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A comparative study of p95-HER2 carboxy terminal fragment (CTF) detected by immunohistochemistry and VeraTag immunoassays in human breast tumors

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Abstract

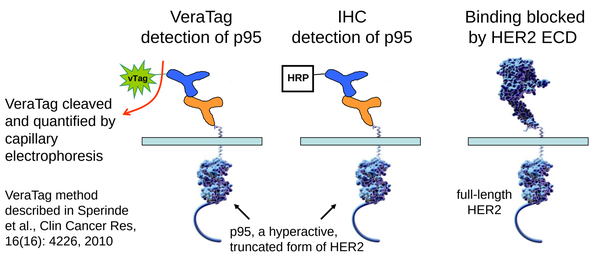
BACKGROUND: C-terminally truncated forms of HER2 (HER2-CTF) have been reported to be highly oncogenic and correlate with node-positive breast cancer, poor prognosis, and attenuated response to trastuzumab in patients with HER2+ metastatic breast cancer (MBC). A quantitative, sensitive and specific immunoassay using the VeraTag™ technology has been developed to detect the active Met611-HER2 CTF (p95) in FFPE tumor samples and has been used to identify HER2+ MBC patients who have a reduced response to trastuzumab in two independent cohorts. Recently, an IHC assay using an antibody directed to a similar p95 epitope was reported to detect a high percentage of HER2+ samples (79%) as p95 positive (#530, ASCO 2011).

METHODS: To verify the cellular specificity and accuracy of the p95 VeraTag assay, and evaluate the prevalence of p95 in breast tumors, an IHC assay was developed which utilizes the same p95 antibody as the VeraTag assay. The IHC and VeraTag assay formats were compared in 120 human breast tumor excisional and core biopsy samples submitted to central clinical reference lab testing, and having different levels of HER2 measured by HercepTest™ (Dako, Inc.) IHC: 0 (n=20); 1+ (n=20), 2+ (n=20), and 3+ (n=60). Replicate slides were run in both VeraTag and IHC assays to demonstrate reproducibility. Nearly all p95 IHC signal was associated with tumor cells only.

RESULTS: Consistent with earlier p95 VeraTag data, a general correlation was observed between p95 and HER2 levels, with tumors expressing normal levels of HER2 rarely expressing high p95 levels. However, within the subset of HER2+ samples (IHC 3+ or FISH > 2.2; n=63), a ~30-fold range of p95 VeraTag signal was observed. A similar relationship was observed for samples assayed by p95 IHC. All samples measured as p95 IHC 3+ (≥10% cells with 3+ membranous staining) were HER2+ by central lab testing and represented nearly a third of the HER2+ samples. The p95 VeraTag assay showed a significant but weak correlation with p95 IHC.

CONCLUSIONS: These results indicate that the tumor-specific p95 signal detected by the p95 VeraTag assay is in general agreement with an IHC assay using the same antibody; however a number of cases with p95 VeraTag scores above the clinical cutoff do not fall into the p95 IHC 3+ category. Additionally, the prevalence of p95 positivity by VeraTag assay and by IHC 3+ scores is consistent with earlier peer-reviewed studies and lower than recently reported using a different IHC assay. The clinical significance of using the p95 IHC assay to complement the p95 VeraTag score is being evaluated.

Methods - p95 VeraTag and p95 IHC



1. p95 is detected by a p95-specific Ab followed by a VeraTag-labeled secondary Ab.
2. VeraTag is cleaved from Ab by reduction of disulfide linker.
3. Solution is collected and analyzed by capillary electrophoresis.
4. Integral of VeraTag signal (RPA) is normalized to tumor area (TA).
5. All steps of the p95 IHC assay were run on a Ventana Discovery XT.

Cohort selection and study plan

The current cohort was selected by HER2 IHC to span the entire distribution of HER2 expression, overweighting the HER2-positives.

HER2 IHC	Number of cases
0	20
1+	20
2+	20
3+	60

120 cases
4 slides per case

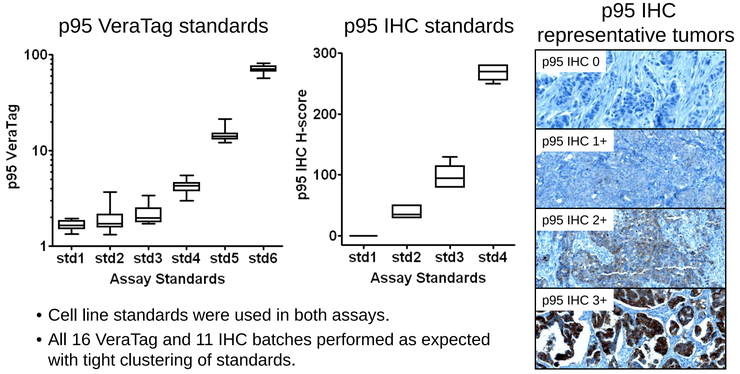
p95 VeraTag tumor-average expression	p95 IHC peak membrane expression
2 replicate assays	2 replicate assays
• Run on different days • Varied equipment • Varied operators	• Run on different days • Varied equipment • Varied pathologists

Goals

Assess **reproducibility** of p95 VeraTag and IHC assays
- Typical p95 expression levels are much lower than amplified HER2
- Reproducibility will be critical for a clinically useful assay

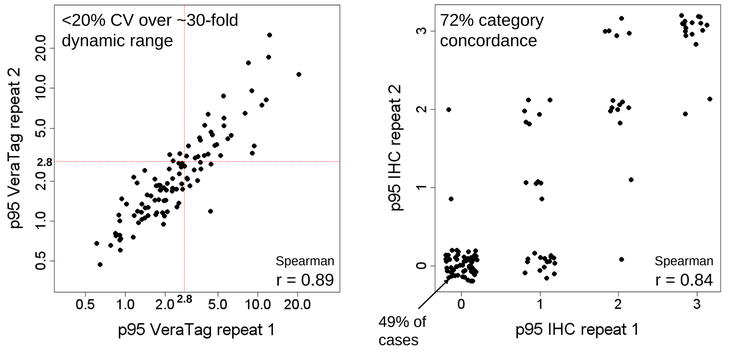
Assess **concordance** of p95 VeraTag and IHC assays
- IHC quantification methods often focus on peak, membranous expression.
- The VeraTag method provides a tumor average expression level.
- Method of p95 quantification may be clinically important.

Assay Performance



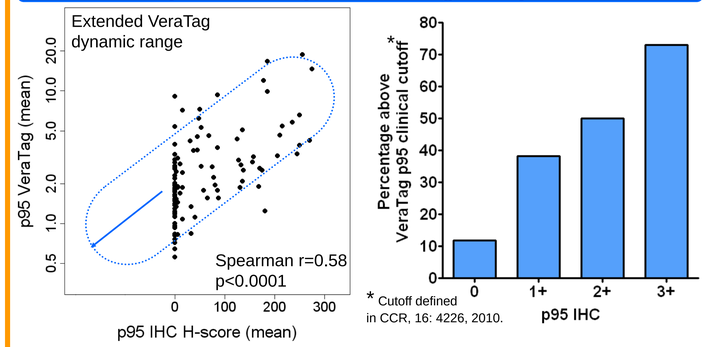
- Cell line standards were used in both assays.
- All 16 VeraTag and 11 IHC batches performed as expected with tight clustering of standards.

Assay Reproducibility



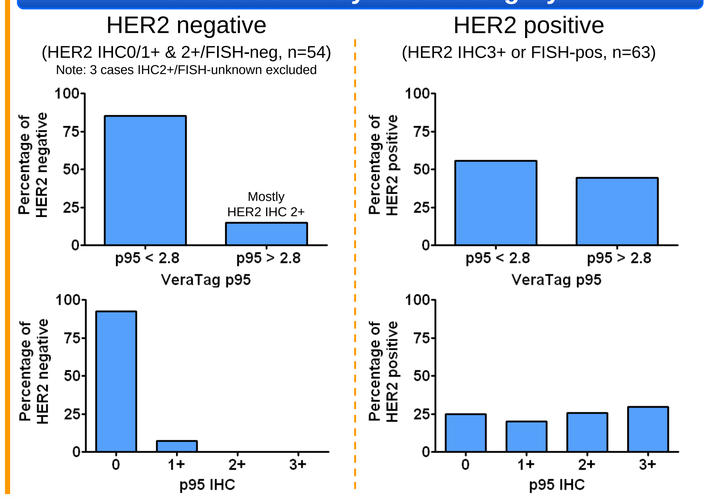
- Both p95 VeraTag and p95 IHC displayed high concordance between replicates.
- In contrast to the VeraTag distribution, 49% of cases were clustered at the bottom of the p95 IHC range (replicate IHC=0), reflecting the sensitivity limitations of the IHC format.

Comparison of p95 VeraTag and p95 IHC



p95 VeraTag vs. IHC H-score: $R^2 = 0.33$ → Significant but weak correlation

Distributions by HER2 category



Summary

- Both p95 VeraTag and p95 IHC assays produced reproducible measurements of p95.
- p95 VeraTag assay shows good reproducibility around working clinical cutoff of 2.8.
- Equivalent p95 IHC cutoff would be at 1+ or 2+ where reproducibility isn't as high.
- p95 VeraTag and p95 IHC may provide complementary measures of p95 expression.
- The clinical significance of using the p95 IHC assay to complement the p95 VeraTag score is being evaluated.