LAB LOCATIONS

California

Integrated Oncology
2601 Campus Drive
Irvine, California 92612
(800) 710-1800
Fax (949) 450-0146

Monogram Biosciences
345 Oyster Point Blvd,
South San Francisco, CA 94080
(800) 777-0177

New York

Integrated Oncology
521 West 57th Street, Sixth Floor
New York, New York 10019
(800) 447-8881
Fax (212) 258-2137

North Carolina

LabCorp Center for Molecular
Biology and Pathology
1912 Alexander Drive
Research Triangle Park,
North Carolina 27709
(800) 345-4363
Fax (919) 361-7201

Tennessee

Integrated Oncology
201 Summit View Drive, Suite 100
Brentwood, Tennessee 37027
(800) 874-8532
Fax (615) 370-8074

Integrated ONCOLOGY
LabCorp Specialty Testing Group

Monogram BIOSCIENCES
LabCorp Specialty Testing Group

onc-700-v1-1212

Correlation of quantitative p95HER2 and total HER2 levels with clinical outcomes in a combined analysis of two cohorts of trastuzumab-treated metastatic breast cancer patients

R Duchnowska[1], J Sperinde[2], K Leitzel[3], B Szostakiewicz[4], A Paquet[2], SM Ali[3,5], T Jankowski[6], M Haddad[2], E Fuchs[7], B Arłukowicz-Czartoryska[8], J Winslow[2], C F Singer[9], PJ Wysocki[10], Y Lie[2], R Horvat[11], M Foszczynska-Kłoda[12], C Petropoulos[2], B Radecka[13], MM Litwiniuk[10], S Dębska[14], J Weidler[2], W Huang[2], W Biernat[4], WJ Köstler[7], J Jassem[4], A Lipton[3]

[1]Military Institute of Medicine, Warsaw, Poland; [2]Monogram Biosciences/Integrated Oncology-LabCorp, Inc., South San Francisco, CA; [3]Penn State / Hershey Medical Center, Hershey, PA; [4]Medical University of Gdańsk, Gdańsk, Poland; [5]Lebanon VA Medical Center, Lebanon, PA; [6]Regional Cancer Center, Lublin, Poland; [7]Clinical Division of Oncology, Department of Medicine I and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [8]Regional Cancer Center, Białystok, Poland; [9]Department of Obstetrics and Gynecology and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [10]Greater Poland Cancer Center, Poznań, Poland; [11]Department of Clinical Pathology, Medical University of Vienna, Vienna, Austria; [12]West Pomeranian Oncology Center, Szczecin, Poland; [13]Opole Oncological Center, Opole, Poland; [14]Regional Cancer Center, Łódź, Poland

Abstract

Background: Expression of p95HER2 (p95), a truncated form of HER2 also known as p110 or M611-CTF, is a possible trastuzumab resistance mechanism and has been associated with poor prognosis in trastuzumab-treated HER2-positive metastatic breast cancer (MBC). Previously we reported on optimal clinical cutoffs for quantitative p95 (Clin Cancer Res, 16:4226, 2010) and quantitative HER2 protein expression (H2T) by HERmark® (Cancer, 116:5168, 2010) that defined patient subsets with different progression-free survival (PFS). These cutoffs were confirmed in an independent trastuzumab-treated MBC cohort (ASCO 2011, #586). Here, using individual patient data, we performed an analysis on the combined data set of 243 cases from the discovery and validation cohorts to derive optimal cutoffs for quantitative p95 and H2T.

Methods: Both quantitative H2T (HERmark®, Monogram Biosciences) and p95 assays employed the VeraTag® method to quantify protein expression in formalin-fixed, paraffin-embedded tumor samples from two cohorts of 101 and 142 cases of trastuzumab-treated MBC with 7.4 and 9.2 months median PFS, respectively. All analyses were stratified by hormone receptor status, tumor grade (3 vs. 1+2) and cohort. H2T measurements were compared to pre-specified cutoffs for HERmark negative (H2T<10.5 Relative Fluorescence / mm^2 tumor [RF/mm^2]) and HERmark positive (H2T>17.8 RF/mm^2), derived from the <5^{th} percentile of centrally determined HER2-positives and the >95^{th} percentile of centrally determined HER2-negatives, respectively, within a reference database of 1,090 breast cancer patient samples.

Results: Patients classified as HERmark-positive had longer PFS than those classified as HERmark-negative (HR=0.52; p=0.0006; medians 10.0 and 5.9 months). The previously determined optimal H2T cutoff of 13.8 RF/mm^2 in the center of the HERmark-equivocal zone, gave a similar result (HR=0.54; p=0.0005). This was close to the optimal cutoff of 12.75 RF/mm^2 (HR=0.48; p<0.0001, unadjusted) for the combined data set. The PFS for the small group of patients in the HERmark-equivocal zone (n=20) was more similar to the HERmark-negatives (equivocal vs. negative: HR=0.98; p=0.9) than the HERmark-positives (positive vs. equivocal: HR=0.57; p=0.057). The pre-specified p95 cutoff at 2.8 RF/mm^2 separated the 174 HERmark-positive cases into two groups of longer (p95<2.8 RF/mm^2) vs. shorter PFS (HR=1.9; p=0.0014; medians 13.1 and 7.4 months). Increasing continuous p95 also correlated with shorter PFS (HR=1.9/log; p=0.022) in the HERmark-positive subset. An optimal p95 cutoff was identified at 2.7 RF/mm^2 (HR=2.0; p=0.0009, unadjusted), although a slightly higher local HR maximum was found at 1.55 RF/mm^2 (HR=2.3; p=0.0004, unadjusted).

Conclusions: HERmark positive and negative categories, defined by analytical comparison with centrally determined HER2 status, were confirmed to have significantly different PFS in trastuzumab-treated MBC patients. The optimal H2T clinical cutoff for this combined analysis was centered in the HERmark analytical equivocal zone. An optimal p95 clinical cutoff of 2.7 RF/mm^2 derived from this combined analysis was nearly identical to the previously established cutoff of 2.8 RF/mm^2.

Correlation of quantitative p95HER2 and total HER2 levels with clinical outcomes in a combined analysis of two cohorts of trastuzumab-treated metastatic breast cancer patients

R Duchnowska[1], J Sperinde[2], K Leitzel[3], B Szostakiewicz[4], A Paquet[2], SM Ali[3,5], T Jankowski[6], M Haddad[2], E Fuchs[7], B Arłukowicz-Czartoryska[8], J Winslow[2], C F Singer[9], PJ Wysocki[10], Y Lie[2], R Horvat[11], M Foszczynska-Kłoda[12], C Petropoulos[2], B. Radecka[13], MM Litwiniuk[10], S Dębska[14], J Weidler[2], W Huang[2], W Biernat[4], WJ Köstler[7], J Jassem[4], A Lipton[3]

[1]Military Institute of Medicine, Warsaw, Poland; [2]Monogram Biosciences/Integrated Oncology-LabCorp, Inc., South San Francisco, CA; [3]Penn State / Hershey Medical Center, Hershey, PA; [4]Medical University of Gdańsk, Gdańsk, Poland; [5]Lebanon VA Medical Center, Lebanon, PA; [6]Regional Cancer Center, Lublin, Poland; [7]Clinical Division of Oncology, Department of Medicine I and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [8]Regional Cancer Center, Białystok, Poland; [9]Department of Obstetrics and Gynecology and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [10]Greater Poland Cancer Center, Poznań, Poland; [11]Department of Clinical Pathology, Medical University of Vienna, Vienna, Austria; [12]West Pomeranian Oncology Center, Szczecin, Poland; [13]Opole Oncological Center, Opole, Poland; [14]Regional Cancer Center, Łódź, Poland

SABCS 2012
P2-10-31

Background

Expression of p95HER2 (p95), a truncated form of HER2 also known as p110 or M611-CTF, is a possible trastuzumab resistance mechanism and has been associated with poor prognosis in trastuzumab-treated HER2-positive metastatic breast cancer (MBC). Previously we reported on optimal clinical cutoffs for quantitative p95 (Clin Cancer Res, 16:4226, 2010) and quantitative HER2 protein expression (Cancer, 116:5168, 2010) that defined patient subsets with different progression-free survival (PFS). These cutoffs were confirmed in an independent trastuzumab-treated MBC cohort (ASCO 2011, #586). Here, using individual patient data, we performed an analysis on the combined data set of 243 cases from the discovery and validation cohorts to derive optimal cutoffs for quantitative p95 and H2T.

Methods

Both quantitative HER2 (H2T) and p95 assays employed the VeraTag® method (Monogram Biosciences, So. San Francisco, CA) to quantify protein expression in formalin-fixed, paraffin-embedded (FFPE) tumor samples from two cohorts of 101 and 142 cases of trastuzumab-treated MBC with 7.4 and 9.2 months median PFS, respectively. All analyses were stratified by hormone receptor status, tumor grade (3 vs. 1+2) and cohort.

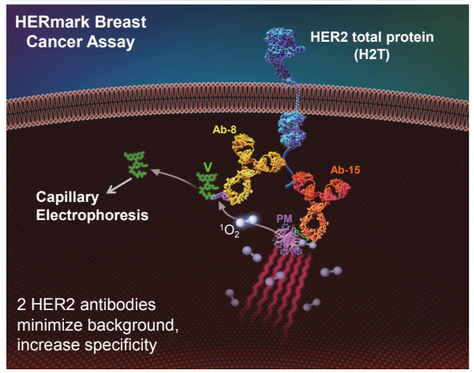
Quantitative HER2 Assay

Total HER2 protein expression (H2T) was quantified using the HERmark® assay as previously described (*Huang et al. Am J Clin Pathol 134:303, 2010*). H2T was quantified through the release of a fluorescent tag (*V* for "VeraTag® reporter", see Figure) conjugated to a HER2 monoclonal antibody (mAb). The antibody is paired with a biotinylated second HER2 mAb. An avidin-linked photosensitizer molecule (*PM*) produces singlet O_2 ($^1O_2$) upon illumination with red light. Signal (*V*) quantified by capillary electrophoresis is normalized to invasive tumor area on the FFPE tissue section. H2T measurements are compared to pre-specified cutoffs for HERmark negative (H2T≤10.5 Relative Fluorescence / mm^2 tumor [RF/mm^2]) and HERmark positive (H2T>17.8 RF/mm^2) with Equivocal defined as 10.5<H2T≤17.8, derived from the <5th percentile of centrally determined HER2-positives and the >95th percentile of centrally determined HER2-negatives, respectively, within a reference database of 1,090 breast cancer patient samples.

Quantitative p95 Assay

P95HER2 (p95) was quantified using the VeraTag platform with a proprietary mAb specific for the M611-CTF form of p95 as described in Clin Cancer Res, 16:4226, 2010.

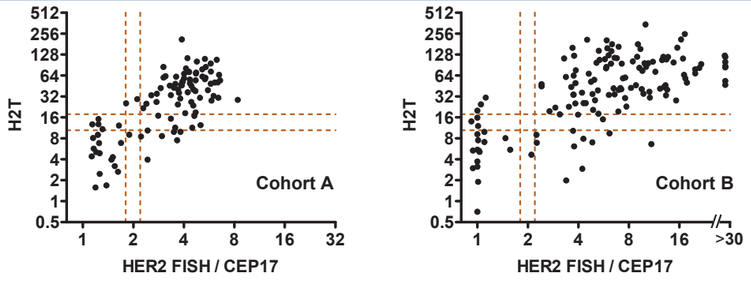
Quantitative HER2 Assay



Patient/Tumor Characteristics

Characteristic	No. (range or %)
Median age at diagnosis	54 (25–85 yr)
HERmark category for primary tumor	
Negative (H2T ≤ 10.5)	49 (20%)
Equivocal (10.5 < H2T ≤ 17.8)	20 (8.2%)
Positive (H2T > 17.8)	174 (72%)
Tumor grade	
Grade 1 (well differentiated)	5 (2.1%)
Grade 2 (moderately differentiated)	72 (30%)
Grade 3 (poorly differentiated)	166 (68%)
N/A (data not available)	*1*
Hormone receptor status	
Negative (ER and PgR negative)	139 (57%)
Positive (ER or PgR positive)	103 (43%)
N/A (data not available)	*2*

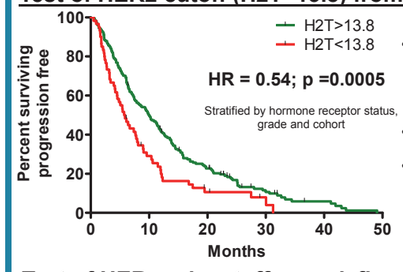
HER2 expression level in two cohorts



Cohort B had higher quantitative HER2 protein expression than **Cohort A**, but this was consistent with higher HER2 gene copy numbers.

Progression free survival vs. quantitative HER2

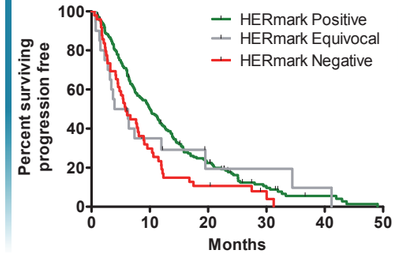
Test of HER2 cutoff (H2T=13.8) from Cancer, 116:5168 (2010)



- The cutoff derived from the training cohort and verified in the test cohort (ASCO 2011, #586) was applied to the combined data set.
- H2T<13.8: n=60. H2T>13.8: n=183.
- The optimal cutoff for the combined data set was H2T=12.75 RF/mm^2 (HR=0.48; p<0.0001, unadjusted).

Test of HERmark cutoffs pre-defined per central HER2 determination*

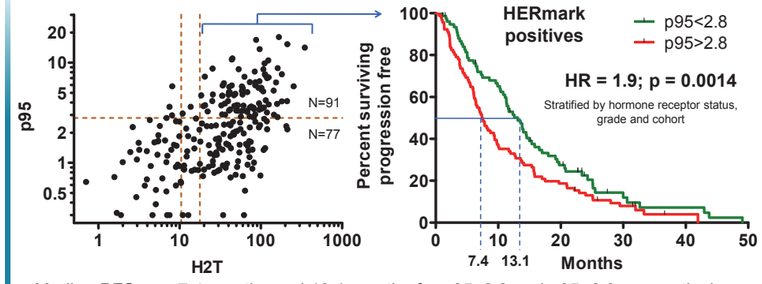
*See Methods section for derivation of analytical HERmark cutoffs from database of 1,090 tumors.



HERmark category (Stratified by hormone receptor status, grade and cohort)	N	PFS vs. HERmark negative group: HR	PFS vs. HERmark negative group: p-value
Negative	49	1	1
Equivocal	20	0.98	0.94
Positive	174	0.52	0.0006

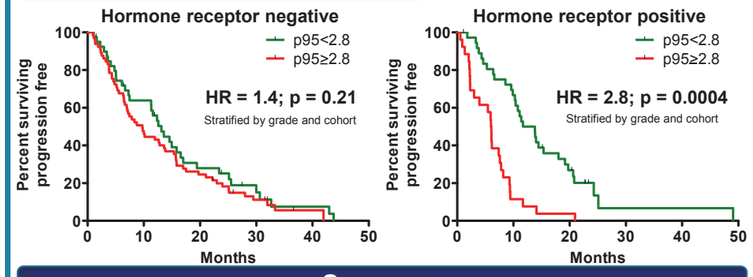
HERmark-positive vs. HERmark-equivocal: HR=0.57; p=0.057

Progression free survival vs. quantitative p95



- Median PFS was 7.4 months and 13.1 months for p95≥2.8 and p95<2.8, respectively.
- Increasing continuous p95 was also correlated with shorter PFS (HR=1.9/log; p=0.022).
- The cutoff of p95=2.8 gave similar results in the HER2 FISH positive population (HR=1.7; p=0.0047).
- The optimal cutoff for the combined set was p95=2.7 (HR=2.0, p=0.0009, unadjusted).

Influence of hormone receptor status



Summary

- The H2T=13.8 cutoff derived from cohort A (Cancer, 116:5168, 2010) and tested in cohort B (ASCO 2011, #586) gave a similar difference in PFS (HR~0.5) to pre-defined HERmark cutoffs derived from concordance studies with central HER2 status.
- The p95=2.8 cutoff derived from cohort A (CCR, 16:4226, 2010) and tested in cohort B (ASCO 2011, #586) was prognostic in both the HERmark and FISH-positive populations.
- The shorter PFS observed for cases with p95≥2.8 was strongly influenced by the hormone receptor positive subgroup.
- These results are consistent with other studies in the metastatic setting. The role of p95 in the neo-adjuvant and adjuvant settings is yet to be determined.

Presented at the 2012 San Antonio Breast Cancer Symposium
December 4–8, 2012, San Antonio, TX