Reviewer’s report

Title: Concerted down-regulation of immune-system related genes predicts metastasis in colorectal carcinoma

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Reviewer: Andreas Scorilas

Reviewer’s report:

This is a very interesting original research manuscript describing the discovery of a panel of genes that can efficiently predict the development of distant metastases in colorectal carcinoma patients. One of the major advantages of this study is the high sample homogeneity that the authors achieved by introducing important criteria. For instance, they decided to include only colorectal carcinoma samples with chromosome instability and excluded all tumors with microsatellite instability, as these two sample subgroups differ in many aspects including survival of the patients. Moreover, all samples included in the current study were characterized as early-stage sample (mostly stage II). Another advantage of this study is that most of the genes that compose this gene signature – informative for patients’ metastasis – were related to the immune system.

Overall, this is a well-designed and well-performed study. However, there are a few issues that should be addressed, in order that this manuscript becomes acceptable:

Major Compulsory Revisions

1. Which calibrator did the authors use in qPCR? This should be stated in the Materials and Methods section.

2. In the Materials and Methods section, the authors mention: “Correlations between clinical parameters and gene expression as measured by qPCR were analyzed by Mann-Whitney test.” This is false: Mann-Whitney U test cannot be used for testing the existence of correlations. Mann-Whitney U test is used for the comparison of the distribution of a variable between two cohorts of samples. The existence of correlations is tested using Spearman’s analysis. Therefore, this should be corrected appropriately.

3. Using qPCR, the authors validated their microarray results regarding only 5 genes of the gene signature that they propose. They should also verify the rest of the genes of this signature in the same number of samples.

4. Figure 2B does not make sense. In more detail: The “sum of normalized expression values of the 14 immune response genes in non-recurrent (n = 33) and metastatic (n = 12) cases; p = 1.69×10^-4 (Mann-Whitney Test)” does not have any biological sense, as expression levels of different genes cannot be added to give a sum. This should be completely changed or removed.

Minor Essential Revisions
5. The order of figures is currently wrong. Figure 3 appears first (not third) in the text. Moreover, Figure 3 bears the legend “Figure 3. ROC curves”. Nevertheless, Figure 3B is NOT a ROC curve, but a Kaplan-Meier Curve. Thus, it would be better to rename Figure 3B into Figure 4, write the appropriate legend, and state it separately in the text.

6. The whole manuscript should be carefully checked for English language (grammatical and syntax) errors and these should be corrected.

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

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

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.