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New Insights in Prognosis and Therapy of Chronic Lymphocytic Leukaemia

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Additional information is available at the end of the chapter

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Abstract

Chronic lymphocytic leukaemia (CLL) is a heterogeneous disease with a very variable clinical outcome. New biological markers, such as cytogenetic abnormalities or mutation status, have become important prognostic factors. Whole-genome sequencing studies have revealed novel genomic mutations, *NOTCH1*, *SF3B1*, *BIRC3*, *TP53* and *MYD88* being the most important. All these mutations have also been associated with the disease outcome. The treatment of CLL has evolved favourably in recent years. However, adverse events or chemorefractoriness occurs in some cases. Luckily, an increasing number of compounds are under development with promising results. Some of these new targeted therapies include B-cell receptor inhibitors, new anti-CD20 antibodies, Bcl-2 inhibitors, immunomodulatory drugs or chimeric antigen receptors (CARs). In this chapter, we will conduct a review of the new prognostic markers of CLL, the relationship they have with each other to build prognostic scores, the role they have in guiding treatment decisions and the novel therapies that have emerged recently with immunologic, biochemical and genetic targets.

Keywords: Chronic lymphocytic leukaemia, genetic abnormalities, recurrent mutations, targeted therapy, signalling pathway

1. Introduction

Chronic lymphocytic leukaemia (CLL) is a neoplasm characterized by the proliferation and accumulation of monoclonal mature B lymphocytes in the peripheral blood, bone marrow,

spleen and lymph nodes. It is the most frequent type of leukaemia in adults from Western countries, showing a predilection for the elderly, with a median age at diagnosis of 72 years old. It has been more prevalent in men than in women [1, 2].

The clinical course of CLL is highly heterogeneous. Some patients require treatment at the time of diagnosis, while others remain asymptomatic and may even never be treated. Therefore, median survival times range from a few months to many decades [3,4]. In order to define disease extent and prognosis, Rai and Binet staging systems were designed around 35–40 years ago and remain widely used in clinical practice [5,6]. They are based on physical examination and blood counts and are therefore inexpensive and easy to apply. However, some patients with early stages promptly progress and do not respond to therapy. For these reasons, over the last ten years, several biological markers such as immunoglobulin heavy-chain variable region (*IGHV*) mutation status, cytogenetic abnormalities or expression of specific proteins on CLL cells have become important prognostic factors for this disease [7–10]. They are available in routine clinical practice and may even guide treatment decisions. Recently, the improvements in the next-generation sequencing technologies have revealed novel genomic markers with important prognostic value highlighting *NOTCH1* (neurogenic locus notch homolog protein 1), *SF3B1* (splicing factor 3B subunit 1), *BIRC3* (baculoviral IAP repeat-containing protein 3), *TP53* (tumour protein p53) and *MYD88* (myeloid differentiation primary response 88). In the first part of this chapter, we will conduct a review of these markers, its implications on pathophysiology and prognosis of CLL, the relationship they have with other prognostic factors in order to establish new scores for clinical practice, and the role they have in guiding therapeutic choices.

CLL remains an incurable disease with the exception of allogeneic transplantation. Besides, some patients without treatment present survival rates similar to the normal population, and no benefit has been found when early treatment has been applied in this subset of patients. In addition, spontaneous cure has been reported in rare occasions [11]. For these reasons, only half of the patients diagnosed with CLL will require treatment during follow-up. Fortunately, survival rates of patients with CLL have improved significantly, thanks to the great advances in treatment over the past decades [12]. Glucocorticoids and alkylating drugs were the first treatments introduced, followed by purine analogues. Later, the arrival of targeted antibody therapy led by rituximab (anti-CD20) was the most revolutionary progress. Bendamustine, another alkylating agent used in Germany for more than 30 years, has also been approved for the treatment of CLL after showing its benefits for this disease in clinical trials [13,14]. All these drugs remain widely used in routine clinical practice, mainly in combination regimens. In fact, chemo-immunotherapy regimens are nowadays the standard approach to therapy of most patients with CLL, as they have demonstrated to produce a survival benefit with durable remissions [15–17]. Table 1 sums up approved drugs for the treatment of CLL by categories; and Table 2 summarizes the combination of suggested treatment regimens used for the treatment of CLL recommended by the NCCN (National Comprehensive Cancer Network) [17].

Alkylating agents

Chlorambucil

Cyclophosphamide

Bendamustine

Purine analogues

Fludarabine

Pentostatin

Cladribine

Monoclonal antibodies

Anti-CD20

Rituximab

Ofatumumab

Obinutuzumab

Anti-CD52

Alemtuzumab

B-cell receptor inhibitors

Ibrutinib

Idelalisib

Table 1. Approved drugs by categories for the treatment of CLL

Acronym	Drugs
FCR	Fludarabine, cyclophosphamide, rituximab
FR	Fludarabine, rituximab
PCR	Pentostatin, cyclophosphamide, rituximab
BR	Bendamustine, rituximab
Chlorambucil + anti-CD20	Rituximab/obinutuzumab/ofatumumab
HDMP + rituximab	High-dose methylprednisolone, rituximab
RR	Lenalidomide, rituximab
RCHOP	Rituximab, cyclophosphamide, doxorubicine, vincristine, prednisone
Idelalisib + rituximab	Idelalisib, rituximab
Alemtuzumab + rituximab	Alemtuzumab, rituximab

Table 2. Combination suggested treatment regimens used for the treatment of CLL recommended by the National Comprehensive Cancer Network (NCCN) Version 1-2015

Nevertheless, some of these regimens are not exempted from adverse events that limit their use in frail patients, especially the elderly. Moreover, some patients are chemo-resistant to these drugs, and a curative therapy is still absent for them. Knowledge of the pathogenesis of B-cell receptor (BCR) has led to the investigation of novel molecular targets like Bruton tyrosine kinase inhibitors or phosphatidylinositol 3-kinases (PI3Ks). New anti-CD20 antibodies such as ofatumumab or obinutuzumab have also been shown to have a promising activity in CLL. Other new target therapies under study include Bcl-2 inhibitors, immunomodulatory drugs (lenalidomide) or chimeric antigen receptors. The second part of this chapter will be dedicated to review novel therapies that have emerged recently with immunologic, biochemical and genetic targets.

2. Prognostic markers and risk stratification of CLL

The clinical course of CLL is extremely variable. Prognostic markers are important not only for patient management but also in understanding the disease biology. Many biological factors have been added to the classic staging systems of Rai and Binet with the intention to establish prognostic groups. However, as novel cytogenetic and molecular findings are discovered, our understanding on its prognostic value keeps in constant evolution. In this section, we will conduct a review of these “new” prognostic markers, focusing on genetic markers.

2.1. Genetic markers in CLL

2.1.1. Recurrent genomic abnormalities detected by interface fluorescence in situ hybridization (FISH)

FISH studies are able to detect clonal genomic aberrations in the majority (>80 %) of CLL patients. The most common recurrent chromosomal abnormalities include 13q deletion (13q-), 11q deletion (11q-), trisomy 12 (+12) and 17p deletion (17p-), defining five prognostic categories with different survival times [18].

2.1.1.1. 13q-

13q- is the most frequent chromosome aberration in CLL, observed in approximately 55 % of the cases, and entails the group of patients with a better prognosis. However, the deletions that occur at this chromosome are not homogeneous and neither is the prognosis for this subgroup of patients. The size of the deletion varies thumping. Two types of deletion have been proposed in accordance to their extent: 13q- type I or short deletions not comprising the *RB1* (retinoblastoma 1) locus and 13q- type II or large deletions including the *RB1* locus. The latter have been associated with a more aggressive clinical course [19,20]. 13q deletions are monoallelic in most cases, but biallelic losses have also been described in nearly 30 % of patients, and although it has been controversial, they do not seem to entail a worse clinical outcome [21–24]. The size of the clone harbouring 13q- has also implications on prognosis, with a significantly shorter time to first treatment (TTFT) and overall survival (OS), if a high proportion of cells carry 13q- [22,25,26]. With intent to explain the pathogenesis and clinical

heterogeneity of 13q-, several genes located at 13q have been identified. Among them stand microRNAs (miRNAs) *miR15a* and *miR16-1*, *DLEU2*, *DLEU7* or *RB1*. *MiR15a* and *miR16-1* exhibit a tumour suppressor function in CLL by targeting the *BCL-2* oncogene, being absent or downregulated in the majority of the cases [27,28].

2.1.1.2. 11q-

Prevalence of 11q deletions is estimated below 20 % [18]. The presence of 11q- entails bad prognosis, and often patients present with progressive disease, B symptoms, bulky lymphadenopathy, short TTFT and a reduced OS. In addition, 11q- is associated with unmutated *IGHV* status (U-CLL), which is consistent with the poor prognostic factors mentioned [29,30]. However, some evidence points out that the addition of immunotherapy to classic chemotherapy may overcome the effect of 11q- in previously untreated patients [15,31]. Analogously to 13q-, a high percentage of cells with 11q- has been associated with a worse outcome among 11q- patients [32,33]. 11q- usually implies the loss of the *ATM* (ataxia telangiectasia mutated) gene, albeit *ATM* mutations have been found in <30 % of 11q- cases. *ATM* gene is involved in the repair of damaged DNA; hence, its deficiency causes genomic instability and allows the accumulation of additional genetic mutations during disease course [34]. *BIRC3* gene is also located on 11q close to the *ATM* gene, and deletions and mutations of this gene lead to an unfortunate outcome as well, as they tend to appear in fludarabine-refractory patients [35].

2.1.1.3. +12

Trisomy 12 is the third most frequent cytogenetic aberration, occurring in up to 15 % of CLL cases. Patients with this aberration have been classically considered to have an intermediate prognosis; however, further work has considered this trisomy as a clinical heterogeneous entity [36]. +12 has been associated with an atypical morphology and immunophenotype [37] and has been connected with concurrent trisomy of chromosomes 18 and 19 [38]. Critical genes involved in this aberration remain unknown. *NOTCH-1* mutations were identified in 30–40 % of patients carrying +12, conferring a worse clinical outcome when present in this subset of patients [39–41]. Similarly to deletions, among patients with +12, a high percentage of cells carrying +12 is associated with a worse OS and TTFT [42].

2.1.1.4. 17p-

17p deletion is observed in around 7 % of untreated CLL cases, but its incidence may amount up to 45 % in cases of relapsed or refractory CLL [43]. 17p- is invariably associated with a very poor outcome because of the loss of *TP53* gene. In more than 75 % of the cases with 17p-, mutations in *TP53* are observed in the remaining allele [44]. In spite of that, monoallelic inactivation of *TP53* may be enough to confer a poor prognosis [45]. The tumour suppression p53 acts by inducing apoptosis or cell cycle arrest when DNA is damaged, and consequently genomic complexity is not a rare find in this setting [46]. As in other cytogenetic abnormalities, a high percentage of deleted cells has been associated with a worse outcome within 17p- patients [47]. Patients with 17p- usually do not respond to conventional therapies (fludarabine or alkylating agents), as they are based on p53-dependent mechanisms. Thus, these patients

relapse more frequently and have a shorter OS [48]. Nowadays, 17p- is the only cytogenetic abnormality that defines a different treatment approach. Rational options for these patients include new B-cell receptor inhibitors or methylprednisolone +/- alemtuzumab or rituximab followed by allogeneic bone marrow transplantation in candidates for this procedure.

2.1.2. *IGHV* mutation status

The somatic hypermutation of the variable region of the immunoglobulin heavy-chain genes (*IGHV*) has become one of the most stable and reliable indicators of clinical outcome in CLL. Based on the cutoff value of 98 % identity with the closest germ line *IGHV*, two different subsets of CLL can be indentified: mutated CLL (M-CLL) and unmutated CLL (U-CLL) [8,9]. Somatic mutations of *IGHV* occur in approximately half of the cases and usually present with non-progressive disease, in contrast to patients with U-CLL who have a more aggressive disease with a shorter progression-free survival (PFS), TTFT and OS. U-CLL is also associated with unfavourable prognostic factors such as 11q-, 17p- or ZAP-70 positivity. Furthermore, irrespective of mutation status, some heavy-chain variable regions have been associated with specific clinical features, outcome and varying occurrences from country to country. For example, *IGHV1-69* gene has been observed to be one of the most frequently rearranged genes in Western patients and is almost always associated with the subset of U-CLL [49]. Other subgroups reported to be frequently used in Western patients are *IGHV3-23*, *IGHV4-34* and *IGHV3-07*. Moreover, an overrepresentation of the *IGHV3-21* gene has been reported in northern European countries compared to the Mediterranean region, and it has been associated with a worse prognosis despite the mutation status [50].

2.1.3. Mutations of key tumour suppressor genes

2.1.3.1. *TP53* mutations

CLL harbours *TP53* mutations in around 5–10 % of the cases at diagnosis, but the incidence increases up to 40–50 % in refractory patients or Richter transformation. *TP53* mutations separate a group of patients with critical importance in CLL. This mutation is associated with an ominous outcome due to chemo-refractoriness and therefore is the only biomarker that currently drives treatment decisions in CLL. *TP53* mutations have a detrimental impact on therapy response, PFS and OS [45,48]. *TP53* is a tumour suppressor gene that induces apoptosis or cell cycle arrest after DNA damage. Chemo-refractoriness in patients with these mutations is explained by the mechanism of action of fludarabine or alkylant agents, based on a p53-dependent mechanism. Thus, analogously to 17p-, these patients should be treated, avoiding DNA-damaging chemotherapy agents. Very limited efficient options have been available in the last years in spite of allogeneic stem cell transplantation. Luckily, a number of novel biological drugs such as B-cell receptor inhibitors have been developed and incorporated in the treatment of these patients with encouraging results [51]. Although *TP53* mutations are usually accompanied by 17p-, some patients carry *TP53* mutations in the absence of 17p-. However, the monoallelic *TP53* alteration has the same negative impact in prognosis than biallelic defects [44]. *TP53* mutations may appear in a nondominant clone of CLL cells. Ultra-

deep next-generation techniques were used with the purpose of identifying these subclones, and *TP53* mutations were observed in around 9 % of the cases. These patients showed the same poor survival than patients with *TP53* mutations in the predominant clone [52].

2.1.3.2. *ATM* gene mutations

ATM gene encodes for the ATM protein kinase, a member of the *PI3K* (phosphatidylinositol 3-kinase) family. *ATM* protein takes part in the DNA damage repair mechanism, mediating cellular response to DNA damage in the form of double-strand breaks [53]. The *ATM* gene is located on chromosome 11, and mutations in this gene occur in around 12 % of CLL cases [54]. As mentioned before, 11q- is not frequently associated with *ATM* mutations (30 % of cases), although 11q- almost always implies the loss of *ATM*. Similarly to TP53 and 17p-, mutations in the *ATM* gene entail bad prognosis despite its association with 11q-. Therefore, *ATM* mutations entail short OS, with a more aggressive disease and poor response to chemotherapy, and are related to U-CLL [54]. Another relevant finding concerning *ATM* gene is that biallelic inactivation carries a worse clinical outcome, with more treatment resistance compared to monoallelic alterations [55].

2.1.4. Novel gene mutations

New deep sequencing technologies have discovered in the last five years novel recurrent mutations in CLL, *NOTCH1*, *SF3B1*, *BIRC3* and *MYD88* being the most frequent [56–58]. These mutations can be observed in approximately 10–15 % of CLLs. Table 3 summarizes the most common mutations with their principal features.

Gene mutations	Chromosome	Association	Biologic function	Clinical outcome	Frequency
<i>NOTCH1</i>	9	+12	NOTCH1 signalling	Poor	10–15 %
<i>SF3B1</i>	2	11q-	mRNA splicing	Poor	5–10 %
<i>BIRC3</i>	11	11q-	NF-κB pathway	Poor	4 %
<i>MYD88</i>	3	13q-	NF-κB pathway	Good	3 %
<i>ATM</i>	11	11q-	DNA repair	Poor	12 %
<i>TP53</i>	17	17p-	DNA repair	Dreadful	5–10 %

Table 3. Most frequent recurrent mutations in CLL

2.1.4.1. *NOTCH 1*

NOTCH1 mutations occur in around 10–15 % of de novo CLL cases, being more frequent in advanced disease or Richter transformation [59]. These mutations result in the generation of a truncated protein which lacks the C-domain, becoming more stable in activating the *NOTCH-1* signalling pathway. This protein in its normal conformation regulates the transcription of *MYC*, *TP53* genes and molecules of the NF-κB pathway. Therefore, *NOTCH1*

mutations allow, an up-regulation of the cell cycle, and consequently, an advantage in cell survival and apoptosis resistance [41,58]. *NOTCH1* mutations confer to CLL a worse clinical outcome with a shorter TTF, PFS, OS and less response to treatment even in the context of a rituximab-based regimen. In connection with this, bad prognostic markers have been associated with *NOTCH1* mutations. Among them, ZAP-70, CD38 and U-CLL stand out [60]. It is worth noting that approximately 30 % of cases with Richter transformation harbour *NOTCH1* mutations. However, it is unclear whether this mutation is acquired during progression disease, as a consequence of clonal evolution or can be observed in early stages of CLL [41,59]. *NOTCH1* mutations have also been associated with +12, and when both alterations cluster, *NOTCH1* separates a subgroup of patients with poor outcome [40]. *NOTCH1* mutations have also been identified in 60 % of cases with T-acute lymphoblastic leukaemia and other non-haematological neoplasms, and its role among them can be ambiguous, acting not only as an oncogene but also as a tumour suppression gene. The relevance of these mutations is even greater as they can be used as a target for therapy. In fact, drugs for this purpose are under development [61].

2.1.4.2. *SF3B1*

SF3B1 gene is located at chromosome 2q33.1 and encodes for a splicing factor. Different mutations related to the splicing machinery have been associated with oncogenesis, pointing out that aberrant splicing constitutes an important pathway in malignant transformation. Nevertheless, the real mechanism underlying *SF3B1* dysfunction in CLL remains unknown. It is speculated that *SF3B1* mutations may trigger aberrant splicing of important proteins for the tumourigenesis process including cellular cycle control, angiogenesis or apoptosis. Somatic mutations of *SF3B1* can be found in around 10 % of CLL cases, but the incidence increases during disease evolution, probably determining the appearance of aggressive subclones [56]. *SF3B1* mutations in CLL entail bad prognosis with a more aggressive disease, advanced clinical stage at diagnosis and a worse OS, being more frequently observed in fludarabine refractory patients [62]. Bad prognosis markers such as ZAP-70 and U-CLL have also been associated with these mutations. In contrast, Richter transformation is not an often issue among *SF3B1* mutations [59]. In around 50 % of the cases, *SF3B1* mutations occur accompanied by 11q- or ATM mutations, and although unusual, mutations in *SF3B1* and *ATM* have been observed without 11q-. Other haematological diseases such as myelodysplastic syndromes and non-haematologic tumours may harbour *SF3B1* mutations, but they seem mutually exclusive from other lymphoid neoplasms. In contrast to CLL, *SF3B1* mutations confer a more favourable prognosis in myelodysplasia and identify a particular entity: refractory anaemia with ring sideroblasts [63,64].

2.1.4.3. *BIRC3*

BIRC3 mutations are unusual in CLL, with an incidence of 4 % approximately. The *BIRC3* gene, located near *ATM* gene at 11q33, together with *TRAF2* and *TRAF3* conforms a protein that negatively regulates the activation of the NF- κ B signalling pathway. *BIRC3* mutations result in an activation of the NF- κ B pathway, hence resistance to apoptosis and increased prolifera-

tion of cells. *BIRC3* mutations were found in cases of relapse or fludarabine resistance, especially in the absence of TP53 mutations. In this setting, the incidence of these mutations rises up to 25 %. Anyway, when they appear at diagnosis, a poor clinical outcome is also observed [35]. NF- κ B inhibitors are under development in CLL cases harbouring *BIRC3* mutations with encouraging preclinical results [65].

2.1.4.4. *MYD88*

MYD88 protein takes part in the homeostasis of B human cells working as an adaptor protein of the toll-like/interleukine-1 receptor. *MYD88* mutations cause resistance to apoptosis after they activate the NF- κ B pathway. *MYD88* mutations have been found in several lymphoid neoplasms, distinguishing lymphoplasmacytic lymphoma [66,67]. In CLL, they have been reported in a low proportion of cases (3–5 %) [57,58]. In contrast to previous recurrent mutations, mutations in *MYD88* have been associated with a good outcome in CLL patients. They have also been observed more frequently in young patients and detected in association with 13q- and M-CLL, maintaining the favourable prognosis in these populations [68].

2.1.5. *Other genetic abnormalities with prognostic relevance*

Other genetic abnormalities have been reported in CLL in a very low proportion of cases. These include deletion of 6q, trisomy 3, trisomy 8, trisomy 18, trisomy 19, deletion of 5q and gains of 2p, 3q, 17q and 8q. These alterations have also been related to disease outcome in a recent study that used array comparative genomic hybridization to identify genomic imbalance. Three groups of patients were made according to their prognostic outcome: good outcome (13q- without any of these alterations: gain, 1p, 7p, 12, 18p, 18q, 19, and loss, 4p, 5p, 6q, 7p), adverse outcome (gain, 2p, 3q, 8q, 17q, and loss, 7q, 8p, 11q, 17p, 18p) or intermediate outcome (remainder). This study also identified gain of 3q, 8q and 17p- as independent unfavourable prognostic biomarkers [69]. Translocations are also rare in CLL, but when present, they entail a negative prognostic impact [70]. Finally, complex karyotypes, defined by 3 or more alterations, whether deletions or gains, have also been associated with progressive or refractory disease and *ATM* and *TP53* defects [10,38].

2.2. **Other prognostic biomarkers in CLL**

Many non-genetic markers predict disease outcome in CLL. A brief enumeration of them and their implication in prognosis will be detailed below. Serum markers LDH and β_2 -microglobulin have been widely used, indicating a more advanced disease when their levels are high, and although they are not specific for CLL, they can be easily measured in clinical practice [71]. Lymphocyte double time, defined as the number of months that takes the lymphocyte count to double, is another classic prognostic marker that can be easily used in CLL. Hence, it has been proposed as one of the criteria to indicate therapy [72]. Several protein markers were investigated with the intention to find substitute markers for the arduous *IGHV* mutation status. Among them, CD38, ZAP70 (zeta-chain-associated protein) and CD49d stand out. CD38 and ZAP70 were first described as surrogate markers for U-CLL, but this has been controversial [7,9]. However, further studies demonstrated that both are independently associated with poor

outcome regardless of mutation status [9,73,74]. CD49d has also been correlated with shorter survival times when expressed at high levels and has recently emerged as the strongest flow cytometry predictor for OS [75]. MicroRNAs are small non-coding RNA molecules which act as posttranscriptional regulators of gene expression. Its dysregulation is associated with tumour development, and in CLL, they might be used as prognostic markers, as some studies have demonstrated. For instance, reduced expression of *miR-29*, *mir-223*, *miR-34a* and *mir-181* or increased expression of *miR-21* and *mir-221/222* has been associated with poor prognosis [38]. However, to validate this data, corroboration in prospective studies is necessary.

2.3. Prognostic systems

Numerous prognostic markers have individually shown correlations with survival over the last decades. The current challenge is to build a prognostic model that is clinically relevant, easily applicable, oriented to take therapeutic decisions and feasible in the clinical practice setting. With these goals, some attempts have been done over time. A review of some of these models is summarized in Table 4 and detailed next [76].

Authors	Markers used	Prognostic groups
<i>Rai et al.</i>	-Haematological blood counts -Physical examination	Low: lymphocytosis Intermediate: lymphadenopathy, visceromegaly High: anaemia or thrombocytopenia
<i>Binet et al.</i>	-Haematological blood counts -Physical examination	A: < 3 areas lymphadenopathy B: no A, no B C: anaemia or thrombocytopenia
<i>Döhner et al.</i>	-FISH	13q-: median overall survival (OS) of 133 months Normal karyotype: 111 months +12: 114 months 11q-: 72 months 17p-: 32 months
<i>Wierda et al.</i>	-Age -Absolute lymphocyte count - β_2 -microglobulin levels -Rai stage -Sex -Number of lymph node regions	Calculate the 5-year and 10-year survival probability with specific nomogram
<i>Wierda et al.</i>	-LDH -Number of lymph node regions -Size of lymph nodes on the neck -IGHV mutation status -FISH aberrations	Calculate the 2-year and 4-year treatment-free probability with specific nomogram

Authors	Markers used	Prognostic groups
<i>Pflug et al.</i>	-Sex -Age -ECOG status -17p- (six points) -11q- -IGHV mutation status -β ₂ -microglobulin levels -Thymidine kinase	Male, 1 point; TK >10 U/L, 2 points; β ₂ -microglobulin 1.7–3.5 mg/dL, 1 point; β ₂ -microglobulin >3.5 mg/dL, 2 points; unmutated-CLL (U-CLL), 1 point; ECOG >0, 1 point; 11q-, 1 point; age >60 years, 1 point Low risk: score 0 to 2 Intermediate risk: score 3 to 5 High risk: score 6 to 10 Very high: score 11 to 14
<i>Rossi et al.</i>	-FISH -New mutations	Very low risk: 13q- only Low risk: +12 or a normal FISH Intermediate risk: NOTCH1 and/or SF3B1 and/or 11q- High risk: TP53 and/or BIRC3 abnormalities
<i>Haferlach et al.</i>	-Age -Leucocyte count -IGHV mutation status -IgH translocations -Number of cytogenetic aberrations -TP53 deletion -ATM mutations	Predicting OS: Age ≥ 65 years, 1 point; leucocyte count ≥20 × 10 ⁹ /l, 1 point; U-CLL, 1 point; TP53 deletion, 2 points; translocation involving the IGH locus on 14q32, 2 points; number of chromosome aberrations based on CBA 0, 0 points; 1 or 2, 1 point; and ≥3, 2 points Favourable risk: 0–3 points Intermediate risk: 4–5 points Unfavourable risk: >5 points Predicting TTFT: U-CLL, 1 point; ATM deletion, 1 point; translocation involving the IGH locus on 14q32, 2 points; number of chromosome aberrations based on CBA 0 or 1, 0 points; 2–4, 1 point; and ≥5, 2 points Favourable risk: 0–2 points Intermediate-1 risk: 3 points Intermediate-2 risk: 4 points Unfavourable risk: 5 points

Table 4. Summary of published prognostic indexes for CLL

Rai and Binet prognostic systems [5,6] were the initial scores intended to predict prognosis in patients with CLL. These score systems still stand in routine clinical practice nowadays as they are good predictors for prognosis, inexpensive and able to identify the need for therapy. The biggest disadvantage of them is that a notable amount of patients with early stages progress. *Wierda et al.* [71] developed a nomogram including age and absolute lymphocyte count, β₂-microglobulin levels, Rai stage, sex and number of involved lymph node regions. A specific punctuation was given to each independent covariate, and the summation of the points hurls a new score that can be extrapolated to calculate the 5- and

10-year probability of survival. This model may be useful for previously untreated patients, but its limitations include that it was performed in a young cohort of patients, not extrapolated to all CLL patients, and that new biological markers were not included. However, this nomogram has been validated later in an independent multicentre CLL population and improved by simplifying it into a four-variable model (age, sex, Binet staging, β -2-microglobulin) [77]. The development of FISH studies identified genomic aberrations in around 80 % of CLL patients and led to the stratification into a hierarchy of five prognostic subgroups with decreasing survival times: CLL cases with 17p- had the worst prognosis (median survival of 32 months), followed by cases harbouring 11q- (median survival of 72 months), +12 (median survival of 114 months), normal karyotype (median survival of 111 months) and 13q- (median survival of 133 months) [18]. This model supposed a great achievement to powerfully complement the Rai and Binet prognostic scores and allows to make therapeutic decisions such as to offer a different treatment to 17p- patients. The next step was to include classical and new biological and genetic markers in the same prognostic score. In line with this, *Wierda et al.* [78] proposed a new nomogram, this time including new prognostic markers, with the purpose to calculate the 2- and 4-year probability of TTFT. Along with LDH, the number of involved lymph node sites and large size of lymph nodes of the neck, new biological factors such as *IGHV* mutation status and FISH aberrations according to the hierarchal model were included. This model has not been validated in independent cohorts and has not been used to calculate OS. Nevertheless, it can be useful to identify patients at high risk for progression to treatment. The German CLL Study Group came up with another prognostic system based on 23 classical and novel biological and genetic markers [79]. Eight factors were finally independently associated with inferior survival: sex, age, ECOG status, 17p-, 11q-, *IGHV* mutation status, serum β -2-microglobulin levels and serum thymidine kinase. According to these factors, four risk categories could be separated with different survival times. This prognostic score was validated in an independent cohort. Comparing with classic staging systems, this prognostic score allows to identify early-stage patients who will sooner progress and to separate ultrahigh-risk patients. However, there are some barriers to adopt this score into clinical practice including that thymidine kinase is not available routinely; new data regarding novel mutations such as *NOTCH1*, *SF3B1* or *BIRC3* has not been incorporated; and p53 mutations in patients without 17p- were not represented. *Haferlach et al.* proposed two new scoring systems with a combination of genetic and classical markers. The parameters incorporated were age, leukocyte count, *IGHV* mutation status, IgH translocations, number of chromosomal aberrations detected with CBA (chromosome banding analysis) and *TP53* and *ATM* mutations. The applicability of these scores in the current setting is limited, as CBA is not commonly performed in clinical practice. *Rossi et al.* [80] were the first to propose a scoring system integrating both cytogenetic and mutational markers. This scoring system separates CLL patients into four prognostic risk groups: high risk, harbouring *TP53* and/or *BIRC3* abnormalities (10-year OS: 29 %); intermediate risk, harbouring *NOTCH1* and/or *SF3B1* mutations and/or 11q- (10-year OS: 37 %); low risk, harbouring +12 or a normal genetics (10-year OS: 57 %); and very low risk, harbouring 13q- only, whose 10-year OS

(69.3 %) did not significantly differ from a matched general population. This model exhibits superiority in discrimination patient outcome to the FISH-based model and can be used at any time point from diagnosis, maintaining its prognostic relevance. It emphasizes the importance of including new recurrent mutations, as these may reclassify patients into a higher-risk group. Other authors partially replicated this scoring system although finding some discrepancies that suggested that *NOTCH1* and *SF3B1* mutations should be considered as higher-risk alterations [81,82].

3. Targeted therapy for CLL

Immuno-chemotherapy is the initial approach to the majority of CLL patients who require therapy nowadays. However, some patients relapse, become refractory or suffer important secondary adverse events. For these reasons, the emergence of new targeted treatments has and will revolutionize the treatment model for CLL. Hopefully, targeted therapy will be not only more effective in improving survival of CLL patients but also less toxic, ameliorating quality of life of patients under treatment. This part of the chapter will be dedicated to review the novel targeted treatments that have been already approved or are being studied for the treatment of CLL.

3.1. Second-generation anti-CD20 monoclonal antibodies

CLL cells express antigen CD20 with a low intensity, but enough for the chimeric mouse anti-human CD20 monoclonal antibody rituximab to lyse these cells. Rituximab acts by different mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity and direct toxicity. When used in combination with chemotherapy, rituximab has demonstrated superiority over chemotherapy regimens and has therefore become a standard of care in the treatment of CLL patients either in front-line or salvage therapy [14,15].

3.1.1. Ofatumumab

Ofatumumab is a fully humanized anti-CD20 that binds to a different CD20 epitope than rituximab, generating a greater cytotoxic potential than rituximab by complement-mediated cytotoxicity with the same ADCC. It has been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of fludarabine and alemtuzumab refractory CLL patients and recently by the FDA for previously untreated patients in combination with chlorambucil (Clb) in whom fludarabine-based therapy is considered inappropriate. The study that gave the indication of ofatumumab for previously treated patients consisted of a phase II trial including 138 patients with either fludarabine and alemtuzumab refractoriness or fludarabine refractoriness and bulky disease. Overall response (OR) rates were 58 % and 47 %, and median OS were 13.7 and 15.4 months, respectively. The most common adverse events were infusion reactions and infections, which were primarily grade 1 or 2 events [83]. The FDA approval of ofatumumab in combination with chlorambucil

for previously untreated CLL patients was based on the results of a phase III trial only preliminary reported, comparing ofatumumab in combination with chlorambucil to single-agent chlorambucil in 447 patients in whom fludarabine-based therapy was considered to be inappropriate. The ofatumumab + chlorambucil arm demonstrated superiority with a higher OR (82 % vs. 69 %), CR (12 % vs. 1 %) and PFS (22.4 vs. 13.1 months) [84]. Anyway, a formal comparative trial of ofatumumab versus rituximab is missing, and therefore, the real value of ofatumumab remains to be determined.

3.1.2. *Obinutuzumab (GA101)*

Obinutuzumab is a new second-generation recombinant humanized anti-CD20, glycoengineered to increase its affinity in binding the type 2 CD20 epitope. Obinutuzumab produces an increased ADCC and direct cytotoxicity, with lower complement-mediated toxicity. The FDA approved this drug for its use in combination with chlorambucil for the treatment of patients with previously untreated CLL, in view of the primary results of the CLL-11 trial [85]. This study consisted of a phase III international clinical trial that compared chlorambucil (Clb) versus chlorambucil plus rituximab (R-Clb) versus chlorambucil plus obinutuzumab (O-Clb) in previously untreated CLL patients not candidates to receive fludarabine. Patients in the O-Clb arm achieved a higher OR rate and a prolonged PFS compared to patients in the R-Clb arm and a benefit in OS, PFS and OR rate than patients receiving Clb alone. Grade 3 and 4 neutropenia and infusion reactions were more common with O-Clb than with R-Clb, but the risk of infection was not increased. An updated analysis of this trial has been recently published, confirming the PFS benefit in the arm of O-Clb vs. R-Clb (29.2 versus 15.4 months, $P < 0.001$) and reporting a longer TTFT in the arm of O-Clb vs. R-Clb (42.7 versus 32.7 months, $P < 0.001$). The previously observed OS benefit of G-Clb over Clb monotherapy was confirmed [86].

3.2. B-Cell receptor signalling pathway

The B-cell receptor (BCR) signalling pathway is vital for CLL cell survival and proliferation and therefore constitutes an important new strategy for targeted therapy in CLL. Bruton tyrosine kinase (BTK) or phosphoinositide 3'-kinase (PI3K) constitutes some of the key kinases in this pathway. Inhibitors of these kinases have been under investigation in patients with CLL with promising clinical results and minimal toxicity. In fact, ibrutinib and idelalisib, two oral compounds given as continuous treatment, have been recently approved for treatment of CLL patients. They have demonstrated high efficacy even in the higher-risk patient subgroup. One important aspect of treatment with BCR inhibitors is the development of lymphocytosis, often transient, mediated by the migration of CLL cells from the bone marrow and lymph nodes to the peripheral blood, where cell survival is decreased.

3.2.1. *Ibrutinib*

Ibrutinib is an oral small molecule that acts as an irreversible covalent inhibitor of the BTK resulting in an inhibition of the BCR signalling pathway. It is the first BCR inhibitor approved

by the FDA and EMA for the treatment of relapsed or refractory CLL and as a first-line treatment in cases of 17p- or *TP53* mutations in patients unsuitable for chemo-immunotherapy. Ibrutinib was approved in light of the results of a phase Ib/II multicentre clinical trial that included 85 refractory or relapsed CLL patients to receive ibrutinib at different doses [87]. The population represented at this trial had a high-risk disease with 33 % of 17p-, 36 % of 11q- and a median of four prior therapies. The results were encouraging with an OR rate independent of adverse risk factors of 71 % and an additional 15–20 % of patients with partial response with persistent lymphocytosis but reduced lymph nodes. The estimated 26-month PFS was 75 %, suggesting that ibrutinib responses are quite durable. Ibrutinib was well tolerated with predominant adverse events being grade 1 or 2 including transient diarrhoea, fatigue and upper respiratory tract infections. Subsequently, a multicentric randomized phase III compared ibrutinib with ofatumumab in a group of previously treated patients not candidates for purine analogues [88]. The trial was stopped early after interim analysis because of a significant improvement in OS and PFS in the ibrutinib arm. The promising results of these trials prompted out combination studies that are currently ongoing or have been completed. Combinations of ibrutinib plus rituximab or ibrutinib plus bendamustine and rituximab have also been explored in different trials that enrolled high-risk, refractory or relapsed CLL patients, obtaining OR rates between 93 % and 95%, with an acceptable toxicity profile and being effective even in the subgroups of patients with 17p- or *TP53* mutations [89,90]. In summary, ibrutinib is a well-tolerated drug that has been demonstrated to produce high response rates even in high-risk CLLs. The combination of ibrutinib with other drugs is under investigation and will hopefully change the actual paradigm of CLL treatment due to the encouraging preliminary results. Unfortunately, resistances and progressions to Richter syndrome have been described with a dismal prognosis in these cases.

3.2.2. Idelalisib (CAL-101)

Idelalisib is an oral selective inhibitor of PI3K that produces apoptosis in CLL cells. It has been approved by the FDA and EMA for use in combination with rituximab for patients who have received at least one prior therapy or as first-line treatment in the presence of 17p deletion or *TP53* mutation in patients unsuitable for chemo-immunotherapy. The efficacy of idelalisib in combination with rituximab was assessed in a multicentre randomized phase III trial that compared idelalisib plus rituximab with idelalisib plus placebo in patients with relapsed CLL. Most of the patients enrolled had adverse prognostic factors including 17p-, *TP53* mutations or M-CLL. Idelalisib obtained a higher OR rate than placebo (81 % vs. 13 %, $P > 0.001$) and a superior OS (92 % vs. 80 % at 12 months, $P = 0.02$). PFS at 24 weeks was 93 % in the idelalisib group compared with 46 % in the placebo group ($P < 0.001$). Serious adverse events occurred in both groups in a proportion of 40 % (idelalisib) and 35 % (placebo). These included pneumonia, pyrexia and febrile neutropenia. The benefit of idelalisib was maintained among patients with adverse genetic features. Analogously to ibrutinib, lymphocytosis with node response was observed in some patients [91]. In view of this exciting results, further trials with idelalisib in combination with bendamustine, fludarabine or chlorambucil in patients with relapsed or refractory CLL are being performed, confirming the feasibility and benefits of these combinations [92].

3.3. Bcl-2 inhibitors

B-cell lymphoma-2 (Bcl-2) proteins, encoded by the Bcl-2 gene, are expressed at high levels in CLL cells. These proteins contribute to the regulation of the apoptotic process and therefore constitute an important therapeutic target for CLL.

3.3.1. ABT-263 (*navitoclax*)

Navitoclax is an orally bioavailable BCL-2 inhibitor that binds to several antiapoptotic BCL-2 family proteins including BCL-2, BCL-XL, BCL-x and BCL-B. A phase I trial conducted to evaluate the biologic activity, safety and pharmacokinetics of ABT-263 demonstrated encouraging results for this molecule as a single agent, even in patients with fludarabine-refractory disease, bulky adenopathy or 17p-. However, its therapeutic use was limited because of severe thrombocytopenia, observed as an important adverse effect in 28 % of the patients due to the inhibition of BCL-XL.

3.3.2. GDC-0199/ABT-199 (*venetoclax*)

GDC-0199/ABT-199 is a small molecule reengineered to decrease the thrombocytopenia side effect of navitoclax. ABT-199 produces a selective inhibition BCL-2 with a reduced effect on BCL-XL. It has shown promising results in a phase I trial that enrolled 56 refractory or relapsed CLL patients, 29 % with 17p- and 32 % with fludarabine resistance [93]. The major side effects included tumour lysis syndrome and neutropenia. Interestingly, ABT-199 yielded an OR rate of 85 %, with 13 % of complete responses and 72% of partial responses. These encouraging results were also observed in high-risk patients, with a response rate of 88 % and 75 % in patients with 17p- and fludarabine refractory, respectively. Clearly, these data indicate that Bcl-2 inhibitors will play an important role in the future for the treatment of CLL patients, and therefore, ABT-199 is currently being investigated in combination with immuno-chemotherapy.

3.4. Immunomodulatory drugs

Changes in the microenvironment of tumour cells promote the selective survival of malignant CLL cells preventing apoptosis. Immunomodulatory drugs act by altering cellular features and the cytokines of tumour microenvironment. In fact, lenalidomide has been shown to be effective in CLL.

3.4.1. Lenalidomide

Lenalidomide is an oral second-generation immunomodulatory drug with antiangiogenic, cytokine activity modulating, and immunomodulatory properties. It has been demonstrated to be active in MDS, multiple myeloma and lymphoproliferative disorders. The first phase II trials that tested lenalidomide in relapsed or refractory CLL obtained an OR rate between 32 % and 47 %. The major adverse effects reported were myelotoxicity, tumour flare and tumour lysis syndrome, all of them ameliorated with lower doses of lenalidomide [94,95]. Lenalidomide was also tested as a front-line therapy for CLL patients in another two trials with a different

dosing, but both with a low initial dose (2.5–5 mg daily) and a further escalation to a target of 25 mg. These trials obtained an OR rate between 56 % and 65 %. A high proportion of tumour flare reactions were observed in one of them, although they were mild. Grade 3 or 4 neutropenia was also frequent but without serious consequences. A recent update of one of these studies showed that at a median follow-up of 4 years, time to treatment failure had not been reached and OS was 86 %. These studies showed that lenalidomide is effective as first-line therapy for CLL and is well tolerated when administered in a dose escalation plan [96–98]. Lenalidomide has also been tested in combination with other drugs. In a phase II trial, patients with relapsed or refractory CLL received a combination of rituximab and lenalidomide obtaining an OR rate of 66 % including 12 % of complete responses. Seventy-three percent of patients showed neutropenia (grade 3 or 4), and only 1 episode of grade 3 tumour lysis was reported. Another phase II study of lenalidomide and rituximab was performed, this time as a first-line therapy. The OR rate was 88 %, including 15 % of complete responses. Again, neutropenia was the most common adverse event [99,100]. Additional combinations are currently under study, as well as maintenance therapy after chemo-immunotherapy.

3.5. Chimeric antigen receptors

Chimeric antigen receptors (CARs) are engineered constructs that combine the antigen recognition domain of an antibody with intracellular signalling domains into a single chimeric protein. CD19 antigen is exclusively expressed in B-cells and therefore is a very suitable target for the treatment of CLL with CARs. Indeed, a pivot clinical trial proved the important antitumor activity of this CAR-modified autologous T-cells targeted to CD19 (CART19 cells) in three patients with refractory CLL [101]. Two out of the three patients achieved a complete response lasting longer than two years and the other patient a partial stable response. Toxicities included hypogammaglobulinemia, decreased number of plasma-cells and B-cell aplasia. The CART19 cells expanded > 1,000-fold in vivo and expressed functional CARs for at least six months, and a proportion of them persisted as memory CART19 cells. On average, each infused CAR-expressing T-cell was calculated to eradicate at least 1,000 CLL cells. In another study, ten patients with refractory CLL or relapsed B-cell acute lymphoblastic leukaemia (ALL) were treated with CART19 modified to express a second-generation CAR anti-CD19. Three of the four evaluable patients with bulky CLL who received prior treatment with cyclophosphamide experienced either a significant reduction or a mixed response in lymphadenopathy without development of B-cell aplasia. The short-term persistence of infused T-cells was enhanced by previous administration of cyclophosphamide and was inversely proportional to the tumour burden in peripheral blood [102]. In addition, a longer follow-up from ten patients treated with CART19 was reported. The study included nine adults with relapse or refractory CLL, three patients with p53 deletion and a child with relapsed and refractory ALL [103]. CLL patients received chemotherapy regimens 4–6 days before CART19 infusions. Four of the nine evaluable patients achieved a complete response, including three patients with CLL. Two additional patients from the CLL group had a partial response lasting from three to five months, and three patients did not respond. In the four patients who achieved complete response, maximal expanded cells in peripheral blood were detected at an average of 27-fold higher than the infused dose. No patients with complete response relapsed. Patients who responded devel-

oped a cytokine release syndrome manifested by fever, as well as variable degrees of anorexia, nausea, transient hypotension and hypoxia. In responding CLL patients, cytokine levels were increased, and five patients with cytokine release required treatment. In summary, CART19 can induce potent and sustained responses in patients with advance, refractory and high-risk CLL. However, further research is needed to ascertain the efficacy of this therapy and minimize associated cytokine-mediated toxicities.

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