Abstract and Introduction

Abstract

Chronic lymphocytic leukaemia (CLL) has several unique features that distinguish it from other cancers. Most CLL tumour cells are inert and arrested in G0/G1 of the cell cycle and there is only a small proliferative compartment; however, the progressive accumulation of malignant cells will ultimately lead to symptomatic disease. Pathogenic mechanisms have been elucidated that involve multiple external (for example, microenvironmental stimuli and antigenic drive) and internal (genetic and epigenetic) events that are crucial in the transformation, progression and evolution of CLL. Our growing understanding of CLL biology is allowing the translation of targets and biological classifiers into clinical practice.

Introduction

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in the Western world. It is characterized by the accumulation of small B lymphocytes with a mature appearance in blood, bone marrow, lymph nodes or other lymphoid tissues. CLL has a distinctive immunological marker repertoire and can therefore be differentiated from other B cell lymphomas (Box 1).

Box 1. Definition, diagnostic criteria and cardinal features of CLL

<table>
<thead>
<tr>
<th>Chronic lymphocytic leukaemia (CLL) is a malignant lymphoproliferative disorder of mature B lymphocytes.</th>
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<tbody>
<tr>
<td>The progressive accumulation of monoclonal B lymphocytes leads to leukocytosis, lymphadenopathy, hepatosplenomegaly, bone marrow failure, recurrent infection and is sometimes associated with autoimmune disease (for example, haemolytic anaemia).</td>
</tr>
<tr>
<td>CLL can be diagnosed if the blood clonal B lymphocyte count is &gt;5,000/μL. Below this value monoclonal B lymphocytosis can be diagnosed in asymptomatic individuals without organ involvement.</td>
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<tr>
<td>The immunological profile of CLL cells is defined by weak surface membrane immunoglobulin (Ig) levels (most often IgM or both IgM and IgD) and expression of the B cell antigens CD23, CD19 and CD20 (weak), with co-expression of CD5. CLL cells are usually negative for cyclin D1 and CD10 expression, and there is also usually no or weak...</td>
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Registry data estimate the U.S. incidence of CLL to be 3.9 per 100,000 people, with a median age at diagnosis of 72 years (reviewed in Ref. [1]). The incidence rates in men are nearly twice as high as in women. CLL is less common among people of African or Asian origin. [1] There is substantial geographic variation in CLL incidence, with high incidences in North America and Europe. [1,2] Advanced age and a family history of leukaemia or lymphoma are additional risk factors. [1,2] Common symptoms include lymph node enlargement, constitutional symptoms and bone marrow failure. Most patients are asymptomatic at diagnosis. [3–6]

The understanding of the pathological mechanisms involved in CLL has helped to divide the disease into subgroups, and this has had a profound impact on prognostication and treatment. Major findings have included the demonstration that CLL can be subdivided into cases with or without somatic mutations in the variable regions of the immunoglobulin (Ig) heavy chain (IGHV) genes. [7–9] Similarly important was the finding that CLL cells have biased IGHV usage and carry stereotyped B cell receptors (BCRs), which suggests that antigenic drive contributes to CLL pathogenesis. [7,10–14] The understanding of transforming events in CLL continues to evolve as genomic aberrations, epigenetic modifications, gene mutations and deregulated microRNAs have been identified (Refs [15–19]) (Fig. 1). These factors are of pathogenic and prognostic relevance. [17,20–23] One of the most exciting aspects of this area of research is the translation of these findings into clinical practice. The evolving link between biology and clinical translation will be the focus of this Review.
Figure 1. **CLL pathogenic mechanisms and examples of targeted treatment options.**  

**a** The B cell receptor (BCR) is composed of two immunoglobulin (Ig) heavy and light chains (variable and constant regions), and CD79a and CD79b, which contain an intracellular activation motif that transmits signals to intracellular tyrosine kinases (for example, SYK and LYN). The ability of these kinases to activate downstream pathways varies in chronic lymphocytic leukaemia (CLL) subgroups and is correlated with Ig heavy chain variable region (IGHV) mutational status, zeta-associated protein 70 (ZAP70) and CD38 expression.\(^4,40,41\) These pathways could be targeted by small molecule inhibitors, the most promising of which might be SYK inhibitors.  

**b** Multiple epitopes on the CLL cell are targets for antibody (ab)-based therapies.  

**c** The most common genetic lesions in CLL include deletion of 13q14 and the downregulation of death-associated protein kinase 1 (DAPK1, a stress-activated tumour suppressor protein\(^18,99\)) by DNA methylation.\(^18\) miR-15a and miR-16-1 (encoded by genes located on 13q14) have been shown to target BCL2, and may increase its expression in CLL.\(^143\) This pathway can be targeted at multiple levels, including through using small molecule BH3 mimetics.\(^136\)  

**d** Stromal and T cell interactions also contribute to CLL pathogenesis. Although not fully understood, some drugs (immune-modulating drugs; Imids) in use in CLL have been shown to target the interaction with T cells.\(^109\)
Ag, antigen; BLNK, B cell linker protein; BTK, Bruton tyrosine kinase; CDK, cyclin-dependent kinase; CXCR4, chemokine receptor 4; HDAC, histone deacetylase; IL-4, interleukin 4; Me, methyl group; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T cells; PLC-γ, phospholipase C-γ; SDF1, stromal cell-derived factor 1; VEGFA, vascular endothelial growth factor A.

BCR Response and IGHV Mutation

The B cell response to antigen stimulation is mediated through the BCR in normal and malignant B cells. Each B cell displays a distinct BCR that is formed through variable combinations of V, D and J segments for the Ig heavy chain and V and J gene segments for the light chain. In addition to the combinatorial diversity of different segments, the BCR repertoire is increased by the introduction of somatic mutations through the somatic hypermutation (SHM) process during the germinal centre (GC) reaction ([Box 2](#)) (reviewed in Ref. [24]). Functional BCRs are expressed in most B cell lymphomas but BCR surface expression is usually weak in CLL (reviewed in Ref. [25]). In contrast to most other B cell lymphomas, CLLs that have mutated IGHV genes can be differentiated from those that have unmutated IGHV genes (the term IGHV is used here as usually only the heavy chain V genes are sequenced to assess the mutation status of a CLL, although the light chain V genes are also mutated in IGHV-mutated cases),[7–9] and the two groups follow a different clinical course with patients that have CLL with unmutated IGHVs showing poorer survival (Refs [8, 9, 26]) (Fig. 2). The 98% sequence homology of IGHVs in CLL with the germline copies of IGHVs that is typically used to define this separation is arbitrary, and 'grey-zone cases' (for example, those that have a IGHV homology of 97–98%) seem to have an intermediate prognosis.[27] Although the definition of CLLs with mutated and unmutated IGHVs rests on the presence or absence of clonal mutations in all the CLL cells of a case, some intraclonal diversification of IGHV genes has been reported.[28] However, the level of ongoing hypermutation is rather low, and this intraclonal diversification may be due to the antigenic stimulation of CLL cells, which can induce activation-induced cytidine deaminase (AID, also known as AICDA), the key enzyme for SHM. AID is indeed expressed in a subset of CLL cells, and AID-mediated DNA alterations may occur.[29]

**Box 2. Germinal centre reaction and normal B cell counterpart of CLL**

During T cell-dependent (TD) immune responses, antigen-activated B cells enter B cell follicles in secondary lymphoid organs and establish histological structures called germinal centres (GCs), in which these cells undergo massive clonal expansion. This proliferation takes place in the GC dark zone and is accompanied by the activation of somatic hypermutation (SHM), which introduces mutations at a very high rate into the immunoglobulin (Ig) variable region genes.[24] The mutated GC B cells then migrate to the GC light zone, which is rich in CD4+ T helper (T_H) cells and follicular dendritic cells (FDCs). By interacting with these cells, GC B cells that have acquired B cell receptor (BCR) affinity-increasing mutations are selected. GC B cells that have unfavourable mutations undergo apoptosis. Many GC B cells also undergo class switch recombination of their Ig heavy chain constant region genes. GC B cells intensively migrate between and in the dark and light zones.[142] Positively selected GC B cells usually undergo multiple rounds of proliferation, mutation and selection until they finally differentiate either into memory B cells or plasma cells and leave the GC.

Which is the cellular precursor of the chronic lymphocytic leukaemia (CLL) clone? The current knowledge supports a derivation of Ig heavy chain variable region (IGHV)-mutated CLLs from post-GC memory B cells, although a derivation from B cells that accumulate mutations in a T cell-independent (TI) immune response is also discussed (see the main text). IGHV-unmutated CLLs most likely derive from antigen-activated B cells, but it is unclear whether these are activated conventional naive B cells, CD5+ B cells or marginal zone (MZ)-like B cells. It is also unresolved whether this activation takes place as part of a TI or TD immune response; the TD immune response might involve an abortive GC reaction of autoreactive B cells.
Figure 2. CLLs with unmutated or mutated IGHV genes show markedly different biological and clinical behaviours. Based on the degree of somatic hypermutation, chronic lymphocytic leukaemias (CLLs) can be differentiated into those with mutated and those with unmutated immunoglobulin heavy chain variable region (IGHV) genes.\textsuperscript{[7–9]} The non-random use of IGHV genes and stereotyped B cell receptor (BCR) structures provides evidence for antigenic stimulation and drive. a | There are important biological differences between the two groups. IGHV-unmutated CLLs have higher levels of the protein tyrosine kinase zeta-associated protein 70 (ZAP70) and CD38 expression (shown in dark green) than IGHV-mutated CLLs (shown in light green). IGHV-unmutated CLLs activate key signal transduction pathways in response to BCR activation (for example, LYN, SYK, ERK and AKT) and show a different gene expression profile from IGHV-mutated CLLs.\textsuperscript{[31,32,145]} Reduced signalling in IGHV-mutated CLLs is indicated by grey text and dotted arrows. There is growing evidence that
IGHV-unmutated CLLs have significantly greater proliferative capacity than IGHV-mutated CLLs, and that greater capacity is supported by different telomere lengths. IGHV-unmutated CLLs have a greater likelihood of carrying and acquiring detrimental genetic lesions (11q23 and 17p13 deletion) than IGHV-mutated CLLs. The duration of the presence of the initial and the acquired aberrations are indicated in blue and red, respectively. Overall, 11 out of 64 patients showed clonal evolution, which was only observed in IGHV-unmutated cases. These biological differences may explain the different clinical courses associated with IGHV-unmutated and mutated CLLs. In a retrospective cohort from our centre (n = 365), the estimated median survival was not reached for the group with mutated IGHV versus 111 months for the group with unmutated IGHV (p < 0.001). These survival data are unpublished but are representative of published data (for example, Refs [26, 140]). Part b is reproduced, with permission, from Ref. [35] © (2007) European Hematology Association and Ferrata Storti Foundation. BLNK, B cell linker protein; BTK, Bruton tyrosine kinase; Ca, calcium; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T cells; PLC-γ, phospholipase C-γ; PKC, protein kinase C.

As expected based on the clinical diversity between CLLs with mutated and unmutated IGHV genes, there are important biological differences between the subgroups. These groups show different levels of the protein tyrosine kinase zeta-associated protein 70 (ZAP70) and CD38 expression, differential activity of key signal transduction pathways, different telomere lengths and a markedly different likelihood of carrying or acquiring detrimental genetic lesions (Refs [8, 26, 30–35]) (Fig. 2). After BCR stimulation in CLL, key molecules of the BCR signalling cascade, such as SYK and ZAP70 are recruited, resulting in the phosphorylation of B cell linker protein (BLNK), a central node for intracellular signalling (reviewed by Kipps [36]). This pathway is more readily activated in CLL cells that express ZAP70 and have unmutated IGHVs. IGHV-mutated CLLs show weak BCR signalling and are relatively anergic (Refs [37–41]) (Fig. 1, Fig. 2).

The structure of the BCR is different between IGHV-mutated and unmutated CLLs. A higher proportion of IGHV-unmutated CLL cases carry stereotyped rearrangements of the V, D and J segments that have very similar complementarity-determining region (CDR) 3 regions. IGHV-unmutated CLLs are also characterized by stereotyped light chains and biased somatic mutation patterns. The striking degree of structural restriction of the entire BCR in CLL suggests that common antigens are recognized by CLL cells and supports the hypothesis that an antigen-driven process contributes to CLL pathogenesis. More than 20% of CLL cases carry stereotyped BCRs and approximately 1% carry virtually identical Igs, which is particularly striking because it would be statistically unexpected to find 2 cases with such similar BCRs in 1 million patients. In CLLs with unmutated IGHVs the BCR is usually polyreactive to autoantigens derived from endogenous or exogenous proteins or lipids generated by, for example, oxidative stress.

CLLs that have mutated IGHVs show more restricted antigen binding with oligo- or monoreactive BCRs. Stereotyped BCRs are less likely to occur in mutated-IGHV CLLs. However, if BCR sequences that have IGHV mutations (which are non-autoreactive) are reverted to their unmutated IGHV counterparts, they become auto- and polyreactive, suggesting that IGHV-unmutated and mutated CLL cases may originate from a common precursor with autoreactivity. However, other theories propose that IGHV-unmutated and mutated CLL have different cells of origin (discussed below).

The role of antigens in CLL pathogenesis is further underlined by the fact that the presence of stereotyped BCRs seems to affect prognosis. This has been most convincingly demonstrated for a specific IGHV subgroup, the IGHV3-21 cases, but is likely to exist in other subgroups. [10,12,13,46,50,51]

IGHV1-69 was among the first genes that were shown to be overrepresented in patients that have CLL with unmutated IGHV genes. In these patients there was the use of specific diversity and joining gene segments, a longer than average CDR3 and certain common amino acid motifs. [7,52,53] The use of the IGHV3-21 gene segment was associated with highly restricted Ig heavy and light chain use (IGKV/IGLV) and an aggressive clinical course that was independent of the IGHV mutation status. Both IGHV1-69 and IGHV3-21 have the highest frequencies (>40%) of stereotyped VHδH rearrangements. [13] The correlations between clinical course and IGHV gene mutation and/or other biological
parameters are not absolute, and antigens seem to have a crucial role.

The role of IGHV mutation status in guiding therapy is currently unresolved and treatment decisions should not be based on it outside of clinical trials.\textsuperscript{[3,54,55]} Future treatment strategies may target deregulated signalling pathways (such as SYK, nuclear factor-κB (NF-κB) or ERK) specifically in IGHV-unmutated or mutated CLLs.\textsuperscript{[36]} An attractive translation of the above findings would also be to target antigenic drive; for example, by reducing antigen exposure as has been documented for mucosa-associated lymphoid tissue lymphomas. However, this strategy will be difficult, given the frequent autoreactivity of BCRs with the constant presence of autoantigens.\textsuperscript{[49,56,57]}

**Normal B Cell Counterpart**

The cellular origin of CLL cells is still debated. Originally, it was thought that CLL derives from CD5\textsuperscript{+} B cells because CLL cells invariably express the CD5 antigen and certain mouse strains regularly develop CLL-like malignancies from an accumulation of CD5\textsuperscript{+} B cells with increasing age.\textsuperscript{[58]} Although murine CD5\textsuperscript{+} B cells are established as a distinct B cell subset (B1a B cells) that has self-replenishing features, a propensity to produce autoreactive antibodies and functions mainly in T-independent (TI) immune responses,\textsuperscript{[59]} it is unclear whether they are analogous to human CD5\textsuperscript{+} B cells. Indeed, human CD5\textsuperscript{+} B cells are mostly unresponsive to TI antigens and rarely produce autoreactive antibodies.\textsuperscript{[47,48]} A CD5\textsuperscript{+} B cell origin of CLL was further questioned when it was shown that approximately half of CLL cases carry somatically mutated IGHV genes,\textsuperscript{[8,9]} whereas normal CD5\textsuperscript{+} B cells rarely have IGHV mutations.\textsuperscript{[60]}

As SHM was thought to be strictly linked to GC B cells, CLLs that have mutated IGHV genes were proposed to be derived from B cells that had undergone a GC reaction; that is, post-GC memory B cells. Because CLLs are mostly derived from non-class switched B cells, IGHV-mutated CLLs would derive from IgM\textsuperscript{+}IgD\textsuperscript{+}CD27\textsuperscript{+} B cells that carry mutated IGHV genes and may be non-class switched memory B cells.\textsuperscript{[61]} However, it is controversial whether IgM\textsuperscript{+}IgD\textsuperscript{+}CD27\textsuperscript{+} B cells are post-GC B cells, or whether they acquire their IGHV mutations during TI immune responses that do not involve the GC or during a primary, antigen-independent BCR diversification process.\textsuperscript{[62]} IgM\textsuperscript{+}IgD\textsuperscript{+}CD27\textsuperscript{+} peripheral blood B cells seem to be closely related to splenic marginal zone B cells, and these cells play an important part in such TI immune responses.\textsuperscript{[63]} Consequently, IGHV-mutated CLLs were proposed to derive from marginal zone-like B cells that underwent SHM during TI immune responses.\textsuperscript{[47]} It is, however, still unclear where this process happens as AID, which is essential for SHM, is not expressed in marginal zone B cells.\textsuperscript{[63]}

Several findings argue that IGHV-mutated CLLs are derived from post-GC B cells. First, when global gene expression profiles of CLL cells were compared to naive, GC, memory and cord blood CD5\textsuperscript{+} B cells, it was shown that IGHV-mutated CLLs are most similar in their gene expression patterns to memory B cells\textsuperscript{[31]} (however, a caveat of this analysis is that a mixture of class-switched memory B cells and IgM\textsuperscript{+}IgD\textsuperscript{+}lowCD27\textsuperscript{+} B cells were used as memory B cells\textsuperscript{[61]}). Second, approximately one-third of IGHV-mutated CLL cases carry mutations in \textit{BCL6}, which is the master regulator of the GC B cell differentiation programme.\textsuperscript{[64,65]} \textit{BCL6} is targeted by the SHM machinery, although at an approximately 50-fold lower level than IGHV genes.\textsuperscript{[66]} As SHM is strictly dependent on high levels of target gene transcription\textsuperscript{[67]} and, as high-level \textit{BCL6} transcription is restricted to GC B cells,\textsuperscript{[68]} \textit{BCL6} should remain unmutated in B cells that are undergoing IgV gene hypermutation in a TI immune response. Therefore, CLL cases with \textit{BCL6} mutations are most likely derived from B cells that have experienced the GC processes. Moreover, as the frequency of IGHV gene-mutated CLLs that have mutated \textit{BCL6} is the same as the frequency of classical post-GC B cells with \textit{BCL6} mutations (30%),\textsuperscript{[64–66]} most, if not all, CLLs that have mutated IGHV genes may derive from precursors that underwent SHM in the GC. Third, a subset of CLLs expresses IgG, indicating that these cases derive from class-switched B cells. These CLLs mostly express IgG1 or IgG3 subclasses, which are typical of GC-associated class switching, whereas they rarely express IgG2 (which is typical of class switching in TI immune responses).\textsuperscript{[69,70]} Fourth, we recently showed that IgM\textsuperscript{+}IgD\textsuperscript{+}CD27\textsuperscript{+} B cells are often clonally related to classical class-switched memory B cells and derive from common GC B cell clones, further reinforcing the notion that at least a large fraction of these IGHV-mutated IgM\textsuperscript{+}IgD\textsuperscript{+} B cells descend from GC B cells.\textsuperscript{[71]}
CLLs that have unmutated IGHVs also surprisingly showed a gene expression pattern that was more similar to post-GC memory B cells than to naive or CD5⁺ B cells, so it was speculated that IGHV-unmutated CLLs may also derive from memory B cells.[31] It has, however, recently been shown that a substantial fraction of human CD5⁺ B cells are transitional B cells.[72] Consequently, the potential similarities between CLL cells and mature CD5⁺ B cells might have been blurred in the gene expression studies by using a mixture of transitional and mature CD5⁺ B cells. Nevertheless, there is evidence that GC reactions generate some memory B cells that lack somatic mutations.[71] Importantly, many IGHV-unmutated CLLs express poly- and autoreactive antibodies,[49,56,57] and the CLL cells show an activated phenotype.[33] This evidence strongly argues that IGHV-unmutated CLLs are derived from antigen-experienced B cells. The antigenic specificities of these B cells seem to include both TI and T-dependent (TD) (auto) antigens.[49,56,57] Concerning TD antigens, there are some indications that autoreactive B cells are prevented from undergoing full GC reactions.[73] Therefore, one may speculate that IGHV-unmutated CLLs derive from antigen-stimulated B cells, including both TI and TD stimulations (see the figure in Box 2). As these B cells are chronically stimulated because of their autoreactivity, but may be prevented from undergoing apoptosis owing to their acquisition of primary transforming events, the B cells might acquire features of antigen-experienced memory B cells without undergoing a GC reaction.

Box 2. Germinal centre reaction and normal B cell counterpart of CLL

| During T cell-dependent (TD) immune responses, antigen-activated B cells enter B cell follicles in secondary lymphoid organs and establish histological structures called germinal centres (GCs), in which these cells undergo massive clonal expansion. This proliferation takes place in the GC dark zone and is accompanied by the activation of somatic hypermutation (SHM), which introduces mutations at a very high rate into the immunoglobulin (Ig) variable region genes.[24] The mutated GC B cells then migrate to the GC light zone, which is rich in CD4⁺ T helper (T_H) cells and follicular dendritic cells (FDCs). By interacting with these cells, GC B cells that have acquired B cell receptor (BCR) affinity-increasing mutations are selected. GC B cells that have unfavourable mutations undergo apoptosis. Many GC B cells also undergo class switch recombination of their Ig heavy chain constant region genes. GC B cells intensively migrate between and in the dark and light zones.[142] Positively selected GC B cells usually undergo multiple rounds of proliferation, mutation and selection until they finally differentiate either into memory B cells or plasma cells and leave the GC. |
| Which is the cellular precursor of the chronic lymphocytic leukaemia (CLL) clone? The current knowledge supports a derivation of Ig heavy chain variable region (IGHV)-mutated CLLs from post-GC memory B cells, although a derivation from B cells that accumulate mutations in a T cell-independent (TI) immune response is also discussed (see the main text). IGHV-unmutated CLLs most likely derive from antigen-activated B cells, but it is unclear whether these are activated conventional naive B cells, CD5⁺ B cells or marginal zone (MZ)-like B cells. It is also unresolved whether this activation takes place as part of a TI or TD immune response; the TD immune response might involve an abortive GC reaction of autoreactive B cells. |
Taken together, although the cellular origin of CLL cells has not been finally clarified, there is strong evidence that IGHV-mutated CLLs (mostly) stem from antigen-experienced post-GC memory B cells. IGHV-unmutated CLLs also originate from antigen-experienced B cells that acquire features of memory B cells. Whether CLL cells derive from a particular subset of B cells (naive, marginal zone or CD5+) is less clear. As some features of CLL cells resemble the still enigmatic regulatory B cells (for example, the expression of CD25 and the secretion of interleukin 10 (IL-10)), the possibility that CLL cells are derived from such regulatory B cells is also currently being discussed.[74]

**MBL, a CLL Precursor State?**

Clonal B cell populations with a CLL immunophenotype have been detected in approximately 3.5% of healthy individuals who have normal or slightly increased lymphocyte counts (monoclonal B cell lymphocytosis; MBL).[75] The occurrence of such clones increases with age, (8% aged >70).[75] Using more sensitive multicolour flow cytometry, MBL was detectable in 12% of healthy individuals (aged >40).[76] In most instances the CLL-like clones carry somatically mutated IGHV genes, which suggests that MBL is predominantly a precursor of IGHV-mutated CLL.

Is MBL a precursor state of CLL? In favour of this idea is the fact that CLL-like B cells appear at an increased frequency in relatives of patients with CLL, indicating a common genetic predisposition for such clonal expansions.[77] Furthermore, a recent study suggested that virtually all cases with CLL are preceded by MBL.[78] Most important is the finding that MBL clones often carry genetic lesions that are typical for CLL, such as the 13q14 deletion[79] (discussed below). This shows that MBL is often not simply an (age-related) expansion of normal B cells, but that the clones have already acquired initial genetic lesions, which are a hallmark of a pre-malignant tumour precursor cell. Regarding other features, however, at first glance MBL may not resemble CLL. MBL clones carry somatically mutated IGHV genes much more frequently than CLL,[78,79] and it has been reported that the IGHV gene use in MBL is untypical for CLL and that stereotyped BCRs are rare.[80] However, other studies have reported the frequent use of IGHV genes that are typical of CLL in MBL.[78,79] The overrepresentation of B cell clones that have mutated IGHV genes in MBL might be related to[81]
the more aggressive behaviour of IGHV-unmutated CLLs, which might not persist as long in a stable MBL stage.

In conclusion, although there are still unresolved issues about MBL, several key features of MBL support the idea that it is a precursor stage of CLL (with mutated IGHVs). It must, however, be stressed that only a small fraction of MBL will ever progress to CLL, as the frequency of MBL is approximately 100-fold higher than the frequency of CLL.\[81\]

Genomic Aberrations and Gene Mutations

Approximately 80% of CLL cases show aberrations in a few recurrently affected chromosomal regions (Ref. \[22\]) (Table 1). IGHV-mutated and unmutated CLLs share genomic aberrations, but the incidence of high-risk aberrations is higher in CLLs that have unmutated IGHVs.

Table 1. Summary of genetic profiles: different clinical CLL cohorts and MBL

<table>
<thead>
<tr>
<th>IGHV mutation status or genetic aberration</th>
<th>MBL (%)[79]</th>
<th>MBL or lymphocytosis (%)[79]</th>
<th>Early-stage CLL (%)[150]</th>
<th>Unselected (%)[22,26]</th>
<th>At first-line treatment (%)[150]</th>
<th>Refractory CLL (%)[149,151]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IGHV mutations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutated IGHV</td>
<td>85</td>
<td>90</td>
<td>59</td>
<td>44</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>Unmutated IGHV</td>
<td>15</td>
<td>10</td>
<td>41</td>
<td>56</td>
<td>69</td>
<td>76</td>
</tr>
<tr>
<td><strong>Copy number changes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deletion 13q14</td>
<td>39</td>
<td>58</td>
<td>40*</td>
<td>36*</td>
<td>34*</td>
<td>22*</td>
</tr>
<tr>
<td>Trisomy 12q13</td>
<td>18</td>
<td>21</td>
<td>13</td>
<td>16</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Deletion 11q23†</td>
<td>0</td>
<td>6</td>
<td>10</td>
<td>18</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Deletion 17p13†</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td><strong>Mutation of genes other than IGHV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 mutation†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>8–10 (REFS[23, 129])</td>
<td>8-12[</td>
<td></td>
</tr>
</tbody>
</table>

*Deletion of 13q14 that occurs as the sole abnormality in CLL or MBL when assessed by fluorescence in situ hybridization. †17p13 and 11q23 deletions and mutation of TP53 occur at an increased incidence as disease advances. Even if these occur as secondary genetic lesions, they have a profound effect on the clinical course and outcome of disease. §High-risk genetic aberrations can also be found in early-stage and untreated patients. Unpublished data from our group (the German CLL Study Group (GCLLSG) CLL4 and CLL8 trials). CLL, chronic lymphocytic leukaemia; IGHV, immunoglobulin heavy chain variable region genes; MBL, monoclonal B cell lymphocytosis; ND, not determined.

Deletions in Band 13q14

Deletion of 13q14 is the structural aberration most frequently found in CLL\[22\] CLLs that have a 13q14 deletion as the sole abnormality have a favourable course of disease (Ref. \[22\]) (Table 2). Various groups have attempted to identify a tumour suppressor gene in this region. No inactivation of candidate genes by mutation has been demonstrated, but a complex epigenetic regulatory tumour suppressor mechanism has been described that controls the expression of the
whole region (also in cases without deletion of 13q14).[82]

Table 2. Biological and clinical impact of common genomic aberrations in CLL

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Incidence (%)[22]</th>
<th>Genes involved†</th>
<th>PFS (months)[22]</th>
<th>OS (months)[22]</th>
</tr>
</thead>
<tbody>
<tr>
<td>No aberration</td>
<td>18</td>
<td>?</td>
<td>49</td>
<td>111</td>
</tr>
<tr>
<td>Deletion 13q14</td>
<td>55 (36)*</td>
<td>*mir-15a, mir-16-1 and non-coding RNAs[15,82,143]</td>
<td>92*</td>
<td>133*</td>
</tr>
<tr>
<td>Deletion 11q23</td>
<td>18</td>
<td>*ATM, ?[19–21,89,132]</td>
<td>13</td>
<td>79</td>
</tr>
<tr>
<td>Trisomy 12q13</td>
<td>16</td>
<td>Gene dosage of unknown genes,[88] ?</td>
<td>33</td>
<td>114</td>
</tr>
<tr>
<td>Deletion 17p13</td>
<td>7</td>
<td>*TP53(REFS[17, 23])</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>t14q32</td>
<td>4</td>
<td>IgH, ?</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

*Deletion of 13q14 as the sole abnormality when assessed by fluorescence in situ hybridization.†Confirmed or potentially important pathways that are targeted by these aberrations are included.§The clinical impact of these aberrations is shown by the progression-free and overall survival data from a previously published series.[22] ATM, ataxia telangiectasia-mutated; CLL, chronic lymphocytic leukaemia; IgH, immunoglobulin heavy locus; ND, not determined; OS, overall survival; PFS, progression-free survival (time to first treatment).

Two microRNA genes, *mir-15a* and *mir-16-1*, located in the crucial 13q14 region have been implicated in CLL pathogenesis (Refs[15, 16, 83]) (Fig. 1; Table 2). A mouse model with a targeted deletion of the *mir-15a–mir-16-1* locus recapitulates many features of CLL (U. Klein and R. Dalla-Favera, personal communication). This suggests that miR-15a and miR-16-1 have a direct pathogenetic role in CLL.

Table 2. Biological and clinical impact of common genomic aberrations in CLL

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Incidence (%)[22]</th>
<th>Genes involved†</th>
<th>PFS (months)[22]</th>
<th>OS (months)[22]</th>
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<tbody>
<tr>
<td>No aberration</td>
<td>18</td>
<td>?</td>
<td>49</td>
<td>111</td>
</tr>
<tr>
<td>Deletion 13q14</td>
<td>55 (36)*</td>
<td>*mir-15a, mir-16-1 and non-coding RNAs[15,82,143]</td>
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<tr>
<td>Deletion 11q23</td>
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</table>
Deletions at 13q14 occur at high frequencies in other lymphomas and solid tumours, and a recent study has implicated miR-15a and miR-16-1 in the pathogenesis of prostate cancer through their targeting of cyclin D1 and WNT3A, which promote survival and proliferation.\[84]\n
Deletions of ATM (11q22–q23)

Although they are rarely found in early-stage disease, approximately one-quarter of patients with advanced CLL have 11q23 deletions (Table 1). Correspondingly, patients who have 11q23 deletions have a more rapid disease progression and extensive lymphadenopathy.\[22,54,55,85]\n
In studies that aimed to delineate the 11q23 deletions in CLL, all aberrations affected a minimal consensus region in chromosome bands 11q22.3–q23.1. This region harbours the ataxia telangiectasia-mutated (\textit{ATM}) gene in almost all cases. ATM mutations have been shown to be present in 12% of all patients with CLL and in approximately one-third of the cases with a 11q23 deletion.\[20]\n
The ATM protein kinase is a central component of the DNA damage pathway and mediates cellular responses to DNA double-strand breaks (DSBs). ATM deficiency leads to ataxia–telangiectasia, which is characterized by extreme sensitivity to irradiation, genomic instability and a predisposition to lymphoid malignancies.\[86]\n
ATM activates cell cycle checkpoints, can induce apoptosis in response to DNA breaks and functions directly in the repair of DNA DSBs by maintaining DNA ends in repair complexes.\[87]\n
Other disease-associated genes in 11q22–q23 have been investigated, and a gene dosage effect has been documented. The pathogenic role of other genes in the region remains unresolved (Refs\[88, 89]\) (Table 2).

### Table 1. Summary of genetic profiles: different clinical CLL cohorts and MBL

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Trisomy 12 is among the most frequent aberrations in CLL (10–20%) (Table 1), but the genes involved in the pathogenesis of CLL with trisomy 12 are unknown. A previously described association with poor outcome has not been confirmed.

Consistent with these observations, the incidence of trisomy 12 does not increase with advanced stage or progression to refractory disease (Table 1). Fluorescence in situ hybridization. 17p13 and 11q23 deletions and mutation of TP53 occur at an increased incidence as disease advances. Even if these occur as secondary genetic lesions, they have a profound effect on the clinical course and outcome of disease. High-risk genetic aberrations can also be found in early-stage and untreated patients. Unpublished data from our group (the German CLL Study Group (GCLLSG) CLL4 and CLL8 trials). CLL, chronic lymphocytic leukaemia; IGHV, immunoglobulin heavy chain variable region genes; MBL, monoclonal B cell lymphocytosis; ND, not determined.

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Trisomy 12

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**Mutation of genes other than IGHV**

| TP53 mutation‡ | ND | ND | ND | 8–10 (REFS[23, 129]) | 8-12|| 37 |

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**Mutation of genes other than IGHV**

| TP53 mutation‡ | ND | ND | ND | 8–10 (REFS[23, 129]) | 8-12|| 37 |

*Deletion of 13q14 that occurs as the sole abnormality in CLL or MBL when assessed by fluorescence in situ hybridization. ‡17p13 and 11q23 deletions and mutation of TP53 occur at an increased incidence as disease advances. Even if these occur as secondary genetic lesions, they have a profound effect on the clinical course and outcome of disease. §High-risk genetic aberrations can also be found in early-stage and untreated patients. ||Unpublished data from our group (the German CLL Study Group (GCLLSG) CLL4 and CLL8 trials). CLL, chronic lymphocytic leukaemia; IGHV, immunoglobulin heavy chain variable region genes; MBL, monoclonal B cell lymphocytosis; ND, not determined.
Deletions in Band 17p13 or TP53 Mutations

Deletion of 17p13 is found in 4–9% of CLLs at diagnosis or at initiation of the first treatment.\cite{17,22,54,55} Although the 17p13 deletion usually encompasses most of the short arm of chromosome 17p, the deletion always includes band 17p13, where the tumour suppressor \textit{TP53} (which encodes p53) is located (Fig. 3). Among CLL cases that have monoallelic 17p13 deletions, the majority show mutations in the remaining \textit{TP53} allele (>80%).\cite{23,90} Among cases without 17p13 deletion, \textit{TP53} mutations are much rarer\cite{23} (see below).
Figure 3. TP53 mutations in CLL. Comparison of overall survival stratified by the absence or presence of 17p13 deletions and TP53 mutations. Patients with a 17p13 deletion or TP53 mutation have a poor outcome and are therefore prime candidates for experimental strategies with novel agents and stem cell transplantation.\[126] b 17p13 deletion is invariably associated with loss of TP53 as confirmed by fluorescence in situ hybridization (FISH) (‘+’ denotes a TP53 deletion). The red bars indicate the extent of the 17p13 deletion, which often includes most of the chromosome 17 short arm (data are unpublished but are representative of published results\[148]). c Multiple genomic aberrations target the p53 pathway in chronic lymphocytic leukaemia (CLL). The figure shows a simplified schematic of the p53 pathway and response to DNA damage. In the absence of stress, p53 levels are low as p53 levels are partly mediated through the ubiquitin ligase MDM2, which binds to p53 and promotes its degradation. MDM2 is also induced by p53 in a regulatory loop. MDM2 is located on chromosome 12, so CLL cases with
trisomy 12 have low p53 levels and favourable overall survival. In response to stress (for example, DNA damage), p53 is activated through various pathways, including ataxia telangiectasia-mutated (ATM). ATM is deleted in approximately 20% of CLL cases and is associated with a poor outcome. TP53 loss or mutation is central to refractory CLL. The microRNA miR-34a is associated with p53 defects and refractory CLL. Part a is reproduced, with permission, from Ref. [23] © (2008) American Society of Hematology. PUMA, p53 upregulated modulator of apoptosis (also known as BBC3).

Recurrent Translocations

In contrast to other types of leukaemia or B cell lymphoma in which specific and recurrent Ig locus-associated translocations deregulate known oncogenes, recurrent balanced translocations are rare in CLL. As many Ig-associated chromosomal translocations happen as errors during SHM or class-switch recombination, the lack of such translocations may be easily understood in non-class-switched IGHV-unmutated CLLs, in which the precursors have apparently not been exposed to these Ig gene-remodelling processes. However, this argument does not hold true for IGHV-mutated CLLs, in which the precursors were exposed to SHM and, in a fraction of cases, also had undergone class switching. The lack of Ig-associated translocations may support the notion that CLL is derived from post-GC B cells, in which SHM and class switching are silenced. However, most CLLs also lack translocations that are associated with errors during V(D)J recombination — a process that is active in the CLL precursor cells. Therefore, the lack of frequent Ig locus-associated translocations may also reflect the fact that the specific combination of transforming events that are involved in CLL pathogenesis does not include an oncogene that is a typical target for Ig-associated chromosomal translocations.

A recent study of metaphases from CLL cells suggested that (mostly unbalanced) translocations occurred in 34% of CLLs. In a larger series of over 500 patients, genomic aberrations (primarily genomic imbalances) were detected in 83% of cases by chromosome banding and in 78% of cases by fluorescence in situ hybridization, by screening for a limited number of aberrations. Recurrent reciprocal translocations were rare and mostly targeted the IgH locus in band 14q32 or the 13q14 region with the concomitant loss of genomic material.

Genetic Susceptibility to CLL

Approximately 5–10% of patients with CLL report a family history of leukaemia and lymphoma. The relative risk of first-degree relatives with CLL has been estimated to be 7.5-fold in the United States and Europe. MBL has been found in 13.5–18% of first-degree relatives. Recently, a genome-wide association study identified 6 previously unreported 'CLL risk loci' at bands 2q13, 2q37.1, 6p25.3, 11q24.1, 15q23 and 19q13.32 (Ref. [95]). These data provide the first evidence for the existence of common, low-penetrance susceptibility loci for CLL. The strongest evidence for an association between CLL and a particular gene was found with linkage disequilibrium at 6p25.3, which encompasses interferon regulatory factor 4 (IRF4). IRF4 is particularly interesting in the context of B cell lymphomas because it is a key regulator of B lymphocyte development and proliferation and has important functions in the GC reaction.

Epigenetic Alterations in CLL Pathogenesis

Global and gene-specific aberrant DNA methylation has been detected in CLL. An analysis of the global methylation profile in CLL found that 2.5–8.1% of the CpG islands in CLL samples were aberrantly methylated. Aberrant methylation has been described for genes that are specifically deregulated in CLL (for example, hypomethylation of BCL2 and T cell leukaemia/lymphoma 1 (TCL1)), and the expression levels of ZAP70 correlate closely with the methylation of specific CpG islands in ZAP70 (Ref. [98]).

The loss or reduced expression of death-associated protein kinase 1 (DAPK1) owing to a rare mutation was reported in a large CLL kindred. However, almost all sporadic CLL cases also show epigenetic silencing of DAPK1 by promoter methylation, suggesting that DAPK1 deregulation is a key pathogenic event in CLL (Ref. [18]) (Fig. 1). Nevertheless, the
precise function of DAPK1 remains unclear. DAPK1 links cellular stresses, such as starvation or growth factor deprivation, to the induction of either apoptosis or autophagy, depending on the cellular context and the external stimuli.[99]

Epigenetic modifications are an attractive therapeutic target because they are reversible. Several compounds, including histone deacetylase inhibitors, have been tested on CLL cells in vitro and in clinical studies. Although these compounds have been shown to be biologically and clinically active, the results in CLL have been less encouraging compared with other leukaemias.[100]

Microenvironment

CLL cells interact with and seem to shape their microenvironment, which consists of T cells, stromal cells and soluble factors.[101] Some of these interactions are located in specific compartments[102] (for example, proliferation centres, discussed below). The importance of the microenvironment in CLL is easily appreciated by the fact that CLL cells rapidly undergo apoptosis when they are removed from patients.[103] This process can be prevented by adding a number of cytokines or other cell types to the CLL cell culture.

In the lymph node, the microenvironment provides anti-apoptotic signals and proliferative stimuli, resulting in the formation of proliferation centres of CLL cells (pseudofollicles) that are not found in other lymphomas. CLL cells seem to recruit accessory cells[104,105] and thereby create a microenvironment that supports their own survival. There is an increase of CD3⁺ T cells, most of which are CD40L⁺ CD4⁺, which cluster in and around pseudofollicles.[106] These cells can stimulate CLL cells through the interaction of CD40 and CD40L, and this stimulus synergizes with BCR signalling.[107] Several anti-apoptotic signalling pathways are induced, including the caspase inhibitor survivin (also known as BIRC5), which is specifically expressed at high levels in proliferation centres,[106] and NF-κB, the activation status of which was found to be a prognostic marker in CLL.[108] The CD40–CD40L interaction or NF-κB may be suited to therapeutic intervention (for example, using monoclonal antibodies, CD40 ligation or specific inhibitors) (Fig. 1). CLL cells also induce phenotypical changes in T cells, the most prominent of which is a defective and reduced formation of the immunological synapse.[109]

CLL cells that are in close proximity and in contact with activated CD4⁺ T cells show expression of the cell surface marker CD38 (Ref. [110]). This is of interest because CD38 has been linked to the proliferation of CLL cells. The presence of high numbers of CD38⁺ CLL cells in the blood is associated with a poor prognosis.[8,26,111] In addition, CD38 is an interaction partner for CD31 expressed on 'nurse-like cells' (NLCs).[112] NLCs can develop in vitro from CD14⁺ monocytes by interaction with CLL cells,[113] and secrete stromal cell-derived factor 1 (SDF1) and the TNF family ligands a proliferation-inducing ligand (APRIL; also known as TNFSF13) and B cell activating factor (BAFF; also known as TNFSF13B), which protect CLL cells from apoptosis.[114–116] A potential role of APRIL in the pathology of CLL was suggested by the demonstration that transgenic mice overexpressing APRIL develop a CLL-like disease.[117] The dependency of CLL cells on their microenvironment is exploited by novel therapeutic compounds that target the supporting non-malignant T and natural killer cells and show efficacy in CLL.[118,119]

Clinical Management, Therapy and Prognosis

In contrast to most other leukaemias, CLL is not necessarily treated at diagnosis, but rather after symptomatic disease.[3] This strategy is mainly based on the results of randomized trials that compared early and late treatment that showed no benefit of early treatment.[120] However, early intervention trials are currently selecting high-risk subgroups of CLL based on biological and clinical criteria to reassess early intervention.

Over the past decades, there has been a transition from single-agent alkylator-based therapies to nucleoside analogues, combinations of both alkylators and nucleoside analogues and, most recently, chemo-immunotherapy. Complete response rates have improved from 7% to a maximum of 70%.[54,121] Historical comparisons suggest that the
overall survival has improved with the application of chemo-immunotherapy. However, to date none of the randomized trials of combination chemotherapy showed a survival benefit for a specific treatment in CLL when only the treatment arms were compared. Data from our group published in abstract form suggests that 'modern' therapies are changing the outcome of CLL. Multivariate analysis in the prospective German CLL Study Group (GCLLSG) CLL4 trial suggests that overall survival may be altered when the genetic profile is considered in addition to treatment arms. The logical approach to improve outcome is therefore the development of prognostic and predictive models, which would lead to stratified treatment strategies for specific disease subgroups. It may also be helpful to identify biological low-risk groups to decrease their treatment intensity.

Genotype-specific Therapy

The ultimate goal of this approach would be a genotype- or risk factor-adapted therapy in all patients. This depends on the identification of prognostic and predictive factors that have the strongest clinical impact. The most powerful prognostic factors in CLL include age, Binet stage or Rai classification, serum markers and genetic factors (such as genomic aberrations, IGHV mutation status, ZAP70 (Ref. ) and TP53 mutations). Few studies have addressed potential predictive factors that optimize treatment.

The first risk-adapted treatment for patients with CLL has been developed for patients with 17p13 deletions who have a very poor prognosis with alkylator- and purine analogue-based chemo-immunotherapy (Refs ) (Fig. 3). As there is evidence that several 'biological' agents, such as alemtuzumab, corticosteroids, lenalidomide and flavopiridol act independently of functional p53 in CLL, current treatment approaches in clinical trials use these agents upfront with early allogeneic stem cell transplantation.

Mechanisms of Treatment Resistance

In spite of highly effective treatment options, all patients with CLL will eventually relapse after conventional therapy. Patients relapsing within 2 years after intensive first-line therapy have a poor response to salvage treatment and short overall survival. It is important to address both the time to relapse and the intensity of preceding treatment. Obvious obstacles to the investigation of mechanisms that underlie early relapse and refractory disease lie in the clonal heterogeneity of samples before treatment. The existence of subclones is best documented for cases of 17p13 deletion and TP53 mutation; however, they are also likely to exist for other unidentified mutations or epimutations, leading to chemoresistance.

p53 Pathway

TP53 plays a central part in our current understanding of why some patients fail to respond to chemotherapy in CLL. Since the early 1990s, TP53 mutations and 17p13 deletions have been associated with a poor response to alkylating agents and short survival. Data from prospective trials confirm these results. Mutations of TP53 have been found in 4–10% of patients with untreated CLL. TP53 mutations are associated with higher genetic complexity, although the precise effect of complex genetic aberrations in CLL — especially independently of TP53 mutations — are not well defined. Recent data suggest that the clinical behaviour of cases that have only a TP53 mutation is similar to cases that have deletion of one TP53 allele and mutation of the remaining allele (Refs ) (Fig. 3). Although the overall incidence of TP53 mutations is lower than that of most other cancers, the clinical consequences of the mutation are striking. However, it is important to appreciate that TP53 mutation or 17p13 deletion will only explain a proportion of refractory cases (25–50%). Other components of the p53 pathway, including ATM (discussed below) and miR-34a (a microRNA component of the p53 pathway), seem to contribute to drug resistance.

ATM and DNA Repair

Although ATM inactivation has been associated with refractoriness to chemotherapy through failure to activate p53 and p21, data from larger randomized trials indicate that the primary response is independent of the presence of 11q23
deletion.\textsuperscript{[54,55,131,132]} There is evidence from our group published in abstract form that more intensive combination chemotherapy may be particularly beneficial compared to fludarabine alone in patients who have 11q23 deletions, and the addition of the anti-CD20 antibody rituximab may further enhance efficacy.\textsuperscript{[90,133]} This observation is consistent with the concept that inactivation of ATM is more likely to lead to genomic instability and secondary resistance due to an impaired DNA damage response, which may be particularly detrimental in sublethally exposed cells.\textsuperscript{[134]} Small molecules that inhibit the MDM2–p53 interaction and thereby increase p53 levels have been shown to induce apoptosis in CLL cases with deletion of ATM,\textsuperscript{[135]} and similar approaches may be useful therapeutically.

**Treatment Resistance to Other Agents**

Treatment resistance has mostly been studied in relation to chemotherapy. With the advent of small-molecule inhibitors and antibody-based treatments in CLL, the near future will see a wealth of emerging information on specific resistance mechanisms for numerous novel drug classes. Notably, the cellular microenvironment may contribute to drug resistance as CLL cells with stromal support developed 1,000-fold higher resistance to inhibitors of BCL2 and BCL-X\textsubscript{L} than cells cultured without cellular support.\textsuperscript{[136]}

**Translating Biological Insights into Treatment**

The understanding of biological mechanisms in CLL has dramatically developed over recent years, but we are only beginning to use our growing understanding of biological subgroups to alter the clinical management of CLL. One dilemma is that the clinical course is generally benign in the majority of patients and therefore clinical endpoints are slowly reached. This slows down the translation of biological insights into clinical practice. In spite of this, the growing number of approaches that act differently from classical chemotherapy show great promise and some of them are particularly promising for CLL (for example, SYK inhibition).\textsuperscript{[137]} Increasing emphasis is being put on CLL subgroups that are defined by specific biological characteristics with the goal of improving both response rates and overall survival.

**Conclusions and Perspectives**

Unique discoveries have been made that set CLL apart from other cancers. Based on immunological and genetic assessment of BCR function, it is possible to divide CLL into subtypes (such as IGHV-unmutated and mutated, IGHV3-21 and potentially other IGHV gene-defined subsets) that have distinct biological and clinical characteristics. The precise cellular origin of CLL remains a matter of debate, but there is evidence that IGHV-mutated CLLs derive from post-GC B cells, and that IGHV-unmutated CLLs stem from B cells that have been activated by antigens. The dependence and interaction of CLL cells with the microenvironment is of pivotal importance and is being used as a drug target. The most common genetic lesion — deletion 13q14 — may serve as a model of how deregulated non-coding RNAs contribute to cancer initiation, and this may become a ‘druggable’ target in the future. Although epigenetic modification generally leads to downregulation of DAPK1 in CLL, specific aberrations (11q23 and 17p13 deletions) and gene mutations (TP53 and ATM) help to define distinct biological and clinical subgroups of CLL.

Overall, CLL may serve as a model for how microenvironmental stimuli, antigenic drive and epigenetic, as well as genetic, deregulation are combined in cancer pathogenesis. Importantly, there are a growing number of agents that act on specific biological targets and therefore open new therapeutic horizons.

**Sidebar 1**

**At a Glance**

- Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western world. It is characterized by the accumulation of small B lymphocytes that have a mature appearance.
- Two subsets of CLL cases can be differentiated by the degree of somatic hypermutation (mutated and unmutated immunoglobulin heavy chain variable region (IGHV) genes) that have distinct clinical and biological behaviours.
Overall, more than 20% of CLL cases carry stereotyped B cell receptors, suggesting that common antigen(s) are recognized by CLL cells.

Clonal B cell populations with a CLL immunophenotype have been detected in 3.5% of healthy individuals (monoclonal B cell lymphocytosis; MBL). MBL is often a CLL precursor.

Approximately 80% of CLLs show aberrations in a few frequently affected chromosomal regions, including 13q14 (mir.15a and mir16.1), 11q23 (ataxia telangiectasia-mutated; ATM), trisomy 12 and 17p13 (TP53). Recurrent translocations are rare in CLL.

Global and gene-specific aberrant DNA methylation has been detected in CLL. Almost all sporadic CLL cases also show epigenetic silencing of death-associated protein kinase 1.

In lymphoid organs, CLL cells interact with and seem to shape their microenvironment, which consists of T cells, stromal cells and soluble factors. This interaction is emerging as a therapeutic target.

p53 plays a central part in our current understanding of why some patients fail to respond to chemotherapy.

The most powerful prognostic factors include 17p13 deletion, TP53 mutation, 11q23 deletion, IGHV mutation status, serum markers, clinical stage and age.

CLL may serve as a model of how microenvironmental stimuli, antigenic drive and epigenetic, as well as genetic, deregulation are combined in cancer pathogenesis.

Sidebar 2

Stereotyped B cell receptors
Strikingly similar B cell receptors, which often arise from the use of common H and L chain V region gene segments that share CDR3 structural features (such as their length, amino acid composition and unique amino acid residues at recombination junctions).

Antigenic drive
CLL cells seem to be selected by a limited set of antigenic epitopes at some point in their development. CLL cells are stimulated by the binding of these antigens to the BCR.

Somatic hypermutation
A process that modifies the immunoglobulin variable region genes by introducing mutations into them at a high rate.

Anergic
A state in which B or T cells are unresponsive and cannot be activated by antigen.

IGHV1-69
A specific IGHV gene found at a high frequency in CLLs with unmutated IGHV.

CpG island
A region of DNA with a high density of cytosine–phosphoguanine dinucleotides, which are near the transcriptional start sites of 40% of all mammalian genes. Cytosine methylation in CpG islands is generally associated with stable silencing of the associated gene.

Immunological synapse
The supramolecular structure that is established between a T cell and an antigen-presenting cell or B cell.

Binet stage
A clinical staging system most commonly used in Europe based on lymphadenopathy, spleen and liver size and blood count (red cells and platelets).

Rai classification
A clinical staging system most commonly used in the United States.

References


An analysis indicating that CLL cells use a IGHV repertoire that is characteristic of mature B cells, and suggests that antigens may play a part in the pathogenesis of this disease.


This study establishes the link between 13q14 deletion and downregulation of miR-15a and miR-16-1 in CLL.


This study describes a pathogenetic link between downregulation of DAPK1 (by methylation) and CLL.


31. Klein, U. et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J. Exp. Med.* 194, 1625–1638 (2001). This study demonstrates that the global gene expression of IGHV-mutated and unmutated CLLs are more similar to memory B cells than to naive or CD5<sup>+</sup> B cells.

32. Rosenwald, A. et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J. Exp. Med.* 194, 1639–1647 (2001). This study compared gene expression profiles of CLL samples with unmutated and mutated IGHV and discovered that ZAP70 was differentially expressed in these subgroups.


40. Guarini, A. et al. BCR-ligation induced by IgM stimulation results in gene expression and functional changes only
45. Widhopf, G. F. et al. Chronic lymphocytic leukemia B cells of more than 1% of patients express virtually identical immunoglobulins. Blood 104, 2499–2504 (2004). The findings of virtually identical Igs in 1.3% of CLLs provided compelling evidence that the Igs expressed by CLL B cells are highly selected and unlike the Igs expressed by naive B cells.
61. Klein, U., Rajewsky, K. & Küppers, R. Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for


71. Seifert M, Küppers R. Molecular footprints of a germinal center derivation of human IgM*+*IgG*+*CD27*+* B cells and the dynamics of memory B cell generation. *J. Exp. Med.* 16 November 2009 (doi: 10.1084/jem.20091087)


75. Rawstron, A. C. *et al.* Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. *Blood* 100, 635–639 (2002).


79a. The first detailed study on the incidence, genetic profile and clinical course of MBL.


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**Competing interests statement**
The authors declare competing financial interests.