Immunoglobulin heavy chain variable region gene (IGHV) and prediction of time to first treatment (TTFT) in patients with chronic lymphocytic leukemia (CLL): mutational load or mutational status? Analysis of 1003 cases.

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To the Editor:

The mutation status of the immunoglobulin heavy chain variable region gene (IGHV) is a well-recognized prognostic indicator in patients with chronic lymphocytic leukemia (CLL). The occurrence of IGHV somatic hypermutations (SHM) in CLL cells and the predictive value of its presence or absence have been recognized in patients with CLL for roughly 20 years. The cut-off value of a 2% deviation from (or ≤98% identity with) the corresponding IGHV germline sequence has been used in the initial studies to distinguish ‘mutated’ (M-CLL) from ‘unmutated’ (UM-CLL) cases. Based on this cut-off value, it was determined that unmutated cases had a significantly shorter time to first treatment (TTFT) and outcome. This cut-off value was selected based on the consideration that differences of IGHV genes from the germline of up to 2% might have been related also to allele polymorphisms within the immunoglobulin loci. These somewhat conservative parameters were also suggested by the relative scanty number of sequence data available at that time. The frequency of IGHV gene polymorphisms was reported to be 0.0005% by a subsequent study suggesting that a cut-off value of 99.99% homology to the germline would be sufficient to distinguish truly unmutated from mutated cases. Some of the subsequent studies indicated that a 2% cut-off value was adequate to distinguish two subgroups of patients with a different prognostic expectation while others, virtually in equal numbers, proposed different cut-off values. Despite these discrepancies, the 2% cut-off value is generally adopted in the current clinical practice.

Recently, Jain et al demonstrated in patients treated with the fludarabine, cyclophosphamide and rituximab (FCR) regimen that their subsequent course and survival was greatly influenced by the amount of mutations present in the IGHV gene segment. Thus, the higher levels of mutation corresponded to a better outcome, pointing to the potential prognostic relevance of the percentage of IGHV gene mutation as a continuous rather than a dichotomized variable. The particular study design adopted by Jain et al raises the question of whether their criteria for mutation evaluation...
have a universal value or apply only to patients treated with that particular protocol, as pointed out by Gocke and Richardson in a recent commentary.\textsuperscript{14}

We assessed the prognostic significance of the extent of SHM on the TTFT in a large cohort of 467 newly diagnosed Binet stage A patients from several Italian institutions who were prospectively enrolled in the O-CLL protocol (training cohort, median follow-up 84 months; clinicaltrial.gov identifier: 115 NCT00917540). The same analysis was performed on a Mayo Clinic validation cohort of 536 cases (median follow-up of 78 months). Further details and data analyses are described in the Supplementary Appendix.

Among the 141 cases classified as UM-CLL in the O-CLL protocol based on the \( \leq 2\% \) mutation cut-off point, 94 (67\%) did not present any mutation (truly unmutated cases), 31 (22\%) presented a 1\% mutation and 16 (11\%) presented a 2\% mutation (Figure 1A). No significant differences were noted in the TTFT of these three groups of patients (Figure 1B). The remaining 326 cases, classified as mutated in the O-CLL protocol, had a more even distribution among the different mutation categories according to criteria similar to those employed by Jain et al. (Figure 1A). However, no significant differences were observed in terms of risk to be treated in the groups of mutated cases stratified according to the extent of SHM (Figure 1C). Similar results were observed in the 536 cases of the Mayo Clinic cohort (Figures 1D-F), which provided a further validation of the O-CLL data. Thus, a \( \geq 98\% \) homology between the \( IGHV \) genes of CLL clones and their normal counterpart represents a valid discriminant to distinguish groups with a different prognostic likelihood, without a need for a further stratification based upon the extent of \( IGHV \) gene mutation.

With the aim of further validating this result, a Cox multivariable analysis was performed introducing into the model a minimal number of variables which represented a significant discriminant for TTFT in the Cox univariate analysis - i.e., Rai stage (H.R. 2.5, C.I. 2.0-3.2, p<0.0001), \( \beta 2\)M (H.R. 2.3, C.I. 1.8-2.9, p<0.0001) - together with the extent of \( IGHV \) mutation.
leading to the stratification of CLL cases into different groups. This analysis was carried out on the 870 cases in which all the 3 variables were available in both the O-CLL and Mayo Clinic cohorts. Notably, in this minimalistic multivariable model, only cases with a $IGHV$ mutational load of 0%, >0 to <1% and 1 to $\leq$2% maintained an independent prognostic impact on TTFT, together with $\beta_2M$ and Rai stage (Figure 2), while the remaining patient groups, obtained by stratification according to the extent of SHM, failed to maintain an independent prognostic relevance. The same analysis carried out by a bootstrapping Cox regression model provided similar results (Supplementary Table 1).

In conclusion, this study indicates that a $\leq$2% $IGHV$ gene mutation represents a suitable cut-off level capable of discriminating cases with a different prognosis. Hence, the classic distinction of mutated and unmutated cases may be adequate in the current clinical practice. Furthermore, it appears that CLL cases with values below the set cut-off point truly represent the vast majority of unmutated cases. Finally, stratification methods based upon the extent of the $IGHV$ mutation do not seem [superfluous at least] to add in determining the chance for a given patient to be treated, although it remains to be seen whether such stratification may be needed for a more accurate prognostication in special clinical situations as those reported by Jain et al.13.
Figure legends

Figure 1. Frequency of cases grouped by percentage of *IGHV* mutations in the O-CLL training cohort (A); Kaplan-Meier curves depicted by *IGHV* mutations equal 0% (a), >0% -1% (b), >1%-2% (c), and >2% (d) in the training O-CLL cohort (B); Cox univariate analysis according to different percentages of *IGHV* mutations in the O-CLL training cohort. HR, hazard ratio; CI, confidence interval. The bars represent 95% confidence intervals. *IGHV>*9 was considered as reference value (HR=1) (C); Cases grouped by percentage of *IGHV* mutations in the Mayo Clinic validation cohort (D); Kaplan-Meier curves showing *IGHV* mutations equal 0% (a), >0% and ≤1% (b), >1% to ≤2% (c), and >2% (d) in the Mayo Clinic validation cohort (E); Cox univariate analysis according to different percentages of *IGHV* mutations in the Mayo Clinic validation cohort. HR, hazard ratio; CI, confidence interval. *IGHV>*9 was considered as reference value. (HR=1) (F).

Figure 2. Cox multivariate analysis in which Rai stage, β2M and the percentage of *IGHV* mutations combining the O-CLL training and Mayo Clinic validation cohorts, forced in the model. HR, hazard ratio; CI, confidence interval. *IGHV>*9 was considered as reference value. (HR=1).
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