, a negative regulator of cell cycle progression from G1 to S phase, is mutated and/or deleted in ~30–50% of cases, but rarely so in CLL or *de novo* DLBCL[209,211]. Finally, Richter syndrome lymphomas typically do not have mutations in genes encoding proteins that are involved in nuclear factor-κB signalling or in the transcriptional repressors *PRDM1/BLIMP1* or *BCL6*, which are common in *de novo* DLBCL.

but risks further impairment of immune function. Refractory autoimmune haemolytic anaemia or immune thrombocytopenic purpura might require treatment of the underlying CLL, preferably with therapy that does not substantially impair compensatory haematopoiesis.

Secondary cancers. Several large retrospective analyses have demonstrated that patients with CLL have an increased incidence of several secondary primary malignancies compared with an age-matched population, particularly non-melanoma skin cancers, but also for melanoma, sarcomas, and lung, renal and prostate cancers[177]. The immune deficiencies that are associated with CLL might contribute to this increased risk, but the malignancies observed do not mirror those in patients with other immune-deficiency diseases. Exceptions to this observation are Merkel cell carcinoma[178], which is associated with Merkel cell polyomavirus infection, and Bowen disease, which is an aggressive form of squamous cell carcinoma associated with human papillomavirus infection[179]. Although initial studies had suggested that the risk of secondary cancers was increased following chemotherapy, subsequent studies have suggested that the risk is similar in untreated patients who continue on watch and wait[180].

Prolymphocytic transformation. B cell prolymphocytic transformation is a rare event, occurring in <1% of patients. This disease is characterized by symptomatic splenomegaly, rapidly rising numbers of leukaemia cells in the blood, >55% of which have the morphology of a prolymphocyte on a blood smear. Diagnosis of prolymphocytic leukaemia is made by evaluation of the blood smear, immunophenotyping and molecular genetics. The clinical behaviour of prolymphocytic leukaemia is generally more aggressive than CLL, although some patients still might have indolent disease. Patients with prolymphocytic leukaemia are typically treated with combination purine analogue-based chemoimmunotherapy. However, drugs that inhibit BCR signalling, such as ibrutinib or idelalisib, might be effective in the management of some patients, especially those with del(17p) or inactivating mutations in *TP53*.

Richter syndrome. Richter syndrome is the transformation of CLL to an aggressive lymphoma, commonly DLBCL (BOX 2), classic Hodgkin lymphoma or an unusual histology of Hodgkin–Reed–Sternberg-like cells surrounded by CLL cells without the polymorphous reactive infiltrate of classic Hodgkin lymphoma[181]. Approximately 2–7% of patients with CLL develop Richter syndrome, with an incidence rate of ~0.5% per year of observation[182]. Richter syndrome may occur more frequently in patients with CLL cells that harbour *NOTCH1* mutations or that express certain stereotypical immunoglobulin molecules, particularly those with a heavy-chain variable region encoded by *IGHV4-39* and a heavy-chain third complementarity-determining region (HCDR3) encoded by *IGHD6-13* and *IGHJ5*, the so-called 'HCDR3 subset 8' (REF. 183).

Clinical suspicion of Richter syndrome is raised if a patient develops new or worsening symptoms, such as night sweats, fatigue and involuntary weight loss, a sharp increase in the levels of serum lactic dehydrogenase, and/or a rapidly enlarging lymph node (or nodes) or an extra-nodal lymphoid mass (or masses). PET imaging can be used to evaluate these patients[184], including directing where to perform a biopsy, which is required to establish the diagnosis. The mainstay of treatment is chemoimmunotherapy, although newer therapies are being investigated using some of the BCR inhibitors and/or BCL-2 inhibitors or immune checkpoint inhibitors. Nevertheless, the prognosis of patients with Richter syndrome generally is poor, particularly for those who are heavily pretreated for CLL and/or who have transformation involving lymphocytes that are clonally related to the underlying CLL[182]. Younger, fit patients who respond to induction therapy should be considered for allogeneic stem cell transplantation to prolong survival.

Acute leukaemia and myelodysplastic syndrome. Acute leukaemia and myelodysplastic syndrome are uncommon in CLL. Overall prognosis is poor and new treatment approaches are needed[185]. The rates of therapy-related acute myeloid leukaemia or myelodysplastic syndrome following purine analogue-based chemoimmunotherapy are ~5%, and are greatly increased in patients who undergo autologous stem cell transplantation. Studies are underway to evaluate whether the use of novel agents, which do not expose normal haematopoietic cells to genotoxic stress, will decrease the incidence of this serious complication.

Outlook

The outlook for patients with CLL has improved substantially over the past several years. Through research on the immune biology and genetics of CLL, patients can be stratified into subgroups with distinctive clinical features, which has improved our capacity to assess prognosis or govern therapy. However, an understanding of the mechanisms that contribute to immune dysfunction or how it contributes to autoimmune disease, such as autoimmune haemolytic anaemia, therapy resistance or therapy-related complications is unknown. Whether tyrosine kinase inhibitors can affect clonal evolution, induce and/or select for drug resistance, or can achieve complete responses if used earlier in the course of the disease is also unknown.

Future treatments

Several therapies are currently under preclinical and clinical investigation for the treatment of patients with CLL, including new drugs and treatment modalities that can modulate the immune system, and cell transplantation.

Immune-modulatory drugs. Immune-modulatory drugs, such as thalidomide and lenalidomide, are approved for the treatment of patients with multiple myeloma, mantle cell lymphoma or myelodysplastic disease. Although these drugs have clinical activity in patients with CLL, they have had limited application unless used in combination with an anti-CD20 monoclonal antibody[166,186]. In CLL, lenalidomide can induce the expression of $p21^{WAF1/CIP1}$, which inhibits cyclin-dependent kinase and CLL cell proliferation[187], and can improve immune synapse formation, potentially enhancing immune function[188]. In patients with CLL, lenalidomide can mitigate the severity of hypogammaglobulinaemia[189], but myelosuppression is a dose-limiting toxicity. Other dose-limiting toxicities associated with the use of lenalidomide, particularly as a first-line therapy, include tumour flare and tumour lysis syndrome. For unknown reasons, patients with CLL seem to be more sensitive to lenalidomide than patients with other haematological indications, mandating the use of low doses (for example, 2.5–5 mg per day) when initiating therapy. Low-dose aspirin is frequently used to mitigate the risk for thromboembolic complications that are associated with lenalidomide therapy.

Thalidomide has little activity in patients with CLL as a monotherapy, but has shown efficacy when combined with other drugs, such as anti-CD20 monoclonal antibodies[190]. Conversely, lenalidomide monotherapy has an overall response rate of 60% (15% complete response rate) as a first-line therapy and 40% (8% complete response rate) as a salvage treatment[165,189]. However, trials assessing the use of lenalidomide as a monotherapy or combination therapy have yielded mixed results. One phase II study reported that lenalidomide was well tolerated as initial therapy in patients >65 years of age, with an overall response rate of 65% and a complete response rate of 10%[189]. However, a multicentre phase III trial for the same patient population had to be terminated owing to an increased number of deaths in patients receiving lenalidomide compared with those receiving chlorambucil[191]. A 7-month treatment course with lenalidomide in combination with rituximab was well tolerated in a multi-institutional phase II study and yielded higher response rates; for patients <65 years of age, the overall response rate was 95%, with a 20% complete response rate, and for patients ≥65 years of age, the overall response rate was 78%, with an 11% complete response rate[166]. The PFS after completion of therapy was ~20 months for both groups. Therapy with an anti-CD20 monoclonal antibody 9 days before initiation of lenalidomide therapy also seems to mitigate the risk for tumour flare reaction[192,193]. The use of lenalidomide after chemoimmunotherapy has been evaluated in a phase III maintenance trial (Continuum Trial; ClinicalTrials.gov identifier: NCT00774345) and results are forthcoming. Ongoing trials are also examining the activity in CLL of a novel lenalidomide analogue, CC-122 (REF. 194).

Allogeneic stem cell transplantation. Allogeneic stem cell transplantation is a potentially curative strategy for patients with relapsed or refractory CLL, including patients with high-risk features such as del(17p). In two clinical studies with patients who lacked serious medical comorbidities and had a median age of 53 or 58 years, the PFS at 3–5 years was 40–50% and overall survival was 50–70%, but the non-relapse mortality at 3–5 years was 25–40%[195,196]. Research efforts are ongoing to develop better-tolerated cell-based therapy with a similar curative potential that can be used without the immunosuppression and associated long-term morbidity and mortality of allogeneic stem cell transplantation.

Donor availability, advanced patient age, associated toxicities of myelosuppression, graft-versus-host disease and impaired resistance to infections limit the application of allogeneic stem cell transplantation in patients with CLL. In addition, the advent of BCR signalling inhibitors and BCL-2 inhibitors provide multiple treatment options that afford well-tolerated, long-term disease control, making allogeneic stem cell transplantation the least desirable option for most patients. Ongoing discussion exists around who are the appropriate patients for allogeneic stem cell transplantation.

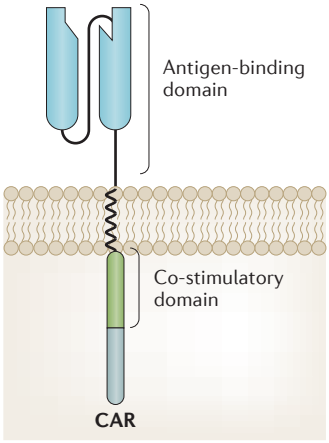
T cell therapy with chimeric antigen receptors. T cells can be modified *ex vivo* to express new surface receptors, known as chimeric antigen receptors (CARs), which have been engineered to target cancer cells, expanded *in vitro* and then reintroduced into the patient as a treatment for CLL (BOX 3).

Both normal and malignant B cells (including CLL cells) express surface CD19, which has been targeted with CAR technology. CD19-targeted CAR T cells have yielded long-term PFS and relapse-free survival durations in patients with CLL; in 14 patients with relapsed or refractory CLL, four patients achieved a complete response and four patients achieved a partial response[197]. None of the complete responders had MRD and none relapsed (a median follow-up of 19 months). However, the efficacy of CAR T cell therapy in patients with CLL

Box 3 | **Chimeric antigen receptors**

Chimeric antigen receptors (CARs) are composed of an antigen-binding domain, a stalk and transmembrane region, an intracellular co-stimulatory signalling domain and CD3ζ (see the figure)[212]. Co-stimulatory signalling domains include CD28 and CD137, which provide the 'second signal' to fully activate and expand T cells upon antigen binding.

Retrovirus vectors are used to introduce the CAR gene into T cells, which integrates into the genome of the T cell for stable expression. When the CAR T cells are exposed to the respective antigen, antigen binding triggers activation and expansion of the CAR T cells and eliminates the cells with target antigen. The binding domain of the CAR can be directed against any surface antigen.



has been modest compared with that in patients with acute lymphoblastic leukaemia; this might be owing to qualitative defects in the T cells of patients with CLL, who are generally older than patients with acute lymphoblastic leukaemia and already have immune dysfunction that reflects disease-associated anergy (see BCR and B cell signalling). Ibrutinib might partially correct some of these defects[198]. Larger phase II trials assessing the use of CAR T cell therapy for CLL are in development, including the use of CAR T cells that can target antigens other than CD19, such as ROR1 (REFS 199,200).

The major adverse effect of CAR T cell therapy is a cytokine release syndrome, which occurs as a result of CAR T cell activation, cytokine production and T cell expansion following target antigen encounter. Cytokine release syndrome is characterized by fever, hypotension and capillary leakage, but neurological toxicity, which can manifest as confusion and seizures, has also been observed in some of the treated patients. Cytokine release syndrome is associated with high cytokine levels, particularly IL-6, and can be managed with an IL-6-binding factor, such as tocilizumab, supportive measures, and glucocorticoids for severe cases. This syndrome seems to be proportionate to the antigen-bearing tumour burden, potentially making CAR T cell therapy more amenable to treatment of patients with MRD.

Immune checkpoint inhibitors. Immune checkpoints are proteins that are expressed on the surface of antigen-presenting cells that regulate the immune system by providing co-stimulatory or co-inhibitory signals to ligands expressed on T cells and other immune effector cells. The finding that cancer cells can evade immune detection and destruction by inhibiting T cells has led to the development of immune checkpoint inhibitors to treat solid tumours, Hodgkin lymphomas and non-Hodgkin lymphomas[201].

The immune checkpoint receptor PD-1, and its ligands PD-L1 and PD-L2, is the most important cognate receptor involved in the suppression of cellular immune activation. CLL cells express high levels of PD-L1 and PD-L2 and can suppress the responses of PD-1-expressing effector T cells[106], leading to an exhausted (that is, no longer functional) T cell phenotype. T cell exhaustion in CLL is also mediated in part by lymphocyte activation gene 3 protein and T cell immunoglobulin mucin receptor 3 (TIM3; also known as HAVCR2). TIM3 negatively regulates the function of type 1 helper T cells and type 1 $CD8^+$ T cells, by triggering cell death upon ligand binding. Other receptors on CLL cells have demonstrated negative immune feedback, including CD276, CD200 and TNF receptor superfamily member 14.

A partial list of immune checkpoint inhibitors that are being evaluated in the therapy of patients with CLL or other cancers include monoclonal antibodies against PD-1, cytotoxic T lymphocyte protein 4, B lymphocyte and T lymphocyte attenuator and its ligand TNF superfamily member 14, the adenosine A2A receptor, indoleamine 2,3-dioxygenase, the V-type immunoglobulin domain-containing suppressor of T cell activation, lymphocyte activation gene 3 protein and TIM3.

Preclinical studies in mouse models have demonstrated that checkpoint inhibitors can reactivate immune effector cells to have anti-leukaemia activity[202]. However, ongoing phase I/II trials of immune checkpoint inhibitors in patients with relapsed CLL have yet to show much clinical activity, possibly reflecting the highly immune suppressive nature of CLL cells and/or the 'exhausted' phenotype of T cells in patients with this disease.

Combination targeted therapy. We can now target the distinctive phenotypic or physiological features of CLL with targeted therapeutic agents, which have a higher therapeutic index than standard chemotherapy. Through the use of combination therapy, which targets different B cell survival signalling pathways and/or achieves better eradication of CLL cells, we might be able to define curative treatments for most patients with this disease.

Research on leukaemia cell survival signalling pathways, such as those governed by interactions between leukaemia cells and cells or secreted factors within the microenvironment (FIG. 4), might identify pathways that are not affected by BCR inhibitors. For example, BCR inhibitors, such as ibrutinib, cannot block ROR1-dependent WNT5A signalling, which enhances CLL cell proliferation, migration and survival[93]; as such, antibodies that block ROR1-dependent signalling could potentially have synergistic activity when used in combination with BCR inhibitors[203]. Furthermore, the interaction between CLL cells and accessory cells in the microenvironment might enhance CLL cell expression of anti-apoptotic proteins other than BCL-2, such as MCL1, thereby contributing to therapy resistance. As such, the therapeutic use of a selective BCL-2 antagonist, such as venetoclax, might be more effective when used in combination with BCL-2 inhibitors[161,162], which also interfere with the homing of CLL cells to the microenvironment. Conceivably, combination target therapy with agents that have synergistic activity will provide highly effective and potentially curative treatment of patients with CLL.

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Competing interests
J.G. has received honoraria for advisory boards from Roche, Genentech, AbbVie, Janssen, Pharmacyclics, Acerta, Gilead, TG Therapeutics and Unum. S.O. has acted as a consultant for Amgen and Celgene, is a scientific advisory board member for CLL Global Research Foundation and has received research support from Acerta, TG Therapeutics, Regeneron, Gilead, Pharmacyclics and ProNAi. K.R. is a member of the medical advisory board for Celgene, Roche/Genetech, Pharmacyclics and Gilead. T.J.K. has received honoraria for Ad boards, meetings, presentations and/or consulting from Gilead, Pharmacyclics, Celgene, Roche and AbbVie, and has received research funding from AbbVie and Celgene. C.J.W. is a co-founder and a member of the scientific advisory board of Neon Therapeutics. W.G.W., G.P., C.M.C. and F.K.S. declare no competing interests.

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