Enhanced sensitivity HERmark® assay (ESHA): a sensitive and quantitative immunoassay superior to immunohistochemistry for the measurement of low HER2 protein levels in formalin-fixed, paraffin-embedded samples.

I. Introduction

Introduction. Breast cancers that express low levels of HER2 that are not considered HER2 positive disease by current HER2 classification standards can still benefit from targeted therapies for many new and promising HER2 targeted therapies, e.g., trastuzumab-eclizumab conjugates, tyrosine kinase inhibitors and vaccines. HER2 status is critically determined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). These technologies may have limited utility to lab the sensitivity required to adequately stratify HER2 positive patients. Following upon the successes of the HERmark Breast Assay, we have developed the Enhanced Sensitivity HERmark® assay to quantify low HER2 protein expression in formalin-fixed, paraffin-embedded (FFPE) samples. The ESHA assay relies on dual-color labeling for the proximity-dependent release of a fluorophore reporter, which is measured with high sensitivity and reproducibility.

Methods. The HER2 fluorescence in situ hybridization (FISH) assay is a label-free, real-time, and HER2 specific assay for low HER2 expression in paraffin-embedded tissues. Low HER2 expression cancer cell lines were used to establish assay conditions that would improve signal strength and optimize the lower limit of the dynamic range of the HERmark® assay. ESHA cancer cell lines and breast tumors were utilized to evaluate assay background, reproducibility, and to compare HER2 assessments based on IHC, V conjugate, and HER2 IHC.

Results. The ESHA format expanded the HER2 assay lower limit of quantification nearly 10-fold, and increased the reported fluorescence signal by an average of 10-fold, which resulted in a decrease in assay variation. ESHA measurements of HER2 protein in a panel of 37 breast cancer cell lines correlated with IHC protein expression levels in the breast cancer cell line panel (R² = 0.95). Reproducibility and assay variance were improved by >100% and >50%, background and good reproducibility.

Conclusions. We have developed an Enhanced Sensitivity HERmark® assay for the quantification of low HER2 protein expression in formalin-fixed, paraffin-embedded human breast tissue samples. This assay can be a particularly useful tool for the development of novel HER2-targeting therapies in patient populations that are not considered HER2 positive by conventional HER2 classification standards. Improving the stratification of HER2 expression may better define, and perhaps expand, the number of patients that are more likely to benefit from HER2-targeted therapies.

II. VeraTag® Assay Workflow

A. VeraTag® workflow

1. Phenotyping and frozen tissue storage
2. Endogenous protein extraction
3. Preparation of reagents
4. VeraTag® enzymatic lysis
5. VeraTag® separation

B. VeraTag® Workflow

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C. VeraTag® workflow

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III. HER2 by HERmark®

Assay Format

Clinical Correlation

IV. HER2 by ESHA

ESHA produced larger signal and improved reproducibility in low HER2 expressing cell lines

V. ESHA Cell Line Controls

Screening FFPE cell lines

VI. HER2 ESHA L.O.Q.

ESHA extended the lower limit of HER2 quantification

VII. ESHA in low HER2 BC

Comparison of ESHA signal to isotype Control in FFPE cell lines and HER2-negative Breast Cancer

VIII. Conclusions

• The HERmark® assay format resulted in an increase in assay signal, a decrease in assay variation, and an extension of the lower end of the dynamic range.

• In low HER2 breast cancer tumors, the ESHA assay demonstrated low background and good reproducibility.

• The ESHA HER2 assay may have utility in the development of novel HER2-targeted therapeutics in patient populations that are not considered HER2-positive by conventional HER2 classification.

• Improving the stratification of HER2 expression may better define, and perhaps expand, the number of patients that are more likely to benefit from HER2-targeted therapy.