Comparative Analysis of Four Immunohistochemical Assays for HER2 Expression in Breast Carcinoma: Correlation with HER2 Gene Amplification and Perspectives for HER2 Low Expression

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Background

Accurate HER2 status based on immunohistochemistry (IHC) is required to select patients for existing and newly developed HER2-targeting treatments. Patients with HER2 gene-amplified and/or HER2 IHC overexpressing breast carcinomas (BCs) are eligible for treatment with anti-HER2 drugs such as trastuzumab. Recently, BC patients with HER2 low expression (IHC 1+ or 2+ without HER2 gene amplification) may be offered new anti-HER2 drug-conjugates such as trastuzumab deruxtecan. Since patient stratification for anti-HER2 drugs primarily is based on IHC, our goal is to compare the analytical accuracy of HER2 overexpression and concordance rate for HER2 low expression in BC amongst four different HER2 IHC assays.

Material and methods

98 formalin-fixed paraffin-embedded human resection BC specimens from five microarrays were analyzed with four different IHC assays: (1) HercepTest SK001, Agilent, (2) HercepTest GE001, Agilent, (3) PATHWAY 4B5, 790-2991, Roche and (4) HER2 EP3, Sakura Finetek USA. The gene amplification status for all specimens were confirmed by fluorescence in-situ hybridization (FISH) analysis (ZytoLight) for HER2 copy number and HER2 gene/Chr.17 ratio. IHC results were evaluated by three reviewers and a consensus result was obtained for each specimen analyzed. IHC and FISH results were scored accordingly to the 2018 ASCO/CAP guidelines. Representative photomics were taken with the Olympus VS200 SlideView.

Results

	SK001	GE001	PATHWAY	HER2 EP3
HER2 low*	19% (19/98)	21% (21/98)	14% (14/98)	21% (21/98)
PPA**	91% (30/33)	94% (31/33)	94% (31/33)	94% (31/33)
NPA**	100% (61/61)	100% (61/61)	100% (61/61)	100% (61/61)

Table 1: Concordance for HER2 Overexpression and HER2 Low Status

*HER2 IHC classified as 1+, 2+ non-amplified (equivocal)

**Positive Predictive Accuracy (PPA) and Negative Predictive Accuracy (NPA) were calculated based on FISH results. IHC 2+ and 3+ included in PPA – 0 and 1+ included in NPA.

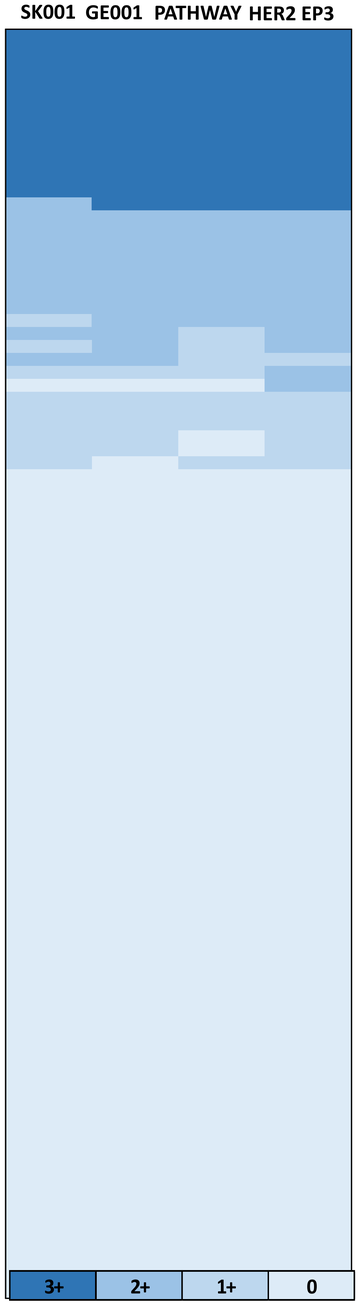

Fig. 1: Distribution of HER2 scores

Map that depicts HER2 IHC scores for the individual BCs (n=98)

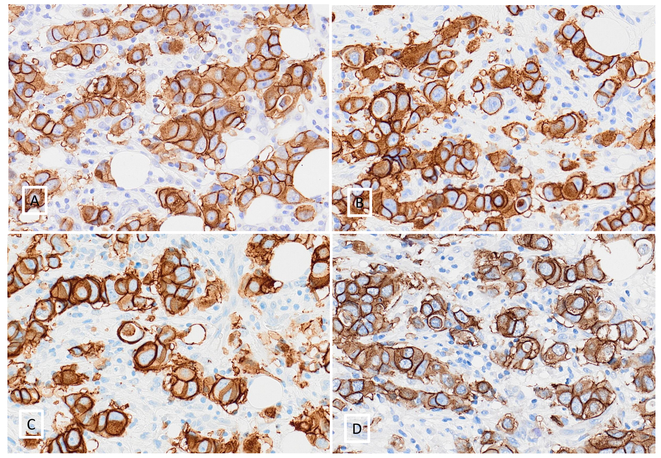

Fig. 2: HER2 IHC Status 3+

Representative photomics (IHC 200x) of HER2 3+ with confirmed FISH amplification for SK001 **(A)**, GE001 **(B)**, PATHWAY **(C)**, and HER2 EP3 **(D)**.

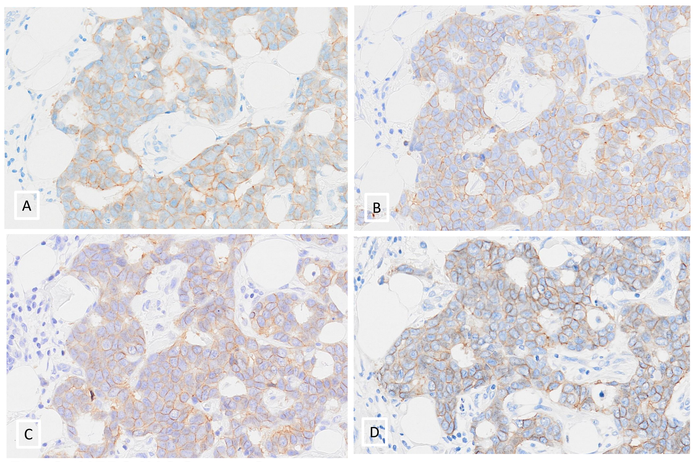

Fig. 3: HER2 IHC Status 2+

Representative photomics (IHC 200x) of HER2 2+ with confirmed FISH unamplification for SK001 **(A)**, GE001 **(B)**, PATHWAY **(C)**, and HER2 EP3 **(D)**.

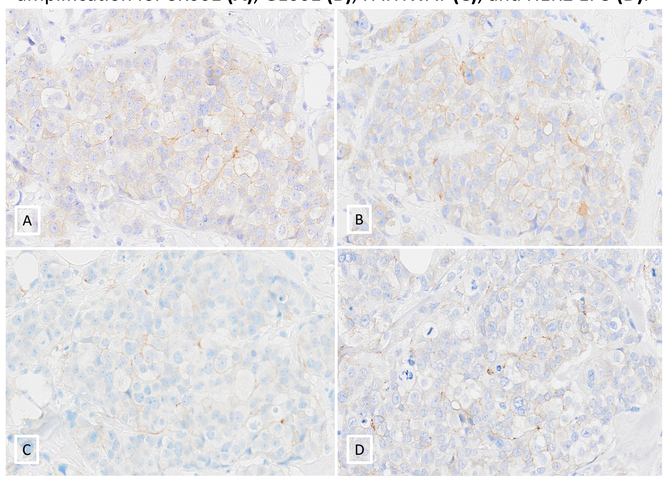

Fig. 4: HER2 IHC Status 1+

Representative photomics (IHC 200x) of HER2 1+ with confirmed FISH unamplification for SK001 **(A)**, GE001 **(B)**, PATHWAY **(C)**, and HER2 EP3 **(D)**.

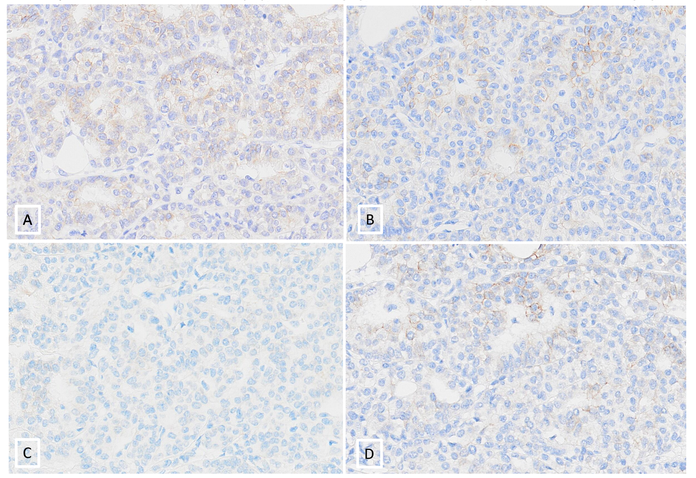

Fig. 5: HER2 IHC Status 0/1+

Representative photomics (IHC 200x) of HER2 0/1+ with confirmed FISH unamplification for SK001 with 1+ **(A)**, GE001 with 1+ **(B)**, PATHWAY with 0 **(C)**, and HER2 EP3 with 1+ **(D)**.

Conclusion

- A high accuracy and concordance for HER2 overexpression was obtained by the four different IHC assays; however, HercepTest SK001 exhibited 3% more false negative results.
- A reduced concordance was seen for HER2 low expression by the different assays.

Disclaimer: This study does not promote any *in-vitro* diagnostic use.

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