

## Abstract of Outstanding Presentation (2)

# Basic Examination of *HER-2/neu* in Breast Carcinoma by Chromogenic in Situ Hybridization: Application to Fine-Needle Aspiration Cytology Specimens

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### Purpose

*HER-2/neu*, a protooncogene located on chromosome 17q, encodes a transmembrane tyrosine kinase with substantial homology to the epidermal growth factor receptor. To determine the efficacy of Herceptin, which is an immunotherapeutic drug for breast carcinoma, it is necessary to analyze the amplification or overexpression of *HER-2/neu* and its protein. In this study, we conducted a basic analysis of the HER2 gene in breast carcinoma by chromogenic in situ hybridization (CISH) using fine-needle aspiration (FNA) cytology specimens.

### Materials and Methods

#### Preparation of Slides

FNA cytology specimens obtained from patients with the same type of carcinoma were examined. Of the patients, 10 carried HER2 protein (3+) and 5 carried HER2 protein (2+) in tissue from an invasive ductal carcinoma as determined with the Hercep test. The specimens obtained by FNA were subjected to direct smearing or wash smearing. After the specimens were fixed with 95% alcohol, tumor cells were identified with Papanicolaou staining. In addition, a region containing many tumors was selected by the cell transfer method.

#### Chromogenic in Situ Hybridization

The slides were dehydrated in a graded alcohol series (80, 90, and 100%) and postfixated in 100% alcohol for 5 min. The detailed protocol recommended by Zymed Laboratories (San Francisco, CA, USA) was as follows. Briefly, denaturation at 95°C was performed.

A digoxigenin-labeled Her-2/neu probe was applied to the slides, and hybridization was carried out at 37°C for 16 to 24 hr.

After hybridization, an appropriate stringency wash (74°C, based on one slide) was performed. The slides were incubated with a mouse anti-digoxigeninantibody and goat anti-mouse antibody conjugated with horseradish peroxidase.

Diaminobenzidine was used as a substrate, and the slides were counterstained with hematoxylin.

#### Evaluation of CISH Results

Results of CISH were observed under a microscope (TE2000-E Nikon Insteck Co., Ltd.; Tokyo, Japan)

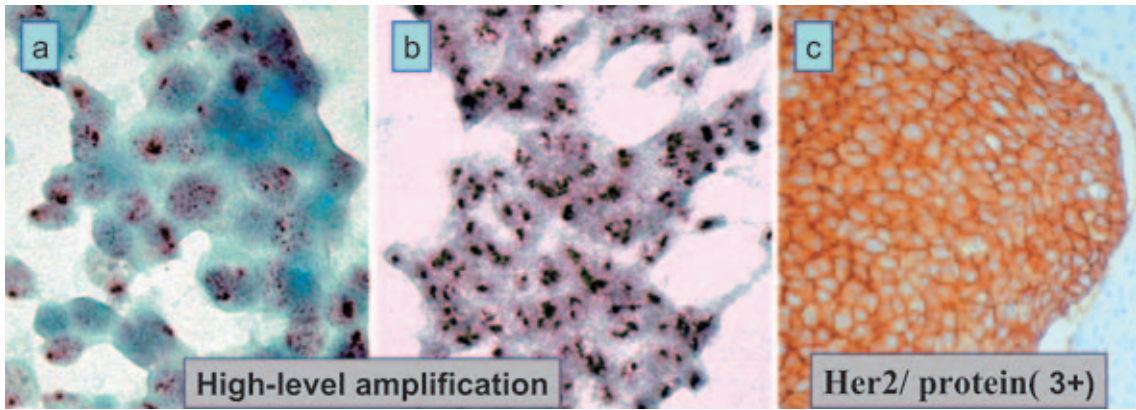


Fig. 1

a, b) Example of FNA cytology specimens with CISH: Her2/oncogene high-level amplification.  
 c) Corresponding Her2/protein(3+) immunostained paraffin section.

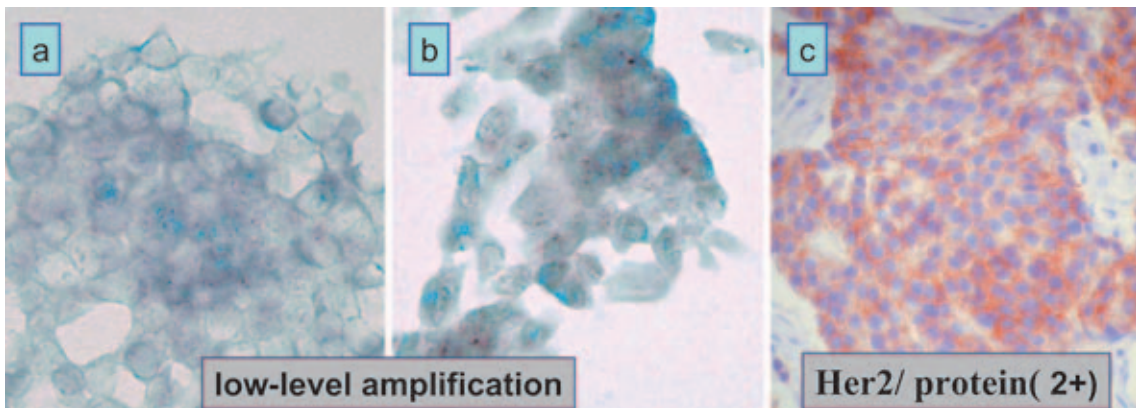


Fig. 2

a, b) Example of FNA cytology specimens with CISH: c) Her2/oncogene low-level amplification.  
 c) Corresponding Her2/protein(2+) immunostained paraffin section.

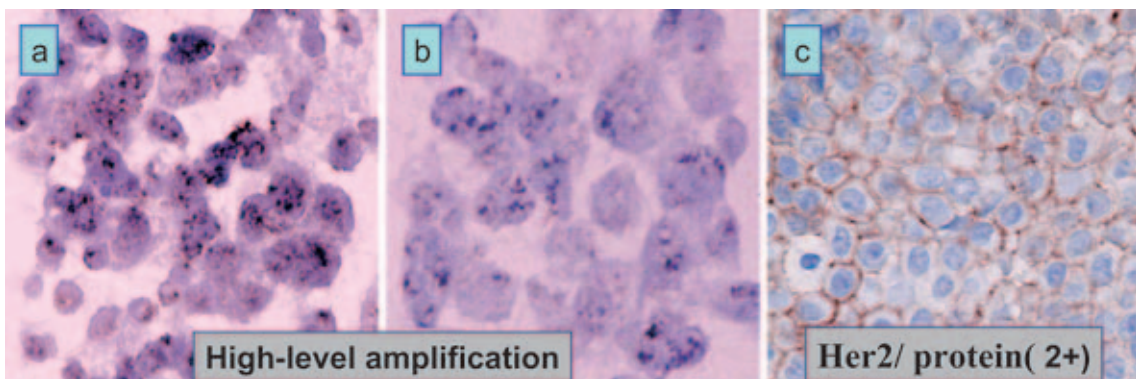


Fig. 3

a, b) Example of FNA cytology specimens with CISH: c) Her2/oncogene high-level amplification.  
 c) Corresponding Her2/protein(2+) immunostained paraffin section.

microscope using a 40 × objective lens. *HER-2/neu* amplification was evaluated with MetaMorph Ver.7 (Molecular Devices Corp.; Downingtown, PA, USA).

The amplification level was considered to be high when more than 10 copies, or a large cluster, of *HER-2/neu* was present in more than 50% of the cancer cell nuclei, whereas the amplification level was considered to be low when 6 to 10 copies or a small cluster of *HER-2/neu* was present in the same percentage of cells.

*HER-2/neu* was not considered to be amplified when 1 to 5 copies of *HER-2/neu* were identified per nucleus.

### **Results (Fig. 1, 2 and 3)**

Hybridization signals were detected in the 10 patients carrying the HER2 protein (3+), and the amplification of *HER-2/neu* was observed. Signals were detected in the 5 patients whose carcinoma cells were determined to carry the HER2 protein (2+); moreover, *HER-2/neu* amplification was observed in 3 of these patients.

### **Conclusions**

CISH using FNA cytology specimens enables the determination of the presence or absence of *HER-2/neu* amplification with light microscopy and allows the permanent preservation of specimens. The specimens treated by wash smearing were easily examined because their cells were in a monolayer.

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