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TP53 disruption as a risk factor in the era of targeted therapies: a multicenter retrospective study of 525 chronic lymphocytic leukemia cases

Running title: *TP53* in CLL patients treated with new drugs

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The addition of the anti CD20 monoclonal antibody (mAb) to fludarabine-cyclophosphamide or bendamustine has led to a remarkable improvement in both progression-free (PFS) and overall survival (OS) of young and elderly CLL patients with favorable prognostic markers (1). However, chemoimmunotherapy (CIT) is ineffective in cases with *TP53* gene disruption, i.e. the deletion of the short arm of chromosome 17 [del(17p)] and/or *TP53* mutations (*TP53*mut) (2).

Ibrutinib (IB) and idelalisib (IDELA), which inhibit the signaling pathway initiated by the B-cell antigen receptor (BCR), or venetoclax (VEN), which facilitates cell apoptosis, have contributed to overcoming the modest efficacy of CIT in patients with del(17p) and/or *TP53*mut (3). However, these patients' treatment still represents a challenge, particularly for long-term leukemia control (4).

Recently, an evaluation of the potential risk factors for OS has been carried out in a pooled, retrospective cohort of a considerable number of RR CLL patients treated with either CIT or with IB, IDELA-R, or VEN in the randomized phase 3 trials (5). The derived BALL score was based on four accessible markers, i.e., β2-microglobulin (β2-M) and lactic dehydrogenase (LDH) serum levels, anemia, and time from the start of the last therapy. The BALL score, recently refined for IB or IDELA (6, 7), proved capable of prognostically segregating RR CLL patients. Notably, del(17p) failed to be classified as a risk factor for OS in this context, although *TP53* inactivation was determined only by measuring del(17p), while the presence of *TP53*mut was not assessed. Therefore, *TP53* disruption may have gone undetected, rendering the interpretation of the data somewhat complex. Recently, a 4-factor model, capable of identifying patients at increased risk of IB failure, was proposed and included *TP53* dysfunction measured by FISH and molecular analysis (8). The above prognostic model was validated in a real-world cohort (9).

The present independent, retrospective, multicenter analysis of patients treated with IB (432 cases), IDELA (85), or VEN (8 cases) in the current clinical practice aimed to assess the value of the prognostic parameters set in previous studies and investigate the extent of *TP53* disruption required to confer a dismal prognosis despite treatment with the new drugs.

Supplementary Table 1 summarizes the main patients' features. The majority of cases were Binet B and C (89.5%), 65.5% were male (65.5%), and the median age was 70.8 years (37.2-88.7). The median range of previous therapies was 2 (range 1-9). The *TP53* dysfunction in our cohort was detected in 201 cases (38.3%).

One hundred and fourteen patients died since starting the new therapies after a median follow-up of 1.7 years. Initially, Cox univariate analysis was used to evaluate the relationships of some predictors and OS. β2-M, LDH levels, anemia, time from the start of the last therapy, Binet stage, and the number of previous

treatments were found to be associated with OS (Supplementary Table 2). Moreover, a significantly shorter OS was observed for cases carrying del(17p) (Hazard Ratio [HR]=1.5, 95% CI 1.1–2.3, *P*=0.024) or *TP53*mut (HR=1.5, 95% CI 1.1–2.1, *P*=0.049) (Figure 1AB and Supplementary Table 2).

To assess the independent relationship between *TP53* disruption and OS, we constructed different Cox multivariate models in which either del(17p) (model 1) or *TP53*mut (model 2) were introduced (Supplementary Table 3). Notably, while del(17p) showed an independent relationship with OS (Supplementary Table 3 model 1), *TP53*mut failed to represent a significant independent risk factor (Supplementary Table 3 model 2). Therefore, for a more precise risk assessment, CLL patients were stratified into four groups, which included cases with *TP53*wt/no-del(17p) (324, 61.7%), *TP53*mut/del(17p) (90, 17.1%), *TP53*mut/no-del(17p) (62, 11.8%), and *TP53*wt/del(17p) (49, 9.3%). OS was significantly shorter in *TP53*mut/del(17p) cases (HR=1.8, 95% CI 1.2–2.8, *P*=0.004), while the Kaplan-Meier curves of both the *TP53*mut/no-del(17p) (HR=0.9, 95% CI 0.4–1.7, *P*=0.7) and of the *TP53*wt/del(17p) (HR=0.8, 95% CI 0.4–1.6, *P*=0.5) group overlapped with that of the *TP53*wt/no-del(17p) group (Figure 1C). Overall, these data demonstrated that simultaneous investigation of both del(17p) and *TP53* led to a more accurate prognostic stratification of patients with both lesions compared with those with a single lesion or no lesions. A sub-analysis of the IB patients' cohort showed similar results (Supplementary Figure 1).

The simultaneous presence of *TP53*mut and del(17p) were subsequently introduced into a further Cox multivariate analysis (Supplementary Table 3 model 3). *TP53*mut/del(17p), remained independently related with OS (HR=1.6, 95% CI 1.1–2.5, *P*=0.026), together with LDH (HR=1.6 95% CI 1.1-2.4, P=0.013), anemia (HR=2.5, 95% CI 1.6–3.9, *P*<0.0001), and previous therapy lines (HR=2.0, 95% CI 1.2–3.2, *P*=0.004).

Finally, facing the different models in which *TP53* alterations were differently defined, i.e., as del(17p) alone (Supplementary Table 3 model 1) and del(17p)/*TP53*mut (Supplementary Table 3 model 3), the Akaike information criterion (AIC) weights indicated that model 3 had a 54% chance to be the best prognostic model for OS, thus supporting the concept that *TP53*mut or del(17p) should not be considered alternative markers but used in parallel to obtain a more precise prognostic evaluation.

However, a limitation of our study is represented by the retrospective nature of *TP53*mut and del(17p) evaluations. A further weakness is represented by the low number of cases treated with VEN. Confirmation studies incorporating a

significantly higher number of patients treated with VEN are warranted to confirm these findings.

Regrettably, data on CK were available in 32.8% (172/525) of cases in our cohort. Thus, CK analysis presents some constraints due to its limited availability in the real-world setting, possibly due to laboratory hitches and the tendency to evaluate cytogenetic, mainly in research centers. However, given the similarities between the cohort with CK data available and the remaining CLL cohort (Supplementary Table 4), including a similar prognostic impact of the *TP53*mut/del(17p) genotype combinations (Supplementary Figure 2), we found of interest to explore the prognostic significance of CK as in association with *TP53* disruption.

CK was detected in 19.7% (34/172) of patients, and it was associated with a significantly higher death risk (HR=2.6; 95% CI 1.2-5.4, P=0.012). The relationship between del(17p), *TP53*mut, and CK is described in Supplementary Table 5. Among the 172 cases, 11 patients (6.4%) showed a triple alteration, while 10 (5.8%) cases showed a CK without any *TP53* aberration. Moreover, 9 (2.3%) and 4 (2.3%) cases presented CK associated with del(17p) and *TP53*mut only, respectively. Overall, CK was significantly linked with del(17p) (20/44 versus 14/128, P<0.0001) and *TP53*mut (15/47 versus 19/125, P=0.05).

Subjects with both a del(17p)/*TP53*mut genotype and CK experienced a significantly higher risk of death (HR=5.5; 95% CI 1.98-15.4, P=0.001) compared with triple-negative cases (i.e., devoid of CK and del(17p) and *TP53*mut) here combined with cases without CK but bearing del(17p) or *TP53*mut only (Supplementary Figure 3).

In contrast, the remaining groups comprising the single CK (i.e., CK without TP53 disruption) as well as CK combined with the del(17p)/*TP53*WT category and the no-del(17p)/*TP53*mut category failed to show any prognostic power in this analysis (Supplementary Figure 3). Notably, cases bearing the del(17p)/*TP53*mut genotype without CK aberration also showed a higher risk of death, of borderline significance compared to the control group (HR=2.8; 95% CI 0.99-7.7, P=0.052) (Supplementary Figure 3).

With the limitation of the relatively low number of cases analyzed, the above-described results indicated that CLL patients with a CK experienced a shorter OS than those free of the cytogenetic aberration. However, the prognosis of CK seems to be dependent on a complete *TP53* alteration. Indeed, cases with both a del(17p)/*TP53*mut genotype and CK have a significantly higher death risk than triple-negative cases, assuming that *TP53*/del(17p) alterations may have extra prognostic value in patients with CK. Nevertheless, these conclusions should be considered with caution and deserve further investigation.

In conclusion, this retrospective, multicenter study indicates the prognostic relevance of *TP53* disruption assessment in patients with RR CLL undergoing treatment with the new molecularly targeted therapies. This assessment is best achieved when both *TP53* mutations and del(17p) are measured, implying that the evaluation of a severe *TP53* impairment allegedly involving both alleles is necessary for the setting of RR CLL treated with new drugs.

Contributions:
F.M., G.D.P., F.R.M., A.G.R., A.C., S.G., D.R., F.D.R., A.C., G.G., A.P., L.V., R.F., M.F., V.G. and M.G. designed the study, analyzed and interpreted data, and wrote the manuscript; F.M. G.T., G.D., and M.G. performed statistical analysis; A.G.R., A.Z., I.D.G., R.B., A.N., G.F., and G.C. performed laboratory tests; G.R., P.S., L.L., M.C., Y.H., M.V., R.M., A.C., A.Co., R.Mo., D.P., G.L., U.C., I.S., F.M.R., V.F., H.J., J.O., E.V., A.R., I.A., A.V., and A.B. provided the patients and collected clinical data. All authors gave final approval for the manuscript.

Conflict-of-interest disclosure:
Nothing to disclose

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request

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Figure legends

Figure 1. Overall survival stratified according to **(A)** del(17p), **(B)** *TP53* mutation, and **(C)** del(17p) and/or *TP53* mutation in CLL subgroups treated with either ibrutinib or idelalisib plus rituximab or venetoclax in a real-world setting.

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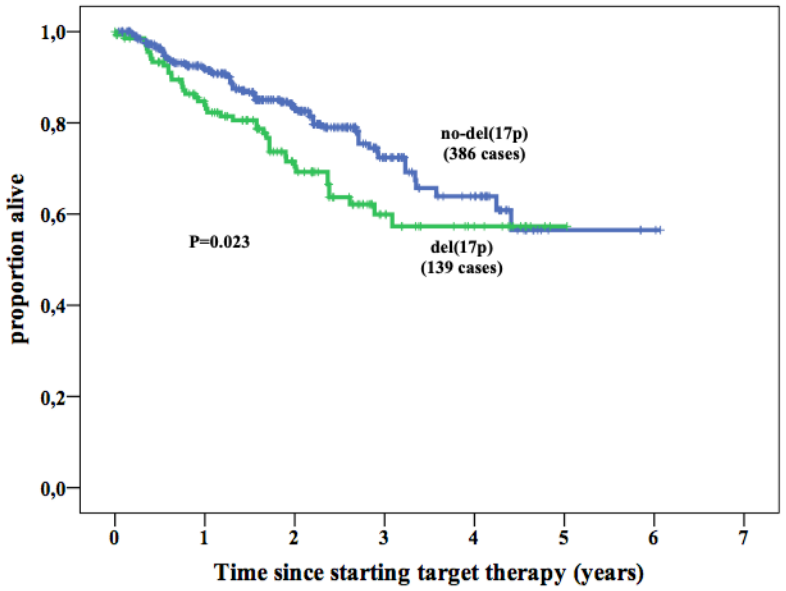
A



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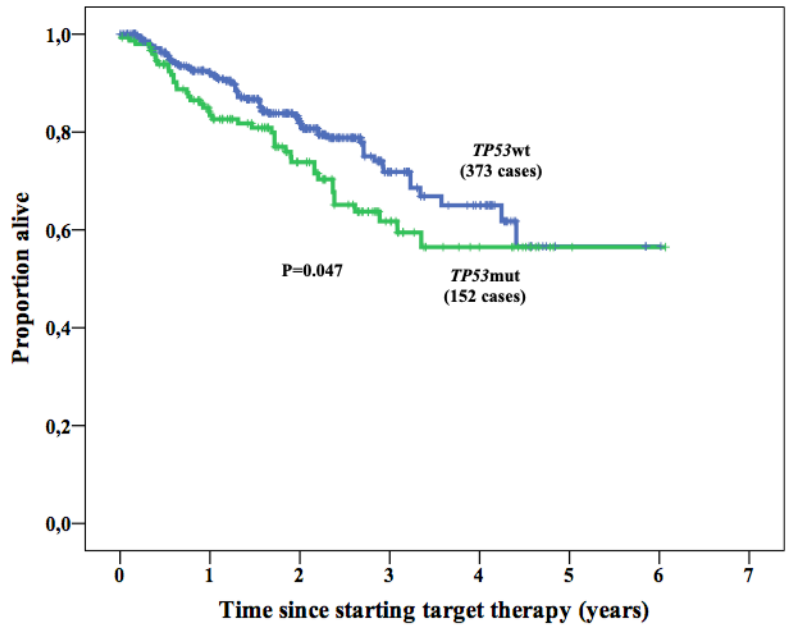

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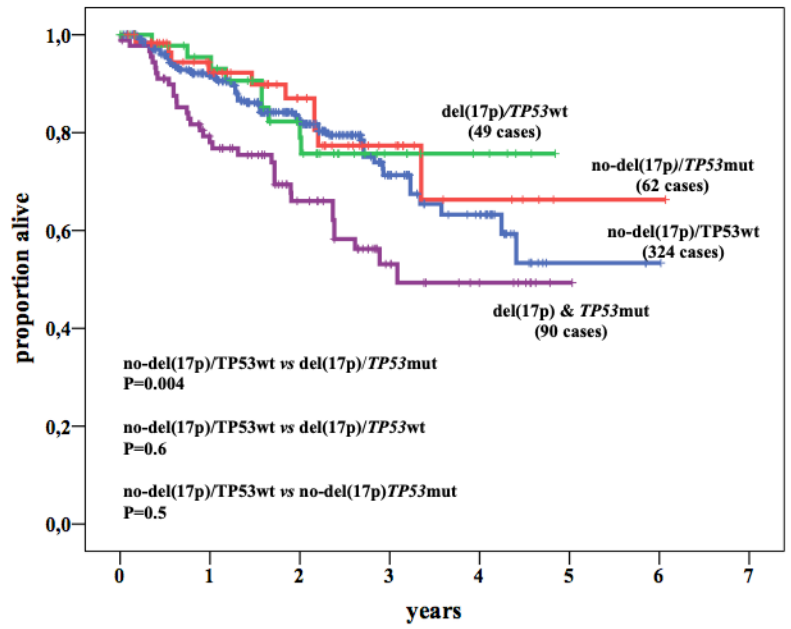

Figure 1.

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