

Abstract

Background: Using IHC or FISH to select patients for trastuzumab-based therapy, only half of HER2-positive patients show evidence of response. In vitro data implicate HER2:HER3 heterodimers and p95HER2 (p95), the truncated 95-kilodalton C-terminal fragment of HER-2 lacking the trastuzumab binding site, as mediators of resistance to trastuzumab at the receptor level. We have previously reported that central FISH-positive patients with low HER2 protein expression by VeraTag had significantly reduced response to trastuzumab compared to patients who had FISH-positive tumors with high HER2 protein expression (Lipton, SABCS 2008). Adding quantitative measurements of HER3 and p95, we offer evidence for the existence of multiple subtypes of HER2-positive tumors that respond differently to trastuzumab.

Methods: Using the VeraTag assay, quantitative protein measurements of HER2, HER3, and p95 were made in FFPE specimens from a cohort of patients with metastatic breast cancer (MBC) and correlated with time to progression (TTP) and overall survival (OS) following treatment with trastuzumab using Kaplan-Meier (KM) and Cox proportional hazards regression analyses.

Results: Measurements of HER2 (H2T), HER3 (H3T) and p95 were made in FFPE tumor samples from 95 patients treated with trastuzumab for metastatic breast cancer. Within the group that over-expressed HER2 by the VeraTag Assay (n=60), a group with highly over-expressed HER2 (n=15) had shorter TTP and OS than those that had moderate HER2 over-expression (median TTP 4.6 vs. 12 mos, HR=2.7; p=0.004; median OS 29 vs. 40 mos, HR=2.0; p=0.047). Within the subgroup with moderate H2T over-expression (n=45), bi-variate Cox analyses demonstrated that p95 and H3T were independent predictors of TTP (p95 HR=2.1; p=0.031; H3T HR=3.5; p=0.0037). For OS, p95 was significant and H3T showed a strong trend (p95 HR=2.5; p=0.025, H3T HR=2.2; p=0.089).

Conclusions: These data suggest the existence of multiple subgroups of HER2-positive patients expressing varying HER2, p95, and HER3 levels that experience different clinical outcomes following treatment with trastuzumab. Furthermore, the association of HER3 and p95 over-expression with poor response to trastuzumab in otherwise HER2-positive tumors suggests possible treatment approaches with combinations of targeted therapies.

Methods

Patient selection: Patients had HER2-positive metastatic breast cancer (IHC 3+ by Herceptest, or IHC 2+, FISH+ by Vysis) at a single institution between 1999-2006, ECOG 0-2 performance status, and life expectancy \geq 12 weeks. They had received no prior trastuzumab, and were treated with either trastuzumab + chemotherapy or trastuzumab alone.

VeraTag Assays make quantitative measurements of HER family proteins using formalin-fixed, paraffin-embedded (FFPE) tissue specimens. Proximity-based assays (HER2 (H2T), HER3 (H3T)) employ two monoclonal antibodies that minimize background and enhance specificity. The p95 assay is not proximity-based, but rather relies on a novel monoclonal antibody to specifically recognize the truncated form of the HER2 receptor (see figure 4).

Statistics: Primary tumor levels of HER family proteins (H2T, H3T and p95) were correlated with response to trastuzumab (TTP and OS). Methods included Test for trend, Kaplan Meier using log rank, and Cox Proportional Hazards regression analyses. The optimal cutpoints for the HER family protein analyses were selected using positional scanning for the lowest p-value (above vs. below the optimal cutpoint) associated with significant differences in progression-free survival (PFS).

Patient selection

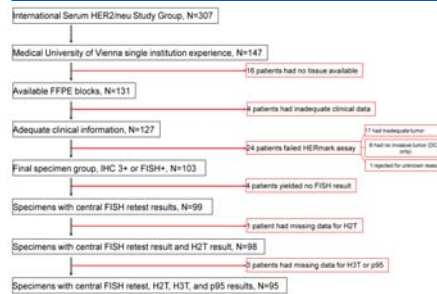


Figure 1: REMARK flow diagram showing how patients were selected for inclusion in this study.

Cohort characteristics

Total number of patients	95
Mean age (range)	55.0 (27.6 - 80.8)
Number of metastatic sites	
< 3	55 (58%)
> 3	40 (42%)
Treatment	
Trastuzumab + chemotherapy	83 (87%)
Trastuzumab only	12 (13%)
Line of chemotherapy	
First line	69 (72%)
Second line	17 (18%)
Third line	8 (8%)
Unknown	1 (1%)
Mean follow-up (months, range)	33.5 (11.8 - 77.9)
Mean treatment length (months, range)	5.9 (0.7 - 33.6)

Figure 2: Clinical characteristics of the cohort of patients included in this study.

Subgroups for comparison

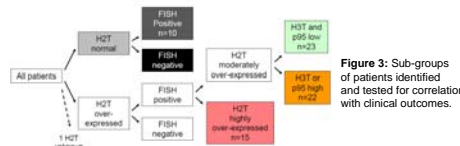


Figure 3: Sub-groups of patients identified and tested for correlation with clinical outcomes.

VeraTag assays for quantitative protein measurements

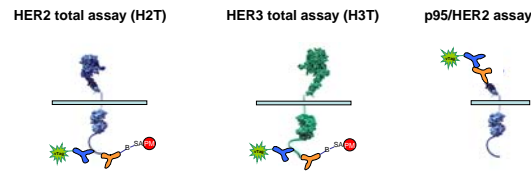


Figure 4: VeraTag assay designs for measurement of different protein analytes. For HER2, commercially available antibodies recognizing two distinct epitopes in the intracellular domain (ICD) of the receptor have been adapted for use in the VeraTag assay. Subsequent to antibody binding and activation of the photostabilizer molecule (PM), the liberated vTags are collected and quantified by capillary electrophoresis. For HER3, a proprietary monoclonal antibody was generated and characterized at Monogram Biosciences. For p95, a proprietary antibody was generated at Monogram that specifically recognizes a conformational epitope in the extracellular tail of the truncated HER2 receptor which is not recognized in the full length HER2.

Subsets of patients experiencing sub-optimal responses following treatment with trastuzumab

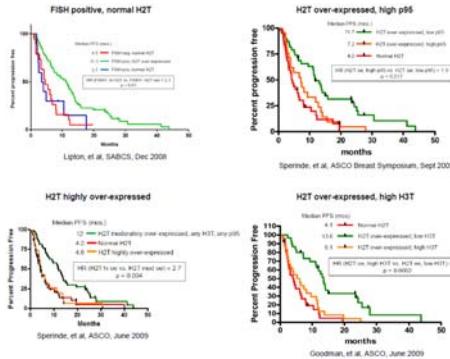
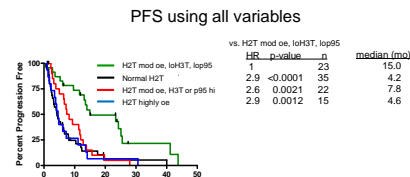


Figure 5: When analyzed individually, and as previously reported, sub-groups of patients defined by quantitative levels of the measured protein analytes H2T, H3T, and p95, were observed to experience different clinical outcomes following treatment with trastuzumab as measured by progression-free survival (PFS). All patients had previously been characterized as HER2-positive by IHC. The particular sub-groups were defined by a) HER2 normal (H2T < 13.8), b) HER2 highly over-expressed (H2T > 68.5), c) HER2 over-expressed (H2T > 13.8) and high p95 (p95 > 140), d) HER2 over-expressed (H2T > 13.8) and high H3T (H3T > 3.5), and e) HER2 moderately over-expressed (13.8 < H2T < 68.5) and both low p95 (p95 < 140) and low H3T (H3T < 3.5).

Multiple sub-types of HER2-positive MBC



OS using all variables

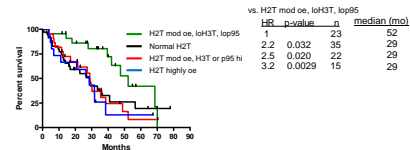


Figure 6: Kaplan-Meier analyses examining the relationship between quantitative levels of HER2, HER3, and p95 determined using FFPE tissue specimens and the clinical outcomes PFS and OS (overall survival). Among patients originally classified by IHC or FISH as HER2-positive, five sub-groups of patients can be discerned:

1. Patients with normal H2T levels, irrespective of FISH status (black)
2. Patients with highly over-expressed H2T (blue)
- 3 and 4. Patients with moderately over-expressed H2T and either high p95 or high H3T (red)
5. Patients with moderately over-expressed H2T and neither high p95 nor high H3T (green)

Summary

These data suggest the possible existence of multiple subgroups of patients with HER2-positive metastatic breast cancer that express varying levels of HER2, p95, and HER3 and that experience different clinical outcomes following treatment with trastuzumab.

The association of HER3 and p95 over-expression with poor response to trastuzumab in otherwise HER2-positive tumors (gene amplified by FISH and also HER2 over-expressors by VeraTag) suggests possible roles in trastuzumab resistance as well as potential treatment approaches using combinations of targeted therapies.

These data are hypothesis-generating, and require confirmation in independent datasets. Experiments designed to test the hypotheses suggested by these results are in progress.

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