

Introduction

Breast cancer is one of the most common cancers in the United States.¹ Approximately 15-20% of invasive intraductal carcinomas (IDCs) exhibit human epidermal growth factor receptor 2 (HER2) overexpression.² Currently, HER2 status of IDC is screened using immunohistochemistry (IHC) and equivocal (2+) cases are confirmed with *in-situ* hybridization (ISH) or fluorescence *in-situ* hybridization (FISH). However, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines for HER2 status have been recently updated to accurately assess IDC HER2 status.³

Objectives:

- To compare the IHC staining patterns and intensities of three different HER2 clones (EP3, RM228 and GR011) to the FDA-approved HER2 clone 4B5 predictive test.
- To determine the concordance rates between HER2 protein expression by IHC and gene amplification by FISH. This study does not promote any *in-vitro* diagnostic use.

Materials & Methods

- A series of 25 human formalin fixed paraffin embedded (FFPE) IDC cases from five microarrays composed of HER2-negative (n=7), HER2-equivocal (n=9) and HER2-positive (n=9) were provided by NordiQC. HER2-equivocal amplified (n=3) and HER2-equivocal unamplified (n=6) were confirmed with Zytolight FISH analysis.
- The following HER2 clones (EP3, RM228, GR011) were run on Tissue-Tek Genie® Advanced Staining System using various pretreatment solutions and the Tissue-Tek Genie® Pro Detection Kit DAB.
- Evaluation of immunostains was performed by a pathologist and an external reviewer and compared to the FDA-approved HER2 4B5 using ASCO/CAP (2018) guidelines.

Results

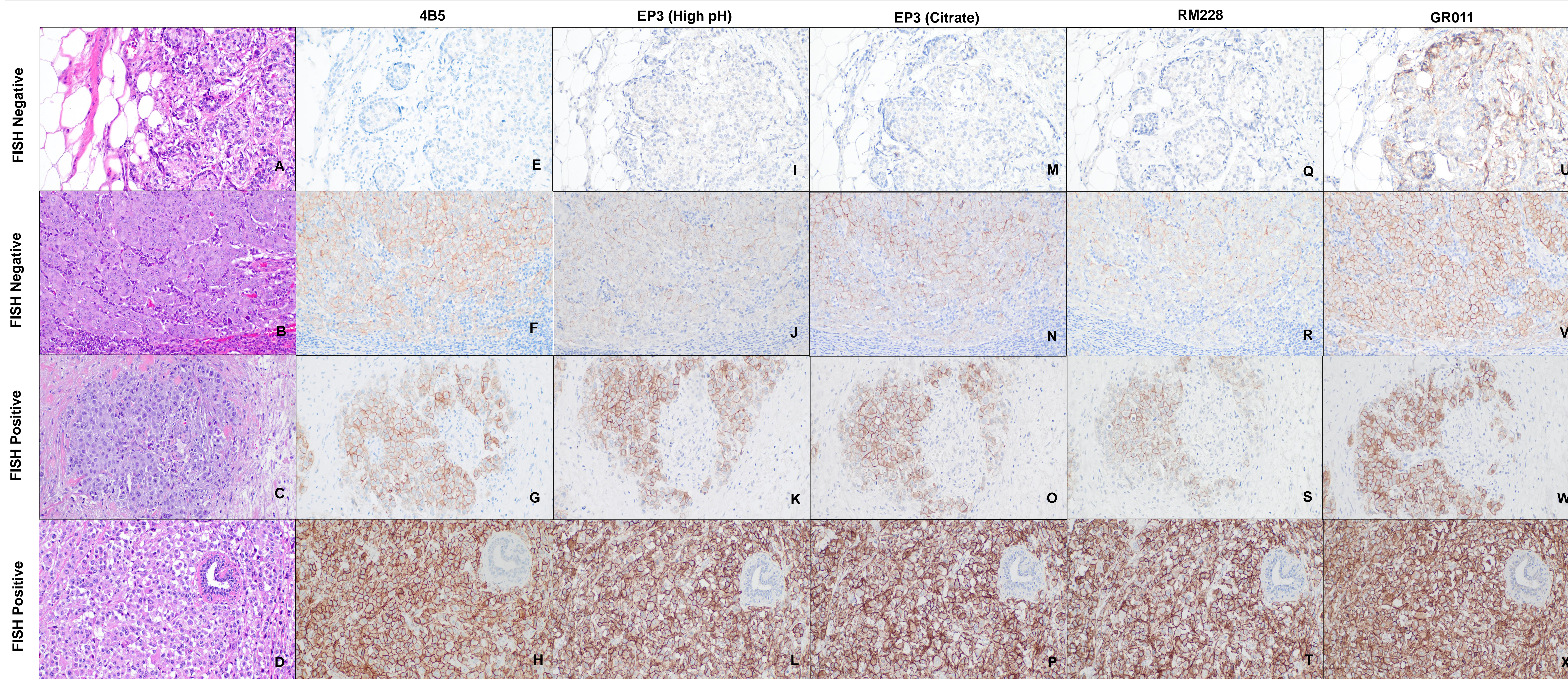


Fig. 1 Representative histological images of 4 different human intraductal carcinomas (H&E, 200X) (A-D) with corresponding confirmed Zytolight FISH analysis and FDA-approved HER2 4B5 IHC scores (IHC, 200X) of 0-1+ unamplified (E), 2+ unamplified (F), 2+ amplified (G) and 3+ amplified (H). HER2 EP3 (high pH antigen retrieval) exhibited IHC scores (IHC, 200X) of 0-1+ (I), 1+ (J), 1-2+ (K) and 3+ (L). HER2 EP3 (citrate) exhibited IHC scores (IHC, 200X) of 0-1+ (I), 1-2+ (J), 2+ (K) and 3+ (L). HER2 RM228 exhibited IHC scores (IHC, 200X) of 0+ (Q), 1-2+ (R), 2+ (S) and 3+ (T). HER2 GR011 exhibited IHC scores (IHC, 200X) of 2+ (U), 2-3+ (V), 2+ (W) and 3+ (X).

Table 1. Correlation of FISH and IHC Results

| FISH | IHC N(%) | | | | | | | | | | | | | | |
|------------------------|----------|----------|---------|---------------|----------|---------|---------------|----------|---------|----------|----------|---------|----------|----------|---------|
| | 4B5 | | | EP3 (High pH) | | | EP3 (Citrate) | | | RM228 | | | GR011 | | |
| | 0/1+ | 2+ | 3+ | 0/1+ | 2+ | 3+ | 0/1+ | 2+ | 3+ | 0/1+ | 2+ | 3+ | 0/1+ | 2+ | 3+ |
| Negative (unamplified) | 7 (100) | 6 (66.7) | 0 (0) | 7 (87.5) | 6 (75.0) | 0 (0) | 8 (88.9) | 5 (75.0) | 0 (0) | 9 (81.8) | 4 (80.0) | 0 (0) | 7 (77.8) | 6 (85.7) | 0 (0) |
| Positive (amplified) | 0 (0) | 3 (33.3) | 9 (100) | 1 (12.5) | 2 (25.0) | 9 (100) | 1 (11.1) | 2 (25.0) | 9 (100) | 2 (18.2) | 1 (20.0) | 9 (100) | 2 (22.2) | 1 (14.3) | 9 (100) |
| Total | 7 | 9 | 9 | 8 | 8 | 9 | 9 | 7 | 9 | 11 | 5 | 9 | 9 | 7 | 9 |

FISH, fluorescent *in situ* hybridization; IHC, immunohistochemistry

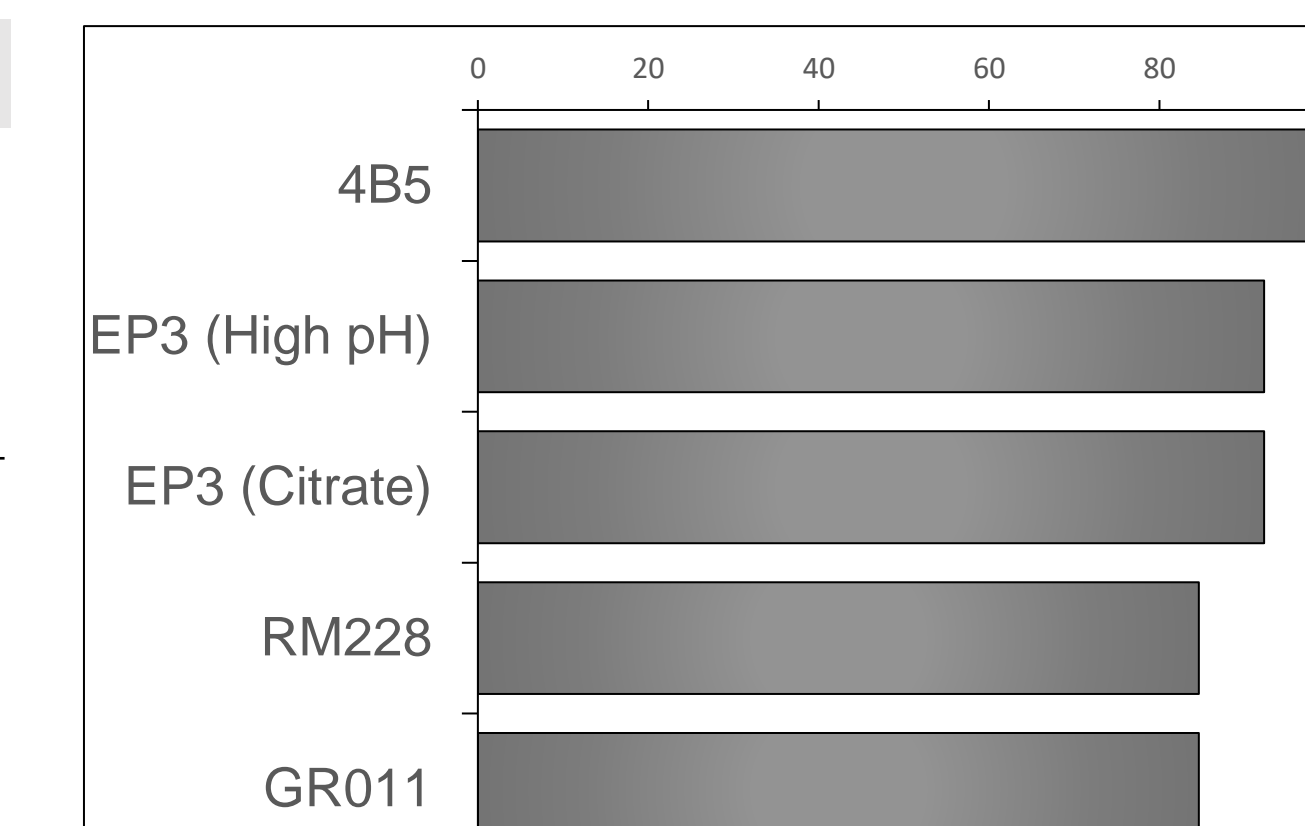


Fig. 2 Concordance Rate of FISH and IHC Scores for Positive HER2 Cases

Conclusion

HER2 EP3 performs similarly to 4B5

