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REVIEW

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Novel prognostic molecular factors: a quantum leap in the field of chronic lymphocytic leukemia

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Abstract
Cytogenetic lesions do not completely explain clinical heterogeneity of chronic lymphocytic leukemia (CLL). The 2016 revision of the World Health Organization classification 2008 indicated that molecular lesions of *TP53*, *NOTCH1*, *SF3B1* and *BIRC3* have potential clinical relevance and could be integrated into an updated risk profile. The negative clinical implications of *TP53* disruptions are well constituted and patients with these mutations should be considered for novel, small molecule signal transduction inhibitors therapies. Mutations of *NOTCH1*, *SF3B1* and *BIRC3* are associated with poor prognosis. Patients with mutated *SF3B1* or *NOTCH1* genes present shorter time to first treatment compared to unmutated group. *NOTCH1* mutations are related to a high risk of Richter's syndrome transformation, especially in case of *TP53* disruptions' coexistence. Large studies on *MYD88* mutations in CLL have not explained clearly their clinical importance.
The aim of this paper is to provide a comprehensive review on novel molecular aberrations identified in CLL.
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Key words: chronic lymphocytic leukemia; *TP53*; *NOTCH1*; *SF3B1*; *BIRC3*; *MYD88*; prognostic factors

Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common types of leukemia in adults and is characterized by the accumulation of malignant B CD5+ lymphocytes in the peripheral blood (PB) and lymphoid organs [1]. CLL is a highly heterogeneous disease presenting either stable course with above 15 years survival or rapidly progressive one leading to death within a year of diagnosis or to transformation to an aggressive lymphoma, known as Richter's syndrome (RS) [2–5]. Clinical heterogeneity of CLL explains the need for identification of prognostic and predictive factors.

In recent years, our knowledge of the genetics of CLL has significantly increased and provided many clinical biomarkers. The currently used ones include immunophenotype markers such as CD38 and ZAP-70 expression on B lymphocytes surface and molecular lesions of well-established prognostic value: mutational status of *IGHV* (immunoglobulin heavy chain variable region) gene or *TP53* mutations [6]. Furthermore, in the year 2000 Döhner *et al.* [7] applied FISH (interphase fluorescence *in situ* hybridization) cytogenetic analysis to evaluate cytogenetic lesions in CLL, finding chromosomal abnormalities in over 80% of patients. By correlating FISH lesions with the course of the disease, a hierarchical model based on five risk categories was designed. Patients with the 17p13 deletion were assigned the worst prognosis, followed by cases carrying the 11q22-q23 deletion, trisomy 12, normal karyotype and 13q14 deletion [7].

Recent studies based on NGS (next generation sequencing) technology have revealed previously unknown genomic alterations in CLL, such as mutations of *NOTCH1* (neurogenic locus notch homolog protein 1), *SF3B1* (splicing factor 3B subunit 1), *BIRC3* (baculoviral IAP repeat-containing protein 3) and *MYD88* (myeloid differentiation primary response gene 88), which provide additional information of

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CLL prognosis [8–11]. Rossi *et al.* [12] regarded that integration of these new mutational disruptions with cytogenetic model results in more precise prediction of survival compared to the Döhner model alone. The 2016 revision of the World Health Organization classification reported that novel molecular lesions have a potential clinical relevance and could be integrated into an updated risk profile [13]. However, in the same year, the International Chronic Lymphocytic Leukemia–International Prognostic Index (CLL-IPI) working group created a model which did not include other than *IGHV* and *TP53* molecular mutations, recognizing the others as showing no independent prognostic value. Therefore, there is a need to define clinical significance of novel prognostic factors besides *TP53* and *IGHV* [14].

This review summarizes the available data concerning molecular lesions found in CLL cells with a broad reference to their importance for the pathogenesis of the disease and clinical prognostic value.

TP53

TP53 (tumor protein p53) gene is located on chromosome 17 (17p13.1) and consists of 11 exons and 10 introns [15]. The translation product of this gene is a phosphoprotein with a molecular weight of 53 kDa (containing 393 amino acids divided into the three domains). It functions as the main tumor suppressor in the human cells. The protein is a transcription factor composed of typical domains: N-terminal, core domain and C-terminal, with specific functions [16]. The N-terminal domain contains a region rich in prolines residues (proline-rich region, 61–94), made up of multiple PXXP motifs (where P is proline and X any other amino acid) and also the transactivation domain (transactivating domain TAD1 and TAD2, amino acids 1–42). Due to this unique domain, *TP53* is responsible for the induction of apoptosis through interactions with other proteins while transactivation of genes is not necessary. Core protein of *TP53* consists mainly of the DNA binding domain (DBD, 102–292) [17]. C-terminal domain is responsible for *TP53* tetramerization, non-specific interaction with DNA, and has a protein binding site enhancing the transcriptional activity of *TP53* [18]. The human *TP53* gene expresses 12 different *TP53* proteins (isoforms) as the effect of alternative splicing [19].

TP53 plays a key role in regulating cell proliferation, mainly by inducing cell cycle arrest, apoptosis or DNA repair mechanisms activation [20]. DNA damage initiates overexpression of *TP53* which induces a phase G1 arrest providing the integrity of the genome. Under extensive damage, where DNA cannot be repaired, *TP53* transactivates genes involved in apoptosis. *TP53* mutations inhibit the cell cycle arrest what causes the deregulation of apoptosis, resulting in malignant transformation and proliferation of damaged cells [21]. Loss of *TP53* function during tumorigenesis triggers deregulation of the cell cycle, genetic instability and resistance to chemotherapy [22].

Total loss of *TP53* function may be caused by coexisting *TP53* mutations with deletion of remaining 17p allele, mutation of both alleles or homozygous mutation resulting from loss of heterozygosity (LOH). Another mechanism limiting the functions of *TP53* is dominant-negative effect: the mutant protein binds with the unchanged form, making a complex which is incapable of DNA binding and inhibits the transactivation of other genes. In addition, it is suggested that *TP53* mutations may also change thermodynamic stability of proteins and result in the acquisition of new properties (gain-of-function, GOF) important for tumor progression or increasing resistance to treatment [23]. The loss of *TP53* function due to mutations or deletions is observed in about 50% of solid tumors [24], with significantly lower proportion in the case of hematological malignancies [25].

TP53 mutations exhibit considerable heterogeneity in terms of both structure and location. Approximately 75% of all mutations represent missense mutations leading to amino-acid changes. The vast majority of point mutations were found in exons 5 to 8 and were clustered in four mutation "hotspots" situated between codons 130 and 280. Less frequent are nonsense mutations, deletions, insertions or mutations in transcription sites [26].

Clinically, *TP53* alterations are associated with inferior prognosis in numerous cancers including lymphomas and CLL. Mutations of *TP53* are found in 10–15% of patients with CLL at diagnosis or before first therapy [27–29]. The highest incidence of *TP53* mutations was observed in patients with fludarabine-refractory CLL [30]. About 80% of cases with 17p deletion also hold *TP53* mutations in the remaining allele [27, 28]. *TP53* mutations in the absence of 17p deletion concerns 3% of patients in the first-line treatment and are associated with significantly worse outcome, especially in the case of mutations located in the DNA binding domain [29]. Patients with missense mutations localized within the DNA-binding motifs (DBMs), the parts of DNA binding domains that are directly involved in contact with DNA, had largely shorter time to first treatment (TFT) and overall survival (OS) compared with both remaining missense mutations and non-missense alterations [31].

CLL has been found to exhibit *TP53* specific mutation profiles. Multivariate analysis revealed a lower percentage of transitions in CpG sites in CLL

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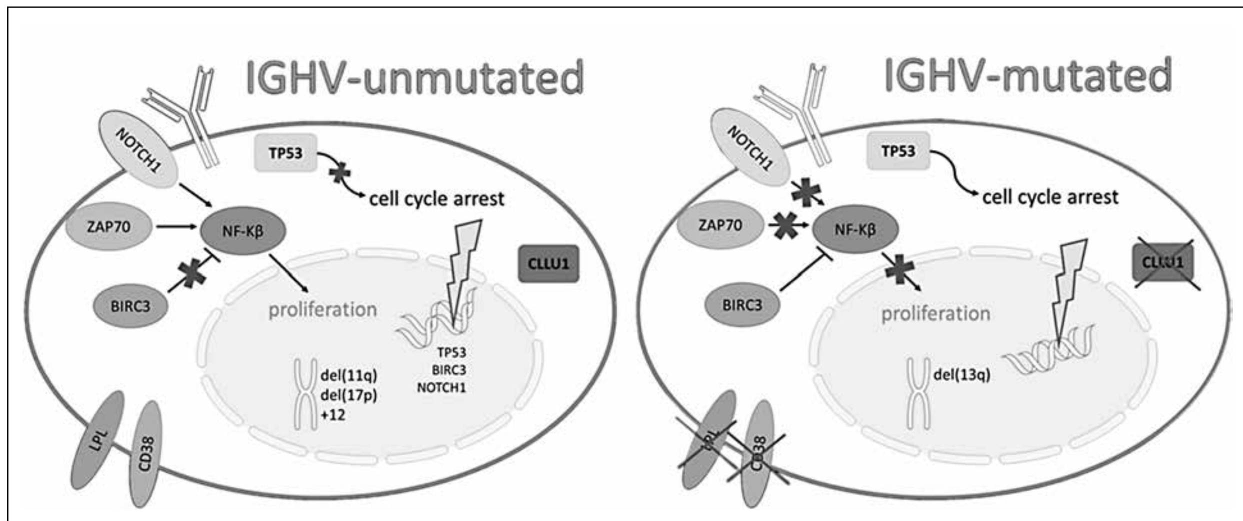

Figure 1. Differential signaling responses in immunoglobulin heavy chain variable (IGHV)-unmutated and mutated chronic lymphocytic leukemia (CLL). Figure presents the most important prognostic factors with regard to the *IGHV* mutational status. *BIRC3* disrupting mutations and *NOTCH1* activating mutations trigger enhanced NF-κB signaling and proliferation in *IGHV*-unmutated CLL. *TP53* deletion and/or mutation is more frequent in *IGHV*-unmutated CLL and results in dysfunctional cell cycle arrest which in consequence leads to enhanced cell survival. ZAP-70 takes part in BcR signaling and its overexpression leads to increased cell proliferation through, *e.g.* NF-κB signaling. Adapted from Rosenquist *et al.* [99].

compared to other cancers. In addition, transitions G→A were more frequent in comparison with C→T, whereas in the other tumors both changes were at a similar level [32].

IGHV

Apart from *TP53* mutations, *IGHV* (immunoglobulin heavy chain variable) gene mutational status is well-known prognostic factor for patients with CLL. Identification of mutational status of *IGHV* genes was a milestone in understanding CLL biology [33]. The presence or absence of mutations in the *IGVH* genes distinguishes two clinical forms of CLL. Patients with *IGHV* mutations display favorable prognosis with long OS while group without the mutations are characterized by an aggressive course of the disease, indicating important role of B-cell receptor (BCR) in the pathogenesis of CLL [33, 34]. Additionally, approximately 20% of untreated patients exhibit almost identical BCR so called stereotyped BCR encoded by different, although phylogenetically related *IGHV* genes [35]. The discovery of stereotyped BCR enabled to assign almost one-third of CLL patients to subsets that represent distinct biological profiles determining similar disease course and outcome [36]. Malcikova *et al.* [37] examined the frequency of *TP53* mutations in relation to *IGHV* gene status and BCR immunoglobulin stereotypy. The study revealed a higher percentage of *TP53* mutation in the unmutated *IGHV* group. Additionally, a different profile of *TP53* mutations in various stereotyped CLL subsets was found pointing to different mechanisms responsible for clinical aggressiveness for each subset [37].

Figure 1 exemplifies the different pathogenic mechanisms involved in *IGHV*-mutated and *IGHV*-unmutated CLL.

NOTCH1

The *NOTCH1* (neurogenic locus notch homolog protein 1) gene, encoded on chromosome 9q34.3, plays a fundamental biological role in hematopoiesis [9]. NOTCH1 receptors have been shown to have an essential role in the pathogenesis of some hematologic and solid malignancies [38, 39]. They are a family of transmembrane proteins belonging to cell surface receptors as well as transcription regulators which are expressed by different tissue [40].

The extracellular domain of *NOTCH1* (N-EC) consists of 36 epidermal growth factorlike repeats (EGFR), 3 cysteine-rich lin12/Notch repeats (LNR) and the heterodimerization domain (HD). In the plasma membrane, *NOTCH1* is cleaved in two units, which are kept together thanks to interactions between the HD domains. After binding to the ligand, *NOTCH1* is further cleaved by the gamma-secretase complex, causing release of the intracellular part (N-IC) [41]. Subsequently, N-IC can transfer to the nucleus where it makes a transcriptional complex. N-IC includes the

RAM domain (R), ankyrine repeats, transactivation domain (TAD) and the PEST sequence that marks N-IC for degradation by FBXW7 [42]. PEST region plays a main role in the proteasomal degradation of the NOTCH receptor by holding to FBXW7, an E3 ubiquitin ligase, which limits the NOTCH activity. Deletion of CT in the C-terminal region leads to removal of the PEST domain, shortening NOTCH protein, altered NOTCH degradation and continuous transcriptional activation of NOTCH target genes in CLL, such as *MYC*, *TP53* and molecules of the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway [10, 43]. Constitutive *NOTCH1* signaling activation was noticed in CLL cells and considered to be involved in apoptosis resistance and increased CLL cells survival [38].

NOTCH1 mutation in CLL patients most frequently concerns C-terminal PEST domain [44]. At the molecular level, these mutations represent mainly frameshift or non-sense events accumulating within exon 34 and including recurrent c.7544_7545delCT deletion (around 80–95% of all mutations) [45]. *NOTCH1* mutations in CLL interfere with the C-terminal PEST domain of the protein, which takes part in the proteasomal degradation of the activated form of *NOTCH1* [44]. In fact, short PEST domain results in stabilization of the active form of *NOTCH1*, the molecule impaired degradation, and thus upregulated *NOTCH1* signaling [46, 47].

NOTCH1 mutations occur in 5–10% newly diagnosed CLL, while their frequency increases to 15–20% in progressive CLL requiring first treatment and in relapsed cases [45, 48]. *NOTCH1* is associated with more aggressive clinical presentation of CLL such as chemorefractoriness and disease progression towards transformation into RS. *NOTCH1* mutations in RS are the second, after *TP53*, most frequent genetic lesions, where they occur in approximately 30% of cases [9, 10, 45]. Analyses on specific and more numerous subgroups of patients have revealed a particularly high frequency of *NOTCH1* mutations in CLL cases that harbor trisomy 12 (+12), as the sole cytogenetic abnormality (30%) [49]. Moreover, patients with *NOTCH1* mutations display a significantly shorter overall survival refining the intermediate prognosis of CLL patients with +12. Importantly, the high frequency of *NOTCH1* mutations in +12 (trisomy 12) CLL patients is associated with a characteristic gene-expression profile characterized by an overexpression of cell cycle related genes located on chromosome 12 [49]. Balatti *et al.* revealed that *NOTCH1* mutations were enriched among *IGHV*-unmutated/ZAP70+ CLL patients harboring +12 (about 42%), as compared to much lower presence in 4% of *IGHV*--unmutated/ZAP70+ cases without +12 [50].

NOTCH1 mutations may constitute potential new biomarker for the selection of poor-risk CLL patients. Patients with *NOTCH1* mutations are characterized by a significantly shorter OS (21–45% at 10 years) and present a more rapidly progressive disease compared to *NOTCH1* wild-type cases (56–66% at 10 years) [9, 10, 45]. According to Mansouri *et al.*, *NOTCH1* mutations similarly as *TP53* mutations seem to be strong, independent prognostic markers of poor prognosis [51]. In the United Kingdom Leukaemia Research Fund Chronic Lymphocytic Leukemia 4 (UK LRF CLL 4) trial study [48] patients with *NOTCH1* mutations had significantly shorter OS compared to wild-type cases (respectively 55 and 83 months) but longer than patients carrying *TP53* abnormalities (26 months). The short OS related to *NOTCH1* mutations could be in part explained by a significantly higher risk (45% in *NOTCH1* mutated *vs*. 4.6% in wild-types) of developing RS in patients harboring *NOTCH1* aberrations [52]. On the basis of preliminary German CLL Study Group (GCLLSG) CLL8 trial exploring the role of new mutations in CLL patients treated with first-line fludarabine-cyclophosphamide (FC) or fludarabine-cyclophosphamide-rituximab (FCR), *NOTCH1* mutations constitute independent predictors of short progression-free survival (PFS) even after FCR treatment [53]. On the contrary, data from the GCLLSG CLL2H trial determining the incidences, associations, and prognostic roles of *NOTCH1*, *SF3B1* and *TP53* mutations in fludarabine-refractory CLL patients treated with alemtuzumab indicate that patients with *NOTCH1* mutations may have longer PFS after treatment with alemtuzumab compared to *NOTCH1* wild-type settings [54]. In multivariable analyses, *NOTCH1* mutations was identified as an independent favorable marker for PFS [55].

SF3B1

The SF3B1 (splicing factor 3b subunit 1) protein is the product of the same-named gene which is composed of 25 exons and located on chromosome 2 in q33.1 region [56]. The protein is considered to be an essential component of the splicing machinery in the process of RNA editing. Splicing consists of the stages of removing introns, which are noncoding sequences, from premessenger RNA and ligating the remaining exons together. The product of *SF3B1* gene is involved in the control of connecting the premessenger RNA with macromolecule, spliceosome, at the beginning of the process. Two types of spliceosome are known: U2-dependent type (classical) and U12 type (alternative), of which each one is composed of five unique nucleoproteins RNA (snRNPs) [57]. SF3B1 is the core protein of snRNP in classical spliceosome. Its

role is to recognize the branch side of premRNA, and, subsequently, to bind it with the spliceosome, what is the initial stadium of splicing [57, 58]. The abnormalities of this regulation, which are associated with the mutations in *SF3B1* gene, may lead to unintended introns retention, and, consequently, to forming alternative, modified transcripts [59]. In the structure of the SF3B1 protein there are two key regions. The first of them is N-terminal end which contains a few binding factors to interact with other spliceosome components forming the complex. The second one is C-terminal end with 22 tandem-repeat domains including HEAT (Huntingtin, Elongation factor 3, protein phosphatase 2A, Targets of rapamycin 1) motifs. The precise role of C-terminus is still unknown [11, 60]. C-terminal HEAT repeats interfere alternatively with U2 snRNP and other spliceosomal components what might regulate splicing activity [61].

In the last years, it becomes evident that mutations in *SF3B1* gene are connected with pathogenesis of hematological disorders, especially with myelodysplastic syndrome [62] and CLL. *SF3B1* mutations in CLL are generally represented by missense substitutions affecting the HEAT domains of the *SF3B1* protein. Most of them are detectable between the fifth to the eighth HEAT repeats (encoded by exons 14–16). The main target, accounting for approximately 40% to 50% of all *SF3B1* mutations, are five hotspots (codons 662, 666, 700, 704 and 742), with the K700E substitution [61, 63–66]. The second most common substitution is G742D (19%), a mutation rarely found in myeloid neoplasm [11, 63].

The role of *SF3B1* mutations at the cellular level remains unknown [67]. Possibly, modified product of the mutated gene interacts incorrectly with RNA and cofactors. However, the small amount of altered transcripts indicates that the mutations do not influence the mechanism of splicing globally [68]. Perhaps, the alternative splicing is the consequence of the mutation, which, in fact, does not influence the pathogenesis of the disease. The recent studies suggest that the mutation results in anomaly of the response to the DNA damage what disturbs genomic stability [65, 66]. Other cellular functions that might be deregulated are telomere maintenance and NOTCH signaling in CLL cells [67].

The *SF3B1* mutations may be subclonal, which is the conclusion of the frequency and the time of their occurrence in comparison with other defects in CLL known as drive mutations (*MYD88*, trisomy 12, del13q). In the study of Landau *et al.* [69], it was disclosed that drive mutations occur in the earlier stage of the CLL and among higher percentage of patients. The incidence of *SF3B1* mutations appears to increase over time and they are correlated with a more advanced clinical stage. Therefore, the *SF3B1* mutations should be taken into account as an important marker of the disease progression or even one of its mechanisms [70].

In the studies conducted by a few research groups *SF3B1* mutations have been observed in CLL cells with frequency accounting from 5 to 20%. Furthermore, it was noticed that *SF3B1* mutations recur rarely in newly diagnosed CLL (5%), while more often (15%) in progressive CLL requiring first treatment and even in 20% relapsed and chemorefractory patients [11, 60, 65, 71, 72]. Consequently, the presence of the *SF3B1* mutations in the CLL cells is concerned with less favorable prognosis. Patients with *SF3B1* mutations were characterized by significantly shorter time to treatment, short PFS after treatment and also low OS rate [65, 73]. Moreover, *SF3B1* mutations are associated with chemoresistance to alkylating agents and fludarabine therapy [71, 73]. The mutations do not limit the survival after allogenetic hematopoietic stem cell transplantation (HSCT),which means it influences negligibly the long term disease control of HSCT [74]. Some correlations between *SF3B1* mutations and other lesions have been described in CLL. It was noticed that *SF3B1* mutations occur more frequently in association with 11q22-q23 deletion, *ATM* mutations and unmutated *IGHV* status while negative correlation was observed with trisomy 12 and isolated del13q [68, 70]. The assessment of *SF3B1* mutation status may contribute to the identification of poor-risk CLL patients and in combination with conventional lesions of CLL may refine the disease prognosis [62, 68].

BIRC3

Baculoviral IAP repeat-containing protein 3 (BIRC3) belongs to the members of the IAP (inhibitor of apoptosis) family which was firstly described in the virus-infected cells. BIRC3 is encoded by gene located in chromosome 11 (11q22.2) and composed of 602 amino acids [75]. In adults it is mainly expressed in lymphoid tissue, especially spleen, and peripheral blood lymphocytes. The structure of *BIRC3* protein is characterized by specific motif, zinc finger domain, containing zinc ions coordinated by cysteine and histidine residues. The second specific region is caspase-recruitment domain (CARD) which is commonly found in proteins involved in inflammation process. Furthermore, *BIRC3* protein has three BIR repeats [76, 77].

The basic and earliest known function of *BIRC3* and other IAPs is the regulation of cellular signal pathways controlling the process of apoptosis. They

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are responsible for the inhibition of the proteolytic activities of caspases — proteases required for intracellular protein degradation and execution of cell necrosis. The inhibition of apoptosis is achieved by ubiquitination of caspases 3 and 7, deactivation of pro-caspase 9 and preventing cell death induced by Fas ligand [78]. The independent ubiquitin ligase activity is attributed to the zinc finger domain [79]. The BIR motifs region participates in interaction between *BIRC3* and tumor necrosis factor (TNF) receptor-associated factors (TRAF1, TRAF2). This formed complex regulates negatively MAP3K14 serine-threonine kinase, the central activator of non-canonical NF-κB signaling. Consequently, *BIRC3* prevents from overactivation of NF-κB which might result in uncontrolled transcription [80, 81]. Interaction with TRAFs is required to ubiquitinate the inhibitor of nuclear factor kappa-B kinase (IKK2), degradation of NF-κB inhibitor alpha (IκBα) and, finally, activation of NF-κB. Taken together, the role of *BIRC3* in the regulation of NF-κB signaling is dual: stimulatory and inhibitory [63, 81, 82]. Moreover, *BIRC3* prevents NF-κB-mediated transcriptional and posttranslational modifications of MDM2 disrupting its expression and function.

Recently, it has been observed that *BIRC3* protein plays a role in modulation of inflammatory signaling and immunological processes, which confirms its multifunctional character. Mutations in *BIRC3* might be represented by a single gene disruption or combination of two of them. Most of them are deletions, frameshift disorders and nonsense substitutions, resulting in inactivation of *BIRC3* protein [63]. It is the result either of reduced transcription of the deleted gene or loss of function due to cutoff of its C-terminal zing finger domain. Truncation of this specific domain, which is characterized by ubiquitin ligase activity, excludes the *BIRC3* protein from inhibition of non-canonical NF-κB signaling [83]. Clear functional effect of the mutation in *BIRC3* gene is, therefore, permanent activation of NF-κB [63, 84].

The molecular alterations targeting *BIRC3* gene should be considered as novel important prognostic parameter in CLL. According to classification proposed by Rossi *et al.* [12], the *BIRC3* alterations were associated with high-risk disease, where the estimated 10-years survival was 29%. Furthermore, in retrospective analysis the median OS was comparable to patients with *TP53* abnormalities and reached 3 years [12]. Consequently, the *BIRC3* mutation is associated with shorter PFS and OS [12, 63, 85]. There are reports which attribute the presence of the mutation to chemorefractoriness [83, 86, 87]. In the study of Landau *et al.* [86], 24% of patients who were refractory to fludarabine-therapy harbored mutated *BIRC3* gene. *BIRC3* mutations are rarely described in patients at diagnosis of CLL accounting from 2 to 10% [85–88]. They might be detected between exons 2 and 9 [87]. Interestingly, they occur mainly within 11q22-q23 deletions (49% in the study by Del Poeta *et al.* [85]). It has been suggested that poor outcome of CLL depends not on the *BIRC3* disruption but on the concomitant del11q or *ATM* mutation [83, 84]. Certainly, the functional consequence of the mutations in *BIRC3* gene and their implications for the diagnosis in the patients with CLL should remain under scrutiny.

MYD88

Myeloid differentiation primary response 88 (MYD88) is a protein that plays an essential role in the innate and adaptive immune response and is encoded by the *MYD88* gene which is located on the short (p) arm of chromosome 3 at position 22 (3p22) [89]. MYD88 functions as a signaling adaptor protein that activates the NF-κB pathway after stimulation of toll-like receptors (TLRs) and receptors for IL-1 and IL-18 on dependent and independent signaling pathways [90]. Furthermore, MYD88 coordinates the gathering of a multi-subunit signaling complex which consists of various members of the IRAK family of serine-threonine kinases [91].

Ngo *et al.* found mutations in *MYD88* in 39% of cases of activated B cell type diffuse large B cell lymphoma (ABC-DLBCL), with a single L265P substitution accounting for 75% of the mutations [92]. The L265P mutation occurs in almost 100% of cases of Waldenström's macroglobulinemia [93], and 2–10% of cases of CLL [10, 94]. Other Toll/IL-1R like domain mutations, such as S219C, prevail in germinal center B cell type diffuse large B cell lymphoma (GCB-DLBCL) [92].

The most common mutation is a single-nucleotide change (c.794T.C) that results in a switch of leucine to proline at codon 265 (p.L265P) [94]. That predominant mutation leads to constitutive NF-κB stimulation, thus conferring a proliferation and survival advantage to the mutant cells. *MYD88* mutations reach up 2% to 5% in CLL and are strikingly enriched among patients expressing mutated *IGHV* genes (M-CLL) [88]. Baliakas *et al.* [70] studied the clinical significance of *MYD88* mutations in a collaborative multicenter series of 1039 well-annotated CLL cases. In this research *MYD88* mutations were identified in 24/1080 (2.2%) CLL patients and 92% cases implemented the hotspot p.L265P substitution. In Xia *et al.* [88] study on Chinese population with CLL, mutations in exons 3-5 of *MYD88* were detected in 23 (8%) of 295 analyzed cases. These mutations were more common

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Table 1. Detailed description of *TP53*, *NOTCH1*, *SF3B1*, *MYD88* and *BIRC3* mutations in CLL patients

Gene	Nucleotide change, % of mutation	Amino acid change	Exon (domain)	Evaluation method	References
TP53	p.R158H p.H193L p.H214R p.R249W p.G245S p.P278A p.Q317X	c.473G>A c.578A>T c.641A>G c.745A>T c.733G>A c.832C>G c.949C>T	4–9	— Sanger sequencing — NGS — dHPLC — FASAY — Arrays (Affymetrix/Roche GeneChip Arrays and p53 AmpliChip)	[96, 97]
SF3B1	K700E, 50% p.G742D, 19% K666E, 12% H662Q, 4% H662D, 4%	c.2146A>G c.2273G>A c.2044A>G c.1984C>G c.1986C>G	14–16 (HEAT)	— Sanger sequencing	[87, 88]
NOTCH1	c.7544_7545delCT, 80–95%	p.P2515fs	34 (PEST)	— ARMS PCR — Sanger sequencing	[11, 87, 88]
BIRC3	c.1673_1674del2bp c.1586A>T	p.K558fs p.Q529L	6–9 (RING, CARD)	— Sanger sequencing	[87]
MYD88	p.L265P, 3.2%	c.794T>C	5	— ARMS PCR — Sanger sequencing — NGS	[88, 92]

Abbreviations: ARMS PCR — amplification refractory mutation system; CLL — chronic lymphocytic leukemia; dHPLC — denaturing high performance liquid chromatography; FASAY — functional analysis of separated alleles in yeast; NGS — next generation sequencing.

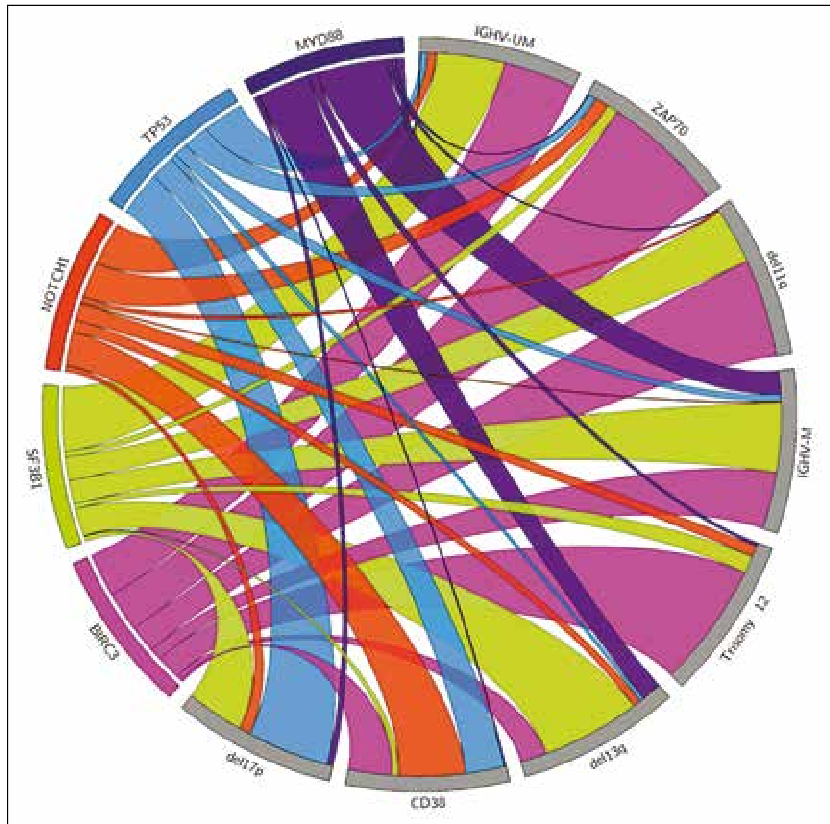

Figure 2. Association between novel gene mutations and established prognostic factors in chronic lymphocytic leukemia (CLL) patients. Graph prepared using Circos program [98]. Circos plots show the pairwise co-occurrence of gene mutations with cytogenetic status, of immunoglobulin heavy chain variable (IGHV) mutational status and expression of CD38 and ZAP-70. The length of the arc corresponds to the frequency of the mutations. The width of the ribbon corresponds to the proportion of co-occurrence with the second marker.

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Table 2. Detailed characteristic of novel genetic mutations in chronic lymphotic leukemia patients

Prognostic factors	Percentage of CLL patients expressing gene mutations				
	SF3B1	***NOTCH1***	***MYD88***	***TP53***	***BIRC3***
IGHV-M	54/202 (26.7%)[70]	2/26 (4%)[87]	19/21 (90%)[95]	5/17 (25%)[87]	5/23 (21.7%)[70]
IGHV-UM	148/202 (73.3%)[70]	24/26 (96%)[87]	2/21 (10%)[95]	12/17 (75%)[87]	18/23 (78.3%)[70]
Del 13q	78/243 (32.1%)[70]	7/26 (26%)[87]	12/18 (67%)[95]	2/17 (12%)[87]	2/23 (8.7%)[70]
Trisomy 12	15/245 (6.1%)[70]	10/26 (38%)[87]	2/17 (12%)[95]	0[87]	10/23 (43.5%)[70]
Del 11q	67/245 (27.3%)[70]	3/26 (12%)[87]	2/18 (6%)[95]	0[87]	11/23 (47.8%)[70]
Del 17p	14/246 (5.7%)[70]	3/26 (12%)[87]	1/18 (6%)[95]	14/17 (82%)[87]	0/23 (0%)[70]
ZAP-70+	7/110 (6.4%)[88]	20/26 (80%)[87]	6/106 (6%)[88]	7/17 (47%)[87]	10/14 (71.4%)[87]
CD38+	5/71 (7%)88	20/26 (77%)87	1/67 (2%)88	7/17 (44%)87	7/14 (50%)87

Superscripts denote references in the main text.

in patients with mutated *IGHV* (2 of 115 *vs.* 21 of 172; p = 0.001). In the other study Jeromin *et al.* [95] analyzed a large cohort of 1160 untreated CLL patients for novel genetic markers including *MYD88*. The mutation was found in 15/969 cases (1.5%) and it was associated with mutated *IGHV* status.

Detailed description of the most frequent novel mutations in CLL and methods of their analysis are summarized in Table 1. Association between novel gene mutations and clinico-biological features of CLL patients present Figure 2 and Table 2.

Summary

Taking into account the clinical heterogeneity in CLL patients, there has been a great need to find novel genetic markers that could improve prognostication. Precise risk profile based on new mutations might contribute to more personalized strategy of treatment and modification of therapeutic algorithms focusing on earlier intervention in patients from high-risk groups. Rossi *et al.* [12] settled that the most accurate survival prediction is achieved by integrating mutational and cytogenetic analyses. On this basis, a hierarchical model consisting of four subgroups was identified, which classifies the patients as follows: (1) high-risk, harboring *TP53* and/or *BIRC3* abnormalities (10-year survival: 29%); (2) intermediate-risk, harboring *NOTCH1* and/or *SF3B1* mutations and/or del11q22-q23 (10-year survival: 37%); (3) low-risk, harboring +12 or a normal karyotype (10-year survival: 57%); and (4) very low-risk, harboring del13q14 only, whose 10-year survival (69.3%) did not significantly differ from a general population. Meanwhile, the International Prognostic Index for CLL (CLL-IPI) from 2016, has integrated only the *IGHV* mutational status and *TP53* aberrations [14]. *TP53* constitutes the only biomarker in CLL that currently guides treatment decisions. Other novel mutations such as *NOTCH1*, *SF3B1* and *BIRC3* do not guide therapeutic choices. Nevertheless, they constitute markers of unfavorable prognosis of CLL, rapid progression and shorter OS [96].

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