Author’s response to reviews

Title: A target based approach identifies genomic predictors of breast cancer patient response to chemotherapy

Authors:

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Dear Dr. Patrick Tan,

We are happy to learn of the positive assessment of our manuscript entitled A target based approach identifies genomic predictors of breast cancer patient response to chemotherapy (8022771326679060). We have addressed the concerns of the referees (in bold) on a point by point basis as requested, as well as provided a revised copy of the manuscript. The changes in the manuscript are highlighted in bold.

Reviewer #1

Summary
This study aimed to identify gene expression indices that were able to predict pathological complete response (pCR) to neoadjuvant anthracycline/taxane-containing chemotherapy regimens in breast cancer. They derive these indices through identifying genes where expression correlates with expression of TOP2A (a target of anthracyclines) and beta-tubulin (a target of taxanes). The indices were used separately and as a combined index. Patients with pCR had higher index scores than patients with residual disease (RD), and index scores were able to predict pCR. The combination index was associated with response in a multivariate analysis.

Comments
Rather than using a top down approach to identify a gene expression signature associated with response, the authors identify genes with expression correlating to expression of two targets of chemotherapy beta-tubulin and TOP2A. The genes selected for the indices are not described in the main text, and are summarised only in a supplementary table. Do any of the genes identified in this approach have a functional connection to the mechanisms of action of
anthracyclines/taxanes? Do any of the genes identified overlap with other
gene-expression based predictors of response to these chemotherapies?

We have added a description of the genes that may have a functional connection
to the mechanism of action of anthracyclines and taxanes in the results section.
We also compared the probeset of the Top2A and B-tubulin indices with the
DLDA30 predictor of response to chemotherapy. The DLDA30 is a well
characterized and validated gene expression based predictor or response to
taxanes and anthracyclines, as well as to 5-fluorouracil and cyclophosphamide
1-3. Interestingly, only one probe set from the TOP2A index overlapped with the
probe sets that comprise the DLDA30 predictor, whereas no probe sets
overlapped between the #-tubulin index and DLDA30 predictor. The probe set
found in common between the TOP2A index and DLDA30 predictor recognizes
transcripts of the MELK gene, which encodes an embryonic leucine zipper
kinase. We have added this data into the results section of our manuscript.
Pages 6-7.

The authors show that increased TOP2A expression is associated with response
to chemotherapy, and that higher TOP2A expression is observed
in patients making pCR compared to those with RD. Is the same true for
beta-tubulin expression in the docetaxel treated cohort?

Beta-tubulin expression was strongly associated with response to docetaxel in
the relatively small docetaxel only treated cohort (AUC: 0.9). However, in larger
datasets comprising patients treated with taxanes in combination with various
other chemotherapeutics, this association appeared to be less strong (AUC:
0.6-0.7). The results section has been modified to reflect these findings. Page 6.

The cohort used to evaluate the association between the b-tubulin index is only
14 patients, profiled in replicate. The authors appear to have treated the
replicates as independent samples. What was the concordance between the
replicates? If concordance was poor then this suggests that intratumour
heterogeneity could confound the utility of this gene expression based approach.
Could the authors address this and comment?

The concordance is quite high between replicates. Based on the expression of
the #-tubulin index probesets, the average correlation (Pearson) between
replicates was quite high and significantly greater than the correlation between
the different primary tumors (Tumor replicates: 0.98, Different tumors: 0.79, p <
0.0001, t-test). While this is a relatively small dataset, these data suggest that
gene expression approaches, such as the one presented here, may not be
confounded by intra-tumoral heterogeneity.
It is not clear how a threshold for these indices could be derived that would show clinical utility prospectively, as the differences in index scores between pCR and RD groups are small. While the authors acknowledge that a prospective study would be needed to confirm clinical utility, no threshold is proposed that would be used in this setting. Could the authors comment on this?

This is a good point. Generally speaking, we would likely need to change technology platforms, from microarray to either qRT-PCR or possibly nanostring, for clinical validation of the target indices. Based on the data presented here, which is all microarray based, it is difficult to extrapolate exactly how we would establish thresholds to stratify patients into likely responder/non-responder groups on different technology platforms. We have now mentioned this issue in the discussion. Page 12.

The specificity of the TOP2A and beta-tubulin indices for anthracycline or taxane response specifically and respectively is unclear.

We have added a section to the results describing the capacity of the TOP2A index to predict response to docetaxel. The TOP2A index does not predict response to docetaxel, suggesting, but not proving, that the TOP2A index is specific to response to anthracycline drugs. Unfortunately, there are no datasets available that include response to only an anthracycline drug in which to test the TOP2A and B-tubulin indices. Page 9.

The manuscript is in general clearly written and is an important contribution. The introduction and discussion are lacking in detail. The authors might want to cite other taxane predictors developed through similar functional-based approaches. We have included additional information on taxane predictors developed with functional approaches in the discussion section. Page 12.

Reviewer #2

Hallett and co-workers revisit in this manuscript the controversial area of target based prediction of response to standard primary chemotherapy in breast cancer. They propose a provocative approach to this issue combining extensive gene expression profiling and individual target aprioristic hypothesis.

The results support authors’ conclusions partly and they acknowledge some of the limitations of the study. While provocative in nature this study faces major limitations in order to qualify the findings as clinically relevant or sounded. The most important limitation of the study refers to its concept. As previously demonstrated, after years of gene profile platforms publication, it is now clear that almost any multi-gene profile strategy overperform the predictive or prognostic value of individual markers. To this extent it has been published that most multigene platforms, while presenting a negligible overlap on individual
genes among them, capture a similar underlying phenomenon, namely, proliferation. Hence, the TOP2A and B-tubulin signatures, presented here, may well predict response to therapy due to its involvement of proliferation related genes more than due to its co-regulation with the theoretical targets of the drugs. This issue is particularly relevant to discuss given the largely controversial literature of the predictive value of the individual targets, in particular of TOP2A.

This way, with the data provided, authors cannot conclude that the predictive value of the signatures is based on the relationship with TOP2A and B-tubulin. In order to do so, authors should present a comparison with other signatures, built following the same methodology, but based in other relevant gens as estrogen receptor, or proliferation related genes. This comparison, and not the comparison to individual genes like the one reported, could base the “target related” nature of the predictive value of the proposed signatures.

This is an excellent point, and did not receive enough discussion in our original manuscript. The notion that multi-gene expression strategies outperform the prognostic/predictive value of clinical variables is well founded for prognostic signatures that seek to predict patient outcome 4-7. Extensive research has revealed that these signatures comprise relatively few overlapping genes, but may generally measure the same underlying phenomenon, proliferation 6, 8, 9. However, these same observations are somewhat less clear for signatures the aim to predict response to chemotherapeutic agents. While it appears that gene signatures which measure proliferation do indeed predict response to chemotherapy 3, 10, it is less clear whether these signatures outperform standard clinical variables. Prospective studies have shown that prediction of response to chemotherapy can be quite accurate using only clinical variables 11, and predictive signatures may not outperform clinical variables at predicting this response 2. This is likely because measurements of clinical variables, such as grade and ER status, are related to the extent of proliferation within a given tumor, and thus perform equally well to a signature that measures proliferation. Indeed, the signatures we present here remained independently predictive of response in a multivariate analysis that included both ER status and tumor grade. That being said, this is still an important issue to address, especially since both the TOP2A and B-tubulin indices include genes that encode proteins involved in proliferation. We have addressed this point in two ways. Firstly, we tested the capacity of the TOP2A index to predict response to the docetaxel-only treated cohort. The TOP2A index was not predictive of response to docetaxel in this cohort. If the TOP2A and B-tubulin indices predicted response to chemotherapy based on their ability to measure proliferation, one would expect the TOP2A index to predict response to docetaxel. Secondly, we generated a ‘proliferation gene’ index, built using the same methodology as the TOP2A and B-tubulin indices, around the well characterized proliferation gene E2F1. Notably, the E2F1 signature was related to chemotherapy response in 3 cohorts of patients treated with chemotherapy. However, the performance of the E2F1 predictor was inferior to either the TOP2A and B-tubulin predictors. Given the observations described above, namely that (i) the TOP2A index was not related to response to docetaxel, (ii) the TOP2A and B-tubulin indices are more predictive of response
than a ‘proliferation index’, we believe these data suggest that there is relevant ‘target related’ predictive value captured by the target indices, rather than the indices simple capturing proliferation as the relevant biological phenomenon. We have modified the manuscript to include this additional data in the results section. Page 9-10.

Other issues that the authors should address to improve the quality of the manuscript are:
- Why results with individual transcripts of TOP2A are reported while the same with B-tubulin are not.

We have expanded the results section to include this data. Beta-tubulin expression was strongly associated with response to docetaxel in the relatively small docetaxel only treated cohort (AUC: 0.9). However, in larger datasets comprising patients treated with taxanes in combination with various other chemotherapeutics, this association appeared to be less strong (AUC: 0.6-0.7). The results section has been re-written to reflect these findings. Page 6.

- Why the TOP2A signature is not tested in the docetaxel-only population (GSE22513) where, if the conclusions of the authors are true, it should not show a relevant predictive value.

We have included an additional section in the results section to address this point. This is described in greater detail above. Page 9-10.

Level of interest: An article of limited interest
Quality of written English: Acceptable
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

In summary, we trust that you find our suitably revised manuscript to be appropriate for publication in BMC Medical Genomics.

Sincerely,
John A. Hassell

2. Lee, J.K., Coutant, C., Kim, Y.C., Qi, Y., Theodorescu, D., Symmans, W.F.,


