TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics

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ABSTRACT

Chronic lymphocytic leukemia is associated with a highly heterogeneous disease course in terms of clinical outcomes and responses to chemoimmunotherapy. This heterogeneity is partly due to genetic aberrations identified in chronic lymphocytic leukemia cells such as mutations of *TP53* and/or deletions in chromosome 17p [del(17p)], resulting in loss of one *TP53* allele. These aberrations are associated with markedly decreased survival and predict impaired response to chemoimmunotherapy thus being among the strongest predictive markers guiding treatment decisions in chronic lymphocytic leukemia. Clinical trials demonstrate the importance of accurately testing for *TP53* aberrations [both del(17p) and *TP53* mutations] before each line of treatment to allow for appropriate treatment decisions that can optimize patients' outcomes. The current report reviews the diagnostic methods to detect *TP53* disruption better, the role of *TP53* aberrations in treatment decisions and current therapies available for patients with chronic lymphocytic leukemia carrying these abnormalities. The standardization in sequencing technologies for accurate identification of *TP53* mutations and the importance of continued evaluation of *TP53* aberrations throughout initial and subsequent lines of therapy remain unmet clinical needs as new therapeutic alternatives become available.

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Introduction

Chronic lymphocytic leukemia (CLL) is associated with a highly heterogeneous disease course, with some patients surviving for more than 10 years without needing treatment, and others experiencing rapid disease progression and poor outcomes despite effective chemoimmunotherapy.[1-3] This heterogeneity is partly explained by the diverse genetic aberrations identified in CLL patients.[4-6] In particular, deletions in chromosome 17p [del(17p)] resulting in loss of the *TP53* gene, which encodes the tumor-suppressor protein p53, are associated with a poor prognosis. Furthermore, mutations of *TP53* are also associated with poor prognosis independently of the presence of del(17p).[7] Collectively, these deletions and mutations will be referred to as *TP53* aberrations.

TP53 aberrations belong to the strongest prognostic and predictive markers guiding treatment decisions in CLL, and are associated with markedly decreased sur-

vival and impaired response to chemoimmunotherapy.[8-12] Until recently, the only effective treatments available for patients with CLL harboring *TP53* aberrations were alemtuzumab and allogeneic hematopoietic stem cell transplantation.[13-17] New small-molecule inhibitors that are efficacious in patients harboring *TP53* aberrations are now available, including the Bruton tyrosine kinase (BTK) inhibitor ibrutinib, the phosphatidylinositol 3-kinase (PI3K) inhibitor idelalisib, and the BCL2 inhibitor venetoclax.[18-26] Identifying *TP53* aberrations is therefore important for determining the most appropriate course of treatment for patients with CLL.[27]

Several diagnostic techniques are currently in routine use for the identification of *TP53* aberrations. A substantial proportion of *TP53* aberrations involve *TP53* mutations in the absence of del(17p).[12,28-31] Therefore, while del(17p) is routinely identified by fluorescence *in situ* hybridization (FISH), FISH testing alone may potentially fail to identify approximately 30-40% of patients with *TP53* aberrations, i.e those carrying only mutations in the gene.[32,33] Thus, it is critical to test for relevant *TP53* mutations, using Sanger sequencing or high-throughput sequencing technologies, in addition to FISH detection of del(17p), and both tests should be performed before each line of therapy to select appropriate treatment, as *TP53* aberrations may emerge during the disease course and after previous treatment.[27,31,34] The European Research Initiative on CLL (ERIC) has implemented a certification program (known as the *TP53* Network) for clinical laboratories performing analysis of *TP53* aberrations in order to improve the reliability of *TP53* mutation analysis and to spread knowledge on testing for *TP53* aberrations in routine clinical practice, with the final aim of optimizing treatment choices and patients' outcomes.[35]

Genetic aberrations in chronic lymphocytic leukemia

Genetic aberrations identified in CLL include genomic abnormalities and specific gene mutations.[6,36] Combinations of these aberrations, along with immunoglobulin heavy variable (IGHV) mutation status, result in biological and clinical subgroups associated with varying outcomes.[10,11,37,38] An overview of the genetic aberrations frequently found in CLL is provided in Table 1.

Chromosomal abnormalities frequently found in CLL include del(13q), trisomy 12, del(11q), and del(17p);[4] other less frequent abnormalities have also been identified such as amplifications of chromosome 2p or 8q, and deletions in chromosomes 8p and 15q.[4,36]

Using conventional karyotyping of stimulated lymphocytes, the presence of three or more chromosomal abnormalities, known as a complex karyotype, has been associated with worse disease outcomes.[39-42] Similar results have been obtained using arrays for DNA copy number alterations to detect genomic complexity.[37,43] There is a strong association of complex karyotype with *TP53* aberrations leading to genetic instability, but a complex karyotype has been demonstrated to be an independent prognostic factor for poor overall survival.[28,39,40,44,45] Chromothripsis-like patterns, defined by tens to hundreds of chromosomal

Table 1. Overview of genetic complexity in chronic lymphocytic leukemia.

Genetic aberration		Frequency in untreated patients	Time to first treatment (median, months)	PFS (median, months)	OS (median, months)	Coexistence with other genetic aberrations	References
Chromosomal abnormalities	del(17p)	4–8.5%	9	-	31–33[a]	*TP53* mutations	(4, 8, 11, 28, 56)
	del(11q)	17–18%	13	-	72–79[a]	*ATM* and/or *SF3B1*, *BIRC3* mutations	(4, 11, 28, 56)
	Trisomy 12	12–16%	33	-	97–114[a]	*NOTCH1* mutations	(4, 11, 28, 56)
	del(13q)	35–55%	92	-	113–133[a]	miRNA 15a/16-1 encoded within DLEU2 intron in 13q23	(4, 11, 28, 56)
	Other (e.g. amp[2p]; amp[8q]; del[8p]; del[15q]; and del[6q])	2–7%	-	-	-	-	(4, 11, 28, 56)
Gene mutation	*TP53*	5–12%	4–58	4–23[b]	21–90[b]	The majority of clonal mutations are associated with del(17p) Mostly associated with U-CLL	(5, 6, 8, 10, 28, 31, 36, 56, 73, 110)
	NOTCH1	10–14%	5–42	18–86[b]	15–34[b]	Mostly in U-CLL (82%) Frequently associated with trisomy 12	(6, 10, 28, 31, 36, 56)
	SF3B1	9–14%	2–86	5–43[b]	28–90[b]	Found together with *TP53* mutations in some studies, but not in others	(5, 6, 28, 31, 36)
	ATM	11–26%	Significantly reduced independently of del(11q)	8–40[b]	26–85[b]	*ATM* and del(11q) occur mostly in U-CLL	(5, 6, 28, 31, 36, 56)
	Other (e.g. *FAT1*, *MYD88*, *POT1*, and *RPS15*)		-	*RPS15*: reduced PFS	*RPS15*: reduced OS	*RPS15* can be exclusive of *TP53* mutations	(36, 52, 54, 73)

U-CLL: IGHV unmutated CLL; [a]In previously untreated patients [b]Across all lines of treatment in chemoimmunotherapy studies. CLL: chronic lymphocytic leukemia; OS: overall survival; PFS: progression-free survival; WT: wild type.

rearrangements in a localized region of the genome, have also been identified in some patients with CLL,[46-48] usually associated with *TP53* and *SETD2* mutations.[6,49]

Apart from *TP53*, the most frequent mutations associated with disease outcomes in CLL are found in the *ATM*, *BIRC3*, *NOTCH1*, and *SF3B1* genes.[6,31,50-53] These and other mutations have been associated with the development of high-risk disease, with a higher incidence of these mutations being found in fludarabine-refractory CLL than in untreated CLL.[6,52,54-56] The impacts of these mutations on outcomes in CLL are outlined in Table 1 but the clinical value of each of them remains to be established.[57]

IGHV gene status

Another important CLL feature that affects prognosis is the IGHV gene mutation status. The clinical course is generally more aggressive in patients with unmutated IGHV genes than in those with mutated IGHV genes.[58,59] *TP53* mutations may be found in both mutated and unmutated CLL, but are usually associated with unmutated CLL.[56] Immunogenetic studies have recently revealed that approximately one third of patients with CLL carry quasi-identical or stereotyped B-cell receptors (BCR) and can be grouped into subsets that share clinico-biological features and outcome.[57]

What is *TP53*?

Over 50% of human cancers carry *TP53* gene mutations,[60] and the importance of *TP53* in tumor development is highlighted by the increased incidence of cancer before the age of 30 in patients with Li-Fraumeni syndrome, which results from germline mutations in the *TP53* gene.[61]

TP53 encodes the tumor-suppressor protein p53, which has numerous cellular activities including regulation of the cell cycle and apoptosis, and promotion of DNA repair in response to cellular stress signals such as DNA damage.[60,62,63] Following DNA damage, p53 triggers either apoptosis or G1 cell-cycle arrest until the cell has completed DNA repair processes, thereby preventing replication of potentially harmful genetic abnormalities.[62]

What are the different types of *TP53* aberration and how do they affect p53 function and pathogenicity?

TP53 aberrations can arise through deletion of the *TP53* locus on chromosome 17 (17p13.1) or gene mutations including missense mutations, insertions or deletions (indels), nonsense mutations or splice-site mutations. Gene mutations are heavily concentrated in the DNA-binding domain, encoded by exons 4–8 of the *TP53* gene, but mutations can also appear in the oligomerization domain or C-terminal domain.[33,63-65] del(17p) and/or *TP53* mutations in various combinations can result in the loss of wildtype p53 function in CLL (Figure 1).[12,28,29,31,33] Six 'hotspot' codons in particular (codons 175, 245, 248, 249, 273, and 282) are affected at elevated frequency.[33,63,66] This is in line with a disease-specific *TP53* mutational profile in CLL.[66]

The most commonly found mutations in *TP53* are missense mutations in the coding region of *TP53*, which lead to an amino acid change in the p53 protein and account for approximately 75% of *TP53* mutations identified.[33,60,63] Missense mutations may result in expression of a mutated p53 protein that cannot activate the p53 tumor-suppressive transcriptional response, have dominant-negative effects over any remaining wildtype p53, and/or could gain oncogenic functions independent of wildtype p53,[5,33,60,64] illustrating their pathogenic and prognostic impact even if occurring in one copy (mono-allelic) of *TP53* with retention of a potentially functional allele.[32] In contrast, del(17p), frameshift mutations, indels, nonsense mutations, and splice-site mutations result in loss of functional p53, and although functional p53 may still be expressed in the presence of a second wildtype allele, this has not been proven to diminish the adverse prognostic impact of such abnormalities (Figure 2).[33]

Based on data obtained from Sanger sequencing, approximately 80% of patients harboring del(17p) also carry *TP53* mutations in the second allele.[8,30,67] Overall, del(17p) associated with *TP53* mutations is the most common abnormality affecting the *TP53* gene in CLL, accounting for approximately two-thirds of cases.[8,10,30,33] The

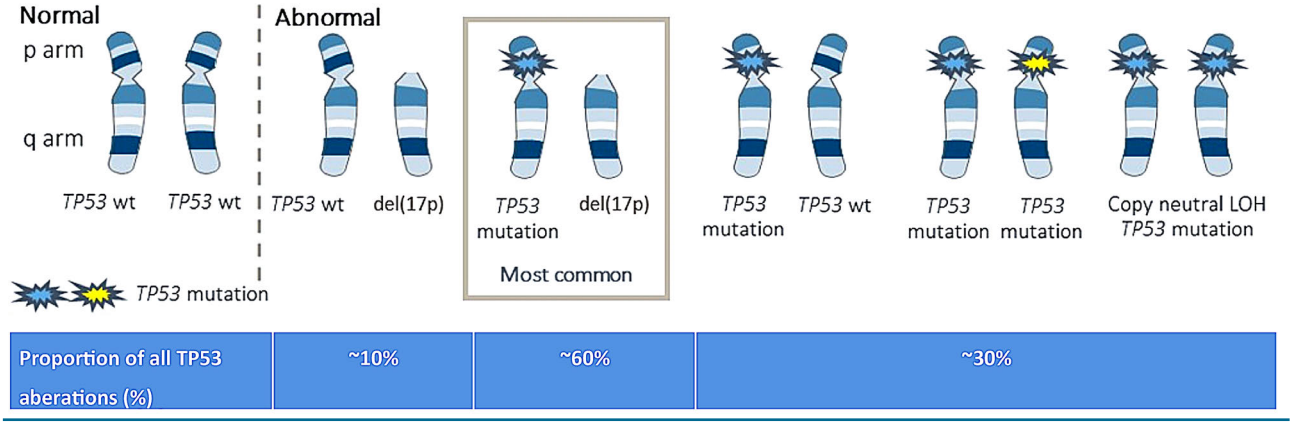

Figure 1. Loss of wildtype (wt) p53 function in chronic lymphocytic leukemia can occur as a result of del(17p) and/or *TP53* mutations.[12,28,29,31,33] The most common cause of *TP53* aberrations is the result of a combination of *TP53* mutation and del(17p), which accounts for up to two-thirds of all *TP53* aberrations.

remaining cases with *TP53* aberration carry either gene mutation(s) or sole del[17p].[28,29,31,33] A *TP53* mutation can be accompanied by a copy-number neutral loss of heterozygosity of the second *TP53* allele.[5,6,30,31]

Clonality and clonal evolution

Individual cancer samples are genetically heterogeneous and contain clonal and subclonal populations.[68,69] These populations may be in equilibrium, with the relative proportions of each subclone remaining stable, or may undergo evolution, with some subclones emerging as dominant.[50] While most untreated CLL, and a minority of treated CLL, maintain stable clonal equilibrium, treatment may shift the architecture in favor of one or more aggressive subclones.[50] This clonal evolution is a key feature of cancer progression and relapse, with tumors likely evolving through competition and interactions between genetically diverse clones (Figure 3).[5] In CLL, clonal evolution after treatment or at the time of relapse has been identified as 'the rule, not the exception'.[5,70] In a study by Landau *et al.*,[5] 47 out of 49 patients with CLL had clonal evolution at the time of relapse. Importantly, chemoimmunotherapy pressure is thought to lead to clonal evolution, most prominently for *TP53* aberrant subclones.[71]

TP53 aberrations are indeed strongly associated with clonal evolution in CLL.[44,72,73] *TP53* aberrations are less frequent at diagnosis (Table 1), while 40–50% of cases with advanced or therapy-refractory CLL harbor aberrations, highlighting the need to re-assess *TP53* status before each line of treatment because the clones could expand at relapse and/or during disease progression.[8,10,56,74] Single or multiple minor subclones harboring *TP53* mutations may be present before therapy or may develop during relapse at any stage. These *TP53*-mutant minor subclones are often present at very low frequencies that may be undetectable by Sanger sequencing and are highly likely to expand to dominant clones under the selective pressure of chemoimmunotherapy.[12,31,51]

How do we test for and report *TP53* aberrations?

Techniques frequently used for assessing *TP53* status in CLL include FISH for del(17p), Sanger sequencing, and next-generation sequencing for *TP53* mutations (Table 2).[27,35,74,75] As *TP53* mutations are associated with a poor prognosis independently of the presence of del(17p),[7] it is important to assess for *TP53* mutation status using a sequencing technique.[27,35]

Sequencing of the *TP53* gene

TP53 sequencing should cover exons 4–10 (corresponding to the DNA binding domain at codons 100–300 and the oligomerization domain at codons 323–365) at a minimum. Sequencing of the whole coding region (exons 2–11) and adjacent splice sites is highly recommended using either bidirectional Sanger sequencing or next-generation sequencing, as studies of the latter have shown that variants can also occur in exons outside the DNA binding domain although their frequency is low (Figure 2).[35]

Sanger sequencing is a widely and routinely used technique to assess *TP53* status in CLL in clinical practice. The technique provides a relatively simple, accessible sequencing approach, but is time-consuming and lacks sensitivity for detecting minor subclones harboring *TP53* mutations, with a detection limit for mutated alleles of 10–

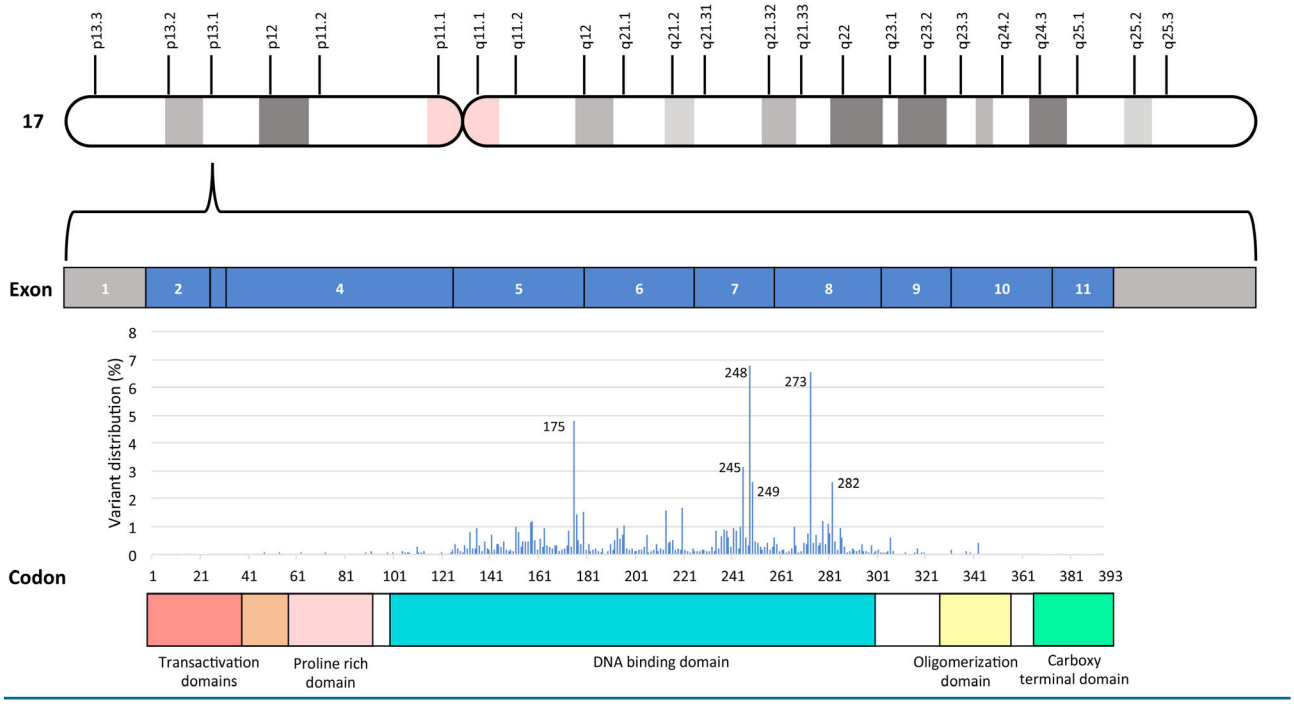

Figure 2. *TP53* gene organization and distribution of mutations by codon.[63,121,122] The *TP53* gene is located at the p13.1 locus on the short arm of chromosome 17 and comprises 11 exon sequences that encode for the p53 protein. While the majority of gene mutations cluster within the DNA-binding domain (codons 100–300, exons 4–8), gene mutations have been detected in almost every codon. Sequencing should, therefore, cover the DNA-binding domain and oligomerization domain as a minimum (exons 4–10), but sequencing of the whole coding region (exons 2–11) is highly recommended.

20%.[27,29,35,76-78] As stated earlier, minor *TP53*-mutant subclones that may be missed by Sanger sequencing also appear to carry the same unfavorable prognostic impact as clonal *TP53* mutations.[7,12,31,51,69]

Next-generation sequencing technologies include targeted next-generation sequencing, which has good correlation with Sanger sequencing in comparison studies[12,28,31,35,75,78] and detects low-frequency mutations below the threshold for Sanger sequencing.[38,79-81] The sensitivity threshold varies depending on a number of variables, including the hardware, methods used for testing and the analytical pipeline, and should be defined by each laboratory using standardized criteria or equivalent medical laboratory standards.[35,75]

Reports of *TP53* mutational analysis should always include the type of analysis and methodology used, the exons analyzed, the limit of detection, and coverage for next-generation sequencing (median and ≥99% minimum).[35] Low-level *TP53* mutations occurring in <10% of DNA that may be subject to further clonal selection are also identified by next-generation sequencing. Recent recommendations on the methodological approaches for *TP53* mutation analysis from The *TP53* Network of ERIC[35] concluded that the clinical importance of mutations in

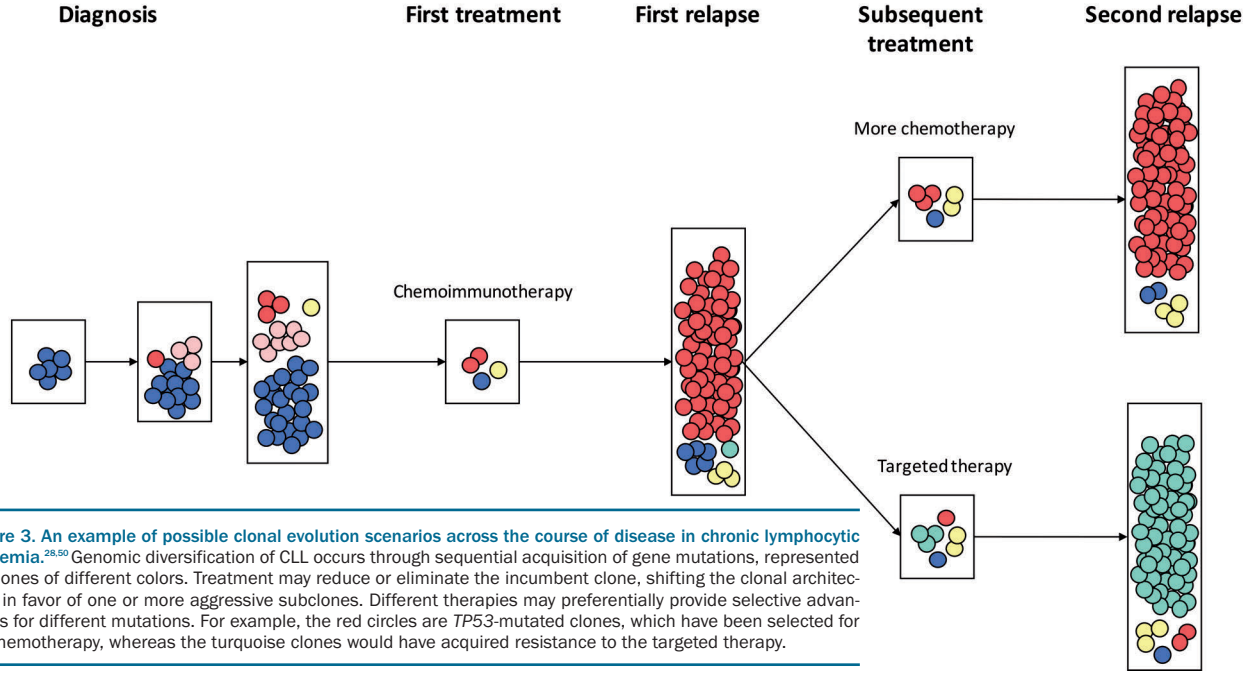

Figure 3. An example of possible clonal evolution scenarios across the course of disease in chronic lymphocytic leukemia.[28,50] Genomic diversification of CLL occurs through sequential acquisition of gene mutations, represented by clones of different colors. Treatment may reduce or eliminate the incumbent clone, shifting the clonal architecture in favor of one or more aggressive subclones. Different therapies may preferentially provide selective advantages for different mutations. For example, the red circles are *TP53*-mutated clones, which have been selected for by chemotherapy, whereas the turquoise clones would have acquired resistance to the targeted therapy.

Table 2. Comparison of methods for the detection of *TP53* aberrations.

Method	Description	Advantages	Disadvantages	References
FISH	FISH uses fluorescent DNA probes to target specific chromosomal locations within the nucleus that can be detected by fluorescence microscopy	• Rapid evaluation of fresh cells or paraffin-embedded interphase nuclei • Widely used in routine clinical practice • High specificity	• Can only detect genetic defects recognized by a specific probe • Cannot detect copy-neutral loss of heterozygosity	(111-114)
Sanger sequencing	Sanger sequencing uses selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during DNA replication, thereby creating sequences of various lengths, which are then separated by size to derive the DNA sequence	• Simple and widely available • Provides direct information on mutation type • Can produce relatively long read lengths • High specificity (~93%)	• Relatively time-consuming • Limited sensitivity (usually approximately 10–20% of mutant alleles) • Limited throughput	(27, 29, 35, 76-78)
NGS	NGS covers a range of technologies that allow high-throughput sequencing of millions or billions of DNA strands in parallel	• High and customizable sensitivity • Simultaneous analysis of large numbers of genes • No PCR with some platforms • Very high specificity (100%)	• Upfront cost of instrumentation, although some NGS sequencers are now cheaper than capillary sequencers (for Sanger) • High throughput needed for cost-effectiveness	(6, 27, 29, 31, 35, 76-78)
Genomic arrays	A technique that allows high-resolution, genome-wide screening of segmental copy number aberrations	• Provides high resolution, genome-wide information • Can detect genomic imbalances (deletions/amplifications) and copy-neutral loss of heterozygosity	• High cost • Cannot detect balanced rearrangements i.e. translocations, balanced insertions, inversions	(43, 44, 48, 115-117)

FISH: fluorescence *in situ* hybridization; NGS: next-generation sequencing; PCR: polymerase chain reaction.

<10% of alleles within the cancer cell population remains an unresolved issue and there is not enough evidence to make therapeutic decisions based on mutations undetectable by Sanger sequencing. This conclusion should be always stated when reporting variants present at a frequency of below 10%.

Outside of the context of research, determination of *TP53* status at diagnosis may not be required; initiation of first-line treatment can be deferred until patients have symptomatic active disease irrespective of *TP53* status.[82-85]

Naming, reporting, and pathogenicity of mutations

The consistent use of nomenclature in managing DNA sequence mutations is essential for concise communication of diagnostic testing and genetic risk assessment.[60] In clinical practice, aberrations are often referred to as mutations, and are referred to as such in clinical reports. However, one must note that the more accurate technical term is 'variant'. It is recommended that mutations are named according to the Human Genome Variation Society guidelines, or according to American College of Medical Genetics guidelines on mutations and mutation pathology in the case of germline mutations.[86,87] Description of mutations at the DNA level using the stable Locus Reference Genomic reference sequence is recommended to enable comparison across studies and databases.[88]

The pathogenicity of more frequent *TP53* mutations is well known, with functional analyses demonstrating that all *TP53* hot-spot mutations result in a clear loss of p53 activity.[5,60] The pathogenicity of some less frequently occurring *TP53* mutations may be less clear, particularly in the case of missense mutations which can have varied functional consequences.[5,33,60,64]

A combination of factors are considered when determining whether a mutation is likely to be pathogenic, including whether the mutation results in an amino acid change, whether the mutation is found in a conserved region of the genome or hotspot region, and whether there is a predicted functional effect of the amino acid splicing change on the protein or post-translational modification.[60] Pathogenicity assessments should be performed by experienced diagnosticians, follow standardized procedures, and be documented. *TP53* locus-specific databases are available and are important tools for analyzing and assessing the pathogenicity of *TP53* mutations. These are the IARC *TP53* database (http://p53.iarc.fr/), the *TP53* website (http://p53.fr/), and the Seshat online software (http://p53.fr/tp53-database/seshat). The Seshat online software, for example, provides a quality check of the mutation nomenclature, generates a description of the mutation, and assesses the pathogenicity of each mutation with the use of specific algorithms. Structural and functional information for each mutation is also produced.[35,89]

Clinical implications of *TP53* aberrations

Patients with del(17p) and/or *TP53* mutations usually respond poorly to the standard first-line chemoimmunotherapy, and have an aggressive disease course.[8-12] In the CLL8 study comparing first-line treatment with fludarabine plus cyclophosphamide or fludarabine plus cyclophosphamide with rituximab, *TP53* aberrations were found to be the strongest prognostic markers in multivariable analyses and were associated with markedly reduced progression-free survival and overall survival (Figure 4).[10] Both in front-line and relapsed/refractory settings, treatment with bendamustine plus rituximab was also shown to be associated with low response rates and poor survival outcomes in patients with CLL harboring *TP53* aberrations.[90] Consequently, chemoimmunotherapy is no longer considered standard therapy for patients with *TP53* aberrations. Until recently, the anti-CD52 antibody alemtuzumab was considered to be the only effective agent available for patients with *TP53* aberrations, despite an

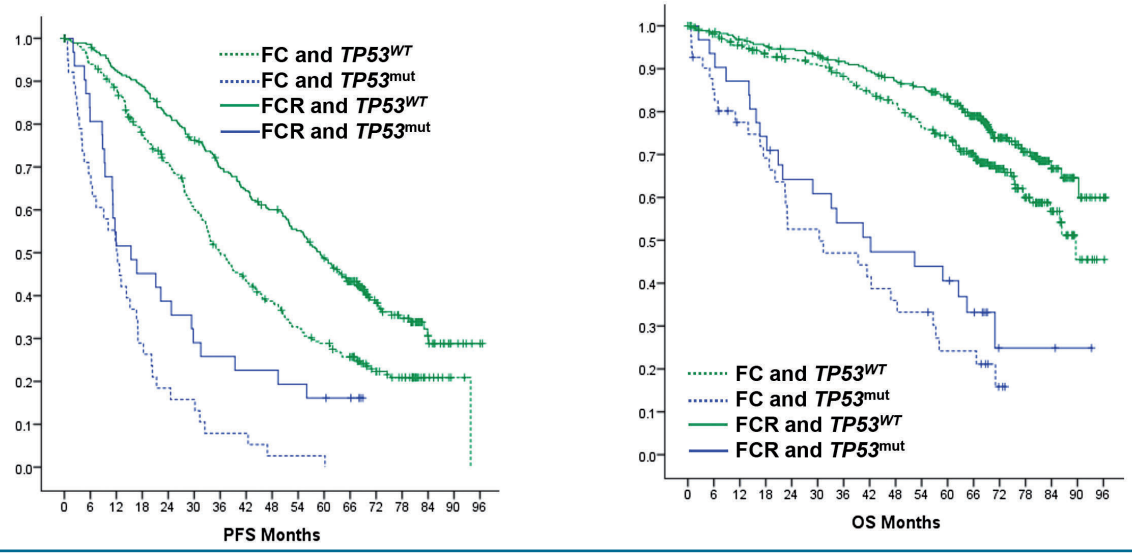

Figure 4. Progression-free and overall survival according to *TP53* status in the CLL8 study.[10] Re-published with permission from The American Society of Hematology, from: Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. Stilgenbauer S *et al.* Blood. 2014;123(21):3247-3254; permission conveyed through Copyright Clearance Center, Inc. FC: fludarabine plus cyclophosphamide; FCR: fludarabine plus cyclophosphamide plus rituximab; mut: mutated; OS: overall survival; PFS: progression-free survival; WT: wild-type.

Table 3. Overview of clinical evidence from phase 2/3 trials for novel treatments in patients with *TP53* aberrations.

Study/treatment Sponsors	Population	*TP53* aberrations at baseline	Overall response in del(17p)/TP53 mutated population	PFS in del(17p)/ *TP53* mutated population	OS in del(17p)/ *TP53* mutated population	Safety (experimental arm, overall population)	Reference
RESONATE-17: A phase 2, open-label, multicenter study of ibrutinib in patients with R/R CLL/SLL and del(17)p Ibrutinib 420 mg OD NCT01744691 Pharmacyclics LLC. Janssen Research & Development, LLC	Adult patients with previously treated del(17p) CLL or SLL (n=144) Median age (range): 64 (57-72) ECOG score: 0: 49 (34%) ≥1: 95 (66%) Median prior regimens (IQR): 2 (1–3)	del(17p) 144/144 (100%) *TP53* mutations 107/116 (92%)	ORR in del(17p) patients was 64% by independent review and 83% by investigator assessment (prespecified primary analysis, median 11.5 months follow-up)	Median PFS (investigator-assessed) not reached at a median follow-up of 11.5 months (prespecified primary analysis)	Median OS not reached at 11.5 months (prespecified primary analysis)	Grade 3–5 AE occurring in >5% of patients: Neutropenia (18%) Pneumonia (13%) Hypertension (13%) Anemia (10%) Thrombocytopenia (8%) Atrial fibrillation (6%) (24-month extended analysis)	(21)
RESONATE: a phase 3, open-label, multicenter study of ibrutinib *versus* ofatumumab in patients with previously treated CLL/SLL Ibrutinib 420 mg OD *versus* ofatumumab NCT01578707 Pharmacyclics LLC. Janssen Research & Development, LLC	Adult patients with R/R CLL/SLL (n=391) Ibrutinib arm. Median age (range): 67 (30–86) ECOG score: 0: 79 (41%) 1: 116 (59%) Median prior regimens: 3 (1–12) Ofatumumab arm. Median age (range): 67 (37–88) ECOG score: 0: 80 (41%) 1:116 (59%) Median prior regimens: 2 (1–13)	del(17p) 127/391 (32%)	ORR in del(17p) patients treated with ibrutinib: 89% ORR in del(17p) patients treated with ofatumumab: 20% (median follow-up 19 months)	Median PFS in del(17p) and/or *TP53* patients not reached at 19 months follow-up in patients treated with ibrutinib Median PFS in del(17p) and/or *TP53* patients 5.8 months in patients treated with ofatumumab Patients with both del17p and *TP53* mutation (n=38) had worse PFS compared with patients with neither of these abnormalities (n=68) (*P*=0.0381) at a median follow-up of 19 months	Median OS in del(17p) or *TP53* patients not reached at 19 months follow-up in patients treated with ibrutinib Median OS not reported in del(17p) or *TP53* patients treated with ofatumumab	Grade 3–5 AE occurred in 56% of patients treated with idelalisib + rituximab and 48% treated with placebo + rituximab Grade 3–5 AE occurred in >5% of patients: Idelalisib + rituximab Neutropenia (34%) Thrombocytopenia (10%) Placebo + rituximab Neutropenia (22%) Thrombocytopenia (16%) Anemia (14%) (overall study population)	(18, 99)
Study 101-08: a phase 2 study of idelalisib plus rituximab in elderly patients with untreated CLL or SLL Idelalisib 150 mg BD plus rituximab NCT01203930 Gilead Sciences	Older patients (≥65 years) with previously untreated CLL or SLL (n=64) Median age (range): 71 (65–90) ECOG score/Karnofsky status: not reported Median prior regimens: 0	del(17p) only: 2/64 (3.1%) *TP53* mutation only: 3/63 (4.7%) Either del(17p) or *TP53* mutation: 9/64 (14.1%) Both del(17p) and *TP53* mutation: 4/64 (6.3%)	ORR in either del(17p) or *TP53* mutation: 100%	Median PFS in del(17p) and/or *TP53* patients not reached after a median 22.4 months on treatment	Median OS in del(17p) and/or *TP53* patients not reached after a median of 22.4 months on treatment	Grade 3–5 AE occurred in 89.1% of patients. Grade 3–5 AE occurred in >5% of patients: Diarrhea and/or colitis (42%) Pneumonia (19%) (overall study population)	(22)
Study 116: a randomized, double-blind, placebo-controlled study of idelalisib in combination with rituximab for previously treated CLL Idelalisib 150 mg BD plus rituximab *versus* placebo plus rituximab NCT01539512 Gilead Sciences	Adult patients with R/R CLL not eligible for cytotoxic agents (n=220); PD within 24 months of last treatment Idelalisib + rituximab Median age (range): 71 (48–90) ECOG score/Karnofsky status: not reported Median prior regimens: 3 (1–12) Placebo + rituximab arm. Median age (range): 71 (47–92) ECOG score/Karnofsky status: not reported Median prior regimens: 3 (1–9)	del(17p) and/or *TP53* mutations Idelalisib + rituximab 46/110 (42%) Rituximab: 50/110 (45%)	ORR in del(17p) and/or *TP53* patients treated with Idelalisib plus rituximab: 77% ORR in del(17p) and/or *TP53* patients treated with rituximab: 15% (second interim analysis, median exposure 5 months with idelalisib, 4 months with rituximab)	Median PFS in del(17p) and/or *TP53* patients treated with idelalisib plus rituximab: not reached Median PFS in del(17p) and/or *TP53* patients treated with rituximab: 4.0 months (second interim analysis)	Not reported in del(17p) and/or *TP53* patients	Grade 3–5 AE occurred in 56% of patients treated with idelalisib + R and 48% treated with placebo + rituximab Grade 3–5 AE occurred in >5% of patients: Idelalisib + rituximab arm: Neutropenia (34%) Thrombocytopenia (10%) Placebo + rituximab arm: Neutropenia (22%) Thrombocytopenia (16%) Anemia (14%) (overall study population)	(19, 23)

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Study/treatment Sponsors	Population	*TP53* aberrations at baseline	Overall response in del(17p)/*TP53* mutated population	PFS in del(17p)/ *TP53* mutated population	OS in del(17p)/ *TP53* mutated population	Safety (experimental arm, overall population)	Reference
Study 115: a randomized, double-blind and placebo-controlled study of idelalisib in combination with bendamustine and rituximab (BR) for previously treated CLL Idelalisib 150 mg BD plus BR *versus* BR NCT01569295 Gilead Sciences	Adult patients with R/R CLL (n=416); PD within 36 months of last treatment Idelalisib + BR Median age (range): 62 (56–69) ECOG score/ Karnofsky status: not reported Median prior regimens: 2 (1–4) Placebo plus BR Median age (range): 64 (56–70) ECOG score/ Karnofsky status: not reported Median prior regimens: 2 (1–4)	del(17p) and/or *TP53* mutations Idelalisib + BR: 69/207 (33%) BR: 68/209 (33%)	ORR in del(17p) patients treated with idelalisib + BR: 22/38 (58%) ORR in del(17p) patients treated with BR: (9/40) 23%	Median PFS in del(17p) and/or *TP53* patients treated with idelalisib + BR: 11.3 months Median PFS in del(17p) and/or *TP53* patients treated with BR: 8.3 months	Median OS in del(17p) and/or *TP53* patients treated with idelalisib + BR: not reached at a median follow-up of 14 months Median OS in del(17p) and/or *TP53* patients treated with BR: 20.3 months	Grade 3–5 AE occurring in ≥5% of patients: Idelalisib + BR: Neutropenia (60%) Febrile neutropenia (23%) Placebo + BR: Neutropenia (47%) Thrombocytopenia (13%) (overall study population)	(118)
Study 119: a phase 3, randomized, controlled study evaluating the efficacy and safety of idelalisib (GS-1101) in combination with ofatumumab for previously treated CLL Idelalisib 150 mg BD + ofatumumab *versus* ofatumumab alone NCT01659021 Gilead Sciences	Adult patients with R/R CLL (n=261); PD within 24 months of last treatment Idelalisib plus ofatumumab Median age (range): 68 (61–74) Karnofsky status: 80 (80–90) Median prior regimens: 3 (2–4) Ofatumumab alone Median age (range): 67 (62–74) Karnofsky status: 80 (80–90) Median prior regimens: 3 (2–5)	del(17p) and/or *TP53* mutations Idelalisib plus ofatumumab: 70/174 (40%) Ofatumumab: 33/87 (38%)	ORR in del(17p) and/or *TP53* patients treated with idelalisib plus ofatumumab: not reported ORR in del(17p) and/or TP53 patients treated with ofatumumab: not reported	Median PFS in del(17p) and/or *TP53* patients treated with idelalisib plus ofatumumab: 15.5 months Median PFS in del(17p) and/or *TP53* patients treated with ofatumumab: 5.8 months	Median OS in del(17p) and/or *TP53* patients treated with idelalisib + ofatumumab: 25.8 months Median OS in del(17p) and/or *TP53* patients treated with ofatumumab: 19.3 months	Grade 3–5 TEAE occurring in ≥5% of patients treated with idelalisib plus ofatumumab: Neutropenia (34%) Diarrhea (20%) Pneumonia (16%) Anemia (14%) Febrile neutropenia (12%) Thrombocytopenia (11%) Hypokalemia (8%) Pyrexia (7%) Dyspnea (6%) Hypertension (5%) Dehydration (5%) Fatigue (5%) Grade 3–5 TEAE occurring in ≥5% of patients treated with ofatumumab: Neutropenia (16%) Pneumonia (8%) Thrombocytopenia (7%) Anemia (6%) Fatigue (5%) (overall study population)	(20, 96)
A phase 2 open-label study of the efficacy of ABT-199 (GDC-0199) in subjects with R/R or previously untreated CLL harboring the 17p deletion Venetoclax 400 mg OD NCT01889186 AbbVie Genentech, Inc.	Adult patients with R/R CLL with del(17p) (n=107) Median age (range): 67 (37–85) ECOG score n (%): 0: 42 (39%) 1: 56 (52%) 2: 9 (8%) Median prior regimens (IQR): 2 (1–4)	del(17p) 100% *TP53* mutated 60/107 (72%)	ORR in del(17p) patients: 79.4% (independent review committee assessment)	Median PFS in del(17p) patients: not reached at a median follow-up of 12.1 months	Median OS in del(17p) patients: not reached at median follow-up of 12.1 months	Grade 3–5 AE in del(17p) patients occurring in 76% of patients Grade 3–5 AE occurring in ≥5% of patients: Neutropenia (40%) Anemia (18%) Thrombocytopenia (15%) Autoimmune hemolytic anemia (7%) Febrile neutropenia (5%) Pneumonia (5%) Immune thrombocytopenic purpura(5%) Tumor lysis syndrome (5%) Leukopenia (5%)	(24, 119)

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Study/treatment Sponsors	Population	*TP53* aberrations at baseline	Overall response in del(17p)/*TP53* mutated population	PFS in del(17p)/ *TP53* mutated population	OS in del(17p)/ *TP53* mutated population	Safety (experimental arm, overall population)	Reference
MURANO: a randomized, open-label, phase 3 trial evaluating venetoclax plus rituximab *versus* bendamustine plus rituximab in R/R CLL NCT02005471 AbbVie Genentech, Inc.	Adult patients with R/R CLL (n=389): Venetoclax plus rituximab (n=194) Median age (range): 64.5 (28–83) years ECOG score n (%) 0: 111 (57.2%) 1: 82 (42.3%) 2: 1 (0.5%) Number of prior therapies n (%): 1: 111 (57.2%) 2: 57 (29.4%) 3: 22 (11.3%) >3: 4 (2.1%) BR (n=195) Median age (range): 66 (22–85) years ECOG score n (%) 0: 108 (55.7%) 1: 84 (43.3%) 2: 2 (1.0%) Number of prior therapies n (%): 1: 117 (60.0%) 2: 43 (22.1%) 3: 34 (17.4%) >3: 1 (0.5%)	del(17p) only Venetoclax plus rituximab: 24 (14%) BR 18 (11.4%) *TP53* mutation only Venetoclax plus rituximab: 19 (11.1%) BR 23 (14.6%) del(17p) and *TP53* mutated Venetoclax plus rituximab: 22 (12.9%) BR 22 (13.9%)	Not reported	del(17p) Median PFS not reached with venetoclax plus rituximab at 2-year follow-up Median PFS 15.4 months with BR *TP53* mutation Median PFS not reached with venetoclax plus rituximab at 2-year follow-up Median PFS 12.9 months with BR	Not reported	Grade 3–4 AE in patients receiving venetoclax plus rituximab: 82.0% Grade 3–4 AE in patients receiving BR 70.2%	(120)

AE: adverse events; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BD: twice daily; BR: bendamustine plus rituximab; CLL: chronic lymphocytic leukemia; ECOG: Eastern Cooperative Oncology Group; HR: hazard ratio; IQR: interquartile range; OD: once daily; ORR: overall response rate; OS: overall survival; PD: progressive disease; PFS: progression-free survival; R/R: relapsed/refractory; SLL, small lymphocytic leukemia; TEAE: treatment emergent adverse events.

overall limited efficacy and a high risk of opportunistic infectious complications.[16] Allogeneic hematopoietic stem cell transplantation is a potentially curative therapeutic option for patients with *TP53* aberrations, but is only feasible for highly selected younger, physically fit patients and those who have obtained a good therapeutic response.[13,15,17]

Therapies with p53-independent mechanisms of action

Recent developments in the treatment options for patients with CLL harboring *TP53* aberrations include small-molecule kinase inhibitors that target the BCR pathway (ibrutinib and idelalisib)[18-22,26] and the anti-apoptotic protein BCL2 (venetoclax).[24,91-93] Ibrutinib is an inhibitor of Bruton tyrosine kinase,[94,95] whereas idelalisib is an inhibitor of the PI3K p110δ isoform,[19,96] both of which are involved in mediating intracellular signaling from several receptors including the BCR. Venetoclax is a BH3-mimetic inhibitor of BCL2, an anti-apoptotic protein with constitutively elevated expression in CLL.[92,97] An overview of the clinical evidence from phase 2/3 trials for these treatments in patients with CLL harboring *TP53* aberrations is shown in Table 3. The studies were carried out in varying patient populations, but overall, these novel therapies produced responses and favorable survival times in a high proportion of patients harboring *TP53* aberrations and represent a significant advance for this high-risk population compared to chemoimmunotherapy regimes.[18-26] It is important to note that such therapies achieved similar responses in patients with relapsed or refractory CLL, irrespective of risk factors that are associated with poorer responses to chemoimmunotherapy.[92,98-100]

Given the improvements seen with these therapies, accelerated approval programs have made the therapies available for CLL treatment in the clinic. Currently in Europe, ibrutinib is licensed as monotherapy for first-line treatment and for relapsed/refractory patients with CLL, or in combination with bendamustine plus rituximab in the relapsed/refractory setting.[94] Idelalisib is indicated in combination with an anti-CD20 monoclonal antibody (rituximab or ofatumumab) for relapsed/refractory CLL therapy, and as first-line therapy in patients with del(17p)/*TP53* mutations not suitable for other therapies.[96] Venetoclax is currently licensed in Europe for patients with relapsed/refractory CLL in whom both chemoimmunotherapy and a BCR inhibitor have failed, or for patients with del(17p) or a *TP53* mutation who are not suitable for BCR inhibitors or in whom BCR inhibitor treatment has failed.[97] Although limited data are available for all these agents in the treatment-naïve setting, the approvals as first-line therapy reflect the high level of unmet need for patients with *TP53* aberrations. Moreover, the development of these novel therapies has produced a change in therapeutic goals. In particular, frail patients with progressive CLL can now be treated with the aim of effectively controlling the disease, whereas previously palliative care would have been the only option.[19]

It has also become evident that patients may develop resistance to these targeted therapies. For example, mutations in the *BTK* and *PLCG2* genes have been associated with resistance to ibrutinib, while upregulation of anti-apoptotic *BCL2* family members has been associated with resistance to venetoclax.[101-104] Mechanisms of resistance to idelalisib have not yet been fully characterized; because idelalisib inhibits the PI3K p110δ isoform, resistance may theoretically involve upregulation of other PI3K isoforms.[105] However, in a whole-exome sequencing analysis of 13 patients with CLL who had progressed while on idelalisib plus anti-CD20 treatment in three phase 3 trials, none of the patients had recurrent progression-associated mutations in the PI3K pathway or other related pathways.[71]

The optimal sequencing of these targeted therapies is currently unknown, but observational studies suggest that patients who discontinue a BCR pathway inhibitor due to toxicity may benefit from an alternative BCR pathway inhibitor. Conversely, those patients who progress under BCR inhibitor therapy fare better with venetoclax than an alternative BCR inhibitor.[106,107] Following progression on one or more therapies, allogeneic hematopoietic stem cell transplantation also remains a valid option, especially because these novel therapies may render patients more fit for this procedure.

It is important to note that, until recently, treatment guidelines for patients with *TP53* aberrations were based on retrospective analyses and subgroup analyses. Patients with *TP53* aberrations are still defined as a high-risk group, despite the development of these newer therapies, but their outcome has greatly improved in recent years. More long-term data and dedicated trials of these new therapies in this population are still needed to understand the long-term prognosis. Nevertheless, these therapies (as monotherapy or in combination) have become the mainstay of treatment in patients with CLL harboring *TP53* mutations or del(17p), as well as in relapsed or refractory CLL and have led to recent updates in treatment guidelines.[34,35,84,85,108,109]

Future considerations

As evidence from clinical trials demonstrates, it is important to test accurately for *TP53* aberrations (both del[17p] and *TP53* mutations) before each line of treatment, thus allowing for appropriate treatment decisions to optimize patients' outcomes. Accurate identification of *TP53* mutations demands standardization in sequencing technologies and pathogenicity assessments. Independent evaluation within prospective clinical trials is still required to determine the clinical impact of minor subclonal mutations (<10%). Similarly, given the continuing evolution of therapeutic agents in CLL, it is important to continue to evaluate *TP53* aberrations as new therapeutic alternatives become available. While allogeneic hematopoietic stem cell transplantation remains the only curative treatment option for patients with CLL harboring *TP53* aberrations, the recent approvals of ibrutinib, idelalisib, and venetoclax have provided significantly improved outcomes for this high-risk group of patients.

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