Reviewer's report

Title: Clinical relevance of DNA microarray analyses using archival formalin-fixed paraffin-embedded breast cancer specimens

Version: 1 Date: 8 February 2011

Reviewer: jame hadfield

Reviewer's report:

The aims of this study as laid out in the background section were met. The manuscript describes a method that has been available for several years but is not yet widely published. The addition of publically available data to analyse the impact of the gene signature reported on patient survival is useful. However the number of samples assayed is quite possibly too low as these are clinical and FFPE preserved. Both of these factors increase the noise in the data and the final subgroups have only 4, 5, 6, and 10 samples each.

- Major Compulsory Revisions
  1) The authors should include a figure of RNA quality traces from their Agilent Bioanalyser analysis of FFPE RNA.
  2) Page7: The authors should state the input amount of RNA into the Illumina Ref8 arrays.

- Minor Essential Revisions
  1) Generally some of the grammar is poor and much of the manuscript could be improved before publication. Example paragraphs:
     1a) Page7: Illumina whole genome direct hybridization and DASL assays: “DASL assays was used for FFPE” and “after the oligonucleotides hybridization“ would better read as “DASL assays were used for FFPE” and “after the oligonucleotide hybridization“
     1b) Page9: Comparison of gene profiling between FFPE and FNAB: “a ANOVA test was performed to look for the genes that most differentially expressed among the four distinct subtypes” would better read as “we performed an ANOVA test to determine the genes that were most differentially expressed among the four distinct subtypes”.
  2) Page5 Line7: become is missing the final ‘s’
  3) Page8 Line6: quintile should read quantile
  4) Page6: Tissue sampling and RNA extraction: I would recommend the inclusion of representative Agilent Bioanalyser trace(s) for the FFPE RNA. These are a useful comparator for readers in comparing their own RNA preparations.
5) Page7: Illumina whole genome direct hybridisation and DASL assays: The authors are using a well cited array for the standard analysis but they do not state the amount of starting RNA, it has been shown that this is an important factor if others are to repeat any experimental procedures (ref: Lynch et al. The cost of reducing starting RNA quantity for Illumina BeadArrays: A bead-level dilution experiment. Bioinformatics BMC Genomics 2010, 11:540)

- Discretionary Revisions
I have no discretionary revisions.

**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, but I do not feel adequately qualified to assess the statistics.

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