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Chapter

New Protein Markers of Chronic Lymphocytic and Acute Lymphocytic Leukemia

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Abstract

There is an urgent need for the application of new protein markers in early and personalized prognostic diagnosis of cancer. As with many other types of malignancies, the number of leukemia-affected patients is on the rise. This requires novel tools when it comes to efficient treatment approaches, specifically those that are preventative and highly precise. Numerous important discoveries have recently been published regarding new proteins and their pathology-related modifications, which may play important roles in the onset and progression of leukemia. Chronic and acute lymphocytic leukemia are represented by important changes in lymphocyte cell metabolism, where many of the regulating trans-membrane protein markers demonstrate altered functions in the regulation of crucial cell transduction signaling pathways. The most notable progress thus far has been achieved in studies concerning CD5, CD10, CD19, CD20, CD22, CD23, and CD52 protein markers and their associated proteins. As such, some of these signals may be applied in specific and personalized diagnostics as well as drug development.

Keywords: chronic lymphocytic leukemia, acute lymphocytic leukemia, protein markers, disease proteomics, personalized cancer diagnosis

1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common malignancy in adults, and acute lymphocytic leukemia (ALL) is the most common pediatric cancer in western countries. These leukemic diseases affect the lymphoid line of blood cells. In most cases, the cause is unknown, hypothesizing that multiple genetic mutations and epigenetic changes are involved. Both diseases are vastly heterogeneous. While CLL is generally considered incurable and progresses slowly in most cases, ALL progresses rapidly and is typically fatal within weeks or months if left untreated. Historically, survival rates have been poor for patients with ALL. Since the introduction of chemotherapy, prognosis for childhood leukemia has improved greatly, and children with ALL are estimated to have a 95% probability of achieving successful remission. However, a total of 10–15% of patients still relapse despite undergoing intensive chemotherapy, and outcomes are far less encouraging in

adults. CLL treatment tends to focus mainly on controlling the state of the disease and its associated symptoms, rather than on its definitive eradication. The specifics of treatment will largely depend on the patient's prognosis and the specific CLL subtype. Therefore, lifelong observation and follow-up are strongly recommended and supported for all the patients. The combination of chemotherapy and non-chemotherapeutic drugs has improved survival of CLL patients overall, leading to long-lasting remissions. The pathology of CLL is complex in that it is influenced by a number of genetic and molecular changes, the CLL microenvironment, as well as various signaling pathways, of which the B-cell receptor (BCR) signaling pathway is central to CLL activation. Signaling pathways that are identified as being affected in CLL patients can provide opportunities for the development of disease-specific drugs to the extent that they may be applicable in future clinical testing and molecular treatments. In any type of cancer, molecular therapy which targets specific regulatory proteins or their disease-associated posttranslational modifications can make way for novel applications which provide even higher specificity and efficiency with regard to treatment. This approach certainly applies to any type of leukemia.

2. Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia in the western world. The disease typically occurs in elderly patients and has a highly variable clinical progression. CLL is characterized by the clonal expansion and accumulation of mature CD19+, CD5+, and CD23+ B lymphocytes in the peripheral blood, bone marrow, and secondary lymphoid organs [1]. CLL cells are phenotypically similar to antigen-experienced B cells and show gene expression profiles similar to memory B cells [2]. The cellular origin of CLL is still debated, but it is assumed that CLL cells originate either from unmutated mature CD5+ B cells or CD5+CD27+ post-germinal center B-cell subsets [3]. CLL cells recirculate between peripheral blood and secondary lymphoid organs, where they proliferate in distinct areas of tissue, termed "pseudofollicles," at a daily birth rate of approximately 1–2% of the entire clone size [4]. Survival of CLL cells strictly depends on a permissive microenvironment composed of cellular components such as monocyte-derived nurse-like cells, T cells, follicular dendritic cells, mesenchymal stromal cells, and endothelial cells. Such dynamic combination of components leads to the presence of molecules such as cytokines, chemokines, and angiogenic factors. Leukemic cells take advantage of these vital proteins by interacting with them via cell-surface receptors or cell adhesion molecules to further facilitate their proliferation and survival [5, 6]. CLL cells are also characterized by an often observed defect in apoptosis which allows peripheral blood B lymphocytes to survive [7].

Autoantigens and/or autonomous mechanisms activate the BCR and its signaling cascade in secondary lymphatic tissues, playing a central pathogenic role in CLL [8]. These events result in activation of multiple downstream regulators in B cells which ultimately mediate changes in cell proliferation, survival, and migration via both transcriptional modulation and phosphorylation. BCR signaling responses in CLL cells are heterogeneous, with effective activation of only a selected set of downstream responses [9]. Another key property of BCRs is that they exhibit somatic mutations in varying amounts; importantly, the degree of mutation has been found to inform the prognosis of disease [2, 10]. Furthermore, many cases of CLL (approximately one third) are characterized by a nearly indistinguishable subset of BCRs exhibiting shared antigens. This suggests a close link between these specific molecules and CLL pathogenesis.

CLL cells usually show constitutive phosphorylation of signaling proteins which promote their proliferation and survival, leading to pathological processes. Protein phosphorylation in lymphocytes is tightly associated with the regulation of a variety of protein activities, functional regulation, and cell signaling and may thus affect initiation and/or progression of the disease. As such, protein phosphorylation may be one of the most promising targets for the discovery of novel cancer-related protein markers and in turn their application in new approaches to molecular therapy. The constitutive activation of proteins by phosphorylation presents its potential for prognostic significance, as the identification of aberrant signal transduction in leukemic cells can become a potential target for novel agents. After BCR stimulation, CLL cells have shown a tendency toward impaired phosphorylation levels. Higher basal phosphorylation levels of PLCγ2 (pY759), p44/42 MAPK (pT202/Y204), p38 MAPK (pT180/Y182), NF-κB p65 (pS529), STAT5 (pY694), and STAT6 (pY641) were detected in CLL cells compared to normal B cells, predicting their impaired function [12]. As such, these markers may represent some of the novel protein targets involved in the development of efficient therapeutics. Cancer cells with constitutive STAT3 activation have been reported to have elevated levels of cell cycle regulation and antiapoptotic proteins, leading to apoptotic resistance. Constitutive serine phosphorylation of STAT1 and STAT3 has also been reported in CLL cells [13]. More recently, new phosphorylations on threonine (pThr314) and two serine residues (pSer254, pSer265) of CD23, which is overexpressed and abnormally regulated in CLL, were reported in B lymphocytes of B-CLL patients [14]. Regulation of these CD23, CLL-associated phosphorylation sites brings new insight to the involvement of this transmembrane protein marker in the onset and progress of CLL.

2.1 Incidence and risk

CLL is the most common leukemia in western countries, with an estimated incidence of about 4.5 new cases per 100,000 individuals annually [1]. It is most frequent in white populations in the United States and the lowest in Eastern Asian populations [15]. Median age at diagnosis is usually 72 years, and more male than female patients (1.7:1) are affected. About 10% of CLL patients are reported to be younger than 55 years of age [16].

The etiology of CLL is still unknown. Genetics and environmental factors may play an important role. Over 25 gene polymorphisms have been identified as contributing to CLL from a familial standpoint. These include genes that play roles in apoptosis, B-cell biology, as well as regulation by microRNAs, all of which have been found to be involved in disease progression [17, 18]. As such, it is important to note that relative to the general population, a six- to ninefold greater risk of developing the disease exists in individuals who have or have had relatives with CLL. Consequent protein synthesis and the involvement of newly synthesized proteins in disease onset and progression are the focus of numerous current studies. Insecticide exposure and farming history have also been associated with a higher environmental risk for developing CLL [19].

2.2 Symptoms and diagnosis

According to the International Workshop on Chronic Lymphocytic Leukemia (iwCLL) 2008 guidelines [20], a CLL diagnosis is established by the presence of more than 5×10^9/L peripheral lymphocytes, which lasts for a duration of at least 3 months, co-expressing CD5-, CD19-, and CD23-positive and weakly expressing CD20- and CD79b-positive as well as surface immunoglobulins.

Immunophenotyping by flow cytometry is required to establish CLL diagnosis based on cell identity, clonality, and quantity [21].

Two clinical staging systems, the Rai et al. [22] and Binet et al. [23] systems, are used to group patients with CLL into risk groups with discrete clinical outcomes. These two staging systems are relatively simple and widely used, relying on a physical examination and standard laboratory tests. Notably, the clinical presentation of CLL at diagnosis is extremely variable. Approximately 60% of patients are asymptomatic, and it is possible to detect the presence of the disease via a routine blood cell count. Lymphadenopathy (80%) and splenomegaly (50%) may be observed. Hepatomegaly is less frequent. As the disease progresses, patients can have B symptoms (weight loss, fever, night sweats, weakness) and exhibit a higher risk of infections. Lymphocytosis is constantly present, but the absolute number of lymphocytes is extremely variable. Anemia and thrombocytopenia may be also observed in 15–30% of patients [22–24]. Monoclonal B lymphocytosis (MBL), which can be observed in 5% of patients who exhibit a regular blood count and no other characteristics of a lymphoproliferative disposition, is characterized by a monoclonal B lymphocyte number of less than 5×10^9/L in circulating blood [25]. Advancement from MBL to CLL is seen in a frequency of 1–2% cases per year [26].

Small lymphocytic lymphoma (SLL), in which the same leukemic cell population is mostly restricted to the bone marrow and lymphoid tissues, is similarly managed but considered to be a single entity [27]. The transformation into Richter syndrome (most commonly diffuse large B-cell lymphoma) occurs in 5–10% of all CLL cases and usually has a very poor prognosis [16].

2.3 Prognostic factors

The most important prognostic factors aside from clinical Rai and Binet staging systems are serum markers including β2 microglobulin levels [28], thymidine kinase levels [29], soluble CD23 levels [30], cellular markers including CD38 [31] and ζ chain associated protein kinase 70 (ZAP70) [32], CD49d [33], chemokines CCL3 a CCL4, genetic parameters including the mutational status of IGHV genes [10], and cytogenetic aberrations [34]. Unfavorable prognostic factors also include the male gender, ≥65 years of age, poor performance status due to medical comorbidities, late-stage disease at diagnosis, an initial white blood cell count above 35×10^9/L, lymphocyte doubling time of less than 6 months, and a diffuse histological pattern in bone marrow infiltration [35]. Elevated levels of beta-2 microglobulin, serum thymidine, and serum CD23 at diagnosis also result in a poor prognosis [36].

ZAP-70 is a cytoplasmic protein tyrosine kinase initially identified in T cells. ZAP-70 expression in CLL is associated with increased BCR signaling capacity and greater responsiveness to chemokines resulting in more pronounced CLL cell migration and activation. Patients with ZAP-70 expression in more than 20% of CLL cells have a relatively shorter median time from diagnosis to initial treatment [37], and ZAP-70 appears to be a risk factor that is closely linked to aggressive CLL [32]. CD38 is a transmembrane protein that supports B-cell interaction and differentiation through the binding of CD31 [38], a cell adhesion molecule expressed by cells of the CLL microenvironment. Patients with high CD38 expression experience faster progression and shorter life expectancy [31]. The expression of the surface molecule CD49d, the α4 subunit of the integrin heterodimer α4β1, promotes microenvironment-mediated proliferation of CLL leukemic cells and has been identified in a subgroup of patients characterized by

progression of disease and short survival [33]. Both CCL3 and CCL4 are members of a cluster of cytokines with function as chemoattractants for monocytes and lymphocytes. They promote the communication of survival and proliferation signals to malignant cells and are associated with worse clinical outcomes in CLL [39, 40].

Immunoglobulin heavy-chain variable region (IGHV) mutation status plays an important role in CLL prognosis. Based on the degree of somatic hypermutation IGHV segments, unmutated IGHV (98% or more sequence homology with the germline sequence) corresponds to CLL originating from B cells that have not undergone a somatic mutation. Such patients can be classified as "unmutated" (U-CLL). Mutated IGHV (less than 98% sequence homology) is referred to as "mutated" (M-CLL) cases [41]. The presence of unmutated IGHV predicts a more aggressive disease type and has traditionally been associated with significantly decreased survival rates compared with mutated IGHV, which is associated with slower disease progression and longer survival [10, 31]. The differences in clinical behavior between M-CLL and U-CLL are determined by differences in responsiveness to external signals (such as BCR responsiveness). U-CLL BCRs are polyreactive and mostly recognize autoantigens and other environmental antigens [42, 43]. In contrast, affinity-matured BCRs from M-CLL cases bind to a restricted set of more specific antigens that either occur infrequently or induce anergy. Consequently, the M-CLL clone remains stable overall or expands at a slower rate [44, 45].

More than 80% of patients with previously untreated CLL have cytogenetic abnormalities, most common of which is a deletion in chromosome del(13q) [del(13q14.1)] (55%), followed by del(11q) [del(11q22-23)] (10–25%), trisomy 12 (10–20%), and del(17p) [del(17p13)] (5–10%) [34, 46]. Recommended analyses include interphase cytogenetic analysis with FISH for the detection of the del(17p), which affects p53 expression. A positive outcome is often seen in individuals who have deletions in 13q. This is likely a result of two missing miRNAs typically found in 13q, miR-15-1, and miR-16-1, which exhibit strong activity in healthy B cells; miR-15-1 and miR-16-1 are thought to play a role in the downregulation of B-cell lymphoma 2 (BCL2), which acts as an antiapoptotic molecule [34].

The association between trisomy 12 and prognosis is still not clear [47]. A deletion in 11q results in the ataxia telangiectasia mutated (ATM) gene, which has shown to be a predictor of poor clinical outcome [34]. Deletions of the short arm of chromosome 17 cause the loss of one tumor protein p53 (TP53) allele and are associated with inactivating mutations in the other allele in 80% of patients with CLL. This cytogenetic aberration is associated with the worst CLL prognosis. Patients have shown marked resistance against genotoxic chemotherapies which has forced clinicians to alter their first-line treatment [34, 48]. Further recurring gene alterations have been found in 5% of cases of CLL samples at time of diagnosis; via whole genome/exome sequencing, genes influencing NOTCH1 and myeloid differentiation primary response (MYD88) [49] have been identified alongside genes coding for splicing factor 3B subunit 1 (SF3B1) [50] and baculoviral IAP repeat containing 3 (BIRC3) [51]. Patients experiencing progressive/refractory CLL and Richter's syndrome were observed to exhibit these mutations in greater frequency [50].

2.4 Therapy

CLL is an incurable disease with a highly heterogeneous clinical course. Previous studies have shown that early treatment with chemotherapeutic agents

was unable to demonstrate a benefit due to these therapeutic interventions in CLL patients [52]. The standard treatment for patients with early disease is a "watch-and-wait" strategy. Treatment should only be initiated in patients with progressive or symptomatic/active disease. In order to determine the best approach to treatment, crucial factors such as the stage of disease, physical status, and cytogenetic risk should be assessed on a per-patient basis [18]. Additionally, the "Go-Go," "Slow-Go," and "No-Go" comorbidity classifications present another important set of factors in determining the optimal avenue for treatment [53].

Monotherapy with alkylating agents (chlorambucil) and purine analogs (fludarabine, pentostatin, cladribine, bendamustine) has served as an initial, frontline therapy for CLL and was the therapeutic "gold standard" for several decades [52]. Compared to monotherapy, the combination of fludarabine with alkylating cyclophosphamide is more widely used, leading to an increased effect on malignant lymphocytes and greater remission inductions [54]. The onset of biological treatment using monoclonal antibodies has led to significant changes in the approach to treatment. As CD20 is expressed on most B-cell malignancies, the introduction of the anti-CD20 antibody rituximab improved the treatment of most CD20-positive non-Hodgkin lymphomas, including CLL. Rituximab is less active as a single agent; however, combinations of rituximab with chemotherapy have shown to be very efficacious therapies for CLL [55]. The combination of rituximab, fludarabine, and cyclophosphamide is considered to be the standard first-line therapy (FCR chemoimmunotherapy) [56]. Ofatumumab and obinutuzumab are another set of CD20 antibodies used for the treatment of patients with relapsed/refractory CLL [57, 58]. Alemtuzumab is a recombinant, fully humanized, monoclonal antibody against the CD52 antigen. Monotherapy with alemtuzumab is used in patients with advanced CLL or relapsed patients after second-line fludarabine therapy and with poor prognostic features [59]. Autologous stem-cell transplantation is not useful in CLL. Maintenance therapy in CLL patients with higher risk of relapse may have some benefit but is not generally recommended [18].

Lenalidomide is an immunomodulatory agent that induces only mild apoptosis of leukemic cells but also reduces CLL proliferation through a cereblon-/p21-dependent mechanism. Lenalidomide has pleiotropic effects on the CLL microenvironment: it increases CD4+ T-mediated antigen presentation, proliferation, and activity and enhances NK and CD4+ T-cell mediated antitumor immune responses [60]. It is active alone, in CLL relapsed/refractory patients, or as an initial treatment for elderly patients or in combination with rituximab [61].

The CXCR4/CXCL12 signaling axis represents another important therapeutic target in CLL. CXCR4 antagonists have been developed, including peptide CXCR4 antagonists (BKT140), small molecule CXCR4 antagonists (AMD3100, plerixafor), and antibodies to CXCR4 (MDX-1338) [62]. Plerixafor inhibits CXCL12-mediated signaling activation on CLL cells and is used in combination with rituximab in relapsed CLL patients [63].

Proteins in the Bcl-2 family are key regulators of the apoptotic process with proapoptotic and prosurvival activities. Venetoclax is a so-called BH3-mimetic drug designed to block the function of the Bcl-2 protein and inhibits the growth of BCL-2-dependent tumors in vivo. Monotherapy with this drug is active and well tolerated in patients with relapsed or refractory del(17p) CLL, providing a new therapeutic option for this very poor prognosis population [64].

B-cell receptor signaling seems to play an important role in the survival of CLL cells. Inhibitors targeting BCR-associated kinases have changed the landscape of

treatment for CLL patients, inducing durable remissions in relapsed/refractory patients, including those carrying unfavorable genetic alterations (e.g., del17p, del11q) [65]. Randomized trials comparing new drugs and/or their combinations with standard chemoimmunotherapy regimens are ongoing and will allow to better define optimal treatment strategies [66]. New light shed onto the mechanisms of BCR activation in CLL has enabled for the design and application of kinase inhibitors targeting BCR signaling kinases BTK, PI3K, and SYK. Bruton's tyrosine kinase, BTK, is a non-receptor tyrosine kinase that plays a central role in downstream activation of cell survival pathways such as NF-κB and MAP kinases via Src family kinases. Ibrutinib is the first human BTK inhibitor. The drug binds irreversibly to a cysteine residue (Cys-481) in the BTK kinase domain [67] and inhibits BTK phosphorylation and its enzymatic activity [68]. Ibrutinib inhibits CLL cell survival and proliferation, as well as leukemia cell migration toward the tissue homing chemokines [69]. Previous tests have shown that ibrutinib yielded durable remissions in CLL/SLL patients with relapsed, refractory, or high-risk disease and in previously untreated older patients [70]. Acalabrutinib, a potentially more selective, irreversible BTK inhibitor has been tested and is currently under early clinical development [71]. PI3Kδ is expressed by hematopoietic cells and plays a critical role in B-cell homeostasis and function. Idelalisib is a highly selective PI3Kδ inhibitor, which antagonizes CLL-survival signals coming from the microenvironment as well as BCR stimulation [72]. This drug inhibits CLL cell chemotaxis toward CXCL12 and CXCL13 and migration beneath stromal cells and also inhibits BCR- and chemokine-receptor-induced AKT and MAP kinase activation [73]. Idelalisib has been tested as single agent or in combination strategies with clinical benefit in patients with relapsed/refractory CLL [74]. Additional PI3K inhibitors are currently under development, including duvelisib, a potent PI3K γδ inhibitor, which antagonizes BCR and microenvironment interactions in vitro [75]. Spleen tyrosine kinase (SYK), which belongs to the SYK/ZAP70 family of non-receptor kinases, has been implicated in tissue homing and retention of activated B cells due to its role as a downstream activator of BCR signaling (chemokine and integrin receptors) [76]. Up to this point, only limited responses have been seen in patients experiencing CLL relapse after introduction of fostamatinib disodium (FosD) to the treatment regimen [77]. FosD is currently the only available inhibitor of SYK on the market, with additional similar drugs being developed [78].

3. Acute lymphocytic leukemia

Acute lymphocytic leukemia (ALL), also known as acute lymphoblastic leukemia or acute lymphoid leukemia, is the most common malignancy in children and the least common type of leukemia in adults. It is an acute type of cancer invading blood and spreading throughout the body to other organs, such as the liver, spleen, lymph nodes, and central nervous system. Without treatment, it can be fatal within a few months. ALL is characterized by a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites, which replace normal blood cells [79]. The exact causes of ALL remain largely unknown, but it is thought to result from genetic alterations such as structural chromosome rearrangements, aneuploidy, and mutations in genes that encode for transcription factors regulating lymphoid development, tumor suppressors, proteins that regulate cell cycle progression, and epigenetic modifiers. Such defects result in abnormal growth [80].

3.1 Classification

ALL is a hematologic malignancy with uncontrolled proliferation of lymphoblasts of B- or T-cell origin. ALL cases are clinically classified as B-cell precursor (BCP), mature B-cell, or T-cell types. BCP-ALL arises in B lymphocytes in the early stages of development in the bone marrow and affects 75–80% of adult patients. Mature B-cell ALL arises in more mature developing lymphocytes. This type of ALL is less common and accounts for around 3–5% of all adult cases. In around 20–25% of cases, ALL arises in developing T cells. This type of ALL can be further classified as early, mid, or late, depending on the maturity of the affected cell. T-cell ALL is commonly presented with a high white blood cell count and involvement of the central nervous system at diagnosis [81] (**Table 1**).

3.2 Incidence and risk

The incidence of ALL is estimated at 1.7 per 100,000 population in the United States [82] and 1.28 per 1 000,000 individuals in Europe [83] each year. ALL is the most frequent cancer in children, accounting for 30% of all cancers and 80% of leukemias, with peak incidence occurring at 2–5 years of age. The incidence decreases with age progression and rises back up with a second peak in patients above the age of 50 years, representing about 15% of leukemias [84]. ALL is more common in males than females. Survival rates were poor 50 years ago, when leukemia was considered to be an intractable disease. Currently, pediatric patients with ALL have dramatic cure rates with 80–90% achieving complete remission (CR) [85]. However, prognoses in the elderly remain miserable. Despite a high rate of response to induction chemotherapy, only 30–40% of adult patients with ALL will achieve long-term remission [86].

There are a few risk factors which can increase the possibility for ALL, such as exposure to high levels of radiation, industrial chemicals (such as benzene), pesticides [87], certain types of chemotherapy used to treat other cancers, certain types of viral infections (human T-cell lymphoma/leukemia virus-1 or Epstein-Barr virus) [88], inherited genetic syndromes (such as Down syndrome) [89], and being white and male.

3.3 Symptoms and diagnosis

Most clinical manifestations of ALL exhibit the accumulation of malignant, poorly differentiated lymphoid cells within the bone marrow, peripheral blood, and other tissues. Symptoms of ALL are generally nonspecific with a combination of constitutional symptoms and signs of bone marrow failure (anemia, thrombocytopenia, leukopenia). Common symptoms include “B symptoms”

ALL classification	Subtypes	Ref
B-ALL	B-cell precursor ALL (75–80%)	[81]
	Mature B-cell ALL (3–5%)	
T-ALL	Early T-cell precursor ALL (20%)	
	Mid or late subtypes of T-ALL (5%)	

Table 1.
Classification of ALL subtypes.

(fever, weight loss, night sweats), easy bruising or bleeding, fatigue, dyspnea, and infections. Lymphadenopathy, splenomegaly, or hepatomegaly can be also present [90, 91]. CNS involvement at time of diagnosis occurs in 5–8% of patients and presents most commonly as cranial nerve deficits or meningismus [86]. Current standards for the diagnosis of ALL are based on the classification of lymphoid neoplasms according to the World Health Organization (WHO) 2008 criterion [92]. Diagnosis of ALL is established by the presence of 20% or more lymphoblasts in the bone marrow or peripheral blood [90]. Flow cytometry and cytogenetic testing are needed to confirm the diagnosis and provide risk stratification. Immunophenotyping by flow cytometry has become the standard procedure for ALL diagnosis and subclassification and was also developed as a useful tool for the detection and monitoring of minimal residual disease. In B-lineage ALL, the most important markers for diagnosis, differential diagnosis, and subclassification are CD19, CD10, CD20, CD22, CD24, and CD79a [93, 94]. For T-lineage, they are CD1a, CD2, CD3, CD4, CD5, CD7, and CD8 [95]. Cytogenetics and karyotyping are helpful in the identification of recurrent translocations, chromosomal abnormalities, and numerical alterations. Fluorescence in situ hybridization (FISH) is a useful technique for detecting and localizing the presence or absence of specific DNA sequences on chromosomes, with 99% sensitivity. Finally, array-comparative genomic hybridization (array-CGH, a-CGH) and single-nucleotide polymorphism (SNP) arrays can facilitate the identification of cryptic and/or submicroscopic changes in the genome [96, 97]. Lumbar puncture with CSF analysis is the current standard of care for the diagnosis of CNS involvement. If the CNS is involved, brain MRIs should be performed. Other possible evaluations include a complete blood count alongside cytologic analysis of target cells to evaluate other hematopoietic cell lines, coagulation profiles, and serum chemistries [80].

3.4 Prognostic factors

ALL is a highly heterogeneous disease, and several clinical and biologic characteristics of ALL are used in risk stratification and prognostication. Disease characteristics (e.g., cytogenetics, molecular genetics, immunophenotypes) are substantially different between childhood, young adult, and adult ALL cases. Prognostic factors applied to ALL include age, white blood cell count (WBC), time to achieve a complete hematologic remission, minimal residual disease (MRD) persistence [98], and genetic aberrations. Older age and higher leukocyte count are associated with poor prognosis. Children older than 10 years with a leukocyte count exceeding 50,000/mm^3 are classified as high risk according to the National Cancer Institute criteria (NCI-HR) [99]. ALL in young adults leads to poorer outcomes and exhibits high-risk genomic features (BCR-ABL1, BCR-ABL1-like, ETP-ALL [100], JAK mutation, CRLF2 alteration [101], iAMP21 [102], or DUX4 translocation [103]). The National Cancer Institute defined adolescent and young adults (AYA) to be those aged 15–39 years old. AYAs may benefit from pediatric-inspired regimens and are thus considered separate from adults >40 years [104]. Elderly patients tend to have a form of the disease characterized by intrinsically unfavorable biology (BCR-ABL1, BCR-ABL1-like, hypodiploidy, and complex karyotype), more medical comorbidities, and an inability to tolerate standard chemotherapies. They also experience a higher risk of relapse. As such, patients over the age of 60 have particularly poor outcomes, with only 10–15% surviving long term [105]. Response to chemotherapy is a strong prognostic indicator in ALL. Clearance of leukemic blasts in the early

phase of treatment and the achievement of remission at the end of induction are predictors of relapse risk and have prognostic importance. Gender has also been recognized as a prognostic factor, with females having a better outcome than males overall.

3.4.1 Cytogenetic/genetic risk

Cytogenetic analyses have demonstrated that chromosomal aberrations (insertions, deletions, translocations, and inversions) and numerical alterations (hyperdiploid, pseudodiploid, and hypodiploid) are hallmarks of ALL [106]. The prevalence of genetic subtypes differs with age and is of prognostic relevance. Approximately half of pediatric leukemia cases involve aneuploidy (with changes in chromosome number), including high hyperdiploidy (50–67 chromosomes) or hypodiploidy (44 chromosomes or fewer) [107]. The chromosome most frequently gained in patients with high hyperdiploidy is 21 (>90% cases with trisomy or tetrasomy of chromosome 21) [108]. It is thought that the duplication of specific chromosomes contributes to leukemogenesis, making high hyperdiploidy a stronger prognostic factor than hypodiploidy. Hypodiploidy has been associated with dismal prognosis in all observed cases of ALL. Near-haploid (24–31 chromosomes) and low-hypodiploid (32–39 chromosomes) ALLs exhibit activation of Ras- and PI3K-signaling pathways, suggesting that these pathways may be a target for therapy in aggressive hypodiploid ALLs [109]. Studies in the pediatric population have identified genetic syndromes that are connected to the predisposition in a minority of cases of ALL, such as Down syndrome, Fanconi anemia, Bloom syndrome, ataxia telangiectasia, and Nijmegen breakdown syndrome [89, 110, 111].

Characteristic translocations include erythroblast transformation-specific (ETS) variant 6–Runt-related transcription factor 1 (ETV6-RUNX1), the most common translocation (15–25% of pediatric ALL patients) caused by t(12;21)(p13;q22). The prognosis of ALL with ETV6-RUNX1 is excellent [112]. A second common translocation in pediatric ALL is transcription factor 3-PBX homeobox 1 (TCF3-PBX1), which is caused by t(1;19)(q23;p13) and is observed in 5–10% of ALL cases. Previously, patients with this translocation were considered to have poor prognosis, but a recently improved treatment has resulted in better outcomes [113]. A small percentage of ALL patients (3–5%) exhibit the reciprocal translocation t(9;22)(q34;q11), also referred to as the "Philadelphia (Ph) chromosome." The Ph chromosome is largely prominent in patients suffering from chronic myeloid leukemia (CML) and is molecularly characterized by the creation of a non-receptor tyrosine kinase gene (BCR-ABL1) via the fusion of RhoGEF and GTPase-activating protein (BCR) and ABL proto-oncogene 1 (ABL1) [114].

The prevalence of t(9;22) in adult ALL can range from 15 to 50% and increases with age [115]. Ph chromosome positivity has been widely considered to be a factor for poor prognosis. The development of tyrosine kinase inhibitors (TKI), which directly target BCR-ABL1, has shown to significantly improve the treatment strategy for Ph-ALL. Rearrangement of the mixed-lineage leukemia 1 gene (MML1), also known as KMT2A (lysine [K]-specific methyltransferase 2A), on chromosome 11q23 is found in a unique group of acute leukemias and predicts a very poor outcome [116].

More recently, a variant with a similar gene expression profile to Ph-positive ALL, but without the BCR-ABL1 rearrangement, has been identified. This so-called Ph-like ALL, or BCR-ABL1-like ALL, has been associated with poor response to induction chemotherapy, elevated minimal residual disease, and poor survival [117]. The prevalence of Ph-like ALL is common among all ages, ranging from 10 to 15% in children to over 25% in young adults [118]. Patients with Ph-like ALL

harbor a diverse range of genetic alterations which activate cytokine receptor and kinase signaling pathways. Common genomic features of Ph-like ALL include alterations of B-lymphoid transcription factor genes (particularly IKZF1 deletions) as well as rearrangements and mutations of CRLF2, ABL-class tyrosine kinase genes, EPOR, JAK-STAT signaling, and RAS signaling (NRAS, KRAS, PTPN11, NF1) and other less common kinase alterations (FLT3, NTRK3, BLNK, TYK2, PTK2B) [119]. These mutated genes can be successfully targeted with tyrosine kinase inhibitors [117]. Another new high-risk subtype identified in diagnosis of ALL is B-ALL, which is characterized by intrachromosomal amplification of chromosome 21 (iAMP21) [102].

Genome-wide profiling studies have revealed components of multiple cellular and signaling pathways that are frequently mutated in ALL (referred to as cooperative mutations). Deletions in key transcription factors involved in B-cell development include IKAROS family zinc finger 1 (IKZF1), transcription factor 3 (E2A), early B-cell factor 1 (EBF1), and paired box 5 (PAX5). Kinase-activating mutations include rearrangements involving ABL1, JAK2, PDGFRB, CRLF2 and EPOR, activating mutations of IL7R and FLT3, and deletion of SH2B3, as well as mutations involved in tumor suppression (CDKN2A/CDKN2B, PTEN, and RB1), RAS signaling (NRAS, KRAS, and PTPN11), transcriptional regulation (ETV6, ERG, TBL1XR1, and CREBBP), and epigenetic modification (CREBBP, EP300, SETD2, and NSD2) [117]. In all ALL subtypes, multiple cooperating mutations are acquired or enriched for during leukemia development and progression [120]. TP53 disruption has also been detected in relapsed B-ALL and T-ALL, as well as in newly diagnosed children and adult ALL cases. Correlation with poorer outcome has been illustrated and is associated with refractoriness to chemotherapy in adults [121].

Next-generation sequencing (NGS), most notably transcriptome sequencing, has led to the identification of several novel rearrangements that are not made evident by conventional genetic analysis, including DUX4-rearranged [122], MEF2D-rearranged [103], and ZNF384-rearranged B-ALL and ETV6-RUNX1-like B-ALL [123]. These new ALL subtypes have distinct clinical and biological characteristics. The prognosis of the B-ALL subtypes is shown in **Table 2**.

Molecular subtype	Prognosis	Frequency (%)	References
Hyperdiploid	Favorable	20–30	[107, 108]
ETV6-RUNX1	Favorable	15–25	[112]
TCF3-PBX1	Intermediate	5–10	[113]
KMT2A rearranged	Unfavorable	5	[116]
BCR-ABL1	Unfavorable	5–50	[114, 115]
BCR-ABL1 like	Unfavorable	10–25	[118]
Hypodiploid	Unfavorable	3	[109]
iAMP21	unfavorable	2	[102]
DUX4 rearranged	Favorable	4–5	[122]
MEF2D rearranged	Unfavorable	2–3	[103]
ZNF384 rearranged	Intermediate	2–3	[123]
ETV6-RUNX1 like	Intermediate	2–3	[123]

Favorable, intermediate, and unfavorable prognoses of acute lymphoblastic leukemia (ALL) subtypes are associated with 5-year overall survival of >90%, 70–90%, and <70%, respectively.

Table 2.
Prognosis in B-ALL.

T-ALL is characterized by numerous transcriptional, signaling, and epigenetic factors. Activating mutations in NOTCH1 can be found in the majority of T-ALL cases and predict a favorable prognosis [124]. Deletions of the CDKN2A locus encoding the P16/INK4A and P19/ARF tumor suppressors, responsible for control of cell cycle progression and P53 regulation, respectively, are present in about 70% of T-ALLs [125]. Gene expression profiling has identified major categories of T-ALL associated with gene expression during thymocyte development. Cytokine receptor RAS signaling genes, which include FLT3, have been found to be activated by mutation in early T-cell precursor T-ALL (ETP T-ALL). In addition, alterations in genes which disturb hematopoietic development, such as GATA 3, ETV6, and RUNX1, have been observed. Lastly, mutations in histone-modifying genes (EZH2, SUZ12, and EED) are also a consequence of ETP T-ALL. ETP T-ALL has been associated with poor prognosis [126]. Early cortical thymocyte leukemias are primarily associated with translocations resulting in aberrant expression of TLX1, TLX3, and related homeobox transcription factor oncogenes; these exhibit a characteristically favorable outcome [125, 127]. Late cortical leukemias occur further down in the pattern of gene expression programming related to T-cell development, overexpressing the transcription factor oncogene TAL1 with either LMO1 or LMO2 and PTEN. These are associated with poor prognosis [125, 127].

3.5 Therapy

Typical chemotherapy consists of induction, consolidation, and long-term maintenance, with CNS prophylaxis given at intervals throughout therapy. The goal of induction therapy is to achieve complete remission and to restore a normal blood cell count. Predominantly 85–90% of patients achieve complete remission after 4–6 weeks of this regimen [128]. Several chemotherapeutic agents are currently used in the treatment of CLL, including amascrine, asparaginase, cyclophosphamide, cytarabine, daunorubicin, dexamethasone, and methotrexate. Each utilizes slightly differing mechanisms of action; in the general sense however, these molecules affect the growth and division of cancer cells by inducing DNA damage [129]. Multi-agent cytotoxic chemotherapy has had great success in pediatric age groups [130]. Pediatric-inspired treatment protocols have also shown superior outcomes in young adults [104], but the same success has not been reproduced in adults despite regime modifications. Traditional adult treatment protocols include intensive myelosuppressive agents as well as allogeneic hematopoietic stem cell transplant (allo-SCT) in first remission [104]. After achieving complete response, treatment options include consolidation and maintenance chemotherapy or allo-SCT for eligible patients [131]. For high-risk patients (Ph-positive ALL, elevated WBC count, CNS disease, high-risk gene rearrangements, or hypodiploidy) and patients with relapsed/refractory disease, allo-SCT has long been considered the standard of care. However, the advent of TKIs marked a turning point in the treatment of some high-risk subtypes such as Ph-ALL and Ph-like ALL. After induction therapy, subsequent consolidation therapy begins to eradicate residual leukemic cells. Consolidation varies in different protocols but generally utilizes similar agents for induction (various combinations of cytotoxic agents and high dose of escalating methotrexate) and at times includes intrathecal chemotherapy and cranial radiation for CNS prophylaxis [132]. Maintenance therapy typically lasts 1–2 years. Daily 6-mercaptopurine (6-MP) and weekly MTX are a standard combination, and some maintenance therapies are enhanced with vincristine and steroids [80].

A better understanding of the molecular landscape of ALL and advances in the field of monoclonal antibody therapy have resulted in the development of several new agents, especially in the treatment of adolescent and young adults (AYA) and adult patients. Targeted delivery of monoclonal antibodies based on leukemic cell-surface receptor recognition improves efficacy and minimizes off-target toxicity. The antigens CD19, CD20, CD22, and CD52 are the most common antigens to which monoclonal antibodies in B-cell ALL have been directed. Rituximab is a non-conjugated monoclonal antibody designed to target a single antigen on the tumor cell surface. The combination of rituximab with chemotherapy in the frontline treatment of CD20-positive B-ALL has been shown to increase CR duration, lower relapse rates, and improve event-free survival [133]. A new generation of monoclonal antibodies exists which is characterized by the antibody being conjugated to drug or toxins with the purpose of enhancing the efficiency of cancer cell killing. For example, inotuzumab ozogamicin (IO) is a monoclonal antibody against CD22 linked to the cytotoxic agent, calicheamicin. The use of IO alone, and in combination with chemotherapy, has shown promise in relapsed and refractory B-cell ALL [134]. Other modifications to antibody constructs can also augment immunogenic reactions against leukemia. Blinatumomab is the first approved drug in the BiTE class, a bispecific T-cell receptor engager, which has both a monoclonal antibody against CD19 and an anti-CD3 T cell-binding domain. Monotherapy in relapsed and refractory B-cell ALL has resulted in prolonged relapse-free survival [135]. The effectiveness and safety of several newer monoclonal antibodies including ofatumumab [136], obinutuzumab, epratuzumab [137], and moxetumomab pasudotox [138] as single agents or in combination with a chemotherapeutic are currently under investigation. Chimeric antigen receptor (CAR) therapy has shown remarkable efficacy in B-cell ALL. CAR combines both antigen-binding and T-cell activating functions into a single receptor. CAR-modified T cells involve a mechanism in which a patient's own T cells are genetically programmed to recognize leukemic cells, inducing an antileukemic immune response. Complete remission rates as high as 90% have been reported in children and adults with relapsed and refractory ALL posttreatment with CAR-modified T cells targeting the B cell-specific antigen CD19 [139]. Treatment of the high-risk Ph-like ALL has significantly improved with the identification of genetic alterations which deregulate cytokine receptor and tyrosine kinase signaling, both common features of this subtype of ALL. Tyrosine kinase inhibitors (TKIs) such as imatinib, dasatinib, nilotinib, and ponatinib, NOTCH1 and DOT1L pathway inhibitors, and JAK inhibitors have become novel agents for Ph-like ALL therapy. In addition, 50% of Ph-like ALLs show activation of phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) and mammalian target of rapamycin (mTOR) pathways and could therefore present potential targets for mTOR inhibitors [140]. Inhibition of the PI3K/AKT/mTOR pathways may be an effective treatment for T-ALL.

4. Protein markers of CLL and ALL as a new therapeutic targets

New specific protein markers connected with CLL and ALL which have been discovered in the last 10–15 years represent novel potential targets for highly personalized treatments of leukemia. These proteins, associated with different cellular signaling events, mostly include surface receptors/transmembrane proteins—CD5, CD19, CD20, CD22, CD23, CD52, and many others [9, 11, 31, 33, 38]—where protein phosphorylation may play an important role in protein

activity regulation connected to the progression of disease and regulation of pathological events [12–14]. Focusing on such specific modifications presents key opportunities to further facilitate efficient and precise drug strategy design [55–58]. Inhibition of protein kinases associated with key phosphorylations has been an intense research topic in the last decade [67–69, 72, 73, 75]. Significant progress in protein mass spectrometry techniques, specific antibody design and development, parallel studies of genes, epigenetic proteome, and related proteins including their disease-related modifications altogether open a new horizon for a more sensitive and personalized approach to the diagnosis and treatment methods of CLL and ALL. The combination of such approaches should further facilitate the development of more efficient drugs and approaches which more specifically target the key signaling events concerning the onset and progression of the disease. Based on the fact that proteome maps are unique to each individual, there is an urgent need for personalized diagnostics and a personalized molecular treatment approach. Using the information from the proteins associated with the CLL and ALL, and the misregulation of signaling pathways in associated cell regulation events, the precise and detailed protein signaling outcome can form the base of potential success in the domain of efficient drug design and consequent molecular treatment, without the typical side effects of current conventional methods.

5. Conclusion

Given the diverse molecular and genetic alterations occurring in both CLL and ALL, it is unlikely that a single and unique therapeutic approach will be effective across all patients. Great progress has been made thus far in the identification of oncogenic drivers and therapeutic targets. However, although treatment regimens have advanced significantly, they continue to present many challenges for the majority of patients, including toxicity. Future studies focused on the identification of biomarkers should result in more effective treatments exhibiting antileukemic activity with reduced toxicity. Furthermore, highly targeted therapy can be expected to lead to improvements in remission and survival as part of individualized treatment strategies.

Conflict of interest

The authors declare no conflicts of interest.

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