Reviewer's report

Title: Epstein-Barr virus infection and clinical outcome in breast cancer patients correlate with immune cell TNF-alpha/IFN-gamma response

Version: 3 Date: 30 April 2014

Reviewer: Margaret Gulley

Reviewer's report:

BMC CA Marrao et al, 2014

The authors measure EBV DNA in breast cancer tissue and in PBMCs, and they measure EBV reactivation using serology against EBV lytic protein, and some of these EBV factors seem to correlate with clinical outcome in breast cancer. Relapsed cancer patients had higher EBV levels in blood and to a lesser extent in tumor. Lower EBV levels in blood and/or in tumor, and high EBV ZEBRA titers, correlated with worse survival. EBV infection led to higher IFNG production by stimulated blood cells. The authors speculate that anti-tumor immune response is enhanced by EBV infection.

Discretionary revisions:

1. Reword the introductory section in two areas, a. “definitely demonstrating that EBV is restricted to tumor epithelial cells” and b. “clear that EBV expression is restricted to tumor epithelial cells and that the cellular source of the PCR EBV DNA was the epithelial tumor cell” since other groups found EBV in lymphocytes but not in epithelial cells.

2. Line 51 of abstract, it is not clear you are measuring “latent” virus so leave out this qualifier.

3. Line 50, replicative EBV correlates with worse outcome but does not necessarily have a role in driving that worse outcome.

4. It is confusing to use the term “expression” unless you mean RNA or protein expression, e.g. in line 98 of page 5. So clarify throughout the introduction if you mean DNA detection or RNA/protein expression.

5. Misspelled word on line 128

6. Line 139, was the frozen section checked for tumor proportion by H&E stain prior to extraction of DNA? What was the minimal tumor content input to the assay?

7. Which ribosomal DNA served as the normalizer gene, and was is present at only 2 copies per cell? Concern is that (perhaps abundant) rRNA contaminating the specimen might also amplify by Q-PCR. Another control gene might be a better choice, and results of this human gene quantification might also serve as a normalizer that is more accurate than is spectrophotometry for calculating the denominator (cell number) for viral load.
8. Line 234, add results of EBV load in non cancer control PBMC here.
9. Line 277, spell our Lymp and RE/PR.
10. Line 369, the tumor type is properly named Hodgkin lymphoma, not Hodgkin’s disease.
11. Line 379, remove the word “clear” given that you (properly) use the word suggest.
12. Line 390, change “obvious” to “possible”
13. Line 411, did you measure single cells? If not, change this wording.
14. Line 432, reword since there there is an association with high ZEBRA titer and poor outcome but the high ZEBRA could be the effect of late stage cancer and not the cause of poor outcome.
15. Table 2, clarify which grade.
16. Table 2, clarify the meaning of explicative
17. Table 2, clarify if the entries on this table and what they mean. For example, the first entry is EBV-t+ and the relative risk is 2.36 (poor risk), but in table 1 the univariate risk was 0.795 (better risk). What is table 2 showing???
18. Line 147-148 imply your extracted DNA from serum but no data is presented on this topic. EBV DNA levels in serum (copies per mL) would be useful with respect to this survival analysis.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**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