Reviewer’s report

Title: Systematic assessment of prognostic gene signatures for breast cancer shows distinct influence of time and ER status

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Reviewer: Giampaolo Bianchini

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This is the submission of a revised manuscript which was aimed to assess and compare the prognostic power of nine breast cancer gene signatures (Intrinsic, PAM50, 70-gene, 76-gene, Genomic-Grade-Index, 21-gene-Recurrence-Score, EndoPredict, Wound-Response and Hypoxia) in relation to the ER status and follow-up time.

Major Compulsory Revisions

1) As also pointed out by both the two previous reviewers, “the observation that the performance of gene signatures depends on hormone receptor status is not new” and “is well-known for a long time”.

A review on gene expression profiling in breast cancer (Reis-Filho JS, Pusztai L, Lancet 378:1812-1823) stated as follow:

“Many gene expression prognostic signatures have been described during the past decade.

First, despite differences in the genes that compose each of the signatures, they largely identify the same group of patients as having poor prognosis disease. The unifying characteristic is the high expression of proliferation-related genes. Meta-analyses of large cohorts of breast cancers subjected to gene expression profiling revealed that only the proliferation-related component of prognostic signatures can forecast the outcome of patients with breast cancer.

Second, proliferation and the degree of expression of proliferation-related genes are one of the strongest prognostic factors in ER-positive cancers.

Third, since the levels of expression of proliferation-related genes are usually high in ER-negative cancers, first generation prognostic signatures almost invariably classify ER-negative cancers as of poor prognosis…..

Sixth, the accuracy of the outcome predictions of most prognostic signatures seems to be time-dependent, with more accurate predictions at 5 years than at 10 years after diagnosis.”

The different biological processes associated with prognosis and chemotherapy response in ER-positive and ER-negative breast cancers have been deeply and purposely investigated (Iwamoto T J Natl Cancer Inst 103:264-72, 2011). Accordingly with this concept, nowadays, biomarker studies in breast cancer should investigate and develop biomarkers in a subgroup specific way (i.e. Rody

Therefore, all the assessments of the biomarkers in the unselected overall population should be removed from the manuscript because these analysis can be considered inappropriate, not informative and potentially misleading.

All univariate and multivariate analysis as well as time dependent evaluations should be performed and presented only for the two ER group separately

2) The authors assessed the time dependent effect of the signatures separately in the untreated group of patients (Figure S3). For consistency, the same analysis for the adjuvant treated group should be presented. In fact, it is important to know if the amount of the time dependent effect is influenced by administered treatment or if it is retained in patient treated with adjuvant therapy.

3) The METABRIC dataset has been used to confirm the time-varying effect of biomarkers. However, the outcome information available in the METABRIC dataset (breast cancer specific survival) is significantly different from distant event free survival available in the first cohort. In particular, the time from distant metastasis and death could be strongly influenced by treatment administered and molecular subgroup. This call for caution in interpreting the METABRIC data and should be properly discussed

4) The observation that histologic grade influenced the time-dependent effect of biomarkers is interesting but it is not well supported. First, the analysis of time dependency should be performed in ER-positive and ER-negative separately as described in point 1. Second, because in this series the grade itself seemed to have a strong time dependent effect (Figure S4 B), the time varying effect of biomarker in the ER+ group should be assessed separately in grade 3 and grade 1-2 groups to assess if the time dependency is only masked by grade in the proportional hazard assessment.

Minor Essential Revisions

1) “In the context of long-term survival, incorporating characteristics for tumor aging may help to alleviate the time-dependency….”. “Aging” should be replaced with “grading”

2) “Additionally, immune-related gene modules have been implicated to be prognostic in high-risk ER-positive breast cancers [53] and ER-negative breast cancers [54, 55].” (Discussion pg 20) Reference 53 (Rody A, 2009) is incorrectly referenced. It swoed that an immune-module (T-cell) is associated with good prognosis in HER2-positive and TN groups.

3) “Different normalization procedure from the original study [6] may explain why the 76-gene signature did not predict any good prognosis in our data” (pg 18) This sentence should be explained (the 76-gene signature is actually prognostic in both ER-positive and ER-negative).
4) The title of table S5 is incorrect “Time- & ER-dependent effect assessment of individual gene signatures in predicting Distant Metastasis Free Survival (DMFS) on the METABRIC set.” The outcome used in the METABRIC is the breast cancer specific death.


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

**Quality of written English:** Acceptable

**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.