Reviewer's report

Title: An 8-gene qRT-PCR-based gene expression score that has prognostic value in early breast cancer.

Version: 3 Date: 22 January 2010

Reviewer: Yi Zhang

Reviewer's report:

This manuscript described an interesting study to define an 8-gene signature that can be used on FFPE samples using RT-PCR for hormonal receptor-positive breast cancer risk stratification. The advantage of this study is smaller number of genes used in the assay, FFPE samples used, RT-PCR assays with commercially available reagents.

- Minor Essential Revisions

1. One of the rationales for this study is to show that prognostic signatures can be performed in most laboratories using commercially available reagents. Good idea but difficult to implement. A diagnostic test is easier to implement in a central reference lab due to stringent quality control and quality assurance requirement. This is why both Mammaprint and Oncotype are offered in a central lab so that tests can be reproducibly performed. Point-of-care testing is only possible after stringent and regulated the production and validation of testing reagents and instrument. So the authors should make changes in their statement in the Introduction regarding "commercially available assays" into something like "commercially available assays with stringent diagnostics development".

2. A major drawback of this study is there is no true independent validation of the 8-gene signature using the same RT-PCR assay on another FFPE cohort as acknowledged by authors in Discussion. Using datasets from other microarray expression studies can not be considered as true independent validation due to different assay format and sample types. Therefore, the performance shown in the study is likely an over-estimate of the true performance, and the comparison with other signatures can not be considered objective. The author should discuss this in Results and Discussion.

3. page 5 and 7, what is the poor correlation between fresh frozen and FFPE is used to discard genes?

4. page 7, what is the P value cutoff to select 17 genes from 53 genes?

5. The author mentioned leave-one-out in the Methods section for gene selection, is risk stratification (high- vs. low-risk) done in leave-one-out fashion? Are all the performance measures (log-rank P values, multivariate Cox analysis, Kaplan-Meier curves, etc) based on leave-one-out risk stratification or really just the plug-in performance (i.e. using the formula and cutoff point included in the
Results section)? The author should clarify in the Results section. If latter, I would suggest they include the performance based on leave-one-out or other types of cross-validation.

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

**Quality of written English:** Acceptable

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