Evaluation of HER2 RNA expression as a potential predictive biomarker for anti-HER2 therapy

Abstract #968

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Background

Traditionally, available human epidermal growth factor receptor 2 (HER2)- directed therapies have been ineffective in "HER2-low" breast cancers, with low expression of HER2 defined as a score of 1+ by immunohistochemical (IHC) analysis or as an IHC score of 2+ and negative results on in situ hybridization. In the DESTINY-Breast04 trial (NCT03734029), patients with metastatic breast cancer and who were HER2-low, Modi et al. found that treatment with trastuzumab deruxtecan resulted in an impressive 52.3% of patients having a response, as well as in longer progression-free survival and overall survival than the physician's (protocol-limited) choice of chemotherapy. As such, interest has now turned toward defining a sub-population of IHC 0+ tumors that may have HER2 expression below the limit of IHC detection/quantification and may thus also be responsive. We previously validated a high dynamic range HER2 RNA expression assay run as part of our comprehensive genomic profiling test, StrataNGS.

Methods

The Strata Trial (NCT03061305) is an observational clinical trial evaluating the impact of molecular profiling (comprehensive genomic profiling [CGP] plus quantitative transcriptomic profiling [qTP] for RNA expression) on patients with advanced solid tumors. It has been approved by Advarra IRB (Pro00019183). Herein, we evaluated the HER2 RNA expression data together with copy number and clinical outcome data from the Strata Clinical Molecular Database (SCMD) in advanced breast cancer and other advanced solid tumors. Expression data for patients with breast cancer was compared to clinical IHC to determine qTP low and moderate thresholds in the training cohort. Two independent validation cohorts were used to determine clinical validity of thresholds used for high vs. negative status. The clinical IHC categories were 0, 1, 2, and 3 and the qTP categories were HER2 expression score negative, low, moderate, and high expression.

Conclusions

Given that HER2 RNA High predicted benefit from 1st generation anti-HER2 therapies, future studies should consider HER2 RNA Low as an alternative biomarker to HER2 IHC Low, with the opportunity to further expand trastuzumab deruxtecan use into the IHC 0+ breast cancer population and potentially to additional solid tumors.

Additional Information

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References

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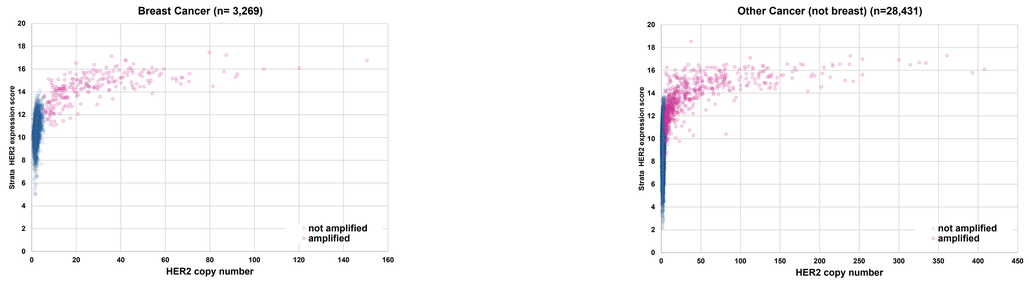
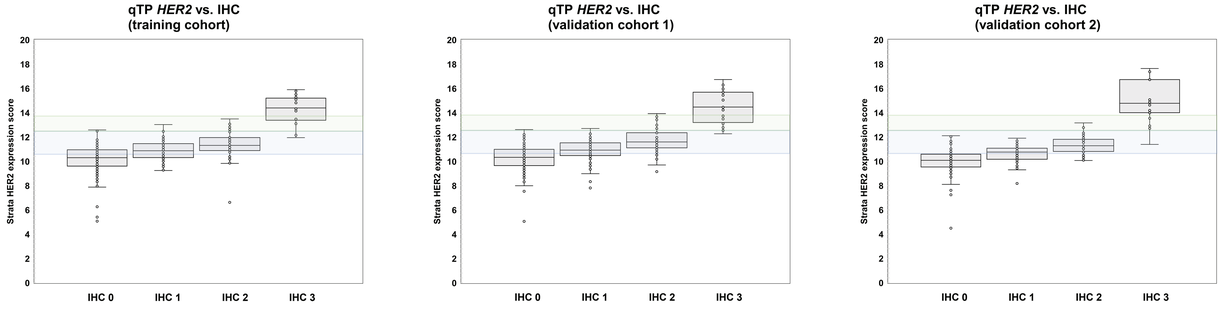
Conflict of Interest Disclosure: All authors are equity holders and/or employees for Strata Oncology. ST and DR are equity holders in Javelin Oncology. ST previously served as a consultant to Strata Oncology and has consulted for Astellas/Medivation and Janssen. He has received research (to University of Michigan) funding from Astellas and has received travel support from the Prostate Cancer Foundation. All authors affirm that this abstract will not be used to sell a particular product or service.

Figure 1. HER2 copy number compared to RNA expression levels in DNA amplified and not-amplified cancer samples



HER2 gene expression was significantly higher in tumors with DNA amplification (>=6 copies; median: 13.9 vs. 10.0 in log2 units; p < 1e-100). Despite similar copy number levels in amplified breast vs. other cancers (median: 21.8 vs. 19.8 copies), HER2 expression levels were ~2-fold higher (median: 14.5 vs. 13.5; p = 1.3e-10). Similarly, HER2 expression levels were higher in non-amplified breast vs. other cancers (median: 10.7 vs. 9.9; p<1e-100), suggesting that DNA amplification and cell lineage affect HER2 expression.

Figure 2. HER2 RNA expression compared to clinical IHC categories

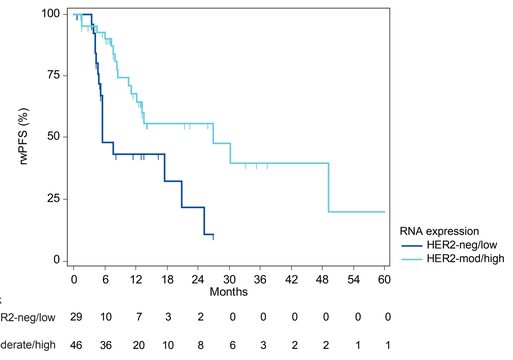


In breast cancer tissue samples with available IHC in a training cohort (n = 273), HER2 RNA expression trended with IHC across the 0-3+ range, while 3+ tumors had distinctly high RNA expression (median: 14.4), 0-2+ tumors had lower expression with overlapping distributions (median: 10.5, 10.9, 11.5, respectively), suggesting that 0-2+ tumors do not represent distinct biological groups, but rather a continuum of low expression. We defined a HER2 RNA Low threshold (bottom blue line) in the training cohort, corresponding to the top 75% of IHC 1-2+ breast cancers (from n=139), which identified 39% of 0+ breast cancers (from n=109) as above that threshold. Importantly, at this threshold, in two independent validation cohorts of 0+ breast cancer samples (n=124 and 92) 34% and 24% were also classified as HER2 RNA Low. Additionally, 7,782/28,431 (27.4%) of all non-breast solid tumors were classified as HER2 RNA Low (data not shown). *HER2 negative (0+) = below bottom blue line; HER2 low (1-2+) = shaded blue area; HER2 moderate (2+) = shaded green area; HER2 high (3+) = above top green line.*

HER2 High vs Negative - Low

Validation Cohort	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)	n
#1	80.0% (51.9-95.7%)	100.0% (98.2-100%)	98.6% (96.0-99.7%)	216
#2	93.8% (69.8-99.8%)	100.0% (97.9-100.0%)	99.5% (97.1-100.0%)	191

Figure 3. 1L or 2L HER2+ therapy determined by clinical IHC/ISH then stratified by HER2 RNA expression



Using our previously validated HER2 Moderate threshold, among 75 eligible SCMD breast cancer patients treated with 1st or 2nd line systemic trastuzumab or pertuzumab containing therapy, HER2 RNA Moderate/High patients (n=46, 59%) had significantly longer time to next therapy (TTNT) compared to HER2 RNA Low/Negative patients (median TTNT 26.9 vs. 5.6 months, adjusted hazard ratio 0.31, p=0.005 when adjusted for 1st vs. 2nd line, pertuzumab inclusion, and inclusion of chemotherapy or hormonal therapy). This suggests there is clinical value in using HER2 RNA expression and may help in the 1L treatment decision.

Tables. HER2 RNA expression categories and clinical considerations

	Training	Validation Cohort 1	Validation Cohort 2
% IHC 1-2+ as HER2 RNA Low (1-2+)	75%	71%	64%
% IHC 0+ as HER2 RNA Low (1-2+)	39%	34%	24%

There is a continuum of HER2 expression in tissue when measured by RNA expression. As such, there are samples that are classified as clinical IHC 0 that have detectable expression of HER2 by RNA expression. Hence, HER2 classification by RNA expression may be an alternative to IHC, and help in therapeutic decisions for cancer patients.

HER2 category	Threshold Rationale
HER2 Negative (0+)	The HER2 Negative (0+) gene expression threshold was set to identify the lowest 25% of HER2 expression in IHC 1-2+ (non HER2 amplified) breast cancers, with 62% of HER2 IHC 0+ samples identified as HER2 Negative (0+) by gene expression. In two separate validation cohorts, 30% & 41% of HER2 IHC 1-2+ (non-amplified) and 62% & 78% of HER2 IHC 0+ samples identified as HER2 Negative (0+) by gene expression. Hence, if the patient is HER2 IHC 1-2+ (non-amplified), then although trastuzumab deruxtecan is still indicated in the >=3rd line, the gene expression is in the lowest 30-41% of such samples, with expression of antibody drug conjugate targets generally being associated with efficacy.
HER2 Low (1-2+)	The HER2 Low (1-2+) gene expression threshold was set to identify the highest 75% of HER2 expression in IHC 1-2+ (non HER2 amplified) breast cancers, given the indication of trastuzumab deruxtecan in patients with HER2 IHC 1-2+ breast cancer in the >=3rd line. In the training cohort, 38% of HER2 IHC 0+ samples were identified as HER2 Low (1-2+) by gene expression. In two separate validation cohorts, 22% &38%of HER2 IHC 0+ samples were identified as HER2 Low (1-2+). Hence, if the patient is HER2 IHC 0+, HER2 gene expression in this sample is similar to the highest 59-70% of HER2 gene expression in 1-2+ IHC (non-amplified) breast cancer in the validation cohorts.
HER2 Moderate (2+)	Based on Moderate (2+) HER2 gene expression status, ERBB2 (HER2) copy number status (amplified/not-amplified) status is used to determine therapeutic implications.
HER2 High (3+)	Based on High (3+) HER2 expression, which showed 100% specificity for 3+ HER2 IHC status in two independent validation cohorts, despite the lack of ERBB2 (HER2) amplification in this sample, we consider this sample HER2 positive for first line treatment selection.

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