bjh commentary

Immunoglobulin heavy variable somatic hyper mutation status in chronic lymphocytic leukaemia: on the threshold of a new era?

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Since the introduction of the *IGHV* gene somatic hypermutation (SHM) status as prognostic marker in chronic lymphocytic leukaemia (CLL) patients in the two 1999 landmark publications from the Stevenson and Chiorazzi groups (Damle *et al.*, 1999; Hamblin *et al.*, 1999), its value has been consistently confirmed in virtually all CLL cohorts analysed thus far. To distinguish good from poor prognostic CLL subgroups, a threshold of 98% identity to the closest germline (=unrearranged) immunoglobulin heavy variable (*IGHV*) gene was introduced. CLL cases showing a clonally rearranged *IGHV* gene with <98% identity to the closest *IGHV* gene (classified as IG-mutated CLL, M-CLL) constitute the more favourable group, while those with ≥98% identity (unmutated CLL, U-CLL) represent the adverse group.

Notably, the 98% threshold is purely mathematical rather than biological, considering that even a single SHM (representing far less than the 2% deviation from germline) could have a major impact on antigen specificity and affinity. Nevertheless, from a haematological perspective, the 98% threshold is useful to define CLL subgroups with statistically distinct outcomes. That said, the 98% threshold has been challenged, although the published data are somewhat conflicting (Davis *et al.*, 2016; Jain *et al.*, 2018; Morabito *et al.*, 2018). In particular, the group of so-called borderline CLL cases (i.e. 97–97·9% *IGHV* identity) has raised interpretation issues as to whether these would be *bona fide* M-CLL cases or not (Davis *et al.*, 2016). Reflecting this uncertainty, the most recent ERIC (European Research Initiative on CLL) guidelines on *IGHV* gene SHM status interpretation consider this borderline group as a category that should be reported with caution (Rosenquist *et al.*, 2017).

In this issue of the *British Journal of Haematology*, a retrospective analysis from the Italian CLL group addresses the prognostic impact of borderline *IGHV* SHM status (Raponi *et al.*, 2020). *IGHV* data from two large cohorts of untreated CLL patients were updated in line with current ERIC guidelines (i.e. repeat analysis with an *IGHV* leader protocol, if required) and using the latest IMGT (ImMunoGeneTics) version for annotation. In doing so, the most accurate definition of percentage of *IGHV* gene identity and SHM determination for the prognostic evaluation could be achieved, still resulting in almost 10% shifts in categories (borderline vs. M-CLL vs. U-CLL), highlighting the critical importance of robust analytical standards. Subsequent evaluation showed that the time-to-first treatment (TFT) of borderline cases appeared very similar to M-CLL but was clearly different from U-CLL, while the same held true even in newly diagnosed and Binet stage A CLL, leading the authors to conclude that borderline cases can be considered favourable-prognostic, albeit with a single striking exception represented by stereotyped subset #2. The latter is very relevant, given that the subset #2 is renowned for its adverse prognosis and is known to be enriched in borderline CLL cases (Baliakas, 2015). Moreover, it raises the question as to whether this is restricted to subset #2 or whether other, as of now non-disclosed, stereotyped subsets with adverse prognosis might also be over-represented in this borderline group, which is especially relevant considering emerging evidence for the existence of 'satellites' to subset #2, i.e. subgroups of cases with similar immunogenetic and clinicobiological features [Gemenetzi *et al.* iwCLL (International Workshop on CLL) 2019]. Hence, arguably, a 'compartmentalised' approach may assist in cleaning up the borderline group to enrich for truly indolent cases, eventually refining risk stratification. Multi-centre studies from collaborative groups collecting enough CLL borderline cases should reveal the impact of more stereotypic subsets being present in the borderline group. More broadly, these findings emphasise the importance of accurate *IGH* gene

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sequence analysis and interpretation in CLL, a fact acknowledged by the inclusion of *IGHV* gene SHM-testing in the list of mandatory tests for all CLL patients requiring front-line treatment (Hallek *et al.*, 2018); as well as the recent decisions/proposals by national CLL study groups [e.g. FILO (French Innovative Leukemia Organisation), GCLLSG (German CLL Study Group)] that subset #2 membership should be used for risk stratification of patients in clinical trials (Fischer *et al.*, 2016).

Raponi *et al.* also made another intriguing observation that perhaps not only the borderline group (97·0–97·9% *IGHV* identity) is relevant for further consideration, but equally so the 98·0–98·9% *IGHV* identity group, which is sometimes also referred to as a borderline group by some authors. In their experience, this CLL group appears indistinguishable from the true borderline group, yet shows a better TFT than the 99–100% group (Raponi *et al.*, 2020). Even though this seems to conflict with other published data (Davis *et al.*, 2016), the observation is interesting and highlights the need for further investigation.

Taken together, these considerations elicit the question as to whether the *IGHV* gene SHM status should be considered as a binary or a continuous marker – in other words, whether using one threshold (98%) is accurate for all situations or whether, perhaps in context-dependent fashion, this parameter should be interpreted as a continuous variable (Jain *et al.*, 2018). This is a most relevant question for CLL today, for at least two important reasons: i) *IGHV* gene SHM status is not only a prognostic factor any more, but also predictive of responses to individual treatments, and so the required accuracy for the individual patient is more demanding; ii) with the introduction of NGS-based strategies for *IGHV* SHM analysis, it remains to be determined if current thresholds for non-NGS strategies are still valid. Twenty years after the first description, *IGHV* SHM analysis is thus still highly valid, and perhaps more relevant than ever before.

References

Damle, R.N., Wasil, T., Fais, F., Ghiotto, F., Valetto, A., Allen, S.L., Buchbinder, A., Budman, D., Dittmar, K., Kolitz, J., Lichtman, S.M., Schulman, P., Vinciguerra, V.P., Rai, K.R., Ferrarini, M. & Chiorazzi, N. (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*, **94**, 1840–1847.

Davis, Z., Forconi, F., Parker, A., Gardiner, A., Thomas, P., Catovsky, D., Rose-Zerilli, M., Strefford, J.C. & Oscier, D. (2016) The outcome of chronic lymphocytic leukaemia patients with 97% IGHV gene identity to germline is distinct from cases with <97% identity and similar to those with 98% identity. *British Journal of Haematology*, **173**, 127–136.

Fischer, K., Bahlo, J., Fink, A.M., Goede, V., Herling, C.D., Cramer, P., Langerbeins, P., von Tresckow, J., Engelke, A., Maurer, C., Kovacs, G., Herling, M., Tausch, E., Kreuzer, K.A., Eichhorst, B., Böttcher, S., Seymour, J.F., Ghia, P., Marlton, P., Kneba, M., Wendtner, C.M., Döhner, H., Stilgenbauer, S. & Hallek, M. (2016) Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*, **127**, 208–215.

Hallek, M., Cheson, B.D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., Hillmen, P., Keating, M., Montserrat, E., Chiorazzi, N., Stilgenbauer, S., Rai, K.R., Byrd, J.C., Eichhorst, B., O'Brien, S., Robak, T., Seymour, J.F. & Kipps, T.J. (2018) iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*, **131**, 2745–2760.

Hamblin, T.J., Davis, Z., Gardiner, A., Oscier, D.G. & Stevenson, F.K. (1999) Unmutated Ig V (H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, **94**, 1848–1854.

Jain, P., Nogueras González, G.M., Kanagal-Shamanna, R., Rozovski, U., Sarwari, N., Tam, C., Wierda, W.G., Thompson, P.A., Jain, N., Luthra, R., Quesada, A., Sanchez-Petitto, G., Ferrajoli, A., Burger, J., Kantarjian, H., Cortes, J., O'Brien, S., Keating, M.J. & Estrov, Z. (2018) The absolute percent deviation of IGHV mutation rather than a 98% cut-off predicts survival of chronic lymphocytic leukaemia patients treated with fludarabine, cyclophosphamide and rituximab. *British Journal of Haematology*, **180**, 33–40.

Morabito, F., Shanafelt, T.D., Gentile, M., Reda, G., Mauro, F.R., Rossi, D., Di Renzo, N., Molica, S., Angrilli, F., Chiarenza, A., Cutrona, G., Chaffee, K.G., Parikh, S.A., Tripepi, G., D'Arrigo, G., Vigna, E., Recchia, A.G., Cortelezzi, A., Gaidano, G., Di Raimondo, F., Fais, F., Foà, R., Neri, A. & Ferrarini, M. (2018) Immunoglobulin heavy chain variable region gene and prediction of time to first treatment in patients with chronic lymphocytic leukemia: mutational load or mutational status? Analysis of 1003 cases. *American Journal of Hematology*, **93**, E216–E219.

Raponi, S., Ilari, C., Della Starza, I., Vincenzo Cappelli, L., Cafforio, L., Piciocchi, A., Arena, V., Mariglia, P., Romana Mauro, F., Gentile, M., Cutrona, G., Moia, R., Favini, C., Morabito, F., Rossi, D., Gaidano, G., Guarini, A., Del Giudice, I. & Foà, R. (2020) Redefining the prognostic likelihood of chronic lymphocytic leukaemia patients with borderline percentage of immunoglobulin variable heavy chain region mutations. *British Journal of Haematology*, **189**, 853–859.

Rosenquist, R., Ghia, P., Hadzidimitriou, A., Sutton, L.A., Agathangelidis, A., Baliakas, P., Darzentas, N., Giudicelli, V., Lefranc, M.P., Langerak, A.W., Belessi, C., Davi, F. & Stamatopoulos, K. (2017) Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. *Leukemia*, **31**, 1477–1481.

