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To cite this article: Fortunato Morabito , Massimo Gentile , Paola Monti , Anna Grazia Recchia , Paola Menichini , Mamdouh Skafi , Moien Atrash , Giuseppa De Luca , Sabrina Bossio , Hamdi Al-Janazreh , Sara Galimberti , Zaidoun Salah , Lucio Morabito , Alham Mujahed , Musa Hindiyeh , Mariella Dono , Franco Fais , Giovanna Cutrona , Antonino Neri , Giovanni Tripepi , Gilberto Fronza & Manlio Ferrarini (2020): TP53 dysfunction in chronic lymphocytic leukemia: clinical relevance in the era of B-cell receptors and BCL-2 inhibitors, Expert Opinion on Investigational Drugs, DOI: [10.1080/13543784.2020.1783239](https://doi.org/10.1080/13543784.2020.1783239)

To link to this article: <https://doi.org/10.1080/13543784.2020.1783239>



Accepted author version posted online: 17 Jun 2020.



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Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Opinion on Investigational Drugs*

DOI: 10.1080/13543784.2020.1783239

TP53 dysfunction in chronic lymphocytic leukemia: clinical relevance in the era of B-cell receptors and BCL-2 inhibitors

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ABSTRACT

Introduction. Patients with *TP53* dysfunction, assessed by del(17p) or *TP53* mutations, respond poorly to chemo-immunotherapy and fare better with the new therapies (BCR and BCL-2 inhibitors); however, it is unclear whether their response is similar to that of patients without anomalies or whether there is currently an adequate determination of *TP53* dysfunction.

Area covered. A literature search was undertaken on clinical trials and real-world experience data on patients with *TP53* dysfunction treated with different protocols. Moreover, data on the *TP53* biological function and on the tests currently employed for its assessment were reviewed.

Expert opinion. Although *TP53* dysfunction has less negative influence on the new biological therapies, patients with these alterations, particularly those with biallelic inactivation of *TP53*, have a worst outcome with these therapies than those without alterations. At present, a determination of *TP53*, particularly with next generation sequencing (NGS) methodologies, may be sufficient for the identifications of the patients unsuitable for chemo-immunotherapy, although integration with del(17p) would be advisable. For the future, more extensive determinations of the *TP53* status, including functional assays, may become part of the current armamentarium for a better patient stratification and treatment with newer protocols.

Keywords: BCR inhibitors, BCL2 inhibitor, Chronic Lymphocytic Leukemia, Clinical outcome, del(17p), Ibrutinib, Idelalisib, Venetoclax, *TP53* mutations.

Article Highlights

- *TP53* dysfunction is operationally defined by the presence of del(17p) and/or *TP53* mutations.
- *TP53* dysfunction is one of the major causes of resistance to chemo-immunotherapy.
- BCR or BCL-2 inhibitors provide an increased likelihood of survival than chemo-immune therapy for patients with *TP53* dysfunction.
- Some exploratory analysis of clinical trials as well as real world evidence, suggest that *TP53* dysfunction, especially in the biallelic inactivation form, continues to have a negative influence on the clinical outcome of patients treated with the new biological therapies.
- New and more stringent laboratory tests to evaluate the residual P53 protein function in patients with *TP53* mutations may plausibly lead to a better risk stratification of patients treated with old and new BCR and BCL-2 inhibitors.

1.0 INTRODUCTION

The strategy of chronic lymphocytic leukemia (CLL) treatment is rapidly changing, since several new biological agents, acting at different levels of leukemic cell metabolism, have been approved or are in advanced experimental phases. These new drugs, with improved efficacy and more favorable toxicity profile, are used alone or are integrated into pre-existing therapeutic protocols [1]. The biological agents currently employed include a glycol-engineered monoclonal anti-CD20 antibody (Obinutuzumab, OB), B-cell receptor (BCR) signaling inhibitors [Ibrutinib (IB) and idelalisib (IDELA)], and the BCL-2 inhibitor venetoclax (Ven). These [2], represent the major subjects of this review.

In the chemotherapy era and even when monoclonal antibodies have been added, it was widely recognized that certain cytogenetic lesions conferred resistance to chemotherapy [3–5]. This was particularly true when a deletion of the short arm of chromosome 17 [del(17p)] was present, suggesting that a *TP53* gene dysfunction was responsible for chemo-immunotherapy resistance, since the site of chromosomal deletion includes the *TP53* locus. Further observations indicated that also *TP53* mutations could confer resistance to chemo-immunotherapy [6] and del(17p) and/or of *TP53* mutations were considered indicative of *TP53* dysfunction. The newer therapies have contributed in part to overcome the poor performance of the chemo-immunotherapy in patients with these alterations, although several issues remain to be clarified. In addition, further studies on *TP53* mutations have indicated the need for refining the current concepts of *TP53* dysfunction. These issues will be the subjects of the present review.

1.0 Chemo-immunotherapy

The addition of the anti-CD20 monoclonal antibody rituximab (R) to fludarabine-cyclophosphamide (FCR) or bendamustine (BR) has led to a remarkable improvement in overall survival (OS) of young and elderly patients, particularly those with a somatically mutated immunoglobulin heavy chain variable region gene (*IGHV*) [7–11], or with an unmutated *IGHV*

gene and a distinctive gene expression profile signature of the leukemic cells [12]. These therapies can induce a minimal residual disease (MRD) negativity [13,14], and a long-term PFS in low-risk CLL patients [1]. The FCR CLL8 clinical trial, demonstrated that patients with del(17p) or *TP53* mutations had a more aggressive clinical course than patients without these abnormalities, with a predicted PFS and OS of roughly 1 and 2-3 years, respectively [4]. A *TP53* dysfunction defined based upon the presence of del(17p) and/or *TP53* mutations also was associated with a markedly inferior outcome in BR treated patients [5]. The independent prognostic role of del(17p) and *TP53* mutation has been underlined by the CLL International Prognostic Index (CLL-IPI), which determined that the risk score of the *TP53* status was 3 times higher than that of age and of clinical stage, and two times higher than that of *IGHV* gene unmutated status [15]. Noteworthy, the *TP53* status was considered as a composite factor [i.e. either del(17p) or *TP53* mutations] based on the absence of a statistical association between the presence of the sole del(17p) *versus* the sole *TP53* mutation or between the presence of a single marker *versus* the presence of both. CLL-IPI has been widely confirmed for patients undergoing chemo-immunotherapy [16–19].

2.0 Targeted new agents

2.1 BCR- inhibitors

Several phase 3 trials have contributed to the switch from chemo-immunotherapy to BCR-inhibitors as summarized in Figure 1. IB proved significantly superior to any comparator drug in terms of PFS and OS, in both treatment naïve [20–25] and relapse/refractory (RR)-CLL patients [26] a finding which changed the treatment paradigm in both clinical settings. Presently, IB continuous use until progression or toxicity is indicated. Besides IB, the current recommendations for RR-CLL case treatment include IDELA in association with R (IDELA-R). A definitive choice between protocols is frequently dictated by comorbidity or expected toxicity profiles. Notably, IDELA-R was superior to R alone [27] commonly used in frail cases unsuitable for chemotherapy (Figure 1).

The addition of IB[28] or IDELA[29] to BR was investigated in two phase 3 randomized trials on RR cases. IB and IDELA in combination with BR were superior to BR alone, reducing the risk of both disease progression and death of about 80% and 40% in the IB trial, and of 67% and 38% in the IDELA protocol, respectively (Figure 1). The absence of an additional control arm (i.e. IB or IDELA-R alone) represented an unfortunate limitation in both studies to indicate the clinical benefit of BR, estimated in previous studies as an 18 month PFS when used as first salvage treatment after FCR, regardless of *TP53* aberrations and/or refractoriness to prior therapy [30]. A real-world indirect comparison study showed that BR and IB could be similarly effective in terms of OS when used as first salvage treatment in patients without del(17p) [31]. The retrospective nature of this study suggests caution, however, and this study requires caution and the effectiveness of BR is effective in a RR setting remains to be ascertained.

Despite of the superior performance of the BCR inhibitors in all experimental settings reported in Figure 1, the question of whether such superior results are observed also in high-risk patients defined by the presence of a *TP53* dysfunction (i.e. del(17p) and/or *TP53* mutations) remains open. Explorative analyses of high-risk groups demonstrated better outcomes in both treatment naïve and RR cases (Figure 2). An exploratory analysis of the IB arm of the Resonate trial (median follow-up 65 months) in the RR setting[32] showed a significantly longer median PFS of 75 patients without *TP53* mutations compared with 79 cases with *TP53* mutations (56.9 versus 40.7 months) (HR: 1.731; 95% CI: 1.156-2.593). Conversely, an analysis of the IDELA-R arm of Study 165 trial[27] indicated no differences between cases with or without *TP53* dysfunction (HR: 1.03; 95% CI: 0.62-1.72). However, the higher incidence of host- and disease-related risk factors in the IDELA-R trial could have played a major role, as these additional confounding factors may have limited, if not overridden, the prognostic impact of the *TP53* dysfunction. Brown et al. [33] focused on an exploratory subset analysis of 38 patients with loss of *TP53* function defined by the both del(17p) and *TP53* mutation compared with 68 cases with one or neither of these abnormalities. Patients with both abnormalities had an inferior PSF ($P=0.0381$), while the PFS of the cases with

either del(17p) or *TP53* mutation was not significantly inferior to that of patients without abnormalities ($P=0.1306$). Further information came from the analysis of RR patients with del(17p) treated with IB in three different studies [34]. The median PFS of cases with del(17p) and complex karyotype (CK) ($n=14$, 67%) was significantly shorter than that cases with del(17p) without CK ($n=7$, 33%) (26 *versus* 52 months), indicating that additional abnormalities, besides *TP53* dysfunction may contribute to the outcome following BCR inhibitors treatment.

2.2 *BCL-2* inhibitor

Up-regulation of the anti-apoptotic *BCL-2* gene, an almost universal feature of CLL clones ([35]) causes overexpression of the *BCL-2* protein, which prevents the activation of the intrinsic/mitochondrial apoptotic pathway [35]. This up-regulation is related to several factors, including the constitutive activation status of CLL cells, the numerous activating signals delivered to CLL cells by the micro-environment and epigenetic regulations. In the CLL cases characterized by del (13q14), the loss of the miR15/miR16 locus, located at the deletion site, contributes to *BCL-2* up-regulation, given the inhibitory effect of these miRs on *BCL-2* expression [36]. Ven binds to the *BCL-2* protein and prevents its subsequent binding to a number of pro-apoptotic proteins, such as BAK1, BAX and BOK [37]. Since the latter proteins can cause mitochondrial damage and induce apoptosis, when freed from *BCL-2* control, Ven has a strong pro-apoptotic effect on CLL cells [37]. CLL cells with or without *TP53* alterations are equally sensitive to Ven *in vitro*[38]. In a phase 1, dose-escalation study, Ven proved capable of inducing ORR in 71% of RR patients with del(17p) [(16% of whom reached complete remission (CR)] *versus* 80% of ORR observed in those without this alteration [39]. Notably, cases with del(17p) experienced a median PFS of 16 months (95% CI, 11-25), whereas 71% of patients (95% CI, 57-81) without del(17p) were progression-free at 15 months [39]. In a phase 2 multicentre study, RR-CLL patients with del(17p) were treated with once daily Ven, with the well-known weekly dose ramp-up schedule. The ORR was 79.4% (95% CI 70.5–86.6), with 1-year PFS and OS of 72% and 86.7%, respectively [40]. A more recent extended and updated analysis showed 2-year PFS and OS of 54% and 73%, respectively[41].

Since R increases the cytotoxic capacity of Ven on CLL cells in vitro [42], the VenR combination was used and compared to BR in a RR-CLL setting in the phase 3 Murano trial[43]. The risk of progressing was significantly reduced in the VenR arm (HR, 0.13; 95% CI, 0.05 to 0.29) compared to the BR arm. Interestingly, the clinical benefit of the BCL-2 inhibitor was maintained in the subgroup of patients with del(17p) (PFS at 2-year 81.5% *versus* 27.8%).

Recently, FDA approved Ven for CLL first-line therapy based on the CLL14 study, in which CLL patients with comorbidities were randomized to receive VenOB or CHBL-OB [44]. The estimated 2-year PFS rate was significantly higher in the VenOB group than in the CHLB-OB group (88.2% [95% CI, 83.7% to 92.6%] vs. 64.1% [95% CI, 57.4% to 70.8%]). A superior clinical benefit was also observed in patients with *TP53* dysfunction [44].

The analysis of the numerous clinical trials reported above may lead to some preliminary conclusions. First, the presence of markers of potential *TP53* dysfunction, such as del(17p) and/or *TP53* mutations, represent negative indicators of response in patients undergoing chemo- or chemo-immuno therapies. Second, BCR or of BCL-2 inhibitors provide an increased likelihood of longer PFS or of OS than chemo-immune therapy for this patient group. Nevertheless, it remains to be determined whether the patients with or without *TP53* dysfunction present a similar outcome when treated with the new drugs. Finally, several considerations raise the issue of whether the methodologies so far universally employed to measure *TP53* dysfunction are adequate to provide information on this complex scenario, as it will be discussed below.

2.3 Real-world experience

Given the difficulties in assessing the exact role of *TP53* dysfunction in the outcome of treatments with the new drugs and particularly with BCR inhibitors, we analyzed the “real-world evidence”, which is becoming progressively more relevant to confirm or disprove the results of clinical trials [45].

Salles and colleagues investigated the efficacy of IB *versus* other chemo-immunotherapies in treatment-naïve and RR-CLL patients [46] although the issue of the *TP53* dysfunction was not

addressed. The real-world data were compared with those of two randomized registration trials. IB reduced significantly the risk of PFS and OS both in treatment-naïve (adjusted HR for PFS: 0.23, 95% CI: 0.14–0.37; $P < 0.0001$; adjusted HR for OS: 0.40, 95% CI: 0.22–0.76; $P = 0.0048$) and in RR setting (adjusted HR for PFS: 0.21, 95% CI: 0.16–0.27; $P < 0.0001$; adjusted HR for OS: 0.29, 95% CI: 0.21–0.41; $P < 0.0001$) *versus* chemo-immunotherapies regimens.

Results on 428 real-world cases, including roughly 45% with either del(17p) and/or *TP53* mutations from 106 centers throughout France treated with IB were recently reported [47]. IB efficacy and safety was confirmed although no specific data on the group with *TP53* dysfunction were reported.

Dimou and colleagues published efficacy and safety real-world data from IB single drug therapy from a single Greek center [48]. Thirty-seven percent of 11 cases treated in first-line and 22% in $\geq 2^{\text{nd}}$ line showed either del(17p) or *TP53* mutation. All cases were still on IB in the 1st line subgroup, while 10% of RR cases progressed.

The Swedish group reported the results of a long-term real-world compassionate IB therapy study on RR-CLL patients [49]. OS was significantly longer in the 42 patients matching the Resonate inclusion criteria ($P = 0.03$) compared with that of the 53 who did not match these criteria. Of interest, in contrast to the early report from the same authors [50], the negative survival impact of del(17p)/*TP53* mutation was no longer significant.

Cases with RR-CLL and del(17p) showed a trend toward significance for OS (HR=1.45, 95% CI 0.97-2.18, $P = 0.07$) compared to cases without del(17p) in a real-word series treated with IB [51]. This result was confirmed in a multivariate model in which the four markers constituting the BALL score ($\beta 2$ -microglobulin and lactate dehydrogenase levels, anemia and time from initiation of last therapy < 24 months) also were introduced [52].

Patients with del(17p) and/or *TP53* mutation had significantly shorter PFS (HR 2.7, 95% CI 1.08-6.7, $P = 0.034$), but not a shorter OS (HR 1.78, 95% CI .55-5.74, $P = 0.332$) than those without these alterations in a series of 141 Ven treated CLL patients, focusing on outcome following Ven

discontinuation[53]. *TP53* dysfunction remained a significant independent predictor for shorter PFS in a multivariate model including *TP53* dysfunction, CK and prior IB therapy (HR 2.8, 95% CI 1.22-6.4, P=0.03).

The role of *TP53* gene dysfunction in predicting OS was also investigated in an independent real-world cohort of 622 CLL cases treated with IB, IDELA or Ven (Morabito et al., in preparation). CLL patients were stratified into three groups [*TP53*mut/del(17p), *TP53*mut/no-del(17p) and *TP53*wt/del(17p) cases]. OS was significantly shorter in *TP53*mut/del(17p) cases, while the Kaplan-Meier curve of the *TP53*wt/del(17p) group overlapped to that of the *TP53*wt/no-del(17p) group, indicating that only the concomitant presence of the two lesions determined an inferior outcome. In a multivariate model, *TP53*mut/del(17p) status remained independently associated with OS together with increased LDH levels, anemia, lines of preceding therapies and the exposure to other new drugs.

2.4 Therapies with new generation biological agents.

Some CLL trials with next generation drugs are still ongoing. Among those reported in Table 1, only a few have available information of specific results on high-risk patients with *TP53* alterations. Acalabrutinib (Acala), a highly selective inhibitor of Bruton's tyrosine kinase (BTK) with negligible off-target activity, showed an interesting safety and efficacy profile in a phase 1–2 multicenter study in patients with RR-CLL. Among the 18 patients with del(17p), the response rate was 100%[54]. Acala monotherapy was superior to IDELA-R or BR in prolonging PFS in patients with RR-CLL; this improvement was also observed in the group of patients with del(17p) (HR 0.21, 95%CI 0.07-0.68) and *TP53* mutation (HR 0.24, 95% CI 0.11-0.56) [55]. In the Phase 3 ELEVATE-TN study, PFS improvement with Acala-OBI or Acala *versus* CHBL-OBI was consistent across subgroups examined including del(17p) (HR 0.13, 95%CI 0.04-0.46) for Acala-OBI; HR 0.20, 95%CI 0.06-0.64 for Acala monotherapy) [56]. Finally, although Ven is currently the only commercially available BCL-2 inhibitor, research dealing with selective inhibitors of other

molecules of the BCL-2 family members, i.e. BCL-xL and MCL-1, is ongoing. However, no clinical trials have been opened yet.

3.0 *TP53* gene, mutational status and P53 protein function.

The definition of *TP53* dysfunction, provided by del(p17) or *TP53* gene mutations may leave some imprecision. Moreover, not all mutations may be equal since different mutations may impact differently on the *TP53* protein function and certain *TP53* gene mutations may also cause impairment of the function of the unmutated allele. Therefore, we have deemed necessary to analyze a number of these aspects in the paragraphs below since they may be relevant for the interpretations of the existing data on clinical trials and for the design of new ones.

3.1 TP53 gene

TP53 is an evolutionary highly conserved tumor suppressor gene [57], located at the telomeric portion of the short arm of chromosome 17 (17p13.1), encoding a protein of 393 amino-acid long with transcription factor activity. Consistent with its tumor suppressor activity is the presence of *TP53* mutations as a hallmark of the hereditary cancer predisposition disorder Li-Fraumeni syndrome [58].

The P53 protein consist of two N-terminal transactivation domains, a proline-rich domain, a central sequence specific DNA-binding domain (DBD), an oligomerization (or tetramerization) domain , and an unstructured C-terminal domain that regulates the binding to the DNA (Figure 3A). *TP53* acts mainly as an inducible TF, which regulates a plethora of target genes involved in cancer suppression [59]. P53 protein is generally undetectable in normal cells, although it can accumulate upon different stress conditions (e.g. DNA damage, oncogenic stress and hypoxia) which also cause P53 activation. Upon detection of DNA lesions by specific sensors, and through the activation of certain signal transduction pathways such as those involving the ATM-kinase, P53 is modified post-translationally, for example by undergoing phosphorylation on Ser15. This specific modification causes P53 release from the ubiquitin-ligase MDM2, which normally leads to P53 degradation

through the proteasome. The undegraded P53 protein then accumulates into the nucleus binds as a tetramer to TP53 response elements (p53-REs) in the promoter regions of effector genes, thus activating their transcription. Hundreds of *TP53* effector genes are involved in regulation of different processes, including cell cycle arrest, DNA repair, apoptosis, senescence, metastasis, autophagy and metabolism.

How *TP53* coordinates the activation of down-stream effector genes is not fully understood. In principle, a promoter-specific activation can depend on i) the intensity of the stress (low/constitutive vs high/acute) [60], ii) the cell type and the cell physiological status which allows the recruitment of specific co-factors iii) the specific features of the p53-RE [61], since REs of different effector genes have variable sequences for which the wt p53 protein has distinct binding affinities.

3.2 Functional effects of TP53 mutations in CLL.

In human cancers, *TP53* is mostly altered by missense mutations (i.e. a mutation causing a single amino-acid substitution) affecting more frequently six residues (known as hotspot codons, i.e. R175, G245, R248, R249, R273 and R282) within the DBD domain [62]. However, the spectrum of *TP53* missense mutations is extremely broad with more than 2,000 different amino-acid changes collectively reported [62], with specific differences in the various tumor types. The *TP53* mutations from CLL studies reported by The Cancer Genome Atlas consortium (TCGA, <http://www.cbioportal.org/>) [63,64] are presented in Figure 3 (Panel A). Mutations affecting codons 175, 179, 220, 248, 273 and 281 represent the majority of identified *TP53* alterations (34%); in addition, the codon 209 was reported as frequently mutated by Zenz et al [65]. Mutant P53 proteins are categorized into DNA contact- or structural- mutants, according to the effect that the amino-acid substitution may have on the direct interaction with DNA (e.g. p.R273H) or on the p53 protein structure structure (e.g. p.R175H), respectively [66]. However, not all *TP53* mutations equally affect the P53 functions, potentially generating a wide range of phenotypic diversity [67]. Over the last 25 years, many data on the functional impact of missense mutations on P53 have been

generated and are publicly available (p53.free.fr/); indeed, functional assays have been performed in yeast and in human cells to measure the properties of single mutant P53 proteins including (i) transactivation potential, (ii) dominant negative effect over the wild-type protein, i.e. the capacity of the mutant protein to inhibit the activity of the wild type counterpart in heterozygous state, and (iii) gain-of-function, a condition indicating that a mutation confers to P53 protein the property of facilitating clonal expansion by acquisition of new properties [62,68]. The emerging picture is that mutant P53 proteins constitute a functional rainbow capable of conferring heterogeneous properties to the cells (Figure 3A)[69,70]. Analyses of genotype/phenotype correlations in germline TP53-associated disorders demonstrated that severely deficient P53 proteins were associated with more severe cancer proneness syndromes (e.g. Li-Fraumeni syndrome), while partial deficient mutant P53s were more frequently found in less severe cancer proneness conditions (e.g. family history)[71]. Consistently, Trbusek et al, [72] found that CLL patients with TP53 missense mutations located in DNA-binding motifs (DBMs), structurally well-defined and essential parts of the P53 DBD, manifested a shorter median survival and TTFT compared with CLL patients having missense mutations outside these DBMs. These results suggested a that TP53 DBMs mutations induced a gain-of-function phenotype, which can determine the expression of genes promoting CLL cell survival, such as the mitogen-activated protein kinase kinase 3 (MAP2K3) [73] and an altered expression of microRNAs [74].

3.3 Dysfunction of the P53 pathway in CLL.

In the last decade assays have been developed to evaluate the function of the whole P53 pathways in purified CLL cells [75]. These are based on the quantification of P53 and P53 target coding or non-coding genes at the RNA level (e.g. p21, bax, puma, fas and mir-34a) by RT-PCR or at the protein level (e.g. P21) by FACS (Fluorescent activated Cell Sorting) and Western Blotting.

Since both the *TP53* and *ATM* genes are integral to the DNA damage response (DDR) pathway, activated by double-stranded DNA breaks, the function of the P53 pathway is evaluated after induction of DDR with appropriate stimuli (e.g. IR, fludarabine/doxorubicin) [76]. If the P53

pathway is functioning, the P53 protein itself, and other known P53 effector gene products, are expected to be induced, while in the absence of such a response a dysfunction is inferred. Interestingly, Cerna et al., [77] identified mir-34a as a positive regulator in the cross-talk between P53 functionality and the B-cell receptor in CLL; in fact, mir-34a is the most prominently up-regulated mir during DDR in CLL cells *in vitro* and *in vivo* following FCR therapy (fludarabine, cyclophosphamide, rituximab), causing the down-regulation of the transcription factor FOXP1 to limit the pro-survival/pro-proliferative signals from BCR.

A specific functional assay using etoposide plus Nutlin-3a, a molecule that releases the P53 protein from the deadly embrace of MDM2, was also developed to detect and distinguish the presence of *ATM* or *TP53* mutations in CLL [78]. Notably, in these assays, the size of the subclone with *TP53* alteration (s) and also the purification of the leukemic cells can influence the results.

4.0 Discussion

The notion of *TP53* dysfunction was introduced long ago to indicate lesions of the *TP53* gene demonstrated by del(17p), detected by FISH, and/or *TP53* mutations, detected by DNA sequencing. This definition raises the issue of whether determination of del(17p) and/or of *TP53* mutations may not represent an oversimplification of the conditions leading to an impaired *TP53* function, both in terms of quality and quantity. The effect of del(17p) may be different in the various cases depending upon the proportion of the cells carrying the deletion, a consideration which also relates to FISH sensitivity. Moreover, the outcome of patients with equivalent proportions of cells with the deletion, can vary depending upon the type of therapy. In addition, del(17p) will have different consequences when accompanied by mutations of the other *TP53* allele. Finally, chemo-immunotherapy is likely to necessitate of a more efficient apoptotic apparatus than BCR and for BCL-2 inhibitors.

The issue becomes even more complex for *TP53* mutations. First, the sensitivity of the sequencing technique is becoming even more relevant. This article is not intended to provide many methodological details, for which we refer to recent ad hoc publications [79–81], nevertheless

clinicians should be aware that the Sanger methodology currently employed has a threshold of detection of variant allelic frequency (VAF) of 10% at best, and that its sensitivity is influenced by the degree of purification of the leukemic cells (which may be in turn influenced by the peripheral lymphocyte count) and by the choice of including or excluding *TP53* exons 2 and 11 in the analysis, in addition to the mandatory exons 4-10. The issue of the sensitivity of the methodology is not trivial. *TP53* mutations are detected in approximately 6% of patients in the early stages (and often confined to minor CLL subclones) and increase with disease progression reaching about 40% among the refractory cases [82,83]. Moreover, patients who fail to respond or respond for a short time to chemo-immunotherapy may have minor subclone(s) carrying *TP53* mutations. These subclone(s) can subsequently expand under the selective pressure of chemotherapy to become the major disease component in later periods [83–85]. It remains to be clarified whether such selection also occurs with the new biological therapies and what are the mechanisms involved. As already mentioned, a dysfunction of the p53 pathway causes a diminished response of the BCR to stimulation [77], although clonal expansion could still be facilitated by signals delivered through the JAK/STAT pathway, as it occurs for IL23 stimulation of CLL cells, which is BCR-independent [86]. BCR inhibitors can be less effective in these conditions and the expansion of subclones with *TP53* dysfunction could be favoured.

To overcome the problems of sensitivity of the Sanger sequence, many laboratories have switched to next generation sequencing (NGS) methodologies, which offer higher sensitivity, although require a careful calibration to prevent false positivity. Moreover, the data of NGS too are influenced by the purification of the leukemic cells and by the choice of the *TP53* gene segments to be analyzed. Finally, and, more important to the clinicians, although NGS allows a higher sensitivity, the optimal threshold of mutation detection has still to be determined based upon evidence-based criteria.

The mutations of the *TP53* gene have a different impact if they are present alone or concomitantly with del(17p), since the presence of the two allelic lesions is likely to have a greater

impact on function. Finally, not all mutations may have a similar effect on P53 protein function. Although both missense and non missense mutations have a negative prognostic impact, the former mutations appear to impact more on the disease course [72]. Moreover, the mutations which affect the *TP53* DNA binding motifs are also those which confer a worst prognosis [72]. Finally, the presence of a mutation in one allele may have a different impact on the function of the remaining allele, depending on the nature of the mutation itself, as discussed above.

Particular conditions have to be considered before drawing possible conclusions on therapy strategies. Patients with CK, identified by chromosome banding, and defined by the presence of >-3 chromosomal alterations may have a dire clinical course [87,88]. In a recent European Research Initiative on CLL (ERIC) collaborative study, involving 5290 cases [89], it was determined that CK occurs in approximately 15% of patients. Among these, the group with the highest cytogenetic complexity, with >- 5 chromosomal alterations, had a particular dismal prognosis. In contrast, patients with a lower number of chromosomal alterations had an adverse clinical course only if there was a concomitant evidence for a *TP53* disfunction. Notably, patients with CK including trisomy 12 and trisomy 19 presented a remarkably indolent course and generally were characterized by the absence of other negative prognostic factors. This group, however, represented approximately 10% of the whole patients with CK. Therefore, an unsatisfactory response to chemo-immunotherapy and also to the newer biological therapies may find an explanation in a CK, irrespective of, or in addition to, other adverse prognostic factors.

A number of patients treated with BCR or BCL-2 inhibitors may respond for a short time and subsequently present a rapid downhill course. This is particularly true for those cases that are characterized by NOTCH mutations and by trisomy 12, in addition to *TP53* dysfunction. These patients most likely developed a Richter transformation (RT), which is defined by the onset of a very aggressive lymphoma in a CLL background [90,91]. Lymph-node biopsy, which may also reveal MYC hyperexpression by malignant cells, a characterizing feature of RT [92], and PET could

confirm the diagnostic hypothesis and the patients may be switched to a suitable therapy for aggressive lymphomas.

A final note concerns a consideration for relapsing patients, in whom the success of any therapy is often conditioned by the number of previous therapy lines (both chemotherapies and biological therapies) irrespective of or in addition to the presence of adverse prognostic factors [52].

5.0 Conclusions

The analysis of clinical trials and of real-life experience demonstrates that *TP53* dysfunction determines the outcome of therapy in CLL. Moreover, the proportion of leukemic cells carrying such dysfunction and the type of *TP53* gene lesion also influences the subsequent fate of patients. Although it is well established that the new biological agents are far superior to chemo-immunotherapy in patients with *TP53* dysfunction, there are a number of issues to be clarified including that of whether the new therapies are equally effective in patients with and without *TP53* dysfunction and whether there are differences for the different agents. Several considerations strongly support a widespread search for *TP53* dysfunction in the patient work-up and possibly suggest the future extension to functional studies. Testing for *TP53* dysfunction should be completed by other investigations aimed at assessing the overall aggressiveness of the disease (e.g. *IGHV* gene mutational status and CK, as well as RT predisposition).

6.0 Expert Opinion

At present, for a patient who is a candidate for a first line therapy, there is the choice for two treatment groups, i.e. chemo-immuno or biological therapies. As already discussed, there is still room for chemo-immunotherapy, which can induce long lasting remissions [13,14]. This result can be achieved only with a careful consideration of the patient risk factors, which primarily include the assessment of the of *TP53* dysfunctions. The ERIC group recommends *TP53* mutational screening as a first step in the algorithm leading to therapeutic decision [79]. This approach appears to be safe and capable of identifying most patients who are not eligible for chemo-immunotherapy. In the near

future, additional advantages will be brought about by the increased use of NGS methodologies with a reasonably high coverage. The choice of the sole determination of *TP53* mutations is based upon considerations that there is a large overlap between patients with *TP53* mutations and those carrying del(17p). Therefore, the two alterations are concomitant in most of leukemic clones and the choice of determining only the *TP53* mutational status will provide an indication of the *TP53* function in most patient except those characterized by the presence of del(17p) only. Although there are variations in the proportions of these patients depending on the different cohorts studied and the disease stage in which the tests were performed, it is possible that these *TP53*-unmutated, del(17p)-positive cases represent 5-10% of patients with *TP53* dysfunction [72,84,93] (Monti et al., under revision). These patients (most likely not eligible for chemo-immunotherapy) will escape detection in the ERIC suggested approach. *TP53* mutations alone, in the absence of del(17p), have a significant impact on chemo-immunotherapy as documented by the clinical trials. Hence all patients with *TP53* mutations should be excluded from chemo-immunotherapy. This exclusion is justified even considering the differences in the biological impact of the various mutations and also the biological consequences of the single mutations on the remaining *TP53* allele (see for example, mutations causing haplo-insufficiency *versus* those determining haplo-sufficiency). Nevertheless, functional analyses of *TP53* mutations are indicated for experimental research only and are so far excluded from the current practice.

The determination of del(17p) and of the *IGHV* gene mutation status, deserves a different consideration, since are feasible in a clinical setting. They are important to detect the few del(17p)-positive *TP53* mutation-negative cases and for an overall evaluation of disease aggressiveness[94], respectively. Notably, the presence of *IGHV* somatic mutations in *TP53*-mutated CLL clones characterize patients that do not have a negative prognosis [95]. Financial rather than medical considerations may eventually suggest or prevent the use of these tests, which in our opinion should be strongly recommended. Likewise, additional laboratory tests, like the determination of NOTCH mutations, of trisomy 12 and of CK would be desirable in the everyday clinical practice, given

their importance for predicting particular clinical conditions such as predisposition to RT or to a dismal outcome. Unfortunately, the utilization of a wide array of molecular and chromosomal diagnostic tests remains a prerogative for a relative minority of patients in the current practice [96].

The next question is whether the above recommendations are applied at least in part in the current practice. In this regards, it comes somehow to a surprise to see the low proportion of cases tested for del(p17) and for *TP53* mutations documented by the inform CLL registry [97]. This information is perhaps even more striking considering that cases with *TP53* mutations (26%) or del(17p) were eventually treated with chemotherapy only. This attitude, although difficult to understand, given the importance of assessing *TP53* dysfunctions in the patient workup and in any further strategy decision, may relate to communication problems and to a certain conservatism of the medical community, which should hopefully change in the near future.

Patients for whom it is intended to use a biological first line therapy or are in a RR following chemo-immunotherapy are all likely to equally respond to biological therapies? Specifically, does the quality of the response to these agents depend upon the presence/absence of *TP53* dysfunction? This is an old question which goes back to time of the alemtuzumab use as an alternative agent for chemotherapy resistant cases, with a likely *TP53* dysfunction [98]. The answer to this question may also determine whether or not the assessment of *TP53* mutations is a mandatory requirement before therapeutic decisions are made, although we would strongly support an extensive diagnostic work-up as stated. Unfortunately, the analysis of relevant trials does not provide unequivocal response as we have already outlined [53]. Clearly the issue deserves exploration in future clinical trials, where new and more stringent laboratory tests to evaluate the residual *TP53* function in patients with *TP53* mutations are possibly employed. This approach may conceivably lead to a new scenario in which patients are stratified in different risk groups based upon residual *TP53* function within the leukemic clone. For most of the groups so classified, it is plausible that the current and maybe the newer biological therapies have a great chance of a durable effect, whereas for other groups, that likely involve a minority of patients, these therapies may

prove ineffective and hence there will be indications for alternative approaches represented by Car-T cell therapies [99] or even by allogeneic bone marrow transplantation [100].

Funding

The work of the authors was funded by unrestricted contribution from GILEAD Sciences Srl. The funding sources had no role in identifying statements, abstracting data, synthesizing results, grading evidence or preparing the manuscript, or in the decision to submit the manuscript for publication (ISR-17-10250). Associazione Italiana Ricerca sul Cancro (AIRC) Grant 5 x mille n.9980, (to F.M., A.N. and M.F.); AIRC and Fondazione CaRiCal co-financed Multi-Unit Regional Grant 2014 n.16695 (to F.M.) Associazione Italiana Ricerca Cancro (AIRC), IG-5506 (to G.F.), IG-14326 (to M.F.), IG-15426 (to F.F.); Compagnia S. Paolo, Turin, Italy (Project 2017.0526 to G.F.) and by the Italian Ministry of Health 5x1000 funds 2015 and 2016 and Current Research 2016 (to G.F., G.C. and to F.F.).

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers

- [1] Khan Y, Lyou Y, El-Masry M, et al. Reassessing the role of chemoimmunotherapy in chronic lymphocytic leukemia. *Expert Rev Hematol*. 2020;13:31–38.
- [2] Fürstenau M, Hallek M, Eichhorst B. Sequential and combination treatments with novel agents in chronic lymphocytic leukemia. *Haematologica*. 2019;104:2144–2154.
- [3] Döhner H, Stilgenbauer S, Benner A, et al. Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia. *N Engl J Med*. 2000;343:1910–1916.
- [4] Stilgenbauer S, Schnaiter A, Paschka P, et al. Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. *Blood*. 2014;123:3247–3254.
- [5] Zaja F, Mian M, Volpetti S, et al. Bendamustine in chronic lymphocytic leukemia: outcome according to different clinical and biological prognostic factors in the everyday clinical practice. *Am J Hematol*. 2013;88:955–960.
- [6] Gaidano G, Rossi D. The mutational landscape of chronic lymphocytic leukemia and its impact on prognosis and treatment. *Hematology Am Soc Hematol Educ Program*. 2017;2017:329–337.
- [7] Rossi D, Terzi-di-Bergamo L, De Paoli L, et al. Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. *Blood*. 2015;126:1921–1924.
- [8] Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood*. 2016;127:303–309.
- [9] Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016;127:208–215.
- [10] Chai-Adisaksopha C, Brown JR. FCR achieves long-term durable remissions in patients with IGHV-mutated CLL. *Blood*. 2017;130:2278–2282.
- [11] Gentile M, Shanafelt TD, Reda G, et al. Validation of a biological score to predict response in chronic lymphocytic leukemia patients treated front-line with bendamustine and rituximab. *Leukemia*. 2018;32:1869–1873.
- [12] Herling CD, Coombes KR, Benner A, et al. Time-to-progression after front-line fludarabine, cyclophosphamide, and rituximab chemoimmunotherapy for chronic lymphocytic leukaemia: a retrospective, multicohort study. *Lancet Oncol*. 2019;20:1576–1586.
- [13] Molica S, Giannarelli D, Montserrat E. Minimal Residual Disease and Survival Outcomes in Patients With Chronic Lymphocytic Leukemia: A Systematic Review and Meta-analysis. *Clin Lymphoma Myeloma Leuk*. 2019;19:423–430.
- [14] Thompson PA, Srivastava J, Peterson C, et al. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood*. 2019;134:1951–1959.

- [15] International CLL-IPI working group. An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data. *Lancet Oncol.* 2016;17:779–790.
- [16] Gentile M, Shanafelt TD, Mauro FR, et al. Predictive value of the CLL-IPI in CLL patients receiving chemo-immunotherapy as first-line treatment. *Eur J Haematol.* 2018;
- [17] Muñoz-Novas C, Poza-Santaella M, González-Gascón Y Marín I, et al. The International Prognostic Index for Patients with Chronic Lymphocytic Leukemia Has the Higher Value in Predicting Overall Outcome Compared with the Barcelona-Brno Biomarkers Only Prognostic Model and the MD Anderson Cancer Center Prognostic Index. *Biomed Res Int.* 2018;2018:9506979.
- [18] Gentile M, Shanafelt TD, Mauro FR, et al. Comparison between the CLL-IPI and the Barcelona-Brno prognostic model: Analysis of 1299 newly diagnosed cases. *Am J Hematol.* 2018;93:E35–E37.
- [19] Molica S, Giannarelli D, Mirabelli R, et al. Chronic lymphocytic leukemia international prognostic index (CLL-IPI) in patients receiving chemoimmuno or targeted therapy: a systematic review and meta-analysis. *Ann Hematol.* 2018;97:2005–2008.
- [20] Burger JA, Tedeschi A, Barr PM, et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. *N Engl J Med.* 2015;373:2425–2437.
- [21] Barr PM, Robak T, Owen C, et al. Sustained efficacy and detailed clinical follow-up of first-line ibrutinib treatment in older patients with chronic lymphocytic leukemia: extended phase 3 results from RESONATE-2. *Haematologica.* 2018;103:1502–1510.
- [22] Burger JA, Barr PM, Robak T, et al. Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. *Leukemia.* 2020;34:787–798.
- [23] Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-Rituximab or Chemoimmunotherapy for Chronic Lymphocytic Leukemia. *N Engl J Med.* 2019;381:432–443.
- [24] Woyach JA, Ruppert AS, Heerema NA, et al. Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL. *N Engl J Med.* 2018;379:2517–2528.
- [25] Moreno C, Greil R, Demirkan F, et al. Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2019;20:43–56.
- [26] Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *N Engl J Med.* 2014;371:213–223.
- [27] Furman RR, Sharman JP, Coutre SE, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. *N Engl J Med.* 2014;370:997–1007.
- [28] Chanan-Khan A, Cramer P, Demirkan F, et al. Ibrutinib combined with bendamustine and rituximab compared with placebo, bendamustine, and rituximab for previously treated

chronic lymphocytic leukaemia or small lymphocytic lymphoma (HELIOS): a randomised, double-blind, phase 3 study. *Lancet Oncol.* 2016;17:200–211.

- [29] Zelenetz AD, Barrientos JC, Brown JR, et al. Idelalisib or placebo in combination with bendamustine and rituximab in patients with relapsed or refractory chronic lymphocytic leukaemia: interim results from a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 2017;18:297–311.
- [30] Fornecker L-M, Aurrant-Schleinitz T, Michallet A-S, et al. Salvage outcomes in patients with first relapse after fludarabine, cyclophosphamide, and rituximab for chronic lymphocytic leukemia: the French intergroup experience. *Am J Hematol.* 2015;90:511–514.
- [31] Cuneo A, Follows G, Rigolin GM, et al. Efficacy of bendamustine and rituximab as first salvage treatment in chronic lymphocytic leukemia and indirect comparison with ibrutinib: a GIMEMA, ERIC and UK CLL FORUM study. *Haematologica.* 2018;103:1209–1217.
- [32] Munir T, Brown JR, O'Brien S, et al. Final analysis from RESONATE: Up to six years of follow-up on ibrutinib in patients with previously treated chronic lymphocytic leukemia or small lymphocytic lymphoma. *Am J Hematol.* 2019;94:1353–1363.
- [33] Brown JR, Hillmen P, O'Brien S, et al. Extended follow-up and impact of high-risk prognostic factors from the phase 3 RESONATE study in patients with previously treated CLL/SLL. *Leukemia.* 2018;32:83–91.
- [34] Jones J, Mato A, Coutre S, et al. Evaluation of 230 patients with relapsed/refractory deletion 17p chronic lymphocytic leukaemia treated with ibrutinib from 3 clinical trials. *Br J Haematol.* 2018;182:504–512.
- [35] Robertson LE, Plunkett W, McConnell K, et al. Bcl-2 expression in chronic lymphocytic leukemia and its correlation with the induction of apoptosis and clinical outcome. *Leukemia.* 1996;10:456–459.
- [36] Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA.* 2005;102:13944–13949.
- [37] Perini GF, Ribeiro GN, Pinto Neto JV, et al. BCL-2 as therapeutic target for hematological malignancies. *J Hematol Oncol.* 2018;11:65.
- [38] Anderson MA, Deng J, Seymour JF, et al. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. *Blood.* 2016;127:3215–3224.
- [39] Roberts AW, Davids MS, Pagel JM, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.* 2016;374:311–322.
- [40] Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study. *Lancet Oncol.* 2016;17:768–778.
- [41] Stilgenbauer S, Eichhorst B, Schetelig J, et al. Venetoclax for Patients With Chronic Lymphocytic Leukemia With 17p Deletion: Results From the Full Population of a Phase II Pivotal Trial. *J Clin Oncol.* 2018;36:1973–1980.

- [42] Souers AJ, Levenson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med.* 2013;19:202–208.
- [43] Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *N Engl J Med.* 2018;378:1107–1120.
- [44] Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. *N Engl J Med.* 2019;380:2225–2236.
- [45] Sherman RE, Anderson SA, Dal Pan GJ, et al. Real-World Evidence - What Is It and What Can It Tell Us? *N Engl J Med.* 2016;375:2293–2297.
- [46] Salles G, Bachy E, Smolej L, et al. Single-agent ibrutinib in RESONATE-2™ and RESONATE™ versus treatments in the real-world PHEDRA databases for patients with chronic lymphocytic leukemia. *Ann Hematol.* 2019;98:2749–2760.
- [47] Ysebaert L, Aurran-Schleinitz T, Dartigeas C, et al. Real-world results of ibrutinib in relapsed/refractory CLL in France: Early results on a large series of 428 patients. *Am J Hematol.* 2017;92:E166–E168.
- [48] Dimou M, Iliakis T, Pardalis V, et al. Safety and efficacy analysis of long-term follow up real-world data with ibrutinib monotherapy in 58 patients with CLL treated in a single-center in Greece. *Leuk Lymphoma.* 2019;60:2939–2945.
- [49] Winqvist M, Andersson P-O, Asklid A, et al. Long-term real-world results of ibrutinib therapy in patients with relapsed or refractory chronic lymphocytic leukemia: 30-month follow up of the Swedish compassionate use cohort. *Haematologica.* 2019;104:e208–e210.
- [50] Winqvist M, Asklid A, Andersson PO, et al. Real-world results of ibrutinib in patients with relapsed or refractory chronic lymphocytic leukemia: data from 95 consecutive patients treated in a compassionate use program. A study from the Swedish Chronic Lymphocytic Leukemia Group. *Haematologica.* 2016;101:1573–1580.
- [51] Gentile M, Morabito F, Del Poeta G, et al. Survival risk score for real-life relapsed/refractory chronic lymphocytic leukemia patients receiving ibrutinib. A campus CLL study. *Leukemia.* 2020;
- [52] Soumerai JD, Ni A, Darif M, et al. Prognostic risk score for patients with relapsed or refractory chronic lymphocytic leukaemia treated with targeted therapies or chemoimmunotherapy: a retrospective, pooled cohort study with external validations. *Lancet Haematol.* 2019;6:e366–e374.
- [53] Mato AR, Thompson M, Allan JN, et al. Real-world outcomes and management strategies for venetoclax-treated chronic lymphocytic leukemia patients in the United States. *Haematologica.* 2018;103:1511–1517.
- [54] Byrd JC, Harrington B, O'Brien S, et al. Acalabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med.* 2016;374:323–332.
- [55] Ghia P, Pluta A, Wach M, et al. ASCEND: Phase III, Randomized Trial of Acalabrutinib Versus Idelalisib Plus Rituximab or Bendamustine Plus Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *J Clin Oncol.* 2020;JCO1903355.

- [56] Sharman JP, Egyed M, Jurczak W, et al. Acalabrutinib with or without obinutuzumab versus chlorambucil and obinutuzumab for treatment-naive chronic lymphocytic leukaemia (ELEVATE TN): a randomised, controlled, phase 3 trial. *Lancet*. 2020;395:1278–1291.
- [57] Soussi T, Caron de Fromental C, May P. Structural aspects of the p53 protein in relation to gene evolution. *Oncogene*. 1990;5:945–952.
- [58] Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*. 1990;250:1233–1238.
- [59] Kasthuber ER, Lowe SW. Putting p53 in Context. *Cell*. 2017;170:1062–1078.
- [60] Vousden KH, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol*. 2007;8:275–283.
- [61] Menendez D, Inga A, Resnick MA. The expanding universe of p53 targets. *Nat Rev Cancer*. 2009;9:724–737.
- [62] Leroy B, Fournier JL, Ishioka C, et al. The TP53 website: an integrative resource centre for the TP53 mutation database and TP53 mutant analysis. *Nucleic Acids Res*. 2013;41:D962–969.
- [63] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401–404.
- [64] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6:p11.
- [65] Zenz T, for the European Research Initiative on CLL (ERIC), Vollmer D, et al. TP53 mutation profile in chronic lymphocytic leukemia: evidence for a disease specific profile from a comprehensive analysis of 268 mutations. *Leukemia*. 2010;24:2072–2079.
- [66] Cho Y, Gorina S, Jeffrey PD, et al. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science*. 1994;265:346–355.
- [67] Oren M, Rotter V. Mutant p53 gain-of-function in cancer. *Cold Spring Harb Perspect Biol*. 2010;2:a001107.
- [68] Kato S, Han S-Y, Liu W, et al. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci USA*. 2003;100:8424–8429.
- [69] Bisio A, Ciribilli Y, Fronza G, et al. TP53 mutants in the tower of babel of cancer progression. *Hum Mutat*. 2014;35:689–701.
- [70] Sabapathy K, Lane DP. Therapeutic targeting of p53: all mutants are equal, but some mutants are more equal than others. *Nat Rev Clin Oncol*. 2018;15:13–30.
- [71] Monti P, Ciribilli Y, Jordan J, et al. Transcriptional functionality of germ line p53 mutants influences cancer phenotype. *Clin Cancer Res*. 2007;13:3789–3795.
- [72] Trbusek M, Smardova J, Malcikova J, et al. Missense Mutations Located in Structural p53 DNA-Binding Motifs Are Associated With Extremely Poor Survival in Chronic Lymphocytic Leukemia. *JCO*. 2011;29:2703–2708.

- [73] Gurtner A, Starace G, Norelli G, et al. Mutant p53-induced up-regulation of mitogen-activated protein kinase kinase 3 contributes to gain of function. *J Biol Chem*. 2010;285:14160–14169.
- [74] Mraz M, Pospisilova S, Malinova K, et al. MicroRNAs in chronic lymphocytic leukemia pathogenesis and disease subtypes. *Leuk Lymphoma*. 2009;50:506–509.
- [75] Te Raa GD, Malčiková J, Mraz M, et al. Assessment of TP53 functionality in chronic lymphocytic leukaemia by different assays; an ERIC-wide approach. *Br J Haematol*. 2014;167:565–569.
- [76] Navrkalova V, Sebejova L, Zemanova J, et al. ATM mutations uniformly lead to ATM dysfunction in chronic lymphocytic leukemia: application of functional test using doxorubicin. *Haematologica*. 2013;98:1124–1131.
- [77] Cerna K, Oppelt J, Chochola V, et al. MicroRNA miR-34a downregulates FOXP1 during DNA damage response to limit BCR signalling in chronic lymphocytic leukaemia B cells. *Leukemia*. 2019;33:403–414.
- [78] Best OG, Gardiner AC, Majid A, et al. A novel functional assay using etoposide plus nutlin-3a detects and distinguishes between ATM and TP53 mutations in CLL. *Leukemia*. 2008;22:1456–1459.
- [79] Malcikova J, on behalf of the European Research Initiative on Chronic Lymphocytic Leukemia (ERIC) — TP53 network, Tausch E, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia—update on methodological approaches and results interpretation. *Leukemia*. 2018;32:1070–1080.
- [80] Campo E, Cymbalista F, Ghia P, et al. TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. *Haematologica*. 2018;103:1956–1968.
- [81] Pavlova S, Smardova J, Tom N, et al. Detection and Functional Analysis of TP53 Mutations in CLL. *Methods Mol Biol*. 2019;1881:63–81.
- [82] Zenz T, Krober A, Scherer K, et al. Monoallelic TP53 inactivation is associated with poor prognosis in chronic lymphocytic leukemia: results from a detailed genetic characterization with long-term follow-up. *Blood*. 2008;112:3322–3329.
- [83] Rossi D, Khiabani H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. *Blood*. 2014;123:2139–2147.
- [84] Rossi D, Cerri M, Deambrogi C, et al. The Prognostic Value of TP53 Mutations in Chronic Lymphocytic Leukemia Is Independent of Del17p13: Implications for Overall Survival and Chemorefractoriness. *Clinical Cancer Research*. 2009;15:995–1004.
- [85] Malcikova J, Stano-Kozubik K, Tichy B, et al. Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia. *Leukemia*. 2015;29:877–885.
- [86] Cutrona G, Tripodo C, Matis S, et al. Microenvironmental regulation of the IL-23R/IL-23 axis overrides chronic lymphocytic leukemia indolence. *Sci Transl Med*. 2018;10.

- [87] Dicker F, Schnittger S, Haferlach T, et al. Immunostimulatory oligonucleotide-induced metaphase cytogenetics detect chromosomal aberrations in 80% of CLL patients: A study of 132 CLL cases with correlation to FISH, IgVH status, and CD38 expression. *Blood*. 2006;108:3152–3160.
- [88] Rigolin GM, Cavallari M, Quaglia FM, et al. In CLL, comorbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI. *Blood*. 2017;129:3495–3498.
- [89] Baliakas P, Jeromin S, Iskas M, et al. Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. *Blood*. 2019;133:1205–1216.
- [90] Rossi D, Spina V, Gaidano G. Biology and treatment of Richter syndrome. *Blood*. 2018;131:2761–2772.
- [91] Anderson MA, Tam C, Lew TE, et al. Clinicopathological features and outcomes of progression of CLL on the BCL2 inhibitor venetoclax. *Blood*. 2017;129:3362–3370.
- [92] Filip D, Mraz M. The role of MYC in the transformation and aggressiveness of “indolent” B-cell malignancies. *Leuk Lymphoma*. 2020;61:510–524.
- [93] Zenz T, Eichhorst B, Busch R, et al. TP53 mutation and survival in chronic lymphocytic leukemia. *J Clin Oncol*. 2010;28:4473–4479.
- [94] Condoluci A, Terzi di Bergamo L, Langerbeins P, et al. International prognostic score for asymptomatic early-stage chronic lymphocytic leukemia. *Blood*. 2020;135:1859–1869.
- [95] Best OG, Gardiner AC, Davis ZA, et al. A subset of Binet stage A CLL patients with TP53 abnormalities and mutated IGHV genes have stable disease. *Leukemia*. 2009;23:212–214.
- [96] Tam CS, Seymour JF. A predictive tool for early-stage CLL. *Blood*. 2020;135:1820–1821.
- [97] Mato AR, Barrientos JC, Ghosh N, et al. Prognostic Testing and Treatment Patterns in Chronic Lymphocytic Leukemia in the Era of Novel Targeted Therapies: Results From the informCLL Registry. *Clin Lymphoma Myeloma Leuk*. 2020;20:174-183.e3.
- [98] Ravandi F, O’Brien S. Alemtuzumab in CLL and other lymphoid neoplasms. *Cancer Invest*. 2006;24:718–725.
- [99] Gauthier J, Hirayama AV, Purushe J, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. *Blood*. 2020;135:1650–1660.
- [100] Kharfan-Dabaja MA, Kumar A, Hamadani M, et al. Clinical Practice Recommendations for Use of Allogeneic Hematopoietic Cell Transplantation in Chronic Lymphocytic Leukemia on Behalf of the Guidelines Committee of the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2016;22:2117–2125.

Legends to Figures

Figure 1.

Forest plots showing the benefit of BCR inhibitors, either alone or in combination, compared with chemotherapy or chemo-immunotherapies assessed by the risk of progressing (PFS) or dying (OS) in several clinical trials.

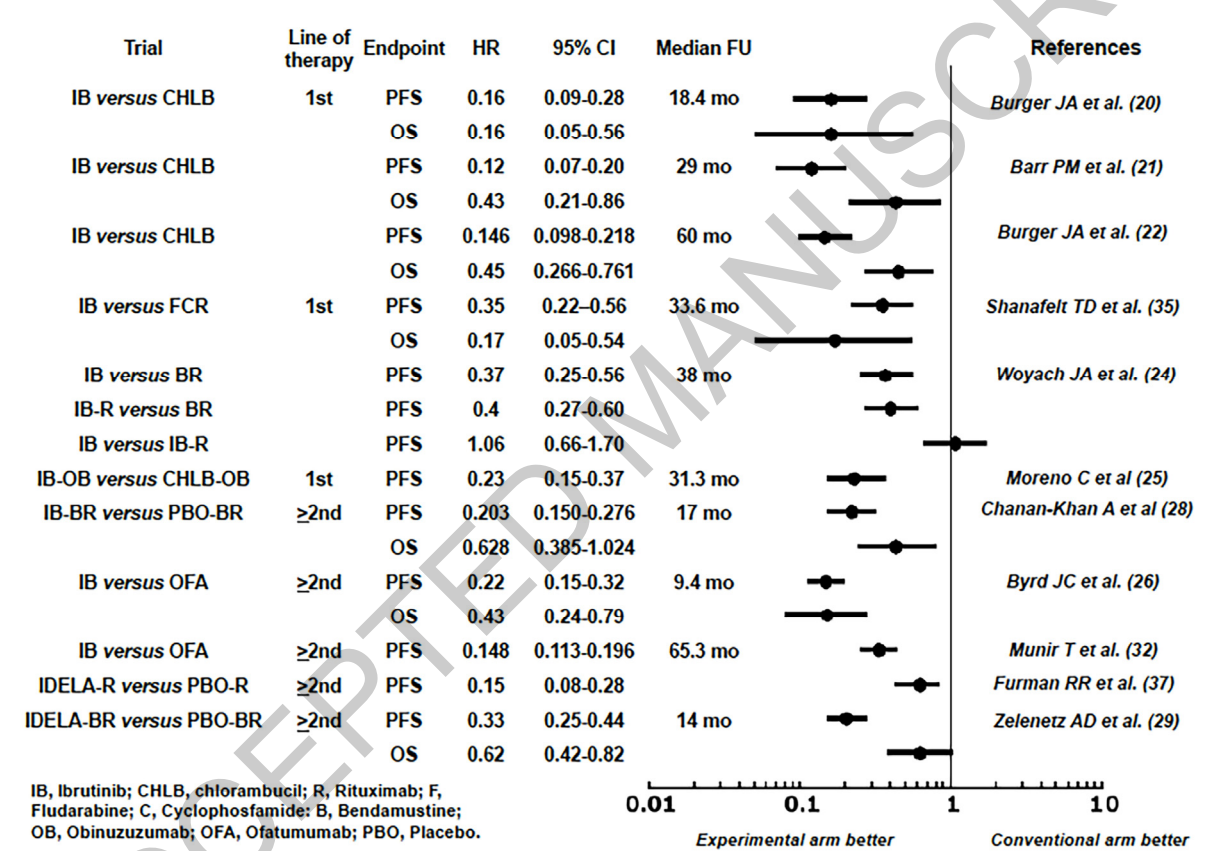


Figure 2.

Forest plots showing the benefit of BCR inhibitors in higher-risk groups in both treatment naïve- and RR-CLL settings, irrespective of the comparator arm, assessed by the risk of progressing (PFS) in several clinical trials.

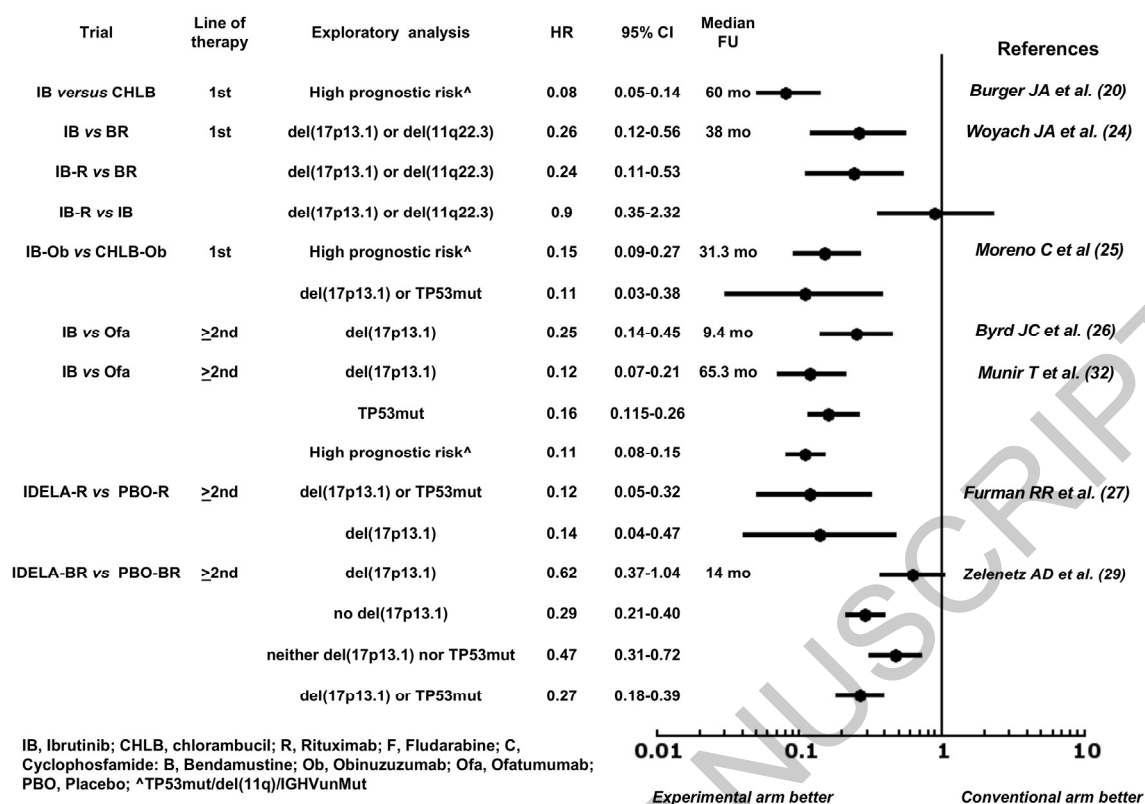


Figure 3.

Panel A). CLL TP53 mutations (missense, truncating and other mutations) from the cBioPortal online tool (The Cancer Genome Atlas consortium, TCGA, <http://www.cbioportal.org/>) are shown according to the frequency (Y axis) and the position of the amino acid hit (X axis). P53 functional domains are indicated as colored boxes. Panel B). Functional heterogeneity of mutants P53 may have variable consequences on CLL clinical phenotypes. Upon the detection of different kind of stress by specific sensors and the activation of signal transduction pathways, such those involving the ATM-kinase, P53 is post-translationally modified. In case of Wild-Type (WT) P53, this modification releases P53 from its negative regulator MDM2 and allows the transcription of P53 target genes responsible for the control of cell proliferation, apoptosis, DNA damage response, autophagy and gene regulation. In case of mutant P53 (MUT), the negative feedback loop with MDM2 is no longer active and mutant P53 proteins will accumulate in the cells regardless any modifications or activation. Moreover, TP53 mutations potentially generate a rainbow of mutant

P53 proteins that can differ for transactivation ability, dominant negative potential and gain of function (e.g. ability to interfere with other TFs including P53 family members). This may lead to a wide range of phenotypic diversity that could impact on important CLL clinical variables, such as tumor aggressiveness, chemo-resistance, and metastatic potential.

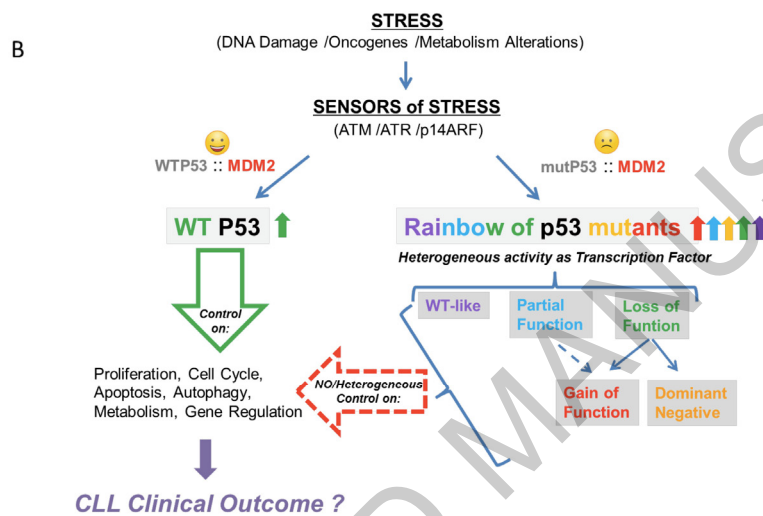
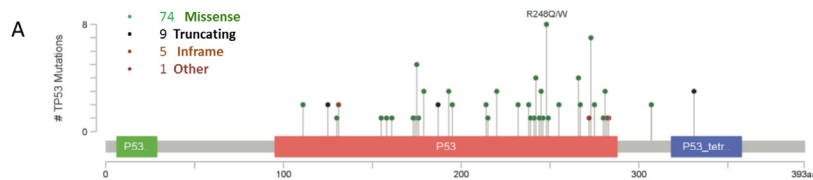


Table 1. Main clinical trials on single novel targeted drugs or drug combinations in CLL patients harboring del(17p) or TP53 mutation.

Drug	Trial	Treatment setting	Del(17p)	TP53 mutation	ORR	Median PFS in patients with TP53 dysfunction
Acalabrutinib	NCT02029443	RR	30% (59)	NR	100%	NR
	ASCEND Phase 3	RR	18% (155)	NR	NR	88% at 1 year
	ELEVATE- TN Phase 3	TN	8.9% (179)	10.6% (179)	NR	NR
Zanubrutinib	NCT02343120 Phase 1	TN/RR	19.1% (94)	NR	100%	NR
	SEQUOIA Phase 3	TN	100% (109)	NR	92.2%	NR
Tirabrutinib	NCT01659255 Phase 1	RR	NR	46.4% (28)	100%	NR
Nivolumab + Ibrutinib	NCT02329847 Phase 1-2	RR	100% ¹ (36)	NR	61%	NR
Pembrolizumab	NCT02332980 Phase 2	RR	38% (16)	NR	0%	NR
Duvelisib	DUO Phase 3	RR	21% (160)	20% (160)	NR	13.8 months
Umbralisib	NCT01767766 Phase 1	RR	NR	NR	NR	NR
Ublituximab + Ibrutinib	GENUINE Phase 3	RR	About 50% (59) 100% high risk	NR	78%	NR
Otlertuzumab + Bendamustine	NCT01188681 Phase 2	RR	5.7% (32)	5.7% (32)	50%	NR
Entospletinib	NCT01799889 Phase 2	RR	24.4% (41)	NR	33.3%	NR