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Comparing Apples to Oranges: A commentary on the Mayo study of MYD88 significance in Waldenstrom's Macroglobulinemia.

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We read with great interest the manuscript by Abeykoon et al. entitled “MYD88 mutation status does not impact overall survival in Waldenstrom macroglobulinemia” [1]. In this paper, the authors sought to evaluate the prognostic value of the MYD88 L265P mutation in Waldenstrom macroglobulinemia (WM) patients. The authors performed a retrospective study that included 558 patients with WM, of whom only 220 (39%) had a formal evaluation for detection of the MYD88 L265P mutation. They were unable to detect the MYD88 L265P mutation in 21% of these patients, and therefore, considered these individuals MYD88 wild type (WT). When evaluating the prognostic value of the MYD88 L265P mutation, patients with and without the mutation had similar overall survival (OS). The authors concluded that the presence of the MYD88 L265P gene mutation did not have a prognostic value in WM and, furthermore, was not a disease defining feature of WM.

We would like to bring a series of important concerns that we identified in this paper, including the methodology used for determination of the MYD88 mutation status, the study design, and conclusions not supported by the author’s current and previous findings. The MYD88 L265P gene mutation was identified by whole genome sequencing as a recurring mutation in 91% patients with WM, and subsequently validated by more sensitive allele-specific polymerase chain reaction (AS-PCR) techniques utilizing CD19-sorted mononuclear cells from bone marrow aspirates of WM patients, wherein the mutation rate was 93% [2, 3]. Subsequently, studies from other academic institutions showed concordant results in which the MYD88 L265P mutation was detected in 90-100% of WM patients [4-6]. A study from the authors’ own institution using

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CD19+CD138+ sorted cells, presumably from the same patient population studied in this report since it included all WM patients seen there from 1995-2016, also showed a 97% prevalence of the MYD88 L265P gene mutation in WM patients [7]. This finding differs greatly from the 79% prevalence reported by Abeykoon et al., and would suggest that most of the patients classified as “MYD88 WT” were in fact MYD88 mutated. A potential explanation for their lower detection rate could stem from using unselected bone marrow aspirates, and lower sensitivity of their PCR technique (approximately 1%). Moreover, other non-L265P MYD88 mutations have been observed in WM patients, and like MYD88 L265P mutations impact ibrutinib response [8]. These non-L265P variants were likely missed by the PCR assay used in this study, and such patients considered “MYD88 WT”. If selected tumor cells, more sensitive AS-PCR testing for MYD88 L265P, and Sanger or next generation sequencing for non-L265P mutations were used, it is likely that most of the “MYD88 WT” patients would have been MYD88 mutated. Embedded MYD88 mutated patients could therefore have made the outcome of the entire “MYD88 WT” group better, and nullified the adverse prognosis associated with MYD88 WT status previously reported by us [9]. Using such a careful approach to determining MYD88 status, we confirmed in a larger population of WM patients that MYD88 mutation status continues to be a highly significant determinant of overall survival by multivariate analysis [10].

Other potential flaws to this study are worth noting. A survival analysis from time of diagnosis to death of any cause was oddly not used. Instead, time from treatment initiation was used by Abeykoon. It is important to note that WM patients who were

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"MYD88 WT" had a shorter time to active disease and treatment initiation versus MYD88 mutated patients, and were more likely to have active disease at diagnosis, making any evaluation of the true prognostic value of MYD88 mutation status problematic. The major argument is that the determination of what makes a patient active is arbitrary given the range of symptomatic complaints, and therefore unlikely to reflect the true biology of the disease. Indeed, the treatments used in the Abeykoon study for "MYD88 WT" and MYD88 mutated differed greatly signifying potential differences in presentation. For example, more "MYD88 WT" patients received alkylators and rituximab combinations, while more MYD88 L265P patients were treated with rituximab alone, thereby signifying the potential for more aggressive disease presentation in the former. No explanation is provided for these differences and no adjustments were made to account for such differences. It is interesting that the rate of transformation to an aggressive lymphoma also differed greatly between MYD88 WT and MYD88 L265P patients, with rates of 16% and 4%, respectively, highlighting differences in disease biology. The higher incidence of disease transformation was also observed by us in MYD88 WT patients [10], making this finding particularly important and arguing against the authors' conclusions that MYD88 mutation status is not disease defining feature.

In addition to the points above, there are several unknown aspects in the study design. It is unclear on what basis just 39% of WM patients were genotyped, as this likely introduced selection bias. The level of tumor involvement at the time of genotyping was not provided. It would have been interesting to have correlated the disease burden with

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the MYD88 mutational status. It is not known if the samples for MYD88 testing were from untreated or previously treated patients, nor if samples were obtained while patients were on active therapy. It is known that the sensitivity for MYD88 detection is impacted by these variables [3]. In as well, there are differences between the diagnostic criteria used for WM patient selection in this study, and that of the WHO and the International Workshops for WM. Finally, no data are provided on type of or time to transformation in the WM patients who transformed.

To summarize, beyond methodological issues pertaining to MYD88 testing, there appear to be fundamental issues with the methodology used to calculate overall survival. The finding that patients with “MYD88 WT” disease had shorter time from diagnosis to activation, were treated more intensively, and had a higher rate of transformation to an aggressive lymphoma highlight differences in WM disease biology based on MYD88 mutation status and appear inconsistent with the author’s conclusions.

References

1. Abeykoon JP, Paludo J, King RL, Ansell SM, Gertz MA, LaPlant BR et al. MYD88 mutation status does not impact overall survival in Waldenstrom macroglobulinemia. Am J Hematol 2017.

2. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med 2012; 367: 826-833.

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3. Xu L, Hunter ZR, Yang G, Cao Y, Liu X, Manning R et al. Detection of MYD88 L265P in peripheral blood of patients with Waldenstrom's Macroglobulinemia and IgM monoclonal gammopathy of undetermined significance. Leukemia 2014; 28: 1698-1704.

4. Poulain S, Roumier C, Decambron A, Renneville A, Herbaux C, Bertrand E et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood 2013; 121: 4504-4511.

5. Schmidt J, Federmann B, Schindler N, Steinhilber J, Bonzheim I, Fend F et al. MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. Br J Haematol 2015; 169: 795-803.

6. Varettoni M, Arcaini L, Zibellini S, Boveri E, Rattotti S, Riboni R et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. Blood 2013; 121: 2522-2528.

7. Ansell SM, Hodge LS, Secreto FJ, Manske M, Braggio E, Price-Troska T et al. Activation of TAK1 by MYD88 L265P drives malignant B-cell Growth in non-Hodgkin lymphoma. Blood Cancer J 2014; 4: e183.

8. Treon SP, Xu L, Hunter Z. MYD88 Mutations and Response to Ibrutinib in Waldenstrom's Macroglobulinemia. N Engl J Med 2015; 373: 584-586.

9. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood 2014; 123: 2791-2796.

10. Treon SP, Gustine J, Xu L, Manning RJ, Tsakmaklis N, Demos M et al. MYD88 wild-type Waldenstrom Macroglobulinaemia: differential diagnosis, risk of histological

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transformation, and overall survival. Br J Haematol. 2017 Nov 27. doi: 10.1111/bjh.15049. [Epub ahead of print]

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