

Therapeutic Options for Patients with TP53 Deficient Chronic Lymphocytic Leukemia: Narrative Review

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Abstract: Chronic lymphocytic leukemia (CLL), which is the most common type of leukemia in western countries in adults, is characterized by heterogeneity in clinical course, prognosis and response to the treatment. Although, in recent years a number of factors with probable prognostic value in CLL have been identified (eg *NOTCH1*, *SF3B1* and *BIRC3* mutations, or evaluation of microRNA expression), *TP53* aberrations are still the most important single factors of poor prognosis. It was found that approximately 30% of all *TP53* defects are mutations lacking 17p13 deletion, whereas sole 17p13 deletion with the absence of *TP53* mutation consists of 10% of all *TP53* defects. The detection of del(17)(p13) and/or *TP53* mutation is not a criterion itself for starting antileukemic therapy, but it is associated with an aggressive course of the disease and poor response to the standard chemoimmunotherapy. Treatment of patients with CLL harbouring *TP53*-deficiency requires drugs that promote cell death independently of *TP53*. Novel and smarter therapies revolutionize the treatment of del(17p) and/or aberrant *TP53* CLL, but development of alternative therapeutic approaches still remains an issue of critical importance.

Keywords: chronic lymphocytic leukemia, p53 protein, molecular aberrations, drug resistance

Introduction

The World Health Organisation defines chronic lymphocytic leukaemia (CLL) as low-grade lymphoproliferative neoplasm with $\geq 5 \times 10^9/L$ clonal B-cells in peripheral circulation.^{1,2} Small, mature-appearing CD5, CD19, dimCD20, and CD23 positive B-lymphocytes accumulate in blood, bone marrow, lymph nodes, and spleen.³ The most common presentation of the disease nowadays is asymptomatic peripheral blood lymphocytosis. Patients may also present with lymphadenopathy, hepatomegaly, splenomegaly, bone marrow failure, recurrent infections, autoimmune hemolytic anemia, or autoimmune thrombocytopenia.⁴

CLL is the most common type of leukaemia in western countries, accounting for approximately 25% of adult leukaemias.^{5,6} In the United States of America, the median age at diagnosis of CLL is 71 years and the age adjusted incidence is 4.5 cases per 100,000 inhabitants. 5-year survival (calculated in 2005–2011) was estimated as 81.7%.⁴ Five year survival was calculated as 67.5% in 1975 and increased to 87.9% in 2007.⁴ It illustrates how novel treatment regimens have revolutionized the management of CLL. As the incidence rate rises with age, the prevalence of CLL is likely to increase due to the demographic changes in western

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countries. As a consequence of more frequent blood testing, the proportion of younger patients with early stage CLL and no symptoms is also likely to increase.⁷ CLL is most common in Caucasians and rarely observed in Asians, even those who migrated to western countries.^{8,9} The incidence of CLL varies between geographical regions, ranging from <0.01% in eastern Asia to 0.06% in Europe and the United States.¹⁰

CLL Prognostic Factors

The clinical course of CLL is highly heterogeneous: some patients require aggressive treatment at the time of the diagnosis, others do not require treatment for many years, if at all. Numerous prognostic factors have been proposed to evaluate the outcome in CLL. Multivariable models, prognostic indexes and nomograms have been developed in order to predict clinical outcome accurately.⁸ Rai's and Binet's classifications,¹¹ although widely used in clinical routine, are not sufficient to determine if the patient will present a rapidly-progressive disease or an indolent one. Nowadays, genetic, epigenetic and molecular markers are the main focus of attention in prognostication in CLL.¹² It is estimated that the typical CLL genome carries approximately 2000 molecular lesions, however only approximately 5 of these are gross structural abnormalities. More than 40 mutated driver genes have been identified in CLL.^{13–15}

Generally, prognostic factors in CLL can be divided into two groups: markers of good and poor prognosis. 13q14 deletion and hypermutation of *IGHV* are associated with good prognosis, whereas del(11q), del(17p); unmutated immunoglobulin heavy-chain (*IGHV*); high expression of ZAP70, CD38, CD49d; and mutations in: *NOTCH1*, *SF3B1* and *BIRC3* are poor prognostic factors.¹³

The most frequent genetic aberration in CLL is deletion of 13q14, occurring in 50% to 60% of patients.^{13,16} The deleted region contains the miR15A and miR16A microRNAs, which inhibit the expression of key regulators of apoptosis and cell cycle.^{13,17} Levels of the miR15A and miR16A microRNAs negatively correlate with the expression of the antiapoptotic gene BCL2, which codes for an antiapoptotic protein and is one of the genes upregulated in CLL.¹³ Elevated expression of the antiapoptotic protein BCL2 makes CLL cells resistant to apoptosis, which results in clonal lymphocytes accumulation.¹⁸ In Rossi et al's study¹⁹ CLL patients harboring solely 13q14 deletion had an expected survival only slightly, not

significantly, poorer than that of the healthy population. The progression rate was very slow (approximately 4% per year) and transformation rate low.¹⁹ The deletion of chromosome 11q22-q23 is observed in 10–20% of CLL cases.²⁰ It always includes the *ATM* gene (ataxia telangiectasia mutated). The *ATM* gene regulates the cell-cycle arrest at G1/S and G2/M to prevent processing of damaged DNA and in case of DNA damage, it alternatively activates the DNA repair pathways or induces apoptosis. *ATM* deletion at the time of the diagnosis identifies an intermediate-risk group of patients. The presence of 11q22-23 deletion is associated with poor response to chemotherapy.^{19,21} Trisomy 12 is observed in 10% to 20% of cases of CLL.⁵ It is the second most common cytogenetic abnormality in patients with CLL.¹⁶ The genes involved in the pathogenesis of CLL with a trisomy 12 are unknown. The prognostic significance of this aberration is not completely understood.^{5,22} Döhner et al considered trisomy 12 an intermediate prognostic risk factor, with a median time to first treatment of 33 months and a median overall survival of 114 months.¹⁶ In contrast, Rossi et al qualified +12 patients as low-risked.¹⁸ +12 is particularly frequent in patients who develop Richter syndrome, especially in patients harbouring *NOTCH1* mutations.^{23,24} Complex karyotype defined as three or more karyotype aberrations, is also considered an independent marker of poor prognosis.^{12,25}

The unmutated immunoglobulin heavy-chain (*IGHV*) genes is one of the most important single factors of poor outcome.¹⁹ Almost all of *IGHV* unmutated patients are expected to progress after chemoimmunotherapy. However, progression-free survival (PFS) of *IGHV*-unmutated patients is similar to that of *IGHV*-mutated patients in the case of treatment with novel drugs.¹³ Condoluci et al conducted a study on a group of 4933 asymptomatic, early-stage, CLL patients, aiming to develop a prognostic score to predict time to first treatment (TTFT). Out of all genetic and molecular factors, taken into account in the study, the *IGHV* unmutated status had the strongest effect on TTFT prognostication. Unmutated *IGHV* along with absolute lymphocyte count higher than 153 109/L and presence of palpable lymph nodes became components of International Prognostic Score for Early-stage CLL (IPS-E). The IPS-E is a simple prognostic model: each of the three covariates, present in the patient is scored 1 point. Score 0 predicts low-risk (5-year cumulative risk for treatment start was 8.4%), score 1 predicts intermediate-risk (5-year cumulative risk for treatment

start was 28.4%), score 2–3 predicts high-risk (cumulative risk for treatment start was 61.2%).²⁶ On the contrary, hypermutation of the rearranged *IGHV* genes is considered a good prognostic factor. Hamblin et al²⁷ observed that CLL patients carrying mutated *IGHV* genes experience an indolent course of the disease in comparison with patients harbouring unmutated *IGHV* genes. Median survival for stage A patients with unmutated *IGHV* genes was 95 months compared with 293 months for patients with mutated *IGHV* genes ($p = 0.0008$).²⁷

High expression of ZAP70, CD38, and CD49d are well-known adverse prognostic factors.^{28,29} CD38 is a transmembrane type 2 glycoprotein, expressed not only in hematological cells, but also in the brain, the eye, bone tissue, smooth muscles and pancreatic isle cells.³⁰ It plays a critical role in regulation of calcium signalling and hence regulates the insulin secretion, oxytocin production, bone tissue metabolism. It also takes part in the immune response: T cell activation, neutrophil chemotaxis, dendritic cell migration, monocyte chemokine production.²⁸ CD38 is also a lymphocyte differentiation antigen, which interacts with: TCR in T-lymphocytes, BCR in B-lymphocytes, CD16/CD81 complex in NK cells. It induces proliferation in mature B cells and blocks the immature B-cell haematopoiesis.^{28,31} In the Damle et al³² study, CD38 expressed in more than 30% of CLL cells predicted shorter overall survival.³² Notwithstanding, some authors propose 20% or 7% cut-off levels.^{28,33,34} CD49d is an alpha-4-integrin, which binds either with beta 7 integrin in order to mediate lymphocyte adhesion or with beta 1 integrin (CD29). The combination CD49d/CD29 forms very late antigen-4 (VLA-4), expressed on leukocytes CD34-positive hematopoietic stem/progenitor cells.²⁸ VLA-4 plays an important role in fetal and adult haematopoiesis.^{28,35,36} Germline CD49d expression superior to the 30% level is associated with shorter OS and PFS.³⁷ It seems that high expression of CD38 and CD49d prolongs CLL cells survival by enhancing the stimulatory signals from microenvironment and blocking apoptotic signals.²⁸ ZAP70 is a protein kinase expressed by T-lymphocytes and NK cells, but also by normal B-cells.^{38,39} Scielzo et al analyzed ZAP expression on different human normal B-lymphocyte subpopulations, including: naïve, germinal center and memory B cells from tonsils, CD19+ CD5+ cells from cord blood and CD19+ lymphocytes from peripheral blood. ZAP-70 protein was expressed in all cells, although at different

levels, probably dependent on stimulation via the B-cell receptor.³⁸ In CLL, patients with ZAP70 positivity show shortened treatment free survival and shorter OS. In contrast, ZAP70 negative patients demonstrate slow progression rate.⁴⁰

The next-generation sequencing technologies led to identification of additional genetic aberrations in CLL, such as mutations in *NOTCH1*, *SF3B1*, and *BIRC3*, which can be considered as prognostic and predictive biomarkers.⁴¹ The *NOTCH* genes encode transmembrane proteins NOTCH1 to NOTCH4, that function as transcription factors, modifying the expression of numerous target genes, such as *MYC* and NF- κ B signalling components.²¹ *NOTCH1* mutations occur in 4% to 11% of CLL patients and are frequently correlated with trisomy 12 and unmutated *IGHV* genes.^{12,15,42} 80% of *NOTCH1* mutations are represented by c.7544_7545delCT deletion.¹⁵ *NOTCH1* mutations in CLL result in NOTCH1 impaired degradation and deregulated NOTCH signalling.²¹ Multivariate analyses has showed that *NOTCH1* is an independent poor prognostic factor, especially in patients treated with chemoimmunotherapy.⁴³ Moreover, patients carrying *NOTCH* mutation have higher risk of Richter transformation.¹² *NOTCH1* mutations can also be viewed as biomarkers of resistance to the anti-CD20 monoclonal antibodies rituximab and ofatumumab in CLL.^{13,44} The lower sensitivity to anti-CD20 treatment in CLL harbouring *NOTCH1* mutations can be associated with dysregulation of epigenetic repression of CD20 expression, when mutations in exon 34 takes place.⁴⁵ *SF3B1* is a component of the mRNA splicing machinery. *SF3B1* mutations, which result in unaltered DNA damage response, and occur with a prevalence from 7 to 10% of CLL patients. *SF3B1* mutations frequently accompany other alterations: unmutated *IGHV* genes and *ATM* deletion or mutations. The presence of *SF3B1* mutations at the time of CLL presentation identifies a group of patients with intermediate-risk disease.²¹ *BIRC3* gene (the Baculoviral IAP repeat containing 3) is mutated in approximately 2% of CLL patients.¹⁹ *BIRC3* mutations disrupt the C-terminal RING domain, which leads to constitutive NF- κ B activation. NF- κ B activation in CLL is considered responsible for resistance to conventional chemotherapy. *BIRC3* mutations identify a subgroup of patients with poor prognosis.^{13,19} Although numerous other gene mutations recurring at lower frequency have been indicated for their association with CLL outcome, including *SAMHD1*, *RPS15*, *NFKBIE*,

Table 1 Genetic and Molecular Prognostic Factors in CLL

Markers of Very Low Risk	Markers of Low Risk	Markers of Intermediate Risk	Markers of Poor Prognosis
13q14 deletion only	Trisomy 12* Normal genetics	11q22-q23 deletion Mutations in SF3B1 Mutations in NOTCH1	TP53 aberrations Complex karyotype Unmutated IGHV genes High expression of ZAP-70, CD-38, CD49d mutations in BIRC3

Notes: Data from references,^{12,13,16,19,21,25,27–29,43} *Döhner et al¹⁶ considered trisomy 12 as a marker of intermediate risk.

Abbreviation: IGHV, immunoglobulin heavy-chain.

EGR2, *KRAS* and *POT1*, their potential role as prognostic biomarkers needs to be further investigated.⁴⁶

Although factors mentioned above are considered helpful in CLL prognostication, so far it is the *TP53* aberrations that seem the most important prognostic and predictive factor in CLL (Table 1).

P53 Protein – Unveiling the “Guardian of the Genome”

TP53 is a gene, located on chromosome 17, that encodes a tumor suppression protein called p53.⁴⁷ The tp53 protein has two N-terminal transactivation domains, a central DNA binding domain (encoded by exons 4–8 of the *TP53* gene), C-terminus involved in downregulation of DNA binding of the central domain and an oligomerization domain, which is crucial for the transcriptional activity. Regulators, for example a p53 ubiquitin ligase MDM2, keep p53 protein at low levels in normal cells.^{48,49} In response to cellular stress, including DNA damage and replication stress, p53 is activated to promote the elimination or repair of damaged cells, in order to reduce the risk of propagating mutations.^{47,48} It allows the ability to decrease the deleterious consequences of mutations in the human genome. It seems that p53 also suppresses chromothripsis - a type of chromosome shattering and rearrangement.⁴⁸ Due to shelterin defects and incomplete telomeric DNA replication, telomere shortening takes place, which can lead to chromosomal and genomic instability. As cells with dysfunctional telomeres are removed in processes regulated by the p53 protein, telomere dysfunctions promote oncogenesis.⁵⁰ For the reasons mentioned above, p53 is widely known as the “guardian of the genome”.⁴⁸ *TP53* is mutated or inactivated in approximately 60% of malignant neoplasms.⁴⁷ Interestingly, other “non-canonical” functions of p53, contributing to its effects, are still being discovered. One of the protection functions of p53 is modulation of autophagy, an essential

process to maintain homeostasis and protein control. Autophagy suppresses p53, which is crucial for tumor promotion.⁵¹ It was proved that p53 can influence mitochondrial functions as well. When stress stimuli occurs, *SCO2* gene (encoding cytochrome C oxidase 2) overexpresses, leading to ROS (reactive oxygen species) generation. Then, p53 can transactivate *SCO2*, to maintain mitochondrial respiration. In cases of irreversible stress or damage, p53 translocate to mitochondria, leading to apoptosis or necrosis.⁵² p53 also suppress pluripotency and cellular plasticity, induces an iron-dependent form of cell death known as ferroptosis, increases glutamine catabolism, supports anti-oxidant activity, downregulates lipid synthesis, increases fatty acid oxidation, and stimulates gluconeogenesis. It can have opposing effects on the same metabolic processes, depending on the cell type.⁴⁸

TP53 aberrations arise alternatively through deletion of the *TP53* locus on chromosome 17 or *TP53* gene mutations. Gene mutations are predominately concentrated in the DNA-binding domain, encoded by exons 4–8 and comprise of missense mutations, insertions, deletions, non-sense mutations or splice-site mutations. 75% of mutations are missense mutations. Codons 175, 245, 248, 249, 273, and 28 are “hotspots”, the regions affected in particular by mutations.^{25,53} Figure 1 schematically shows the p53 structure and functions.

TP53 Gene Aberrations in CLL

In CLL, *TP53* aberrations, associated with either the deletion of chromosome 17p or *TP53* gene mutations, are the most important single poor prognostic factors.¹² The determination of the outcome of patients with del(17p) had become the aim of numerous clinical trials, in which the median OS oscillated between 2 to 3 years from the time of first-line treatment. Moreover, *TP53* abnormality is also clearly associated with poor response to classical chemoimmunotherapy.⁵⁴ In CLL the most frequent

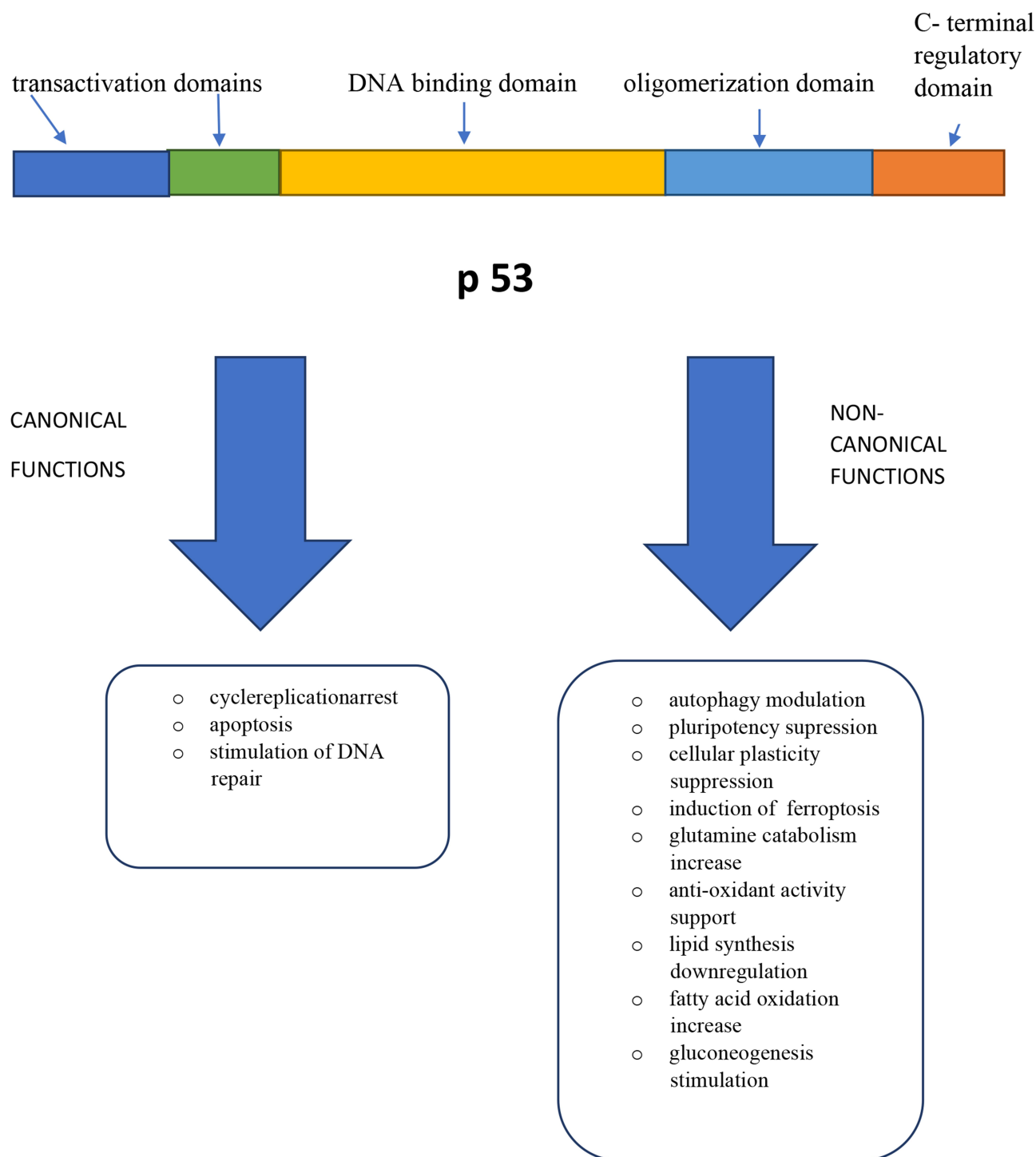


Figure 1 p53 structure and functions. The tp53 protein has two N-terminal transactivation domains, a central DNA binding domain, C-terminus involved in down regulation of DNA binding of the central domain and an oligomerization domain, which is essential for transcriptional activity.^{48,49} In response to cellular stress p53 is activated which leads to promotion of the elimination or repair of damaged cells.^{47,48} Interestingly, other “non-canonical” functions of p53, contributing to its effects, are still being discovered.^{48,52,53}

aberration affecting the TP53 gene is del(17p) accompanied by *TP53* mutations in the second allele.²⁵ Zenz et al⁵⁵ performed the analysis of the pattern of *TP53* mutations in 254 patients with CLL. It has been proved that the most

common are missense mutations (74%), mainly located in the DNA-binding domain. 90% of the mutations were located in exons 5–8. Also the most frequently mutated amino acids were located at positions 175, 179, 248 and

273, which is also observed in all *TP53* mutations in general.⁵⁵ Interestingly, it was suggested in a few studies, that CLL patients with del(17p), show excessive telomere shortening.⁵⁰ Guïèze et al⁵⁰ evaluated the association between *TP53* gene disruptions and impaired telomeres in CLL. *TP53* turned out to be the strongest determinant of telomere status in CLL. Patients with del(17p) had shortened telomeres and higher hTERT (human telomerase reverse transcriptase) (hTERT is a component of telomerase, that prevents the shortening of telomeres).⁵⁰

The issue that also should be raised in the context of *TP53* aberrations in CLL is clonal evolution.²⁵ Clonal evolution can be defined as the process of acquisition of vertically transmissible genetic/epigenetic lesions.⁵⁶ Neoplasm cells are genetically heterogeneous and consist of clonal and subclonal populations, which can either remain in balance or some clones can emerge as dominant. Genetically diverse clones compete and interact with each other, which leads to cancer progression and relapse.²⁵ In CLL clonal evolution is a common phenomenon. *TP53* aberrant subclones are more vulnerable to clonal evolution, especially under chemoimmunotherapy pressure. During the course of the disease, *TP53* mutations can be acquired or selected.²⁵ While at diagnosis, the *TP53* aberrations are observed in 4% to 8% of CLL cases, at the time of first-line treatment the incidence rises to 10%. It is estimated 30–40% in relapsed/refractory CLL and 50–60% in Richter syndrome.¹³ Rossi et al proved that not only *TP53* clonal mutations (represented in >20% leukemic cells), but also the presence of very small *TP53* mutated subclones, predict poor prognosis. In patients with *TP53* mutated subclones OS was significantly shorter (5-year OS: 46.3%; $P = 0.0042$) than in individuals with an unmutated *TP53* gene (5-year OS: 75.1%) and similar to OS in patients with clonal *TP53* mutations (5-year OS: 34.6%; $P = 0.6926$). Thus, the identification of patients with *TP53* subclones might be advisable in order to manage them as high-risk CLL.⁵⁷

In clinical practice, del(17) in CLL is assessed by the FISH technique and *TP53* mutations are evaluated by Sanger sequencing and next-generation sequencing.²⁵ Approximately 30% of all *TP53* defects are mutations lacking 17p13 deletion, whereas sole 17p13 deletion with the absence of a *TP53* mutation consisting of 10% of all *TP53* defects. It is believed that the high proportion of *TP53* mutations with no 17p13 deletion may be caused by the presence of two *TP53* mutations on individual alleles in CLL cells.²¹ That is why it is highly recommended to evaluate both the presence of chromosome 17p13 deletion and *TP53* mutations.⁵⁸ In order to unify the methods of detection of *TP53* mutation/del(17)(p13), in

2012 the European Research Initiative on CLL (ERIC) published recommendations (available at http://www.ericll.org/tp53_aberrations/) on several methodologies suitable for *TP53* analysis. Principal procedures suggested by ERIC for *TP53* mutation detection are Sanger Sequencing and Next Generation Sequencing (NGS).⁴⁹ *TP53* sequencing should at very least cover exons 4–10, including splicing sites. As it has been proved that variants can occur in exons outside the DNA binding domain (although it is very rare), the optimal range recommended goes from the second to the eleventh exon.^{49,54,58} It is crucial to test all patients for del(17)(p13) and *TP53* mutation before treatment initiation and at every relapse.⁵⁴ Testing for those mutations at every relapse is recommended due to the ability of leukaemic clones to evolve.²¹ The detection of del(17)(p13) and *TP53* mutation is not a criterion itself for starting therapy. Treatment in patients with CLL who have *TP53* mutation should be started according to the International Workshop on Chronic Lymphocytic Leukemia criteria, identically like in patients without aggravating mutations.^{54,59} Some authors are of the opinion that determination of *TP53* status should not be performed before the patient meets criteria for starting therapy.^{21,25}

Numerous studies have been performed, proving that del(17p) and/or *TP53* mutations are associated with an aggressive course of the disease and poor response to the standard chemoimmunotherapy.²⁵ One of the most frequently cited studies considering the issue of genetic aberration, including *TP53* mutations as predictive factor in CLL, was performed by S. Stilgenbauer et al⁶⁰ and is called “CLL8 trial”.⁶⁰ The outcome of the first-line treatment with fludarabine+cyclophosphamide (FC) or fludarabine+cyclophosphamide with rituximab (FCR), has been evaluated in 817 patients with different genetical lesions. *TP53* was mutated in 11.5% of patients and interestingly, *TP53* mutated was found in 16.1% *NOTCH1* mutated patients and in 14.0% of *SF3B1* mutated patients. Mutation in *TP53* was the most frequent missense mutation and it was situated in the DNA-binding domain. *TP53* mutation was positively associated with a CIRS (Cumulative Illness Rating Scale) higher than 1. Patients with *TP53* mutated had significantly lower clinical response rates both with FC and FCR treatment. Minimal Residual Disease (MRD) negativity was significantly less frequent in patients with *TP53* mutated treated with both regimens. The presence of *TP53* mutation was significantly related with shorter PFS (FC: HR 4.295, $P < 0.001$; FCR: HR 3.173, $P < 0.001$) and OS (FC: HR 4.642, $P < 0.001$; FCR: HR 4.447, $P < 0.001$). Only 7 out of 46 patients with del(17) had

PFS longer than 24 months. In conclusion, del(17) marked the worst prognosis among subgroups with different genetic aberrations.⁶⁰

Rossi et al¹⁹ reported that median OS in patients harbouring a *TP53* mutation was estimated 3 to 5 years. Only 30% of patients were alive at 10 years observation. This subgroup has 70% reduction of the expected survival in comparison with the general population.¹⁹ It should be highlighted that this data had been collected before the introduction of novel therapies. Parikh et al⁶¹ performed the meta-analysis evaluating the prognostic significance of *IGHV* mutation status and genetic abnormalities determined by FISH in patients with CLL. Patients with high-risk FISH (del17p13 and del11q23) had shorter median PFS and OS than patients with low or intermediate-risk. High-risk FISH has been proved to be an independent predictor of PFS in 8 of 17 studies analyzed.⁶¹

It has also been proved, that among patients with *TP53* abnormalities, the risk of Richter transformation is higher than in the general population of patients with CLL.¹³ Richter transformation, first described by Dr. Maurice Richter in 1928, is defined as the transformation of CLL to an aggressive lymphoma.^{62,63} Most commonly, CLL transforms to the diffuse large B cell lymphoma (DLBCL).⁶⁴ A cumulative incidence of Richter transformation in the natural history of CLL is estimated as 2–10%.^{63,65,66} The factors that increase the probability of the Richter transformation include: advance staged CLL, lymph nodes > 3 cm, unmutated *IGHV*, del(17p), *TP53* mutation, *NOTCH1* mutated, and stereotyped B-cell receptor (BCR).⁶³ In around 60% of patients, inactivation of the *TP53* gene can be observed.⁶⁴ In the Fabbri et al study⁶⁴ del(17p), occurring in 40% of patients has been the most frequent aberration among patients with Richter transformation. Moreover, four *TP53* altered cases displayed intra-chromosomal rearrangements, which suggests chromothripsis.⁶⁴ In the Wang et al study⁶³ 34.4% of patients with Richter transformation had *TP53* disruption. Patients with *TP53* aberration had a worse OS (median OS 8.3 vs 12.8 months).

Management of Patients with *TP53* Deficient CLL in the Era of Novel Agents

On the basis of research described above and clinicians' observations, nowadays chemoimmunotherapy is not considered standard management in patients with *TP53*

aberrations.²⁵ Treatment of patients with CLL harbouring *TP53*-deficiency requires drugs that promote cell death independently of *TP53* (Table 2).

Alemtuzumab

Alemtuzumab is a recombinant, fully humanized, monoclonal antibody against CD52.⁵⁵ The moderate effectiveness of alemtuzumab in patients with high-risk genetic markers such as del(17p) and *TP53* mutations has been proven.⁵ In a randomized Phase 3 trial (CAM307), de novo patients treated with alemtuzumab demonstrated a 3-fold improvement in overall response rate (64% vs 20%, $p = 0.08$), compared with patients treated with chlorambucil. The median PFS has also been improved (10.7 months vs 2.2 months, $p = 0.41$).⁶⁷ The license of alemtuzumab was withdrawn in 2012 due to a decision by Sanofi. At present alemtuzumab is only available through an international compassionate use program.⁵ The usage of alemtuzumab in CLL has also been limited due to significant toxicities, most of all high incidence of neutropenia and cytomegalovirus reactivation. Alemtuzumab had been replaced as the treatment of choice for patients with *TP53* aberrations, by new agents with better effectiveness.⁶⁸

Drugs Targeting BCR Signaling

A better insight in CLL pathophysiology, which led to the development of novel targeted approaches, revolutionized the treatment of CLL in *TP53* deficient patients.⁵⁴ Constitutive BCR signaling is of great significance for the pathogenesis of CLL, as it enhances the proliferation and survival of B lymphocytes.^{69,70} Upon stimuli of the BCR, protein tyrosine kinases, such as spleen tyrosine kinase (SYK) and LYN kinase become activated. SYK and LYN activate the Bruton tyrosine kinase (BTK) and phosphoinositol-3-kinases (PI3Ks), which stimulate downstream cascades resulting in activation of protein kinase B (AKT), extracellular signal-regulated kinases ERK1 and 2, nuclear factor (NF)- κ B, and nuclear factor of activated T-cells (NFAT).⁶⁹ Inhibition of kinases, involved in BCR signaling became an attractive potentiality in the treatment of CLL.⁷⁰

Ibrutinib

Ibrutinib is a small molecule BTK inhibitor.⁴ It binds covalently and irreversibly to cysteine 481 within the ATP binding site. It also cross-reacts with other TEC kinases.^{4,69} In 2014, on the basis of surprisingly good

Table 2 Current Treatment Options for Patients with CLL with TP53 Disrupted (Outside the Context of Clinical Trials)

Drug/Procedure	Indications in CLL and SLL	Date of FDA Approval
Ibrutinib	Frontline therapy in adult patients	First approval in February, 2014 (for patients who have received at least one previous therapy), then the indications have been expanded
Ibrutinib + rituximab	Initial treatment of adult patients	April, 2020
Ibrutinib + obinotuzumab	Treatment-naïve adult patient	January, 2019
Ibrutinib + bendamustine + rituximab	Patients after at least one prior therapy	May, 2016
Idelalisib + rituximab	R/R CLL, in adult patients for whom rituximab alone would be considered appropriate therapy due to other co-morbidities	July, 2014
Acalabrutinib	Adult patients	November, 2019
Duvelisib	Adult patients with R/R CLL after at least two prior therapies	September, 2018
Venetoclax	Adult patients who have relapsed or are not suitable for treatment with BCR inhibitors	First approval in April, 2016 (for patients with CLL who have 17p deletion and who have been treated with at least one prior therapy), then the indications have been expanded
Venetoclax + obinotuzumab	Adult patients who are unsuitable for or have failed a B-cell receptor pathway inhibitors	EMA approval: January, 2020
allo-HSCT	High risk disease and lower transplant risk (young, fit patients with well-matched donors)	

Note: Data from references. ^{18,69,72,91,93,94}

Abbreviations: CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; FDA, Food and Drug Administration; R/R CLL, relapsed/refractory chronic lymphocytic leukemia; EMA, European Medicines Agency; allo-HSCT, allogeneic hematopoietic stem cell transplantation.

results of a phase Ib/II study (PCYC-1102), FDA and EMA approved ibrutinib for previously treated CLL patients. The study has been conducted on 85 patients with relapsed or refractory (R/R) CLL. 28 patients had del17p. Patients were given ibrutinib orally, either 420 mg or 840 mg per day. The ORR was 71% in the 420-mg cohort and also 71% in the 840-mg cohort. The 26-month PFS was estimated at 75%, and OS was 83%. Moreover, PFS was elongated, even despite high-risk genomic features, including 17p13.1 deletion. The estimated 26-month PFS was 57% and the OS: 70%. Toxic effects included grade 1 or 2 diarrhea, fatigue, and upper respiratory tract infection.⁷¹ A latter analysis, on the extended cohort of PCYC-1102 and additional patients, has been performed by O'Brien et al.⁷² Continuous treatment with ibrutinib demonstrated efficiency and tolerability. The ORR has been reached in 87% of treatment-naïve (TN) patients

and 89% of R/R patients after 5-years follow-up. Treatment with ibrutinib has been proved relatively efficient in patients from high-risked subgroups, including patients with del(17p). The ORR for R/R patients with TP53 aberrations was 79%. The median PFS in patients with del(17p) was 26 months and OS was 57 months, both were the shortest compared with other high-risk groups.

A phase 3 randomized trial, called RESONATE, compared the efficacy of ibrutinib to anti-CD20 antibody ofatumumab in patients with R/R CLL or SLL. 32% of patients in the ibrutinib arm and 33% of patients in the ofatumumab arm had del(17p). In the group treated with ibrutinib, PFS was significantly improved (HR = 0.25, 95% CI: 0.14–0.45). The median duration of PFS was 5.8 months in the ofatumumab group and was not reached in the ibrutinib group. At 6 months follow-up, 83% of patients in the ibrutinib arm with del(17p) were alive

with no disease progression compared with 49% in the ofatumumab group.

The results of Farooqui et al⁷³ study, evaluating the efficiency of single-agent treatment with ibrutinib in patients with *TP53* aberrations, are also encouraging. 51 patients with active CLL have been enrolled in the study, 47 of which had deletion 17p13.1 and four carried solely a *TP53* mutation. 35 patients had not ever been treated and 16 had R/R CLL. An objective response was achieved by 97% of previously untreated patients (95% CI 86–100) and by 80% of patients with R/R CLL (95% CI 52–96). 18% of patients discontinued treatment: 10% due to disease progression and 6% accounted for by patient's deaths. Richter's transformation occurred in three patients and prolymphocytic transformation in 2 patients, who discontinued treatment because of disease progression. Median time to transformation was 5.5 months.⁷³ To compare, responses in patients treated with fludarabine, cyclophosphamide, and rituximab were observed in approximately 68% of patients and median PFS was less than 12 months.⁷⁴ The most frequent adverse events in patients treated with ibrutinib were of grade 1 or 2 and included were arthralgia, diarrhoea, rash, nail ridging, bruising, and muscle spasms or cramps. 24% of patients had grade 3 or worse neutropenia, 14% of patients had anemia, 10% of patients had thrombocytopenia. Cytopenia mostly occurred during the first months of treatment, then improved and were not complicated with infections or bleeding. As regards non-hematological treatment-associated adverse effects, three patients had grade 3 pneumonia, one patient had grade 3 rash.⁷³

Treatment with ibrutinib is generally well tolerated. Common adverse effects include: nausea, diarrhea, fatigue, muscle and bone pain, febrile neutropenia, infections, hypertension, atrial fibrillation and flutter and bleeding problems, especially in patients taking anticoagulants. Some authors claim that in a “real-world setting”, adverse events during therapy with ibrutinib are more common than in clinical trials and the discontinuation of therapy occurs in more than 20% of patients.⁷⁵ During the treatment with drugs targeting B-cell receptor signaling, including ibrutinib, a transient increase in lymphocytosis in the peripheral blood is frequently observed. Interestingly, lymphocytosis occurs along with the reduction in lymph node and spleen size. It has been proved that BTK inhibitors might mobilize CLL cells from the bone marrow, lymph node, or spleen to peripheral blood.^{76,77} Burger et al confirmed that treatment with ibrutinib causes

CLL cell redistribution from tissues into the blood. After starting ibrutinib therapy, a continuous improvement in bone marrow infiltration by CLL cells was observed. Moreover, authors proved that in patients with unmutated *IGHV* CLL (which is a poor prognostic factor), the rates of tissue cell death are higher and there is less redistribution after initiation of therapy, as well as faster bone marrow clearance of leukemia cells, compared with patients with mutated *IGHV*.⁷⁶ Ponader et al demonstrated in vitro and in vivo in mice that ibrutinib mobilize CLL cells from the tissues. To evaluate the effects of ibrutinib on the number of circulating and tissue-based lymphocytes, an EμTCL1 adoptive transfer mouse model resembling CLL in patients was used. TCL1 leukemia cells were transferred in SCID mice, having led to accelerated disease. Mice were treated with suboptimal (2.5 mg/kg/d) and optimal (25 mg/kg/d) doses of ibrutinib for three weeks. Mice treated with 25 mg/kg/d ibrutinib had significantly smaller livers and spleens with markedly reduced leukemic infiltration, compared with control mice. The crucial mechanism of ibrutinib, leading to the early redistribution of CLL cells from the tissues into the blood is the inhibition of CLL cells chemotaxis toward the chemokines CXCL12 and CXCL13, probably along with inhibition of adhesion molecule function/signaling.⁷⁷

Lymphocytosis usually normalize while continuing the treatment with ibrutinib. What is important is not to misinterpret elevated lymphocyte counts a sign of progression. For patients meeting the criteria of partial remission except lymphocytosis, the category “partial remission with lymphocytosis” has been established.⁵⁴

Idelalisib

Idelalisib (previously GS-1101 or CAL-101) is a small-molecule inhibitor of PI3K Delta isoform of the catalytic subunit of PI3K, toward which idelalisib is highly selective, and is expressed in hematopoietic cells, particularly in leukocytes.^{54,69} In 2014, FDA and EMA approved idelalisib in combination with rituximab for patients with R/R CLL or for those who have CLL and harbour del(17p) or *TP53* mutated.⁶⁹ The decision was a consequence of encouraging results of studies by Furman et al⁷⁸ and O'Brien et al.⁷⁹ Furman et al⁷⁸ evaluated the efficacy and safety of idelalisib in combination with rituximab in comparison with rituximab plus placebo. The study was conducted on 220 patients with relapsed CLL and comorbidities: decreased renal function, myelosuppression, or other major coexisting conditions. Approximately 80% of the patients had unmutated

IGHV and more than 40% had 17p deletion or *TP53* mutations. The median PFS in patients treated with idelalisib was not reached (HR 0.15; $P < 0.001$) compared with 5.5 months in the placebo group. OR was 81% in the idelalisib group vs 13% in the placebo group (OR 29.92; $P < 0.001$). OS at 12 months was 92% in the idelalisib group vs 80% in the placebo group ($p = 0.02$). 40% of patients from the idelalisib group and 35% of patients from the placebo group experienced serious adverse effects, the most frequent being: pneumonia, pyrexia, and febrile neutropenia. In 8% of patients in the idelalisib group and 10% in the placebo group adverse events led to treatment discontinuation. Although idelalisib, like ibrutinib, causes lymphocytosis, the addition of rituximab blunted and shortened the duration of the elevation of peripheral lymphocyte counts.⁷⁸ O'Brien et al⁷⁹ assessed idelalisib+rituximab as initial therapy in 64 previously untreated elderly patients with CLL or small lymphocytic leukemia. Treatment resulted in durable disease control: the ORR was 97% (100% in 2 patients with del(17p)/*TP53* mutations), PFS at 36 months was 83% and OS at 36 months was 90%. All patients had at least one adverse effect and 89.1% experienced grade ≥ 3 adverse effects. The most common adverse events included diarrhea, rash, pyrexia, nausea, chills, cough and fatigue.⁷⁹ Idelalisib/ofatumumab combo also proved to be effective in CLL. Median PFS was 16.3 months in the idelalisib plus ofatumumab group (95% CI 13.6–17.8) and 8.0 months (5.7–8.2) in the ofatumumab group (adjusted HR 0.27, 95% CI 0.19–0.39, $p < 0.0001$). In the subgroup with del(17p) OS was 25.8 months in the idelalisib/ofatumumab vs 19.3 months in placebo group. However, in idelalisib +ofatumumab group serious infections, including pneumonia and sepsis, were more common. Out of 261 patients, 22 patients in the idelalisib+ofatumumab group and 6 patients in the placebo group, died due to treatment-related adverse events.⁸⁰

The most alarming treatment-associated adverse events, fatal and/or severe are diarrhea, colitis, hepatotoxicity, pneumonitis (especially opportunistic *Pneumocystis jirovecii* pneumonia) and intestinal perforation. Idelalisib should not be used along with other hepatotoxic drugs; strong cytochrome P4503A (CYP3A) inducers, eg rifampicin, and CYP3A substrates, eg midazolam. During idelalisib treatment serum transaminase levels should be assessed on a regular basis and the patient should be monitored for pneumonitis.⁸¹

In addition to drugs already widely used in treatment of CLL, like ibrutinib and idelalisib, several second-

generation BTK inhibitors and PI3K inhibitors are currently being tested in Phase I and II clinical trials.

Acalabrutinib

Acalabrutinib (ACP-196), a second-generation BTK inhibitor, has been developed to overcome limiting toxicities observed during the treatment with ibrutinib, while reaching similar outcomes in patients with CLL. It could be achieved due to the higher selectivity of acalabrutinib, which limits off-target effects on other kinases such as TEC, EGFR, and ITK. In 2019 the FDA approved acalabrutinib for treatment of TN and relapsed refractory CLL or small lymphocytic lymphoma (SLL).⁷⁰ The approval was based on the results of two clinical trials: ELEVATE-TN⁸² and ASCEND.⁸³ In the phase 3 ASCEND trial, conducted on 310 patients with R/R CLL, acalabrutinib monotherapy has been compared to idelalisib+rituximab or bendamustine+rituximab therapies. 16% of patients had del17p. OS at 12 months was 94% for the acalabrutinib group and 91% idelalisib+rituximab or bendamustine+rituximab groups. PFS at 12 months were 88% for acalabrutinib and 68% for idelalisib+rituximab or bendamustine+rituximab. It has been proved that acalabrutinib is better tolerated than idelalisib+rituximab. 49% of patients in the idelalisib+rituximab group had to discontinue the treatment compared to 11% of patients treated with acalabrutinib. During the treatment with acalabrutinib, grade ≥ 3 adverse events included neutropenia (16%), anemia (12%) and pneumonia (5%). Other adverse effects were: headache and cough.⁸³ In phase 3 ELEVATE-TN study⁸² 535 treatment naïve patients with CLL were treated either with acalabrutinib alone or with obinutuzumab or obinutuzumab+chlorambucil. At 28 month follow up, PFS was prolonged in acalabrutinib + obinutuzumab group as well as in acalabrutinib in monotherapy group vs obinutuzumab +chlorambucil. 30-months PFS rates were estimated 90%, 82%, and 34% respectively, with acalabrutinib+obinutuzumab, acalabrutinib in monotherapy, and obinutuzumab +chlorambucil. PFS improvement with acalabrutinib+obinutuzumab or acalabrutinib in comparison with obinutuzumab+chlorambucil was also observed in the del(17p) subgroup (HR [95% CI]; 0.13 [0.04–0.46]; 0.20 [0.06–0.64]). Median OS has not been reached in any group. The ORR for acalabrutinib+obinutuzumab, for acalabrutinib in monotherapy and for obinutuzumab+chlorambucil was 94%, 85%, and 79%, respectively. Adverse events in acalabrutinib - containing schemes included: headache, diarrhea, neutropenia, atrial fibrillation, bleeding events,

hypertension. Adverse events were the reason of treatment discontinuation in 11% of patients on acalabrutinib+obinutuzumab, 9% of patients on acalabrutinib, and 14% of patients on obinutuzumab+chlorambucil.⁸²

Zanubrutinib

Zanubrutinib (BGB-3111) is another second-generation, irreversible BTK inhibitor.⁷⁰ It has greater selectivity compared with ibrutinib and what seems even more important it has a longer half-life in comparison with acalabrutinib and ibrutinib, which results in longer exposure of CLL cells to BTK inhibition.⁸⁴ In November, 2019 zanubrutinib received approval for treatment of R/R mantle cell lymphoma.⁸⁵ Studies evaluating the drug's efficacy and toxicities in CLL are ongoing. In the Phase 1 study, ORR was 96.2% (95% CI, 89.2–99.2), at a median follow-up of 13.7 months. ORR in all 16 patients with del(17p) or *TP53* mutation was 100% (95% CI, 79.4–100).⁸⁶

Duvelisib

Duvelisib (IPI-145) also appears an interesting alternative for other drugs targeting BCR signaling. It is expected to have better efficiency as it inhibits both p110δ and p110γ isoforms of PI3K.⁶⁹ Duvelisib demonstrated better efficiency than ofatumumab in a phase 3 clinical trial conducted on 160 R/R CLL patients. ORR in the duvelisib group was 74% vs 45% with ofatumumab. PFS in duvelisib group was of 13.3 months vs 9.9 months in ofatumumab group. Duvelisib was also superior to ofatumumab in p53-deficient patients. Their median PFS was 12.7 months vs 9 months for patients treated with ofatumumab.⁸⁷ In 2018, duvelisib has been approved in the treatment of R/R CLL.

New BCR Signaling Inhibitors Under Investigation

Results from clinical trials of multiple drugs targeting BCR signaling are eagerly awaited. New PI3K inhibitors and new BTK inhibitors, especially worth tracking include: umbralisib (TGR-1202), acalisib (GS-9820), copanlisib (BAY 80–6946), bimiralisib (PQR309), tirabrutinib (ONO/GS-4059), vecabrutinib (SNS-062), and fenebrutinib (GDC-0853).^{54,69} Currently, the data considering the effectiveness of most of these drugs in patients with *TP53* mutated is scant.

Apart from BTK inhibitors and PI3K inhibitors, SYK (spleen tyrosine kinase) inhibitors is another promising

class of drugs that inhibit BCR signaling.⁶⁹ Entospletinib (GS-9973), selective inhibitor of SYK has been evaluated in combination with idelalisib in Phase 2 study, conducted on patients with R/R CLL.⁸⁸ Although it turned out to be effective (ORR was 60%), the study had to be terminated early due to treatment-emergent pneumonitis in 18% of patients (severe in 11 of 12 cases, 2 fatalities).⁸⁸ Entospletinib and idelalisib combo probably caused excessive mTOR inhibition, which led to immune cell recruitment, T helper 1-type response and consequently, noninfectious pneumonitis. It is suggested, that isolated SYK inhibition may not lead to similar inflammatory effects.⁶⁹ The trial investigating entospletinib and obinutuzumab combination is ongoing, no results have been posted yet.⁸⁹ Cerdulatinib (PRT062070), a dual inhibitor of SYK and JAK1/3 and TAK-659, a dual SYK and FLT3 inhibitor are in phase 2a and 1 clinical trials, respectively.⁸⁸

Inhibiting Bcl-2 with BH3-Mimetics

In numerous studies it has been reported that CLL cells overexpress antiapoptotic protein Bcl2, which results in clonal lymphocytes accumulation that characterize CLL.^{18,69,90} Based on this observation, BH3-mimetic drugs, a new class of agents triggering cancer cells apoptosis, have been developed.^{18,69}

Venetoclax

Venetoclax (ABT-199) is a highly BCL-2-selective, orally available BH3-mimetic.^{69,84} In 2016, it was approved by the EMA as a monotherapy for CLL patients with del(17p) or *TP53* mutation who have relapsed or are not suitable for treatment with BCR inhibitors, and also for patients without del (17p) or *TP53* mutation and who are refractory to chemoimmunotherapy and BCR inhibitors. The decision followed the encouraging results of phase 2 M13-982⁹¹ and M14-032⁹² trials in R/RCLL.⁶⁹ Phase 2 M13-982 study⁹¹ has been conducted on 107 patients with del(17p) from USA, Canada, UK, Germany, Poland, and Australia. At a median follow-up of 12.1 months OR was achieved in 79.4% of patients (95% CI 70.5–86.6). Serious adverse events occurred in 55% of patients and included: pyrexia, autoimmune haemolytic anaemia, pneumonia and febrile neutropenia. Neutropenia, infection, anemia and thrombocytopenia were the most common grade 3–4 adverse events. Seven patients died due to disease progression and four from adverse events, none considered as treatment associated.⁹¹

In 2019, venetoclax in combination with obinutuzumab has been approved for TN patients with CLL and SLL.⁹³ The decision was based on the outcome of a CLL14 (NCT02242942) trial of venetoclax+obinutuzumab vs obinutuzumab+chlorambucil in 432 patients with TN CLL with comorbidities. In total, 13.8% of patients had *TP53* deletion, mutation, or both. venetoclax+obinutuzumab was associated with longer PFS at 24 months than chlorambucil–obinutuzumab 88.2% (95% CI, 83.7 to 92.6) vs 64.1% (95% CI, 57.4 to 70.8). This benefit was also observed in patients with *TP53* deletion, mutation, or both and in patients with unmutated *IGHV*. Adverse events occurred in 94.3% patients treated with venetoclax–obinutuzumab and in 99.5% of patients in the chlorambucil–obinutuzumab group. The treatment had to be discontinued due to adverse events in 16.0% and 15.4% of patients, respectively. The most frequent adverse effect of grade 3 or 4 were neutropenia, occurring in 52.8% of patients in the venetoclax–obinutuzumab group and in 48.1% of patients in the chlorambucil–obinutuzumab group and infections occurring in 17.5% and 15.0%, respectively. 9.3% of patients died in the venetoclax–obinutuzumab group and 7.9% died in the chlorambucil–obinutuzumab group.⁹⁴ Seymour et al⁹⁵ revealed the results of randomized, open-label, phase 3 trial in which 389 patients with R/R CLL treated with venetoclax+rituximab or bendamustine+rituximab took part (MURANO study). After a median follow-up of 23.8 months, PFS was significantly higher in the venetoclax+rituximab group than in the bendamustine+rituximab group. The 2-year rates of PFS were 84.9% and 36.3%, respectively (HR for progression or death, 0.17; 95% confidence interval [CI], 0.11–0.25; $P<0.001$). The benefit was also observed in patients with *TP53* mutated. In the subgroup of patients with chromosome 17p deletion, the 2-year rate of PFS was 81.5% in the venetoclax+rituximab group compared to 27.8% in the bendamustine+rituximab group (HR 0.13; 95% CI, 0.05 to 0.29). The most frequently observed adverse effects grade 3 or 4 was neutropenia in both groups, with higher prevalence in the venetoclax+rituximab group (67% vs 55.3% respectively).⁹⁵ In 4-year follow-up Kater et al⁹⁶ reported the long-term outcomes and the predictive value of molecular and genetic characteristics. Four-year PFS and OS were higher in venetoclax+rituximab group compared to the bendamustine+rituximab group. Undetectable MRD at the end of therapy was associated with higher PFS compared with both low and high MRD positivity. In patients with mutated *TP53*, *NOTCH1*, *XPO1* and *BRAF*,

treated with venetoclax+rituximab, lower undetectable MRD rates were reported.⁹⁶ Roberts et al⁹⁷ also evaluated the efficacy of venetoclax and identified the treatment effect modifiers in patients with CLL and SLL. Objective responses were achieved in 75% of all patients, including 22% complete remissions. Chromosome 17p deletion and/or *TP53* mutation and *NOTCH1* mutation were consistently associated with shorter duration of response, but not the probability of response.⁹⁷

Levin et al,⁹⁸ in HOVON 141/VISION trial, are exploring the effects of the combination treatment with ibrutinib+venetoclax in RR/CLL. After a short induction with ibrutinib, patients with RR/CLL are treated with 12 cycles of venetoclax. Those who reach undetectable MRD after 12 cycles are randomised to continue ibrutinib or treatment discontinuation. PFS after 12 months follow-up is to be the primary endpoint of the study, which has not been completed yet.⁹⁸

What needs to be remembered during managing CLL patients with venetoclax is the possibility of tumor lysis syndrome (TLS).⁵⁴ Roberts et al.¹⁸ and Seymour et al⁹⁹ reported cases of TLS with fatal outcome during therapy with venetoclax. Before starting the therapy, to prevent TLS, a risk assessment for the development of this adverse effect should be performed, based on the patient's renal function and tumour cell load. A stepwise ramp-up of dosage should be introduced (20 mg for a start, then weekly increase to a final dose of 400 mg). Patients should be closely monitored during the treatment in search of TLS.⁵⁴

Chimeric Antigen Receptor—T Cell Therapy in the Treatment of CLL

Chimeric antigen receptor—T cell therapy (CAR-T) is a very recent treatment in CLL, attracting more and more attention.⁴ Genetically modified T cells express chimeric antigen receptor (CAR) targeting specific antigen, which is, in CLL, CD19.^{54,100} Preclinical and clinical trials prove that CAR-T therapy is a promising treatment in R/R CLL. Turtle et al¹⁰¹ evaluated the safety and effectiveness of CAR-T cell therapy in patients with CLL, previously treated with ibrutinib. 23 out of 24 patients had either complex karyotype or del(17p). Four weeks after CAR-T cell infusion, ORR was 71%. 83% of patients developed cytokine release syndrome, 33% developed neurotoxicity which was the reason of one fatal outcome. In patients who achieved the clinical response, 88% had no minimal

residual disease (MRD) evaluated by flow cytometry, and 58% had no detectable disease evaluated by deep IGH sequencing. Then, patients who respond to CAR-T therapy may achieve a deep remission without MRD.¹⁰¹

Common adverse events of CAR-T cell therapy in CLL include cytokine release syndrome (CRS), B cell aplasia, neurotoxicity, and infections.¹⁰⁰ CRS occurs due to the sudden activation of CAR-T cells, after encountering target cells. The cytokines released include most of all IL-6, IL-10, TNF- α , and IFN- γ . Inflammatory factors can be also produced by macrophages, monocytes, natural killer cells, and dendritic cells in the response for stimulation of CAR-T cells, target cells, and inflammatory mediators.¹⁰⁰ CRS occurs in nearly 83% of CLL patients, with a higher frequency in patients who were previously treated with ibrutinib.¹⁰⁰ Symptoms of CRS range from fever and hypoxia to renal failure and, rarely, death. Treatment with tocilizumab (IL-6 receptor antagonist) and corticosteroids can be a CRS management strategy.¹⁰² The limitations of CAR-T consist the complexity of the approach, expenses and toxicities.⁴

Allogenic Stem-Cell Transplantation in High Risk CLL Patients

Despite novel treatment possibilities, allogenic stem-cell transplantation (alloPBSCT) still remains an option for patients with CLL.⁵⁴ However, the cohort of patients that should be qualified to the procedure seem to change. Therapy with novel drugs gives a chance of durable remission in patients with del(17), so one must be careful not to overweight the risks of alloPBSCT over the benefits of therapy with new agents. OS at 6 years from alloPBSCT is 50–60%. Treatment related mortality in CLL was estimated 16–25% and *graft vs host* disease was observed in 50–55% of patients. As alloPBSCT is the only treatment that offers a full recovery, it could be relevant for young, high-risk patients, without comorbidities, with a perfectly matched donor.^{54,84}

Resistance to Novel Therapies

Although novel agents, such as ibrutinib, idelalisib and venetoclax, are significantly efficient in CLL, some patients develop resistance to these drugs and progress.¹⁰³ Sedlarikova et al¹⁰³ provided an overview of the acquired resistance to novel agents. 80% of patients who failed on ibrutinib, had acquired mutations in *BTK* and *PLCG2* genes. In idelalisib failure, no resistance-

associated mutations or deregulated signalling were reported. In patients who had failed on venetoclax, acquired mutations in the *BCL2* gene were found. Resistance-associated mutations in patients who have progressed on ibrutinib and venetoclax are often presented at low allelic frequencies.¹⁰³ One of the studies analysed by Sedlarikova et al was by Woyach et al,¹⁰⁴ who performed the whole-exome sequencing of six patients with R/R CLL, previously treated with ibrutinib. In 5 patients acysteine-to-serine (C481S) mutation in *BTK* at the binding site of ibrutinib was found and in 3 patients mutations in *PLC γ 2* were identified.¹⁰⁴ Acysteine-to-serine mutation of *BTK* results in solely transient inhibition of *BTK*, which results in poor therapeutic outcome. Mutations in *PLC γ 2* possibly lead to autonomous BCR activity, due to gain-of-function.⁷¹ Although acquired resistance to ibrutinib occurs in a small proportion of patients, patients with del(17p13.1), del(11q22.3) or a complex karyotype seem to be at greater risk of relapse. Gángó et al¹⁰⁵ reported that in patients with CLL treated with ibrutinib, with persisting *TP53* mutated subclones, no *BTK* mutations were detected. Conversely, an association between reduction or elimination of *TP53* mutated clones and the presence of *BTK* mutations has been proved. The authors hypothesize that the treatment with ibrutinib may create conditions, in which the survival of subclones with *BTK* mutations is facilitated, by eliminating subclones with *TP53* mutations or that the elimination of *TP53* subclones allow the expansion of subclones harboring *BTK* mutations.^{103,105} As the mutations in *BTK*, *PLCG2* and *BCL2* leading to the treatment failure, are possible to detect a few months before clinical relapse, in patients receiving long-term treatment with BCR and BCL-2 inhibitors, regular genetic testing should be performed, preferably using highly sensitive ultra-deep NGS approaches.¹⁰³

New Perspectives in Treatment of CLL with del(17p) and/or *TP53* Mutation – A Straight Way from Biology to Therapy

Improvements in understanding the biology of CLL keeps resulting in the development of new treatment strategies, which are being evaluated in pre-clinical trials. Here we present a few of the approaches that, in our opinion, seem most promising.

Boudny et al⁴¹ hypothesized that checkpoint kinase 1 (CHK1), which takes part in DNA replication and repair,

could be a suitable target in the treatment of CLL. CHK1 is an important component of the DNA damage response (DDR) pathway, which serves as an anti-cancer barrier.¹⁰⁶ The newly developed agent MU380 is a selective inhibitor of CHK1. In CLL-derived cell lines: *TP53*-mut MEC-1 and MEC-2, but also in *TP53*-wild type OSU-CLL, MU380 altered cell cycle and induced apoptosis. MU380 reduced the viability of CLL cells, both unstimulated (even though their CHK1 level is low) and induced to proliferate by anti-CD40/IL-4 stimuli. Effects were comparable in samples harbouring *p53* pathway dysfunction and samples without such alterations. Only a few samples were resistant to the inhibitor, only 5 of 96 showed viability $\geq 80\%$ after 72 h treatment. It is worth adding that non-cancerous cells were much less sensitive to MU380. Thus, in the future MU380 may represent an attractive option for high-risk CLL patients.⁴¹

Another potential target in the therapy of CLL is USP7-PTEN network. USP7 is an de-ubiquitinase, that is over-expressed in about 70% of CLL CD19+ cells, both at the mRNA and protein levels. It is of great significance in CLL pathogenesis, as it inactivates three major tumor suppressors: *p53*, PTEN and FoxO.¹⁰⁶ Carra et al¹⁰⁷ demonstrated that in CLL cell lines USP7 can be effectively targeted by the USP7 inhibitor P5091, which results in reactivation of tumour suppressive functions and hence promotes the induction of cell growth arrest and apoptosis. These effects were observed both in *TP53*-wild type and *TP53*-null environment. It is very probable that, P5091 effects are *p53*-independent, as P5091 promoted apoptosis in *TP53*-mutated MEC-1 cells.¹⁰⁷

One of the features of CLL cells is their high intrinsic reactive oxygen species (ROS) stress, which is even higher when *p53* function is lost.¹⁰⁸ Liu et al¹⁰⁸ proved that CLL cells with loss of *p53* are sensitive to ROS-mediated cell killing, even when CLL cells are under the protection of bone marrow stromal cells.¹⁰³ The ROS-modulating factor used by the authors was phenethyl isothiocyanate (PEITC), a natural substance, that can be found in cruciferous vegetables.^{108,109} In mouse model, the usage of PEITC resulted in massive cell death in primary 17p-CLL cells and *p53*-null leukemia cells. PEITC at a concentration of 5 μM killed 17p-CLL cells, with only 17% viable cells remained at 24 h after drug incubation. 17p-CLL cells were resistant to standard anti-CLL agents such as F-ara-A and Oxaliplatin. Survival 48 h after treatment with F-ara-A and Oxaliplatin were 53% and 42% respectively. The overall

survival in mice treated with PEITC was 7.8 months vs 3.5 months for untreated mice.¹⁰⁸

Ciardullo et al¹¹⁰ examined the effect of the second-generation MDM2 inhibitor, RG7388, on the induction of *p53*-transcriptional targets in CLL cells and normal cells. MDM2 is an ubiquitin ligase, which controls the levels and activity of *p53* via ubiquitin-dependent proteasomal degradation. RG7388 is a non-genotoxic compound, that binds to MDM2 in the *p53*-binding pocket and can release *p53*, leading to effective stabilization of the *p53* pathway. Ciardullo et al¹¹⁰ proved that RG7388 induces a pro-apoptotic *p53* gene signature in CLL, but not in normal blood and bone marrow cells.¹¹⁰

Conclusions

In recent years we witnessed novel and smarter therapies revolutionise the treatment of del(17p) and/or aberrant *TP53* CLL. As a consequence of the wide availability of ibrutinib, idelalisib, venetoclax and other targeted therapies, nowadays the prognosis of those patients has improved significantly.⁶⁸ However, *TP53* aberrations are still the most important single factors of poor prognosis in CLL.¹² That is due to the early progression of the disease in patients with *TP53* mutations, high risk of Richter transformation, adverse effects mandating treatment discontinuation, acquired resistance to BTK inhibitors. Hence, the development of alternative therapeutic approaches still remains an issue of critical importance.¹⁰¹ Efforts should be focused both on the improvement of the already approved approaches, but also on the development of new strategies. CAR-T cells therapy is one of the most promising advances, that, wide spread, could be another milestone in the treatment of CLL. New technologies, combined with ever-increasing understanding of CLL pathogenesis justify our hopes for new therapies for *TP53*-mutated CLL patients.

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