Reviewer’s report

Title: MicroRNA signature associated with outcome of gastric cancer patients following chemotherapy

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Reviewer: Richie Soong

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The authors report they have identified miRNA indices that can (1) distinguish gastric tumour from normal stomach epithelium and (2) predict chemoresistance to cisplatin-fluorouracil treatment. Unfortunately, much more work is required to make the data in this study more convincing.

Major compulsory revisions

The authors need to rearrange and improve the text to enhance comprehension. Unique descriptions of results can be found in the abstract, methods and discussion sections (eg. validation of chemoresistance signature) making it difficult to follow study design and findings. The discussion of the findings is very abrupt – indeed, the lack of proportional discussion on the index (1) makes it appear a remnant of a past submission version.

The methods seem to have a large arbitrary element, giving the impression of data fitting. Different p-values are used to define significance for miRNA differentially expressed between 90 tumours and 34 normal tissue samples (p<0.001), best performing predictors in the training set (p<0.005), and predictors of chemoresistance (p<0.05). miRNA validated by qPCR, and highlighted for discussion, are not the top ranked miRNA. In fact it is unclear which miRNA are the ‘best performing predictors’, as all that is reported are miRNA significantly differentially expressed between 90 tumours and 34 normal samples. It is difficult to appreciate an index if details on the components, their selection and their relative weightings are not disclosed. The thresholds for defining the number and proportions of cases in chemoresistance risk groups seem quite arbitrary as well. The selection of 8 cases for validation of the chemoresistance index does not comply with norms for dividing series into training and validation sets. The study needs to have more recognized, objective and consistent approaches to threshold setting and sample grouping, or improved justification of choices to improve confidence in the reliability of findings.

It would also be reassuring to know if there is a rational basis for the association between the miRNAs identified in the two signatures and the respective states they predict. For example, are the miRNAs in the index predicting resistance to cisplatin/fluorouracil therapy regulating genes/proteins involved in the pharmacology of these agents? This would help to support that the predictive accuracy of the indices may be reproducible in independent series.
Minor essential revisions

The decision to divide samples series based on patient enrollment used to derive signature (1) should be revisited. Time-related effects including sample stability differences, disease and patient management trends are possible over a 5-year span.

The source of tissue samples (eg. department, hospital) should be disclosed. The source of normal stomach mucosa from ‘healthy individuals’ should be disclosed as well, as states such as gastritis or inflammation could influence miRNA levels.

It is relevant to include confidence intervals and/or p-values in Table 2 to allow appreciation of the rankings

Clarification of quantification procedures for real-time PCR analysis is required. The relationship between Ct differences and fold-change is logarithmic. The scales in Figure 2 and methods text make it unclear if this is appreciated.

It is unclear what the acronym “LMT” stands for. Evidence to support the specifications described for the custom array platform is required.

Discretionary revisions

The statement on sample size estimation needs to be clarified. It is unclear why the sample size estimation was considered valid, as it appears based on mRNA and not miRNA data.

Commentary on the trends in Table 1 should be provided. There appears to be some significant differences in the frequencies of some clinicopathological features between training and test sets. What does relative dose intensity relate to? Why is there no median follow-up time for survivors disclosed for the test set? Why is distant metastasis % displayed when the frequency is 100% in both the training and test sets. The formatting of Table 1 is irregular, making for difficult comprehension.

[Page 4, line 5] “proof of concept” changed to “proof of principle” to be more consistent.

Throughout the manuscript, the text “miRNAs” and “microRNAs” are interchangeably used. The authors should be consistent in their annotation.

[Page 6, line 1] Reference not updated as miR-1 has been reported to be downregulated by Liu et al, Eur J cancer, 2011.

Figure 1. “Does prognostic index increased after chemotherapy” could be changed to “Does predictive index increased after chemotherapy” to be more consistent.

Level of interest: An article whose findings are important to those with closely
related research interests

**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