

STUDY DESIGN

Using a human breast carcinoma TMA, HER2 and Ki-67 immunohistochemistry (IHC) was used to compare digital and manual scoring methods for endpoints of H-score and percentage of proliferating tumor cells.

METHODS

Advanced Staining & Imaging

A commercially available tumor microarray slide containing four breast carcinoma tumor cores (two cores per tumor) was stained for HER2 or Ki-67 by IHC and imaged using an Aperio AT2 whole slide scanner.

Quantification

Slides were quantified for HER2 and Ki-67 immunolabeling through deep learning algorithm (DLA)-assisted tumor recognition and target detection. Analysis was overseen by a board-certified veterinary pathologist.

HER2 Analysis

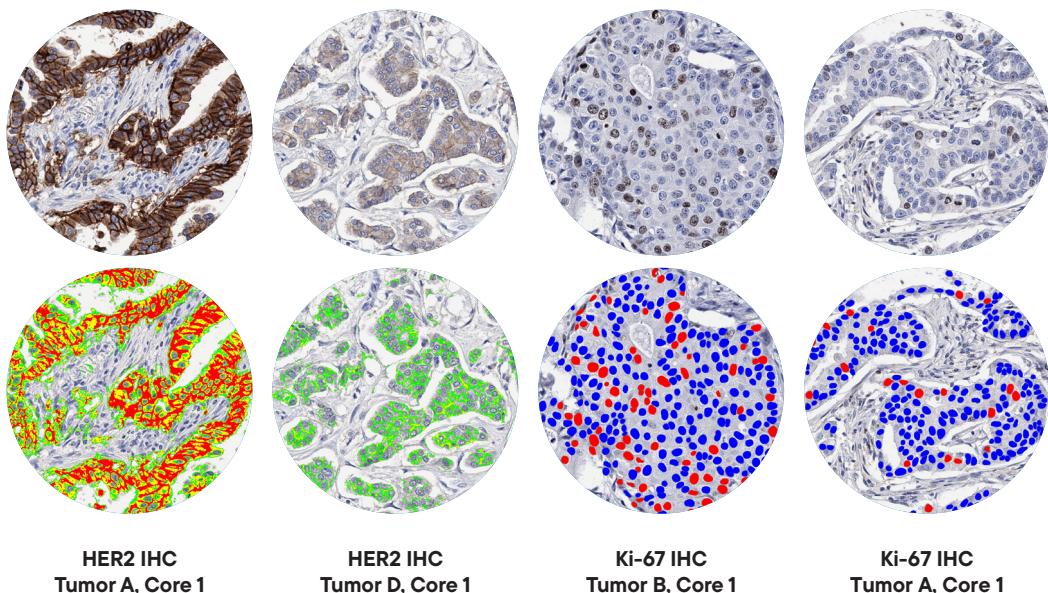
Within the detected tumor cells, a DLA was applied to identify weak, moderate, and strong-intensity HER2 immunolabeling and report each area measurement in μm^2 . A digital H-score was calculated with normalization to the tumor area. Manual scores performed by a pathologist were provided by the TMA vendor and were used for comparison.

H-Score Calculation on a Scale of 0-300: $(1 \times \text{percentage area of weak staining}) + (2 \times \text{percentage area of moderate staining}) + (3 \times \text{percentage area of strong staining}) * 100$

Ki-67 Analysis

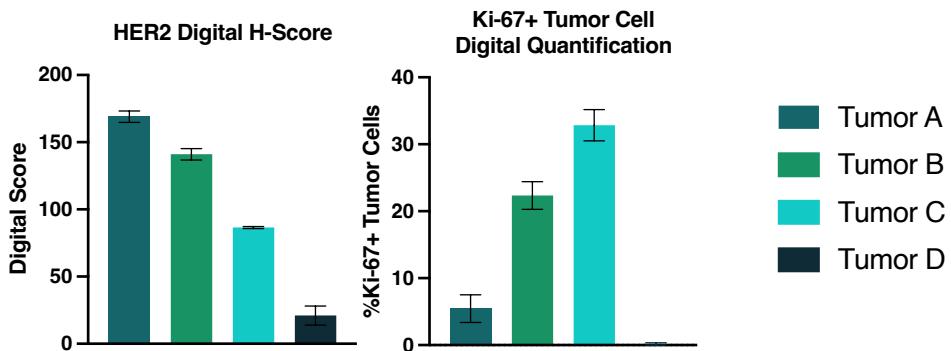
Ki-67 immunolabeling was also detected specifically within the nuclei of tumor cells. The total number of tumor cells was collected and used to calculate a percentage of Ki-67+ tumor cells in each core. This was compared to the manually reported percentage of Ki-67+ tumor cells by pathologist supplied by the vendor.

RESULTS



Representative core images at 40x. A high and low expressing core were selected for both HER2 and Ki-67 to demonstrate the algorithm's performance for each analysis. HER2 threshold designations for each intensity are as follows: strong = red, moderate = yellow, weak = green. Ki-67+ classification of the tumor cell nuclei include red labeled Ki-67+ tumor cells, and blue-labeled Ki-67- tumor cells.

Tumor Core ID	HER2 Digital H-Score	HER2 Manual H-Score	Percentage Ki-67+ Cells (Digital)	Percentage Ki-67+ Cells (Manual)
Tumor A (Core 1)	166	3+	6.9%	<14%
Tumor A (Core 2)	172	3+	4.0%	<14%
Tumor B (Core 1)	138	2-3+	23.8%	23%
Tumor B (Core 2)	144	2-3+	20.9%	25%
Tumor C (Core 1)	86	1+	34.5%	30%
Tumor C (Core 2)	87	1+	31.2%	35%
Tumor D (Core 1)	26	0	0.0%	<14%
Tumor D (Core 2)	16	0	0.3%	<14%



CONCLUSIONS

- HER2 IHC produced weak to strong intensity membranous to cytoplasmic immunolabeling of tumor cells, with varying intensity for each core.
- The digital H-score generated from the algorithm's detection of weak, moderate, and strong intensity labeling, normalized to the tumor area, was positively correlated with the reported manual score by pathologist ($r=0.99$).
- Ki-67 IHC produced nuclear immunolabeling in tumor cells, with variable abundance in each core.
- Detection of Ki-67 immunolabeled (proliferating) cells was positively correlated to the reported positive percentage by pathologist ($r=0.97$).