SAN ANTONIO BREAST CANCER SYMPOSIUM—DECEMBER 6-10, 2022

Deep learning-based assessment of HER2-low expression on breast cancer H&E digital whole slide images

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Background:

Antibody drug conjugates (ADCs) against HER2 have shown meaningful clinical activity in HER2-low breast cancers, defined as 1+ or 2+ expression by immunohistochemistry (IHC) without gene amplification by *in situ* hybridization (ISH) techniques. Given that these methods were originally developed for accurate detection of *HER2* gene amplification (IHC 3+ or ISH+), their sensitivity and robustness for the detection of low and ultra-low levels of HER2 are questionable. We have recently described a deep learning algorithm that can detect signatures of HER2 expression based on training utilizing scanned H&E whole slide images (WSI) of breast cancers for which IHC and mRNA expression levels of HER2 were available. Here, we report the application of our algorithm to two independent breast cancer cohorts.

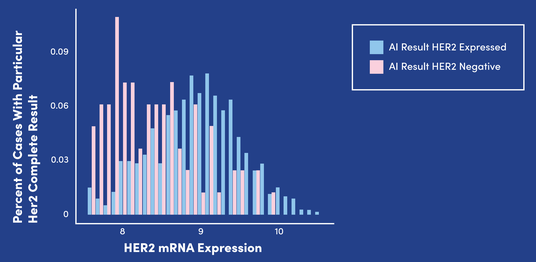
Methods:

A model was developed based on recognition of invasive breast cancer in H&E WSIs, and then trained via a computational neural network with multiple instance learning for binary classification of cases as HER2 "negative" and HER2 "expressed" (low). For training, true negatives were defined as having HER2 IHC-0 and mRNA level <7.6. HER2-low cases were defined as IHC-1+/2+ and mRNA >9 (OncotypeDx HER2 gene specific score). IHC-0 cases with mRNA >7.6 were excluded from the training cohorts. The resulting model (HER2Complete) was able to distinguish HER2-negative from HER2-low cases with an AUC of 0.91 (+/- 0.08). Here we use HER2Complete to assess HER2 in two additional cohorts that include 901 ER+/HER2 IHC-0 and 52 HER2 IHC-0 breast cancers from MSK and TCGA cohorts, respectively. For the TCGA cohort, concomitant transcriptomics data (RNASeq) as a reference for HER2 mRNA expression were retrieved and HER2 "expressed" was defined as RNASeq expression of HER2 greater than the 90th percentile of the geometric mean of expression of three reference genes not expressed in breast tissues (TTN, MUC13, OR10A6). Values less than this reference cut-off in the TCGA cohort were considered HER2 "not expressed."

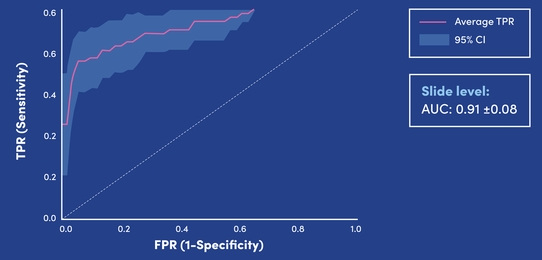
Results:

Among the 901 IHC-0 test cases from the MSK cohort, the model identified 82 as "negative", whereas 819 were found to have features of HER2 expression (HER2-Low). Of the 82 negative cases in the MSK cohort, all except 13 cases expressed mRNA levels <9, and 786/819 of the HER2-low cases expressed mRNA levels >8. Of the 52 IHC-0 cases in the TCGA cohort, 33 also had HER2 "not expressed" by our reference based RNASeq expression cut-off. Our model identified 15 of these 33 as "negative", while 15 of the 19 TCGA cases with IHC 0+ and HER2 "expressed" by our cut-off were identified as HER2-low by our model.

Distribution of Cases by mRNA Expression and AI Characterization of Whole Slide Image in MSK Data Set



AUC for HER2Complete Detection of HER2 Negative (IHC-0 and no RNA Expression of ERBB2) in MSK Dataset HER2-negative v HER2-expressed



Specificity	NPV	Sensitivity	PPV
99%	92%	49.0%	96%

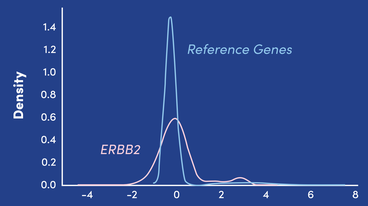
HER2Complete Results (HER2-Low vs HER2 Expressed) For TCGA HER2 IHC-0 Cases With RNAseq Expression or Non Expression Normalized to Reference Genes

HER2 IHC-0	AI Result HER2-Low	AI Result HER2 Negative
ERBB2 mRNA above cut-off (Expressed)	15	4
ERBB2 mRNA below cut-off (No Expression)	18	15

Conclusion:

AI tools based on the analysis of WSIs of routinely prepared H&E sections may predict HER2 status in breast cancer. This work requires further investigation using treatment response data to demonstrate that cases with morphologic features of low level HER2 expression will respond to ADCs.

Quantification of ERBB2 (HER2) mRNA Versus Reference Genes Measured By RNASeq In TCGA Dataset



- Reference profile from housekeeping genes: TTN, MUC13, OR10A6 (geometric mean)
- 90 percentile of the reference profile is used as the threshold for ERBB2 expression

Top Histologic Features Associated With HER2Complete Results In TCGA

HER2 NULL:	HER2 EXPRESSED:
Molecular subtype: Basal-Like	**Histology:** Invasive lobular carcinoma
Histology: Mixed / Invasive ductal carcinoma	**Inflammation:** Absent
Inflammation: Present	**Molecular subtype:** Luminal A
Weak association: ER-?	**Weak association:** ER+ ?

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