Reviewer's report

Title: Whole exome sequencing of microdissected splenic marginal zone lymphoma: a study to discover novel tumor-specific mutations.

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Reviewer: Gianluca Gaidano

Reviewer's report:

The authors have applied whole exome sequencing to microdissected SMZL samples. Their findings are mainly confirmatory of previous studies. Several issues require further care, as detailed below.

MAJOR COMPULSORY REVISIONS

1. The novelty of the paper is limited. Exome sequencing of unselected spleen tissues gave essentially the same results when applied to larger cohorts of patients. Novelty would be higher if the authors performed multiregional sequencing of SMZL cells microdissected from different regions of the spleen. Such approach might reveal the clonal architecture and evolution history of SMZL.

2. Page 3, line 8. The statement "The molecular pathogenesis of SMZL remains unclear" should be rephrased. Indeed, a number of recently published studies have disclosed recurrently mutated genes in SMZL, including NOTCH2 and KLF2.

3. Page 6, line 24. "Somatic variants were called if the allele frequency of the normal tissue was smaller 0.2 and the delta between tumor and normal frequency was at least 0.1." The method the authors used to call somatic variant does not seem as stringent as it would be recommended. Indeed, the variant allele frequency cut-off in the normal tissue is high (20%).

4. The authors assessed the recurrence of specific mutations in the validation set of SMZL cases. This approach has advantages if the mutation is known to recurrently affect a hotspot (i.e. the MYD88 L265P variant). However, in the setting of genes without known mutation hotspots, variants might be dispersed across the entire coding region. Therefore the entire coding region plus splice sites of the discovered genes should be analyzed in the validation panel.

5. The somatic origin of SMYD1 variants should be confirmed by testing the paired normal DNA.

6. Given the relatively high prevalence of MYD88 mutations and the relative scarcity of NOTCH2 mutations, the authors should rule out the inclusion in the study cohort of disorders looking alike SMZL (i.e. LPL).
Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests