TP53 Disruption in Chronic Lymphocytic Leukemia Under Ibrutinib: More is Worse?

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SUMMARY

Patients with chronic lymphocytic leukemia carrying a single *TP53* hit (chromosome 17p deletion or single *TP53* mutation) demonstrate excellent progression-free survival and overall survival on ibrutinib compared with cases harboring multiple *TP53* hits. Testing *TP53* deletion/mutation combining FISH and deep next-generation sequencing should be performed for a correct patient evaluation.

See related article by Brieghel et al., p. 4531

In this issue of *Clinical Cancer Research*, Brieghel and colleagues (1) took advantage of samples from patients with chronic lymphocytic leukemia (CLL) treated with ibrutinib in the context of a clinical trial and in real world, and investigated *TP53* disruption by a combination of FISH and next-generation (deep) sequencing (NGS) analyses. Their results suggest a different clinical behavior if *TP53* disruption is due to a single hit (i.e., either 17p deletion or a single *TP53* mutation, as occurring in the so-called single-hit CLL) or to multiple hits (i.e., simultaneous detection of 17p deletion and *TP53* mutation, or detection of more than one *TP53* mutation, as occurring in multi-hit CLL), the latter associated to significantly shorter overall survival intervals (1).

The *TP53* gene, encoding the transcription factor and tumor suppressor p53, is the most frequently mutated gene across cancer types, with a mutation rate of approximately 50% across all tumors. The *TP53* importance in oncology is underlined by the increased incidence of cancer among patients affected by the Li-Fraumeni syndrome caused by germline mutations in the *TP53* gene. Disruption of *TP53* gene can result from deletion at chromosome 17p13.1 (17p deletion) or gene mutations. *TP53* gene mutations are more frequently located in the DNA-binding domain, encoded by exons 4–8, although they can also be present throughout the entire coding region or may affect the splice sites (**Fig. 1A**). These mutations are missense mutations in the majority of cases, but also frame-shift, nonsense, and splicing mutations can be detected (reviewed in ref. 2).

As summarized in **Fig. 1B** (left), while single-hit CLL are characterized by the presence of either *TP53* deletion or a single *TP53* mutation, in multi-hit CLL both *TP53* deletion and *TP53* mutation (s), or more than one *TP53* mutations are found in the CLL cells populations. The techniques usually employed for *TP53* disruption detection, including those reported in Brieghel and colleagues (1), do not allow to dissect whether the multiple hits disrupting the *TP53* gene actually occur in the context of a single cell, or rather involve different CLL subclones (**Fig. 1B**, right), or are the result of a combination of both.

TP53 disruption represents the most important biomarker in CLL, always included in algorithms with proven prognostic relevance in the context of chemoimmunotherapy (CIT); moreover, its relevance as predictive factor is underscored by the fact that CIT is nowadays contraindicated for patients harboring *TP53* lesions at the time of therapeutic choice.

Even in the context of novel target therapies including ibrutinib, patients with CLL with *TP53* aberrations showed significantly shorter response duration compared with patients without these aberrations. In this regard, a long-term experience (median follow-up: 6 years) on the use of single-agent ibrutinib within the phase II PCYC-1102/1103 study showed that the presence of *TP53* aberrations, although carried out by investigating the 17p deletion only, negatively impact on prognosis in refractory/relapsed (R/R) CLL (3). In particular, the median progression-free survival and overall survival observed in R/R CLL cases with *TP53* aberration were shorter than those seen in other high-risk cytogenetic groups including CLL harboring the 11q deletion (3).

These data are in line with the results of a recent study reported by Ahn and colleagues (4). In this article, the NIH CLL group, analyzed data of patients with CLL treated with ibrutinib in phase II and III trials, through a combination of multivariable analysis and machine-learning algorithms. They confirmed the independent prognostic impact of *TP53* aberration (concurrent *TP53* mutation and 17p deletion, or either one of these lesions) together with prior treatment, elevated levels of β2-microglobulin and lactate dehydrogenase. Combining these four factors, the authors proposed a scoring system which allowed segregating patients with CLL treated with ibrutinib into discrete subgroups with significantly different prognosis (4).

However, both these studies (3, 4) consider *TP53* aberrations as a dichotomized prognosticator (either absent or present), lacking to evaluate the prognostic impact of single versus multiple *TP53* hits. Herein, Brieghel and colleagues made a point that patients treated with single-agent ibrutinib carrying only a single *TP53* hit experienced excellent progression-free survival and overall survival on ibrutinib compared with those with multiple *TP53* hits (1).

Similar results were reported in a recent retrospective analysis generated within the framework of an institutional Italian initiative on CLL (Campus CLL) on 525 R/R CLL cases undergoing targeted therapies (ibrutinib, rituximab-idelalisib, and venetoclax). In this study, the simultaneous presence of 17p deletion and *TP53* mutation negatively impacted on overall survival of patients with CLL treated

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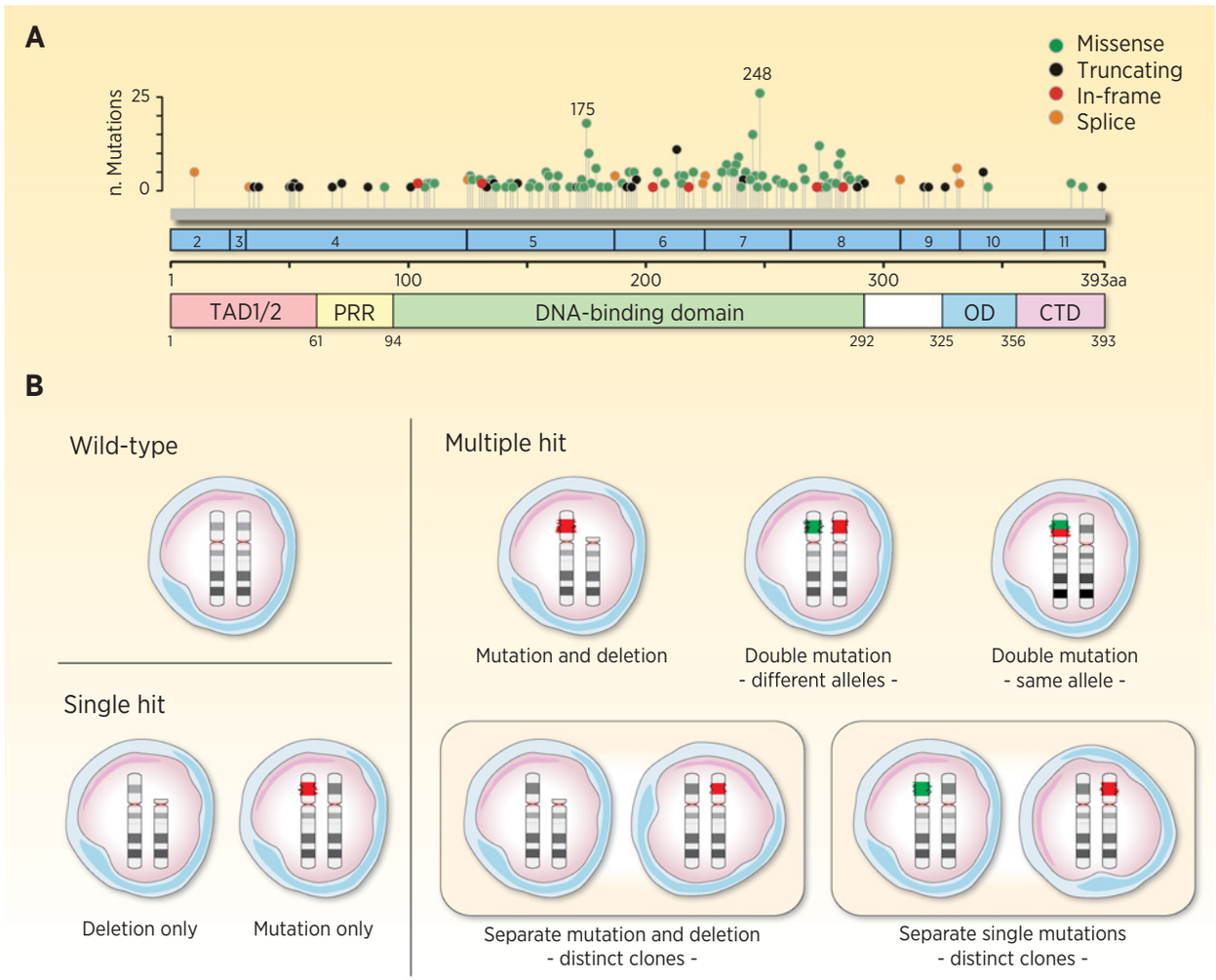

Figure 1.

TP53 organization and aberrations. **A,** *TP53* gene distribution of mutations by codon. Needle plot graph of 384 *TP53* mutations along the TP53 coding sequence. Data were collected from cBioPortal (http://www.cbioportal.org/) selecting all "Lymphoid Neoplasm" samples (n = 5,871) and filtering for *TP53* gene. The vertical bars indicate the number of mutation. Sequences referring to the transactivation domain (TAD), the proline-rich region (PRR), the DNA-binding domain, the tetramerization domain (OD), and the C-Terminal domain (CTD) of the p53 protein are reported. Horizontal bar refers to amino acid numbering of p53 protein. **B,** Loss of wild-type p53 function can occur as a result of 17p deletion and/or *TP53* mutations in different combinations. Single hit refers to the presence of a 17p deletion or a single *TP53* mutation in a mutually exclusive way. Multiple hit refers to the concomitant presence of deletion and mutations or multiple mutations.

with new drugs as salvage therapy compared with *TP53*-mutated/not-17p-deleted or *TP53* wild-type/17p-deleted cases (5). Interestingly, the latter groups, comparable with the single-hit CLL of Brieghel and colleagues (1), showed not statistically different overall survivals respect to the control group (i.e., patients which were both *TP53* wild type and not-17p-deleted), a comparison not addressed by Brieghel and colleagues (1).

Substantial differences can be noted between the design of this study (5), and the study by Brieghel and colleagues (1), including the pure retrospective nature of the former, and the fact that, in the former, *TP53* mutation analyses were performed, either by Sanger or NGS, without computing low burden mutations (1, 5). Despite these discrepancies, their take-home message turns out strikingly similar, that is, targeted therapies, ibrutinib in particular, may overcome the negative impact of a single-hit *TP53* disruption, but not that due to the simultaneous presence of *TP53* mutations and 17p deletion (1, 5).

Altogether, it appears mandatory to interrogate patients undergoing ibrutinib therapy by simultaneously testing *TP53* deletion and mutation to dissect single-hit versus double-hit *TP53*-disrupted patients, thus improving their risk stratification, and eventually prioritize the CLL with multiple *TP53* hits toward clinical trials testing novel treatment approaches or other (combination) therapies.

In this scenario, the choice of the technical approach for *TP53* mutation evaluation is not irrelevant; in particular, a NGS approach utilized in conjunction with a robust bioinformatics pipeline allowing the calling of mutations with low variant allele frequency (VAF, e.g., below 1% VAF) should be always preferred if the technical quality is guaranteed.

Because the coexistence of *TP53* deletion and mutation in CLL is quite common (2), it is reasonable that in cases classified single-hit by 17p deletion, the absence of *TP53* mutations is real only if the limit of detection is particularly low [e.g., less than 1% VAF, as in Brieghel and colleagues (1)]. On the other hand, in cases classified as single hit by the presence of a single *TP53* mutation (especially if at low VAF), it cannot be excluded that some minor subclones harboring 17p deletion might be present at a percentage below the detection limit of FISH (usually 5%–10%).

Therefore, it is conceivable that the group of "single-hit CLL by *TP53* deletion" and the group of "single-hit CLL by *TP53* mutation" would not be equivalent, with the second one being most probably "contaminated" with cases bearing both *TP53* mutation and deletion. In keeping with this line of reasoning, the group of single-hit CLL achieving durable response by ibrutinib treatment is almost exclusively composed by "single-hit by *TP53* deletion" cases (1). An area of uncertainty remains especially on the good clinical course under ibrutinib of the subset of "single-hit by *TP53* mutation" CLL. More in general, confirmation on larger cohorts is needed, also in the light of a recent observation that clonal expansion of low-burden *TP53* mutations does not occur in CLL treated with targeted therapy (6).

Authors' Disclosures

No disclosures were reported.

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