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

Elias Campo,1 Florence Cymbalista,2 Paolo Ghia,3 Ulrich Jäger,4 Sarka Pospisilova,5 Richard Rosenquist,6 Anna Schuh7 and Stephan Stilgenbauer8

1Hospital Clinic of Barcelona, University of Barcelona, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS), Barcelona, and CIBERONC, Spain; 2Hôpital Avicenne, AP-HP, UMR INSERMU978/Paris 13 University, Bobigny, France; 3Università Vita-Salute San Raffaele and IRCCS Ospedale San Raffaele, Milan, Italy; 4Medical University of Vienna, Austria; 5Center of Molecular Medicine, Central European Institute of Technology, Masaryk University, Brno, Czech Republic; 6Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; 7University of Oxford, UK and 8Internal Medicine III, Ulm University, Germany and Innere Medizin I, Universitätsklinikum des Saarlandes, Homburg, Germany

All authors contributed equally to this work

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.

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.1,2 This heterogeneity is partly explained by the diverse genetic aberrations identified in CLL patients.3 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).4 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. \(^{13-15}\) 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. \(^{16-20}\) 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). \(^{22-28}\) 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. \(^{25,28}\) 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. \(^{27,27,31}\) 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. \(^{33}\)

### Genetic aberrations in chronic lymphocytic leukemia

Genetic aberrations identified in CLL include genomic abnormalities and specific gene mutations. \(^{3,5,44}\) Combinations of these aberrations, along with immunoglobulin heavy variable (IGHV) mutation status, result in biological and clinical subgroups associated with varying outcomes. \(^{10,11,57,58}\) An overview of the genetic aberrations frequently found in CLL is provided in Table 1.

Chromosomal aberrations frequently found in CLL include del(13q), trisomy 12, del(11q), and del(17p); \(^{22,23}\) other less frequent abnormalities have also been identified such as amplifications of chromosome 2p or 8q, and deletions in chromosomes 8p and 15q. \(^{3,5,6}\)

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. \(^{36,41}\) Similar results have been obtained using arrays for DNA copy number alterations to detect genomic complexity. \(^{37,42}\) 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. \(^{38-40,44,45}\) 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               | TP53 mutations                             | (4, 8, 11, 28, 56) |
| del(11q)            | 17–18%                          | 13                                    | -                    | 72–79               | ATM and/or SF3B1, BIRC2 mutations           | (4, 11, 28, 56) |
| Trisomy 12          | 12–16%                          | 33                                    | -                    | 97–114              | NOTCH1 mutations                           | (4, 11, 28, 56) |
| del(13q)            | 35–55%                          | 92                                    | -                    | 113–133             | miRNA 15a/16-1 encoded within DLEU2         | (4, 11, 28, 56) |
| Other (e.g. amp[2p]; amp[8q]; del[15q]; del[16q]) | 2–7%                            | -                                     | -                    | -                   |                                            | (4, 11, 28, 56) |
| **Gene mutation**   |                                 |                                        |                      |                     |                                            |            |
| TP53               | 5–12%                           | 4–58                                  | 4–23\(^{c}\)         | 21–80              | The majority of clonal mutations are associated with del(17p) | (5, 6, 8, 10, 28, 31, 36, 56, 73, 110) |
| NOTCH1             | 10–14%                          | 5–42                                  | 18–86\(^{d}\)        | 15–34\(^{d}\)      | Mostly in U-CLL (82%)                       | (6, 10, 28, 31, 36, 56) |
| SF3B1              | 9–14%                           | 2–86                                  | 5–43\(^{d}\)         | 28–90              | Found together with TP53 mutations in some studies, but not in others | (5, 6, 28, 31, 36) |
| ATM                | 11–28%                          | Significantly reduced independently of del(11q) | 8–40\(^{d}\)          | 26–85              | ATM and del(11q) occur mostly in U-CLL     | (5, 6, 28, 31, 36) |
| Other (e.g. FAPI, MYD88, POTI, and RPS15) | -                               | -                                     | -                    | -                   | TP53 can be exclusive of TP53 mutations     | (36, 52, 54, 73) |

UCCL, IGHV unmethylated CLL; \(^{*}\) in previously untreated patients; \(^{\dagger}\) 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, usually associated with TP53 and SETD2 mutations.

Apart from TP53, the most frequent mutations associated with disease outcomes in CLL are found in the ATM, BIRC3, NOTCH1, and SF3B1 genes. 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. 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.

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. TP53 mutations may be found in both mutated and unmutated CLL, but are usually associated with unmutated CLL. 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.

What is TP53?

Over 50% of human cancers carry TP53 gene mutations, 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.

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. 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.

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. del(17p) and/or TP53 mutations in various combinations can result in the loss of wildtype p53 function in CLL (Figure 1).

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. 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, illustrating their pathogenic and prognostic impact even if occurring in one copy (mono-allelic) of TP53 with retention of a potentially functional allele. 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).

Based on data obtained from Sanger sequencing, approximately 80% of patients harboring del(17p) also carry TP53 mutations in the second allele. 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. The
remaining cases with TP53 aberration carry either gene mutation(s) or sole del(17p). A TP53 mutation can be accompanied by a copy-number neutral loss of heterozygosity of the second TP53 allele.

Clonality and clonal evolution

Individual cancer samples are genetically heterogeneous and contain clonal and subclonal populations. 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. 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. 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). In CLL, clonal evolution after treatment or at the time of relapse has been identified as ‘the rule, not the exception’. In a study by Landau et al., 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.

TP53 aberrations are indeed strongly associated with clonal evolution in CLL. 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 reassess TP53 status before each line of treatment because the clones could expand at relapse and/or during disease progression.

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). As TP53 mutations are associated with a poor prognosis independently of the presence of del(17p), it is important to assess for TP53 mutation status using a sequencing technique.

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). 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–
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.35,79-81 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.35,79

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 ERIC35 concluded that the clinical importance of mutations in

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**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                                   | • Can only detect genetic defects recognized by a specific probe                | (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 (~95%) | • Relatively time-consuming • Limited sensitivity (usually approximately 10–20% of mutant alleles) • Limited throughput | (27, 28, 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.80 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.89,90

Clinical implications of TP53 aberrations

Patients with del(17p) and/or TP53 mutations usually respond poorly to the standard first-line chemioimmunotherapy, 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, chemioimmunotherapy 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(17p) | Ibrutinib 420 mg OD | del(17p) 144/144 (100%) | ORR in del(17p) patients treated with ibrutinib: 89% | Median PFS in del(17p)/TP53 mutated population | Median OS not reached at 11.5 months (prespecified primary analysis) | Grade 3–5 AE occurring in >5% of patients: Neutropenia (38%) Pneumonia (13%) Hypertension (13%) Anemia (10%) Thrombocytopenia (8%) Atrial fibrillation (6%) (24-month extended analysis) | (21) |
| NCT01744691 | | | | | | | |
| Pharmacyslics LLC, Janssen Research & Development, LLC | Median prior regimens (RQR): 2 (1–3) | | | | | | |
| RESONATE: a phase 3, open-label, multicenter study of ibrutinib versus ofatumumab in patients with previously treated CLL/SLL | Ibrutinib arm. | del(17p) 127/391 (32%) | ORR in del(17p) patients treated with ofatumumab: 20% (median follow-up 19 months) | Median PFS in del(17p)/TP53 mutated population | Median OS not reached at 11.5 months (prespecified primary analysis) | Grade 3–5 AE occurring in 56% of patients treated with ibrutinib and 48% treated with placebo + rituximab Grade 3–5 AE occurred in >5% of patients: Ibrutinib + rituximab Neutropenia (34%) Thrombocytopenia (10%) Placebo + rituximab Neutropenia (22%) Thrombocytopenia (16%) Anemia (14%) (overall study population) | (18, 99) |
| NCT01757070 | Median prior regimens: 3 (1–12) Ofatumumab arm. | | | | | | |
| Pharmacyslics LLC, Janssen Research & Development, LLC | Median age (range): 67 (38–88) ECOG score: 0.80 (41%) 1: 116 (59%) Median prior regimens: 2 (1–13) | | | | | | |
| Study 101-08: a phase 2 study of idelalisib plus rituximab in elderly patients with untreated CLL or SLL | Ibrutinib + ofatumumab arm. | del(17p) only: 2/64 (3.1%) | ORR in either del(17p) or TP53 mutation: 100% | Median PFS in del(17p) and/or TP53 patients treated with idelalisib and/or TP53 mutation not reached at a median of 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) |
| NCT0120930 | Median prior regimens: 0 | Both del(17p) and TP53 mutation: 4/64 (6.3%) | | | | | |
| Gilead Sciences | Older patients (≥65 years) with previously untreated CLL or SLL (n=64) | del(17p) only: 2/64 (3.1%) | ORR in either del(17p) or TP53 mutation: 100% | Median PFS in del(17p) and/or TP53 patients treated with idelalisib and/or TP53 mutation not reached at a median of 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 HR: a randomized, double-blind, placebo-controlled study of idelalisib in combination with rituximab for previously treated CLL | Ibrutinib + ofatumumab arm. | del(17p) and/or TP53 mutations | ORR in del(17p) and/or TP53 patients treated with idelalisib plus rituximab: 77% | Median PFS in del(17p) and/or TP53 patients treated with idelalisib plus rituximab not reached | Not reported in del(17p) and/or TP53 patients | Grade 3–5 AE occurred in 50% of patients treated with idelalisib + R and 48% treated with placebo + rituximab Grade 3–5 AE occurred in >5% of patients: Ibrutinib + rituximab Neutropenia (34%) Thrombocytopenia (10%) Placebo + rituximab Neutropenia (22%) Thrombocytopenia (16%) Anemia (14%) (overall study population) | (19, 23) |
| NCT0150512 | Median prior regimens: 3 (1–12) Placebo + rituximab arm. | Median age (range): 71 (47–92) ECOG score/Karnofsky status: not reported | | | | | |
| Gilead Sciences | | | | | | | |

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### Study 115: a randomized, double-blind and placebo-controlled study of idelalisib in combination with bendamustine and rituximab (BR) for previously treated CLL

| 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 |
|-----------------|----------|------------|-----------------------------|--------------------------------------------------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------|
| ofatumumab:     | Genentech, Inc. | Adult patients with R/R CLL (n=261); PD within 24 months of last treatment | del(17p) and/or TP53 mutations | ORR in del(17p) and/or TP53 patients treated with idelalisib + BR: 22/38 (58%) | Median PFS in del(17p) and/or TP53 patients treated with idelalisib + BR: 11.3 months | Median OS in del(17p) and/or TP53 patients treated with idelalisib + BR: not reached at a median follow-up of 14 months | Grade 3–5 AE occurring in ≥5% of patients: Neutropenia (60%) | (20, 96) |
|                 | Gilead Sciences | Placebo plus BR | 65% (61–74) ECOG score/ Karnofsky status: not reported | Median prior | 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: Neutropenia (16%) | (118) |
| NCT01569295     |          | Median age (range): 65 (62–74) | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |
| Plus BR versus BR |          | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |

### Study 116: a phase 3, randomized, controlled study evaluating the efficacy and safety of idelalisib (GS-1101) in combination with ofatumumab for previously treated CLL

| 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 |
|-----------------|----------|------------|-----------------------------|--------------------------------------------------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------|
| ofatumumab:     | Genentech, Inc. | Adult patients with R/R CLL (n=261); PD within 24 months of last treatment | del(17p) and/or TP53 mutations | ORR in del(17p) and/or TP53 patients treated with idelalisib + ofatumumab: 15.5 months | Median PFS 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 idelalisib + ofatumumab: 19.3 months | Grade 3–5 TAE occurring in ≥5% of patients: Neutropenia (34%) | (20, 96) |
|                 | Gilead Sciences | Placebo plus BR | 65% (61–74) ECOG score/ Karnofsky status: not reported | Median prior | Median prior | Median prior | Median prior | Median prior |
| NCT01659021     |          | Median age (range): 65 (62–74) | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |
| Plus BR versus BR |          | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |

### 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

| 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 |
|-----------------|----------|------------|-----------------------------|--------------------------------------------------------|------------------------------------------|------------------------------------------|-----------------------------------------------|-----------|
| ofatumumab:     | AbbVie | Adult patients with R/R CLL with del(17p) (n=107) | del(17p) and/or TP53 mutations | 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 | (24, 119) |
|                 | Genentech, Inc. | Median age (range): 67 (37–85) | Median prior | Median prior (IQR): 2 (1–4) | Median prior | Median prior | Median prior | Median prior |
|                 | Venetoclax 400 mg OD | Median age (range): 67 (37–85) | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |
| NCT01889186     |          | Median age (range): 67 (37–85) | Median prior | Median prior | Median prior | Median prior | Median prior | Median prior |

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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.\(^{15,17}\)

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)\(^{16,22,24}\) 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 PISP p110δ isoform,\(^{16,96}\) 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.\(^{97,98}\) 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.\(^{10,24}\) 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.\(^{62,96,100}\)

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.\(^{34}\) 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.\(^{24}\)
It has also become evident that patients may develop resistance to these targeted therapies. For example, mutations in the BTK and PIMC2 genes have been associated with resistance to ibrutinib, while upregulation of anti-apoptotic BCL2 family members has been associated with resistance to venetoclax.\textsuperscript{103-104} Mechanisms of resistance to idelalisib have not yet been fully characterized; because idelalisib inhibits the PI3K p110δ isofrom, resistance may theoretically involve upregulation of other PI3K isoforms.\textsuperscript{103} 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.\textsuperscript{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.\textsuperscript{106,107} Following progression on one or more therapies, allogeneic hematopoietic stem cell transplantation 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.\textsuperscript{34,33,34,20,196,197}

**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 pathogenecity 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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