San Antonio Breast Cancer Symposium® - December 8-11, 2020

Targetable ERBB2 mutation status is an independent marker of adverse prognosis in estrogen receptor positive, ERBB2 non-amplified primary lobular breast carcinoma: validation using a novel gene signature of HER2 activation

Mansour Alsaleem[1], Sasagu Kurozumi[2], Kartikeya Bhardwaj[1], Cintia Monteiro[1], Stacey EP Joosten[3], Andrew R Green[1], Takaaki Fujii[2], Ken Shirabe[2], Ian O Ellis[1], Emad A Rakha[1], Nigel P Mongan[1], David M Heery[1], Wilbert Zwart[3], Steffi Oesterreich[4] and Simon J Johnston[5].

1. University of Nottingham, Nottingham, United Kingdom. 2. Gunma University Graduate School of Medicine, Gunma, Japan. 3. Netherlands Cancer Institute, Amsterdam, Netherlands. 4. Womens Cancer Research Center, UPMC Hillman Cancer Center and Magee-Women Research Institute, Pittsburgh, PA. USA. 5. AstraZeneca, Cambridge, United Kingdom

BACKGROUND

Invasive lobular carcinoma (ILC) accounts for 10-15% of primary breast cancer and is typically ER+ and *ERBB2* non-amplified.

There is preclinical evidence that somatic *ERBB2* mutation may provide an alternative and tractable mechanism for upregulation of HER2 activity in tumors that do not express HER2 by current clinical criteria.

Using large public datasets, we previously demonstrated that targetable *ERBB2* mutations are enriched in ILC versus invasive ductal carcinoma (IDC) and are an independent prognostic factor in ILC (HR=3.7, 95% CI 1.2-11.0; p=0.021)[1].

We next hypothesized that a gene expression signature incorporating HER2 activity due to *ERBB2* mutation (*ERBB2*mut) and / or amplification would validate the prognostic signal we found in ILC.

METHODS

To derive a novel gene expression signature of HER2 activity that accounted for the effect of potentially targetable *ERBB2* mutations in *ERBB2* non-amplified tumors, we applied a weighted average difference (WAD) method to gene expression data in cases from the METABRIC 2012 (N = 1980) and TCGA 2015 (N = 817) cohorts (see Figure A for summary diagram).

Gene expression in *ERBB2*mut cases (N = 38, selected by *ERBB2* non-amplified status and patient age > 50) was compared with the same order of magnitude of *ERBB2* wild-type cases (N = 79, selected by *ERBB2* non-amplified status, grade > 1, stage > I, patient age > 50). We then repeated the same model for known activating *ERBB2* mutated (onc*ERBB2*mut) cases (N = 23) using the same comparator and selection criteria.

To incorporate the effect of HER2 activity via *ERBB2* amplification, the overlap of differentially expressed genes (DEGs) shared by both comparisons (*ERBB2*mut and onc*ERBB2*mut vs. *ERBB2* wild-type) with DEGs from a further comparison of *ERBB2* amplified (N = 247) vs. non-amplified (N = 1733) cases in METABRIC was calculated.

Then, to incorporate clinical HER2 status as the downstream phenotype, the overlap of this list with DEGs from a comparison of HER2+ vs. HER2− cases in TCGA was calculated.

In contrast to *ERBB2*mut cases, matching was not performed for *ERBB2* amplified or HER2+ cases because numbers were higher and within an order of magnitude across groups, such that similar variation in gene expression could reasonably be assumed.

Multiple gene expression signatures of HER2 activity have been derived using cell line models and patient tumors. We compared our novel gene signature with the HER2 activity signature established by Desmedt et al[2] with respect to its ability to detect potentially targetable *ERBB2*mut cases in our ILC/IDC dataset.

This was achieved by multivariate regression modeling of response to neratinib for each gene signature using breast cancer cell line pharmacogenomic data from the BROAD Institute, accessed online via the CellMinerCDB portal.

RESULTS

We show that our novel HER2 pathway signature score uniquely enriches for *ERBB2* mutated tumors (see Figure B). Using a Cox regression model and stratifying gene expression scores into upper versus lower quartiles, we were able to validate the prognostic signal of ERBB2 mutations in ILC tumors (HR for 10-year OS in ILC=2.3, 95% CI 1.04-5.05; p=0.040) (Figure C). In contrast, no relationship was found between ERBB2 mutation status or novel HER2 pathway enrichment score and patient outcome in cases of IDC.

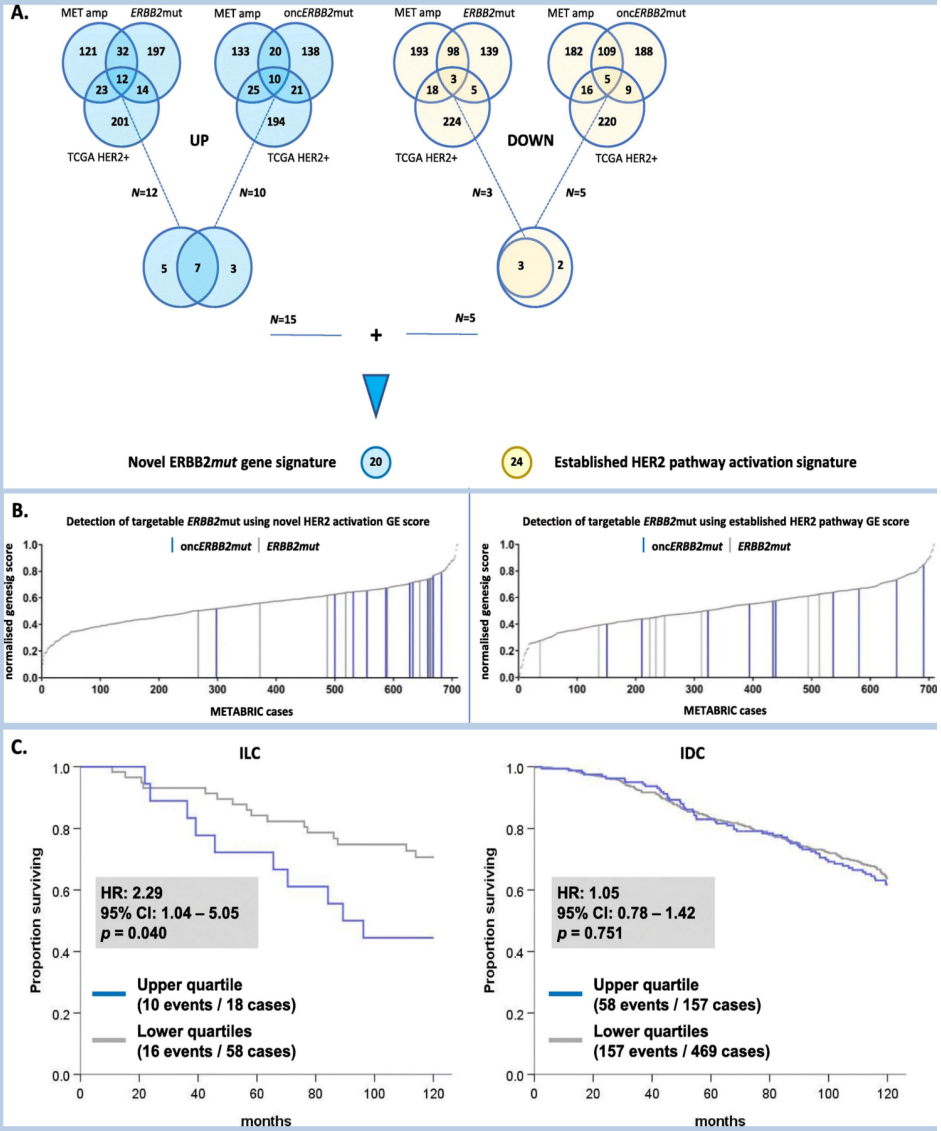

Figure: Novel gene signature of HER2 activity incorporating *ERBB2*mut, *ERBB2* amplification, and clinical HER2 status. (A) Generation of a 20-gene signature of HER2 activity. The upper Venn diagrams show the overlap between the top 500 DEGs by WAD score for METABRIC amplified vs. non-amplified, *ERBB2* mutant vs. wild-type, and TCGA HER2+ vs. HER2−. Upregulated DEGs are shaded blue and down-regulated DEGs yellow. *ERBB2*mut and onc*ERBB2*mut vs. wild-type are analyzed separately, and the overlap combined in the lower Venn diagrams. (B) Comparison with an established 24-gene signature of HER pathway activation, using a gene signature (genesig) score derived from multivariate analysis of response to neratinib in breast cancer cell lines. Cases with *ERBB2*mut (gray lines) and onc*ERBB2*mut (blue lines) clustered in the upper quartile of normalized genesig scores for the novel signature but not the established signature. (C) 10-year OS analysis of cases in the current study stratified by histological subtype and novel genesig score (upper vs. lower quartiles) indicates that *ERBB2*mut-associated DEGs are prognostic in ILC but not IDC. GE, geneset enrichment

DISCUSSION & CONCLUSION

We generated a novel gene signature that reflects HER2 pathway activity more broadly than existing signatures. In the current study we applied the novel signature to validate the prognostic effect of *ERBB2* mutations in ILC. We conclude that *ERBB2* mutations that are enriched in ILC provide a robust biomarker of HER2 pathway activation and could be detected via gene expression signature. Clinical trials of HER2-targeted therapy in *ERBB2* non-amplified primary ILC are warranted. Future translational study of our novel gene signature of HER2 activation may reveal further roles as a biomarker for HER2-targeted therapeutics beyond the clinical context of *ERBB2* amplification.

References

1. Kurozumi et al. Targetable ERBB2 mutation status is an independent marker of adverse prognosis in estrogen receptor positive, ERBB2 nonamplified primary lobular breast carcinoma: a retrospective in silico analysis of public datasets. Breast Cancer Res 2020; 22:85.
2. Desmedt C et al. Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. Clin Cancer Res. 2008;14(16):5158–65.

ACKNOWLEDGMENTS

The first author of this abstract, Mansour Alsaleem would like to express his sincere gratitude to his supervisors, funders, collaborators, and all of his colleagues for their continuous support of his PhD study and research. The first author of this abstract received funding and support from Qassim University, Kingdom of Saudi Arabia.