Current Clinical and Medical Education

Received 17 Aug 2024 | Revised 18 Aug 2024 | Accepted 12 Oct 2024 | Published Online 14 Oct 2024

Published By: Vision Publisher

ISSN 2942-0792

CCME 02 (10), 92-103

Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma: Morphologic, Histologic Features, Clinical and Prognostic Implications of Novel Discoveries

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Abstracts: Two prevalent low-grade, typically indolent B-cell lymphoproliferative disorders are chronic lymphocytic leukaemia (CLL) and small lymphocytic lymphoma (SLL), which are lymph node tumours. Both SLL and CLL can develop from preexisting low-level monoclonal or oligoclonal B-cell expansions; unlike other B-cell malignancies, they do not exhibit the hallmark reciprocal chromosomal translocations. At now, CLL/SLL offers the most promising model among haematopoietic tumours for the application of prognostic and predictive indicators to influence treatment decisions. Additionally, CLL/SLL exemplifies the integration of serum biomarkers with tumour immunophenotyping (FC, IHC), genomic profiling (FISH, etc.), and tumour histogenesis studies (Ig gene expression mutation status, for example) as a case study. Similar to chronic lymphocytic leukaemia (CML) and acute lymphoblastic leukaemia (ALL), chemotherapy-induced disease (CRD) monitoring by FC is now standard practice in post-treatment care

Keyword: Chronic Lymphocytic Leukemia, Morphologic, Clinical and Prognostic Implications.

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Introduction

In chronic lymphoproliferative lymphoma (CLL), tiny, monomorphic B cells clonally accumulate in lymphoid organs, bone marrow (BM), and peripheral blood (PB). This leads to progressive lymphocytosis. The traditional way to diagnose chronic lymphocytic leukaemia (CLL) is with a three-month history of absolute lymphocytosis (³5 × 109/L)

accompanied by a suitable immunophenotype, meaning clonal B cells expressing CD5 and CD23. The 2008 WHO classification now includes updated diagnostic criteria, specifically that chronic lymphocytic leukaemia (CLL) can be diagnosed with lymphocytosis below 5 × 109/L in the presence of cytopenias or disease-related symptoms [1, 3]. The presence of less than 5 × 109 monoclonal B cells/L of blood is called "monoclonal B-lymphocytosis" (MBL), which can progress to frank CLL at a rate of 1-2% per year, unless there is lymphadenopathy, organomegaly (as determined by physical examination or CT scans), cytopenias, or symptoms related to the disease. Most cases of chronic lymphocytic leukaemia (CLL) in the US and EU occur in the elderly. Annual incidence rates range from 2 to 6 cases per 100,000 people, with a peak of 12.8 incidences per 100,000 people aged 65 and up. There are about twice as many men as women. There is strong evidence that constitutional genetic influences play a role in its development, since the incidence is extremely low in Asian countries and in Asian people that have migrated to Western countries. Clinical staging systems are based on the fact that decreased haematopoiesis leading to progressive cytopenias is the principal consequence of aggressive and/or long-standing CLL [3. 4]. Paraproteinemia or immunological dysregulation caused by CLL cells may explain why some CLL patients develop autoimmune haemolytic anaemia. Lymphadenopathy and/or splenomegaly are symptoms of the non-leukemic variant of small lymphocytic lymphoma (SLL), which is defined as lymphocytosis less than 5 × 109/L. While some lymphomas with characteristic immunophenotype and morphology may initially manifest only at extranodal locations (such as the gastrointestinal system), they typically develop into normal CLL/SLL with time.

Characteristics of Chronic Lymphoblastic Leukaemia (CLL/SLL) and Histologic Subtypes

Small, mature lymphocytes with a narrow cytoplasmic border and a highly stained nucleus are the hallmarks of CLL cells found in bone marrow aspirate and peripheral blood smears. Additional distinctive morphologic features observed on smear preparations include Gumprecht nuclear shadows, also known as smudge cells, which are shattered fragments of cells. Prolymphocytes, larger lymphoid cells with round nuclei and prominent nucleoli, are likely the proliferative components and are intermingled with small CLL cells in varying quantities. Additionally, tumour cells in many CLL/SLL cases resemble circulating lymphoma cells; these cells may have nuclear folds and clefts or very basophilic cytoplasm. Cases of CLL showing an increase in the number of bigger or atypical forms could indicate a tumor's progression from typical CLL or represent actual morphologic variations. French American British (FAB) classification was the first to identify and label these cases as either atypical CLL (with a spectrum of small to large pleomorphic lymphocytes but fewer than 10% prolymphocytes) or mixed cell type (with a dimorphic population of small lymphocytes and prolymphocytes) (CLL-PLL). While CLL/SLL typically does not have a substantial serum paraprotein, another variety includes tumour cells that have more abundant amphophilic cytoplasm and a plasmacytoid appearance to the nuclei, which increases the chance of lymphoplasmacytic lymphoma [5-7]. Although there is morphologic heterogeneity, the 2008 WHO classification does not distinguish between these subtypes, even though research has linked certain morphologic variants to a worse prognosis. World Health Organisation and Food and Drug Administration criteria classify as prolymphocytic leukaemia (B-cell PLL) cases that exhibit a CLL-like immunophenotype but have more than 55% prolymphocytes at diagnosis. These cases are extremely rare and warrant a thorough differential diagnosis that includes mantle cell lymphoma and large B-cell lymphoma.

Whether it's interstitial, nodular, or diffuse patterns of infiltration, bone marrow involvement is evident in all cases of CLL and, to a lesser extent, in about 70% of SLL at diagnosis. Advanced disease stages and a worse prognosis are

linked to diffuse involvement. While follicular lymphoma (FL) typically has paratrabecular lymphoid aggregates, CLL is more likely to have intertrabular ones. Although sinusoidal BM involvement is more commonly observed in splenic marginal zone lymphoma (MZL), it is not uncommon for CLL to exhibit this feature. A splenectomy, fine needle aspirate, or biopsy of a lymph node (LN) is the gold standard for diagnosing SLL [8, 9]. Paraimmunoblasts, medium-sized nucleolated cells that are the tissue analogues of prolymphocytes, are found within vaguely nodular and pale-staining proliferation centres or pseudofollicles, which typically obscure the LN architecture. Although SLL typically has moderate mitotic activity overall, mitotic figures are more common in proliferation centres. It is common for the LN sinuses to be completely blocked, and the capsule and perinodal adipose tissue are both invaded. Coincidentally, CLL cells may be seen in biopsies taken for other causes because they travel to areas where inflammation already exists, such as prostatitis or dermatitis.

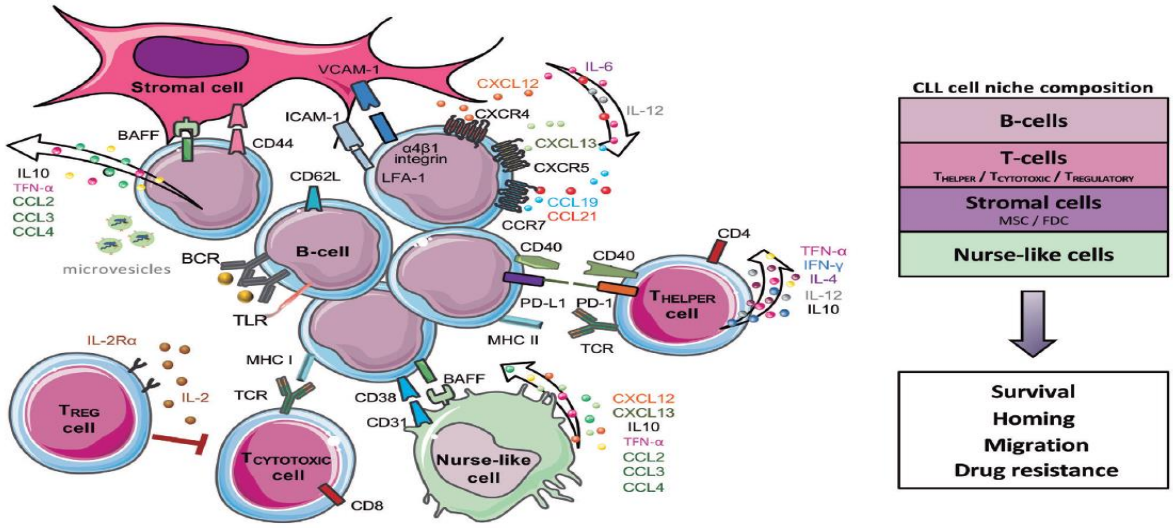

Figure 1. A microenvironment in chronic lymphocytic leukaemia. Cells in chronic lymphocytic leukaemia (CLL) communicate with one another and with stromal cells, T cells, and nurse-like cells (NLC) through direct contacts, adhesion molecules, chemokine/cytokine receptors, and ligand-receptor interactions. Chemokines CXCL12, CXCL13, and CCL19/CCL21 are released by several cell types; these chemokines interact with certain receptors on CLL cells, including CXCR4, CXCR5, and CCR7. Follicle dendritic cells (FDC) release CXCL13, and high endothelial venules release CCL19/CCL21. Tumour cell migration and homing are facilitated by adhesion molecules and their ligands, such as a4β1 integrin and LFA-1, as well as VCAM1 and ICAM, among others. Proliferation of CLL can be accelerated by B-cell receptor (BCR) activation, which is triggered by environmental or auto-/self-antigens as well as homotypic IG interactions.22,138 A key component of antigen presentation and the development of proper B-cell responses involves the interactions between CD40 and CD40 ligand (CD40L) on activated CD4+ T cells. In order to entice T lymphocytes and other stromal cells, activated CLL cells release angiogenic factors and chemokines (CCL2, CCL3, and CCL4).29, IL-10 and other immunosuppressant substances help tumour cells avoid the immune response and stay in a state of tolerance. medication that fights cancer The CD8+ T cells run out of juice when they're constantly exposed to antigens produced by tumours.Forty regulatory T cells (Tregs) secrete suppressive cytokines that restrict CD4+ and CD8+ cell proliferation.142 Inflammation is induced in T cells, monocytes, and stromal cells by tumor-released extracellular vesicles that contain noncoding RNA and proteins.

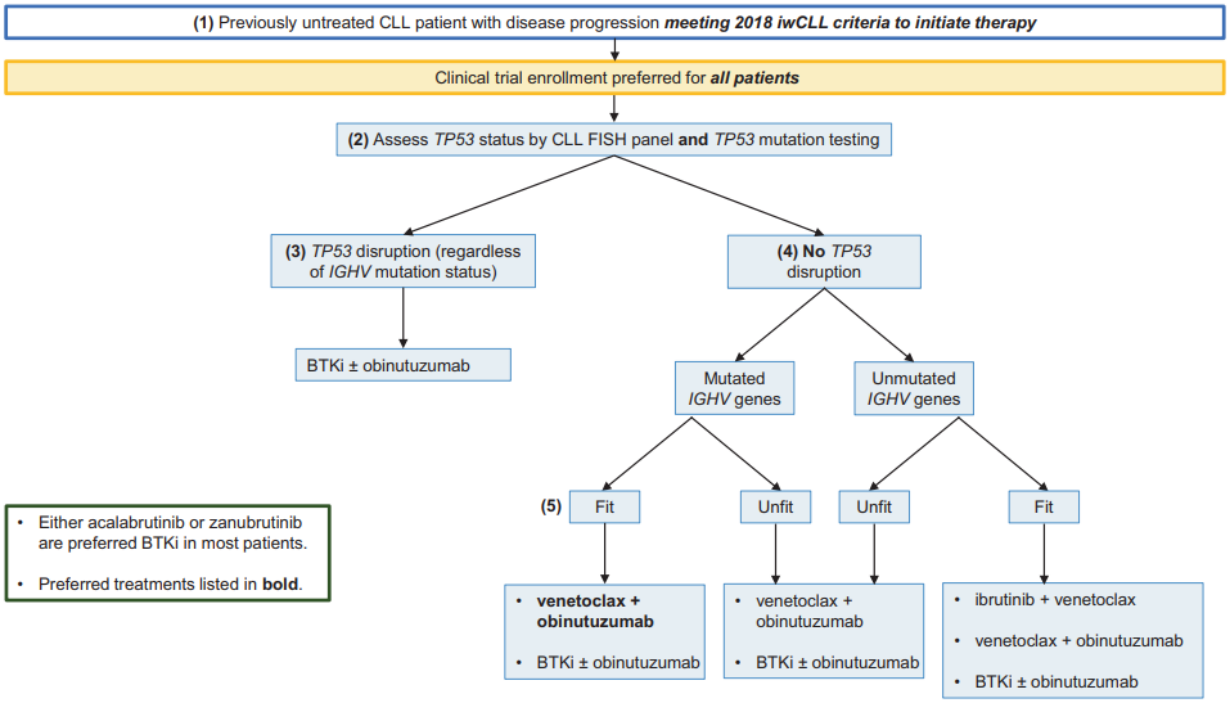

Figure 2. Method for caring for CLL patients who have never had treatment before but who fulfil the treatment criteria set out by the 2018 International Workshop on Chronic Lymphocytic Leukaemia (iwCLL). IGHV immunoglobulin heavy chain gene, BTKi Bruton tyrosine kinase inhibitors.

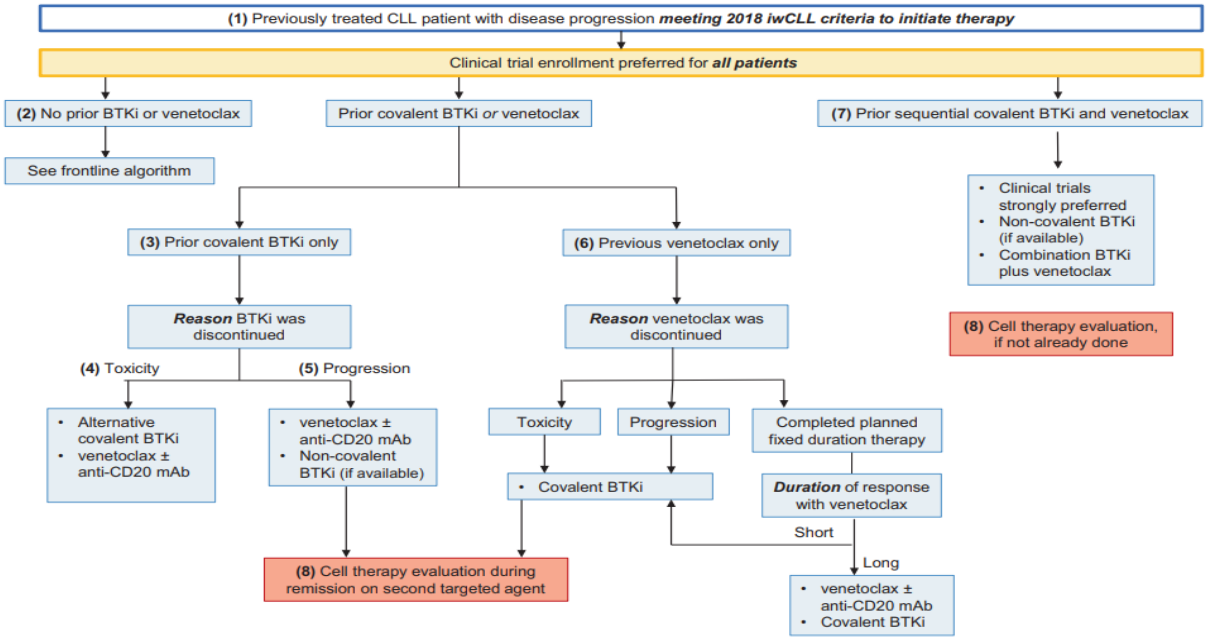

Figure 3. Methods for treating CLL relapses in individuals who fulfil the treatment criteria established by the 2018 International Workshop on Chronic Lymphocytic Leukaemia (iwCLL). antibodies, immunoglobulin heavy chain gene, Bruton tyrosine kinase inhibitors, and mAbs.

Diagnosis Laboratory Testing

According to flow cytometry (FC), CLL cells typically express CD5—which is generally a T-cell antigen—along with the B-cell surface antigens CD19, CD20, and CD23, whereas clonotypic kappa or lambda immunoglobulin light chain expression is restricted. As opposed to normal circulating B cells and other B-cell tumours, these often have low

expression levels of surface immunoglobulin (CD20), CD79b, and typically IgM. Although it is expressed in other B-cell tumours, FMC7, an altered CD20 epitope, is often negative in CLL. Bone marrow biopsy or clot sections can also be routinely used to perform most of these CLL indicators [10]. It is important to consider the clinical, laboratory, and morphologic findings in order to rule out other types of B-cell tumours, even though the immunophenotype may not always follow the conventional pattern for CLL indicators.

Prognostic and Predictive Markers That Are Commonly Used

Current treatment options for chronic lymphocytic leukaemia (CLL) cover a wide spectrum, from observation alone to immunotherapy with anti-CD20 antibodies (such as rituximab), two-, three-, or four-drug combinations, stem cell transplantation, and a wide range of outcomes. Consequently, numerous indicators for CLL prognosis and therapeutic response prediction have been created, with some produced for SLL as well. Nonetheless, the haematologic parameter-based staging approaches continue to hold paramount importance.

Diagnostic and clinical evaluation criteria: The Rai and Binet systems are two popular hematologic-based staging procedures that are utilised in clinical trials and patient care. They are similar to one another. The current Rai classification was simplified from five stages (0-IV) to three groups (low-, intermediate-, and high-risk) based on PB lymphocytosis, platelet count, haemoglobin (Hb) level, and the presence or absence of lymphadenopathy. These groups have different clinical outcomes with most current therapy regimens. If there are organomegaly (lymph nodes that are enlarged to a size larger than 1 cm) and anaemia or thrombocytopenia, then the number of affected areas, as determined by the Binet staging system, is based on that. Any doctor in the globe can use these two stage systems since they are easy [11-14], cheap, and universal. In neither case is a computed tomography (CT) scan, magnetic resonance imaging (MRI), or ultrasound necessary; instead, a thorough physical examination and routine laboratory testing are sufficient.

Genomic study: CLL is characterised by frequent genomic gains and losses but very rare reciprocal chromosomal translocations. Chromosome 13(q14) deletions affect 50% of the population, chr 11(q23) (covering the ATM gene) affects 19%, trisomy affects 20% of the population, and chr 17 changes leading to the loss of the TP53 locus affect 8% of the population. Due to the tiny size or complex patterns of these changes and the low proliferative power of most CLL cells, standard karyotyping of metaphase chromosomes is not the best method for detecting these abnormalities. Therefore, FISH is the preferable method. With the help of standard karyotyping and a small FISH panel that includes the four loci mentioned before, genetic abnormalities can be detected in more than 80% of CLL cases. In advanced or progressed CLL, loss of TP53 and ATM is commonly observed and is strongly correlated with poor prognosis. Recent research has demonstrated that array-based comparative genomic hybridisation (CGH) whole genome profiling may detect all of these abnormalities and more in CLL/SLL at a low cost.

A mutation status analysis of immunoglobulin heavy chain variable region (IgVH) users: As In order to enhance the produced antibody's affinity for antigen, somatic hypermutation of the immunoglobulin variable gene segments takes place in the germinal centre (GC). This process introduces sequence changes in the immunoglobulin genes. Conventionally, this mutation status is ascertained by comparing the results of PCR-based IgVH sequencing from tumour RNA to the reference sequence for that particular IGVH segment. The conventional wisdom holds that antigen-experienced B cells after GC are the likely progenitors of B-cell tumours with somatically altered IgVH genes.

The prevalence of these mutant IgVH genes in CLL is around 50%. Numerous groups across the globe have conducted large-scale investigations since 1999, when research by Hamblin et al. and Damle et al. established a link between mutant IgVH and positive [15-19] clinical result. Median overall survival for patients with CLL and unmutated IgVH genes is often about 10 years, but for patients with tumours and mutated IgVH genes it is typically over 20 years. Furthermore, genomic progression, unusual morphology, ATM and TP53 deletions, and advanced stage illness are all characteristics of "unmutated" or pre-GC CLL.

Biomarkers related with B-cell receptors, include surface CD38, ZAP-70, and TCL1: Identifying surrogate markers that correlate with mutation status and maturation stage is necessary due to the time-consuming, costly, and tumor-RNA isolation required for IgVH mutational research. Among the molecules involved in the surface antibody/B-cell receptor (BCR) signalling pathway, the three most well-characterized are T-cell leukaemia gene 1 (TCL1), 70-kDa zeta-associated protein (ZAP-70), and surface CD38. They all correspond with "unmutated" or pre-GC tumour state, albeit to different extents. While TCL1 and ZAP70 have the highest pre-GC/unmutated connection, they also correlate with the ability of cultured CLL cells to proliferate in response to BCR-crosslinking.

Not Very Popular Prognostic Markers

Markers of proliferation: In addition to tumour load, markers of cell proliferation and turnover can offer important prognostic information. Among these, you can find LDH, beta-2 microglobulin, and lymphocyte doubling time (LDT), which is the amount of months it takes for an untreated patient's absolute lymphocyte count to double. Longer LDTs are associated with longer OS and treatment-free intervals [20, 21], even in early-stage CLL; individuals with shorter LDTs have lower OS and treatment-free intervals. The limitation of LDT evaluation to a retrospective only is a downside of the method. Promising additional progression predictors include telomere length and telomerase activity assessments. A measure of serum levels Prognostic indicators have been suggested to include thrombopoietin (TPO), levels of interleukin (IL)-6, IL-8, and IL-10, levels of secreted or shed soluble (s)CD23, sCD27, sCD44, and sCD138, and levels of circulating (c)CD20. It is believed that these indicators indicate the level of immune activation in CLL and the impact of the microenvironment on growth [22-25]. Measurements of microvessel density in lymph nodes and bone marrow, for example, provide a more direct picture of the tissue microenvironment and its relationship to disease progression and clinical stage. Clinical stage and illness progression have been linked to serum levels of pro-angiogenic substances expressed and released by CLL cells, including vascular endothelial growth factor (VEFG) and basic fibroblast growth factor (bFGF). Angiogenesis pathways are crucial for signalling, and CLL cells express VEFG receptors (VEGFR-1 and -2).

The potential effects of new discoveries on patient care and prognosis

Determining the prognosis of individual patients has long been of great interest due to the clinical heterogeneity of CLL, which can range from a relatively asymptomatic condition that may even regress spontaneously to a progressing disease that ultimately results in the patient's death. While numerous prognostic markers have been discovered in recent decades, only a handful have shown to be truly effective. For a long time, clinical markers including the Rai and Binet staging systems, IGHV mutational status, and numerical aberrations found by FISH were the only ones used to assess CLL patients' prognoses.47 Although these prognostic systems utilised newly found drivers (NOTCH1, SF3B1, BIRC3) made possible by next-generation sequencing, none of these efforts were able to become standard of

care.pages 94 to 96 As a matter of fact [26, 27], the iwCLL recommendations solely call for routinely checking for IGHV status and TP53 abnormalities.86 Age, clinical staging, β2-microglobulin serum concentration, IGHV mutation status, and TP53 abnormalities are all part of the new CLL International Prognostic Index (CLL-IPI), which is a prognostic score that integrates both clinical and cytogenetic/genomic data.97 The CLL-IPI has been verified in retrospective studies and was established in cohorts of patients treated with CIT.pages 98–100. Simplified versions of the CLL-IPI integrating only TP53 and IGHV have been proposed since, out of the five factors, these two have the biggest impact on a patient's outcome. 101 New research shows that individuals with early, asymptomatic disease can be predicted to have a shorter time to first therapy using a score that takes into account the existence of palpable lymph nodes [28, 29], an absolute lymphocyte count greater than 15 x109/L, and unmutated IGHV.102 However, there are a number of organisations who are pushing for new markers to be used in clinical practice, such as epigenetic subsets or complicated karyotypes. Validation in patient cohorts treated with targeted medicines is necessary for all of these new prognostic and predictive models.

Relapsed/refractory disease

Decisions for further treatment for relapsed or resistant disease must take into account both the results of previous treatments and the reasons why those treatments were ineffective (e.g., refractoriness vs. intolerance). Several phase I-III trials have shown that ibrutinib is effective in treating relapsed or resistant cancer, but this is beginning to change for two key reasons: (i) Ibrutinib is already the first line of defence for many patients, and (ii) new, formidable competitors have emerged in the past year. Among the finest options in this case, even for those with high-risk genetic abnormalities, is venetoclax. Multiple phase I-II trials in patients who had previously undergone CIT or BTK/PI3K inhibitors demonstrated the drug's efficacy and safety. The full approval of venetoclax combined with rituximab for patients with relapsed/refractory CLL was based on a phase III trial that officially confirmed this promising activity by showing that it was superior to bendamustine plus rituximab in response rate, progression-free survival, and overall survival. Alternative BTK inhibitors and PI3K inhibitors are two other options [30]. The first PI3K inhibitor to be licensed for the treatment of relapsed/refractory CLL was idelalisib in conjunction with rituximab; however, due to its less favourable adverse event profile, it is seldom utilised. No randomised data have been found to support this potential superiority, but it could be useful in older patients who also have trouble tolerating ibrutinib, or in patients with complex karyotypes, who are more likely to experience progressionand/or transformation when treated with venetoclax or ibrutinib. Another PI3K inhibitor that was authorised by the FDA after a phase III randomised trial was duvelisib.130 The drug's safety profile and effectiveness seem to be similar to, if not somewhat better than, idelalisib. Only acalabrutinib has received FDA approval for the treatment of relapsed or refractory CLL, while there are a number of other BTK inhibitors under development. A phase III research found that compared to bendamustine plus rituximab and idelalisib plus rituximab, acalabrutinib was the best option. A different research found that 85% of patients who had an ibrutinib intolerance were able to receive acalabrutinib, and the response rate was 76%. However, this trial did not permit prior ibrutinib medication. Unfortunately, some patients will still not react to current treatments and may need to be considered for curative procedures. There is currently no treatment option other than allogeneic haematopoietic cell transplantation [31], which has the ability to cure the disease but comes with a high risk of complications and death. Despite a huge decline in referrals for allogeneic haematopoietic cell transplantation over the last several decades, 133 this procedure should still be considered for healthy young patients whose BCR/BCL2

inhibitor treatment has failed, especially for those with TP53 abnormalities or Richter transformation. Also, none of the commercially available treatments have been licensed for this indication yet, even though chimeric antigen receptor T cells could be useful in this case and the results are promising.

The Evolution of Disease

Due to the lack of efficacy of the newly authorised innovative drugs, Richter transformation continues to be a frightening event for CLL patients, especially those whose CLL is clonally related to the original. The fact that these medications do not work is due, in large part, to disease change. Always seek histological confirmation as it can provide insight into prognosis. For example, a better prognosis is associated with Hodgkin lymphoma and tumours that are clonally unrelated [32]. Traditional CIT (e.g., RCHOP: rituximab plus cyclophosphamide, doxorubicin, vincristine, prednisone) should be considered for patients with altered disease. If a response is observed, allogeneic haematopoietic cell transplantation should be considered. As an alternative to allogeneic transplantation, autologous haematopoietic cell transplantation can be explored in certain cases. Chimeric antigen receptor T cells have the potential to be effective as well, however at this time, patients with Richter transformation do not have access to any of the authorised medicines.

Advanced Methods for the Treatment of Chronic Lymphoblastic Leukaemia in the Elderly

Immunoglobulin G Chemoimmunotherapy, a therapeutic method that has become the gold standard for CLL in healthy adults, involves adding a set of chemicals called anti-CD20 antibodies to a patient's chemotherapy regimen. Rituximab is a medicine. Several diseases, such as CLL, aggressive lymphoma, and follicular B-cell lymphoma, have been treated with rituximab, a type I monoclonal anti-CD20 antibody, since its approval in 1997. The binding to the B cell surface CD20 antigen is the mechanism of action. While some research indicated that the antibody was effective when administered alone,18 other studies indicated that it was far more effective when combined with other chemotherapeutic treatments. The side effects of some therapy alternatives may be intolerable for older patients or individuals with comorbidities, so it is important to carefully examine the chemotherapeutic drug that is paired with rituximab. For CLL patients under the age of 65 who are in generally good condition, have low-risk prognostic characteristics, and have not been treated before, FCR is the gold standard treatment. It is possible that FCR might be an option for certain elderly individuals who are in good condition and have favourable prognostic characteristics. Nevertheless, FCR is not well-tolerated by individuals who are 65 and older, as well as those who have many medical conditions. As a result of therapy-induced myelosuppression, bone marrow function decreases, and erythrocyte, neutrophil, and platelet counts drop. It is a common reason why people stop using FCR medication.20 A modified treatment regimen with fewer side effects has proved more beneficial for older patients, since myelosuppression and its consequences following FCR treatment are more common in this age group. Study on CLL10 Alternative, more tolerable therapeutic options are available for patients who, due to age or comorbidities, may not qualify for FCR as initial therapy. In this study, 561 healthy individuals with active CLL who had never received any treatment before were enrolled. Two hundred and eighty-two patients made up the FCR group, whereas 279 patients made up the BR group. Patients with del(17p) were not included in the study, and the age range was 33 to 81 years (median, 61.5 years). As predicted, the median progression-free survival (PFS) for the FCR group was 57.6 months, while for the BR group it was 42.3 months.34 A distinction was observed when PFS was examined by splitting the population into

two categories based on age (<65 years vs. > 65 years). Median progression-free survival (PFS) was 38.5 months for BR and 53.6 months for FCR in the younger age group, indicating a statistically significant difference between the two treatment groups. But there was no discernible change when looking at the elder age bracket. This finding, along with the fact that the elderly patients treated with FCR were more likely to experience toxic side effects (71% vs. 41%) and therapy-related myeloid leukemia/myelodysplastic syndrome was more common in the elderly patients treated with FCR, demonstrated that BR is a superior first-line chemotherapeutic option for patients with chronic lymphocytic leukaemia who are not good candidates for FCR.

Progression and Transformation Patterns

Among the many possible long-term developments in CLL/SLL is the establishment of a genetically separate B-cell neoplasm, which occurs less frequently than the more typical step-wise clonal progression of the same tumour. Two to eight percent of CLL patients will undergo the distinct transition of their cancer into large B-cell lymphoma (LBCL), which is known as Richter's syndrome or Richter's metamorphosis. Although it occurs less frequently, some writers have referred to the secondary occurrence of classical Hodgkin lymphoma or other high-grade lymphoid malignancies as Richter's transformation, which further confuses matters. Rapid growth of one or more nodal groups, especially in the retroperitoneum, is commonly seen alongside new systemic symptoms such as fever and weight loss, which are often accompanied with discrete big cell change. The patient may experience a sudden worsening of their clinical condition. Additional common signs include an elevated blood LDH level, paraproteinemia, hypercalcemia (in the absence of lytic bone lesions), hepatosplenomegaly, and the development of additional involvement sites outside of the lymph nodes. Recent longitudinal research including 186 patients found that factors such as unmutated IgVH status, IGH-V4-39 usage, lack of del(13q14), expression of CD38 and ZAP70, lymph node size and number, advanced Binet stage, and increased LDH were predictive of large cell transformation in univariate analyses. Only an increase in lymph node size (³3 cm) and the lack of chr 13q14 deletion were found to be independent predictors in the multivariate analysis. What makes this set of found characteristics all the more interesting is that they differed from the ones most strongly linked to clinical progression and overall survival. Since CLL is known to impair T-cell immunity (and immunosuppressant treatment effects likely give latent EBV a chance to reemerge and start clonal B-cell expansions that end in lymphoma), the discovery of Epstein-Barr virus (EBV) in certain cases of large cell transformation gives a clear pathogenetic mechanism. Both CLL cells and bystander lymphocytes might become infected with EBV in this experiment. Take Ansell et al.'s study on big cell transformation in CLL patients as an example. Out of 25 patients, 4 had EBV in their converted tumour cells. Among these patients, 3 had a B-cell phenotype that expressed virally-encoded LMP1 and EBV-encoded RNAs (EBERs), and 1 had a T-cell phenotype that was positive for just EBERs. The EBV+ lymphoma cells were found to have originated from the original CLL in two out of three patients, as demonstrated by Thornton and colleagues. While EBV-driven B-cell malignancies often occur in T-cell malignancies (since they both share an immunodeficient condition associated with the tumour), they are uncommon in B-cell neoplasms outside of CLL/SLL. Fludarabine therapy, which is known to reduce T-cell immunity, is likely to blame for the expansion of EBV-associated B-cells.

Unrelated to EBV, CLL disease development is characterised by a cascade of genetic alterations, such as p53 loss and/or ATM dysfunction, which cause cells to proliferate more rapidly. Although this process does not always reach the final stage of prolymphocytic transformation, it does so in situations where the percentage of prolymphocytes is

greater than 55%. The pre-GC subset of CLL/SLL is remarkably the only one affected by this clonal progression pattern, and it is likely responsible for the subgroup's poor prognosis. In order to distinguish between two distinct processes, it is essential to clearly define the mechanism of transformation. This includes distinguishing between conventional clonal progression in the pre-GC fraction and secondary EBV transformation caused by immunosuppression. We therefore urge the replacement of the vague and antiquated phrase "Richter's transformation" with a more accurate histogenetic classification.

Conclusion

Despite a lot of good that has happened in the last 20 years when it comes to treating CLL, the disease is still not curable. Better tolerated treatments with more effective outcomes are quickly expanding the alternatives accessible to older patients with comorbidities and individuals with poor prognostic characteristics. A large portion of the CLL population consists of older adults and people with several chronic diseases, however they are grossly under-represented in treatment trials. This has created a lot of uncertainty and unanswered issues regarding the best way to treat these groups of patients in terms of efficacy and safety. Clinical trials should better reflect the CLL community as a whole by establishing criteria to select trial participants who accurately reflect the illness features required to deliver more reliable information on treatment regimens. Molecular investigations conducted recently have uncovered numerous unique altered genes grouped in various functional pathways, which have shed light on the processes that control the onset and course of CLL. In chronic lymphocytic leukaemia (CLL), certain regulatory areas undergo reprogramming when the disease first manifests, while other regions preserve activities that were already present at earlier phases of B-cell development. Cancer cell survival and treatment resistance can be better understood with the help of data gleaned from studies of the CLL microenvironment. New therapeutic agents and techniques are being developed to take use of the fresh insights provided by this body of information. But there are also fresh questions that have been prompted by these investigations. Little is known about how the disease's striking molecular heterogeneity relates to the wide range of symptoms experienced by patients. A premalignant stage (MBL) always occurs before the disease manifests, and it shares the majority of the molecular causes with overt CLL. Many of the time, these molecular factors don't change. Nevertheless, clonal evolution is a real possibility; it is commonly linked to exponential tumour development, progressive disease, and even disease transformation in rare instances. We still don't know how the microenvironment interacts with the genome and epigenomic changes to impact the progression of the disease, and most studies have only included individuals who have already received treatment. In order to better understand the condition and develop appropriate therapies and management strategies, it will be necessary to gain an integrated perspective of all these elements in order to translate new knowledge into clinical practice.

References

1. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008;111(12):5446–56.
2. Frater JL, McCarron KF, Hammel JP, et al. Typical and atypical chronic lymphocytic leukemia differ clinically and immunophenotypically. Am J Clin Pathol 2001;116(5):655–64.

3. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. J Clin Pathol 1989;42(6):567–84

4. Schwarz J, Mikulenkova D, Cermakova M, et al. Prognostic relevance of the FAB morphological criteria in chronic lymphocytic leukemia: correlations with IgVH gene mutational status and other prognostic markers. Neoplasma 2006;53(3):219–25.

5. Nola M, Pavletic SZ, Weisenburger DD, et al. Prognostic factors influencing survival in patients with B-cell small lymphocytic lymphoma. Am J Hematol 2004;77(1):31–5.

6. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. Nat Rev 2002;2(12):920–32.

7. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 1998;102(8):1515–25.

8. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94(6):1848–54

9. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94(6):1840–7.

10. Herling M, Patel KA, Hsi ED, et al. TCL1 in B-cell tumors retains its normal b-cell pattern of regulation and is a marker of differentiation stage. Am J Surg Pathol 2007;31(7):1123–9

11. Rosenwald A, Alizadeh AA, Widhopf G, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194(11):1639–47.

12. Crespo M, Bosch F, Villamor N, et al. ZAP-70 expression as a surrogate for immunoglobulin-variableregion mutations in chronic lymphocytic leukemia. N Engl J Med 2003;348(18):1764–75.

13. Rassenti LZ, Huynh L, Toy TL, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 2004;351(9):893–901.

14. Herling M, Patel KA, Khalili J, et al. TCL1 shows a regulated expression pattern in chronic lymphocytic leukemia that correlates with molecular subtypes and proliferative state. Leukemia 2006;20(2):280–5.

15. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. Leukemia 2007;21(5):956–64.

16. Hamblin TJ. Richter's syndrome – the downside of fludarabine? Leuk Res 2005;29(10):1103–4.

17. Rossi D, Cerri M, Capello D, et al. Biological and clinical risk factors of chronic lymphocytic leukaemia transformation to Richter syndrome. Br J Haematol 2008

18. Thornton PD, Bellas C, Santon A, et al. Richter's transformation of chronic lymphocytic leukemia. The possible role of fludarabine and the Epstein–Barr virus in its pathogenesis. Leuk Res 2005;29(4):389–95.

19. nsell SM, Li CY, Lloyd RV, Phyliky RL. Epstein–Barr virus infection in Richter's transformation. Am J Hematol 1999;60(2):99–104

20. Keating MJ, O'Brien S, Lerner S, et al. Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. Blood 1998;92(4):1165–71.

21. Timar B, Fulop Z, Csernus B, et al. Relationship between the mutational status of VH genes and pathogenesis of diffuse large B-cell lymphoma in Richter's syndrome. Leukemia 2004;18(2):326–30.

22. Stilgenbauer S, Dohner H. Molecular genetics and its clinical relevance. Hematol Oncol Clin North Am 2004;18(4):827–48. Viii

23. Thorselius M, Krober A, Murray F, et al. Strikingly homologous immunoglobulin gene rearrangements and poor outcome in VH3-21-using chronic lymphocytic leukemia patients independent of geographic origin and mutational status. Blood 2006;107(7):2889–94.

24. Hanna BS, Öztürk S, Seiffert M. Beyond bystanders: myeloid cells in chronic lymphocytic leukemia. Mol Immunol. 2019;110 (1):77-87.

25. Riches JC, Davies JK, McClanahan F, et al. T cells from CLL patients exhibit features of Tcell exhaustion but retain capacity for cytokine production. Blood. 2013;121(9): 1612-1621.

26. Ramsay AG, Evans R, Kiaii S, Svensson L, Hogg N, Gribben JG. Chronic lymphocytic leukemia cells induce defective LFA-1-directed T-cell motility by altering Rho GTPase signaling that is reversible with lenalidomide. Blood. 2013;121(14):2704-2714.

27. Hanna BS, Roessner PM, Yazdanparast H, et al. Control of chronic lymphocytic leukemia development by clonally-expanded CD8+ T-cells that undergo functional exhaustion in secondary lymphoid tissues. Leukemia. 2019;33(3):625-637.

28. Llaó Cid L, Hanna BS, Iskar M, et al. CD8+ T-cells of CLL-bearing mice acquire a transcriptional program of T-cell activation and exhaustion. Leuk Lymphoma. 2020;61(2): 351-356.

29. Kipps TJ, Stevenson FK, Wu CJ, et al. Chronic lymphocytic leukaemia. Nat Rev Dis Prim. 2017;316096.

30. Wiestner A. The role of B-cell receptor inhibitors in the treatment of patients with chronic lymphocytic leukemia. Haematologica. 2015;100(12):1495-1507.

31. Mockridge CI, Potter KN, Wheatley I, Neville LA, Packham G, Stevenson FK. Reversible anergy of sIgM-mediated signaling in the two subsets of CLL defined by VH-gene mutational status. Blood. 2007;109(10):4424-4431.

32. Moore VDG, Brown JR, Certo M, Love TM, Novina CD, Letai A. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J Clin Invest. 2007;117(1):112-121.