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Machine learning based inference of real-time HER2 activity from gene expression profiling in breast cancer to inform HER2 targeted therapy selection

Saumya D. Sisoudiya[1], Jeffrey S. Ross[1,2], Rachel B. Keller-Evans[1], Richard S.P. Huang[1], Alexa B. Schrock[1], Garrett M. Frampton[1], Ericka E. Ebot[1], Ethan S. Sokol[1], Smruthy Sivakumar[1], Adriana M. Kahn[3], Maryam Lustberg[3]

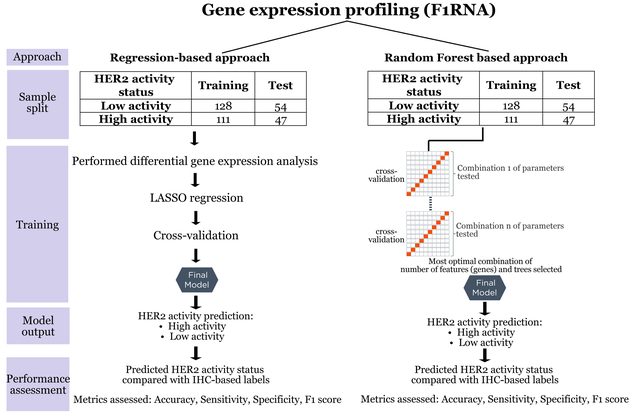
[1]Foundation Medicine, Inc., Boston, MA, USA [2]Upstate Medical University, Syracuse, NY, USA [3]Yale University School of Medicine, CT, USA

BACKGROUND

- Recent clinical trials for HER2 ADCs have led to clinical benefit and approvals of these agents in HER2+ and HER2-low/ultra-low tumors
- However, prior studies have shown that only a fraction of patients with IHC-based HER2-positive tumors will respond to HER2-targeted therapy.
- Orthogonal methods such as those based on gene expression patterns to determine HER2 activity, have the potential to improve patient selection.
- In this study, we utilized gene expression profiling to predict HER2 activity using machine learning models, which may aid the identification of patients most likely to benefit from HER2 targeted therapies, particularly for those with HER2-low tumors.

METHODS

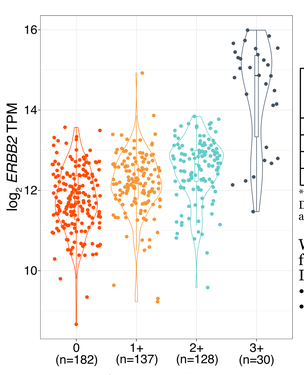
- **Sequencing:** The study cohort comprised 477 patients with breast cancer and RNA-based gene expression (Research Use Only) for 1,517 genes profiled using FoundationOne®RNA (F1RNA), a laboratory developed test by Foundation Medicine, Inc. IHC-based HER2 annotation was extracted from pathology reports. Samples with HER2 IHC 0 were labeled as "low activity" and those with IHC 2+/3+ were labeled as "high activity".
- **Algorithms:** Two algorithms were implemented to obtain HER2 activity status. One approach involved performing differential gene expression analysis for feature selection followed by using LASSO (Least Absolute Shrinkage and Selection Operator) regression in the training set. The second approach utilized a random forest-based machine learning algorithm with hyperparameter tuning through cross-validation in the training set. Performance of both approaches was assessed in independent test sets using the following metrics: accuracy, sensitivity, specificity, and F1 score.



COHORT CHARACTERISTICS

Category	Samples
Number of samples	477
Age median[IQR], years	65 [53-73]
Biopsy site %[n]	
local	38% [182]
metastatic	42% [201]
lymph node	12% [58]
ambiguous	8% [36]
HRDsig positive %[n]	15% [68]
TMB median[IQR]	2.4 [1.2-4.8]
MSS %[n]	95% [429/450]
Disease ontology %[n]	
Breast carcinoma (nos)	49.5% [236]
Breast invasive ductal carcinoma (IDC)	42% [200]
Breast invasive lobular carcinoma (ILC)	7.1% [34]
Breast metaplastic carcinoma	1% [5]
Breast mucinous carcinoma	0.2% [1]
Breast phyllodes tumor	0.2% [1]

Table 1: Cohort summary



HER2 IHC	Number of samples with *ERBB2* amp (F1CDx*)
0	3 (1.6%)
1+	4 (3%)
2+	10 (8%)
3+	24 (80%)

*FoundationOne®CDx (F1CDx®) is a DNA-based comprehensive genomic profiling assay

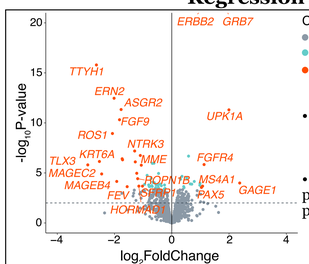
We created HER2 activity labels for samples based on their HER2 IHC status:
- HER2 IHC 0: Low activity
- HER2 IHC 2+/3+: High activity

Figure 1: *ERBB2* RNA expression by IHC status of HER2

Median *ERBB2* expression increased with HER2 IHC categories with 3+ samples having the highest median expression (14.9 $\log_2$TPM). However, samples with IHC 1+ have a range of expression values that overlap the IHC 0 and IHC 2+ samples

RESULTS: HER2 ACTIVITY PREDICTION USING MACHINE LEARNING MODELS

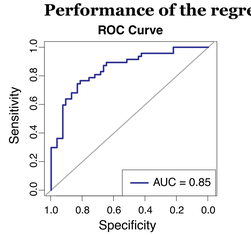
Regression-based model



- Regression model was trained on the TPMs of differentially expressed genes to classify training samples into HER2 low vs high activity classes
- The model was then run on test samples and performance was evaluated by comparing the model predictions with IHC-based labels

Figure 2: Differential gene expression analysis between low vs high activity samples

Performance of the regression model on the test set



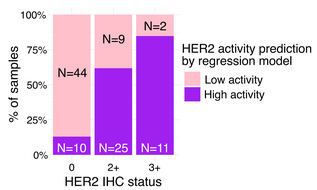


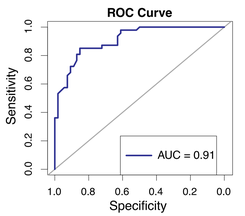
Figure 3: Regression-based HER2 activity prediction across different HER2 IHC categories

Prediction \ Reference (IHC)	low activity	high activity
low activity	44	11
high activity	10	36

Accuracy	Sensitivity	Specificity	F1 score
79%	81%	77%	81%

Random forest model

Performance of random forest model on the test set



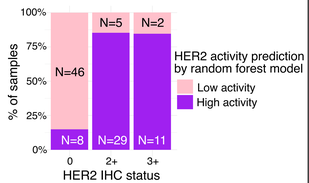


Figure 4: Random forest-based HER2 activity prediction across different HER2 IHC categories

Prediction \ Reference (IHC)	low activity	high activity
low activity	46	7
high activity	8	40

Accuracy	Sensitivity	Specificity	F1 score
85%	85%	85%	86%

Random forest model outperformed regression model with higher accuracy, sensitivity and specificity

Application to HER2 IHC 1+ tumors

- The random forest model was then used to predict HER2 activity of IHC 1+ samples (these were not included in the training or testing samples)
  - Almost 60% of the IHC 1+ samples were predicted to have low HER2 activity
  - N=3 samples had a DNA-based *ERBB2* amplification, all of which were predicted to be high activity. In contrast, none of the low activity samples had *ERBB2* amplifications.

Low activity	High activity
82	55

CONCLUSION

- Despite HER2 IHC status being the current standard for patient selection, only a subset of HER2 therapy-qualifying cases respond to ADC/HER2-targeted therapy, highlighting the urgent need for orthogonal strategies to more accurately identify responders. A multi-gene expression signature could identify signaling pathways that may better inform HER2 dependency.
- *ERBB2* expression is correlated with the extreme HER2 IHC categories (IHC 0 vs IHC 3+). However, samples with IHC 1+ have bimodal distribution of *ERBB2* expression.
- Two approaches were compared to predict HER2 activity using gene expression of >1,400 genes: Regression-based model and random forest-based model
- The random forest model was better at classifying tumors based on HER2 activity compared to the regression-based approach.
- Further validation is warranted to assess clinical utility and to influence trial enrollment strategies.

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