LAB LOCATIONS

California

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

Integrated Oncology
5300 McConnell Avenue
Los Angeles, California 90066
(800) 447-5816
Fax (310) 482-5787

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

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Quantitative HER2 measurement and PI3K mutation profile in matched primary and metastatic breast cancer tissues

W. Huang[1], J. Lara[2], R. Michaelson[2], X. Sun[2], P. Conte[3], V. Guarneri[3], E. Barbieri[3], S. Ali[4,5], K. Leitzel[4], J. Weidler[1], Y. Lie[1], M. Haddad[1], A. Paquet[1], J. Sperinde[1], J. Howitt[6], M. Eisenberg[6], L. Hurley[6], C. Petropoulos[1], and A. Lipton[4]

[1]Monogram Biosciences Inc., South San Francisco, CA; [2]Saint Barnabas Medical Center, Livingston, NJ; [3]University of Modena, Modena, Italy; [4]Penn State /Hershey Medical Center, Hershey, PA; [5]Lebanon VA Medical Center, Lebanon, PA; [6]Laboratory Corporation of America, Research Triangle Park, NC.

Abstract

Background: HER2 status of primary breast cancer (PBC) is routinely used to determine systemic treatment for metastatic breast cancer (MBC) patients. Discordance rates of HER2 status between PBC and MBC range from 5.5% to 29% based on published meta-analyses. The clinical benefit of re-assessing HER2 in MBC tissues remains controversial. In this study, we measured quantitative HER2 expression in matched PBC and MBC tissues and correlated changes of HER2 with mutations in the catalytic domain of PI3 kinase (PIK3CA).

Methods: Total HER2 protein expression (H2T) was quantified by the HERmark® assay in 41 matched PBC and MBC formalin-fixed, paraffin-embedded specimens. PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) was determined using a validated pyrosequencing assay.

Results: MBC samples included 5 lymph node, 13 viscera, 6 brain, and 17 soft tissue lesions (N=41). 27 (66%) cases showed higher H2T in MBC than in matched PBC; and 14 (34%) cases had higher H2T in PBC than in matched MBC, indicating an overall increase of H2T in matched MBC lesions (fold change 0.25-17.57; p=0.005, paired Wilcoxon rank sum test). HER2 positive conversion (HERmark negative/equivocal in PBC, but positive in matched MBC) was found in 6 (15%) cases, while HER2 negative conversion (HERmark positive in PBC, but negative/equivocal in matched MBC) was seen in 2 (5%) cases. HER2 status was unchanged in 33 (80%) cases. PIK3CA mutations were detected in 13 (32%) of PBC and 19 (46%) of MBC samples. Among the HER2 positive conversion cases, PIK3CA mutation was identified in 50% (3/6) PBC and 67% (4/6) MBC, compared to 0% (0/2, PBC or MBC) in the HER2 negative conversion cases. Among cases with unchanged HER2 status, PIK3CA mutation was observed in 30% (10/33) PBC and 42% (14/33) MBC.

Conclusions: Quantitative HER2 assessment revealed a 20% discordance in HER2 status between matched PBC and MBC tissues, with more frequent conversion from low HER2 in PBC to high HER2 in MBC. PIK3CA mutation was observed more frequently in patients who converted from HER2 negative PBC to HER2 positive MBC. These results suggest that reassessment of biomarkers in MBC tissues may better inform the selection of therapeutic options for patients with MBC.

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Quantitative HER2 measurement and PI3K mutation profile in matched primary and metastatic breast cancer tissues

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Integrated ONCOLOGY LabCorp Specialty Testing Group

Weidong Huang[1], Jonathan Lara[2], Richard Michaelson[2], Xiu Sun[2], Pierfranco Conte[3], Valentina Guarneri[3], Elena Barbieri[3], Suhail Ali[4,5], Kim Leitzel[4], Jodi Weidler[1], Yolanda Lie[1], Mojgan Haddad[1], Agnes Paquet[1], Jeff Sperinde[1], John Howitt[6], Marcia Eisenberg[6], Laura Hurley[6], Christos Petropoulos[1], Allan Lipton[4]

[1]Monogram Biosciences/Integrated Oncology-LabCorp, South San Francisco, CA; [2]Saint Barnabas Medical Center, Livingston, NJ; [3]University of Modena, Modena, Italy; [4]Penn State /Hershey Medical Center, Hershey, PA; [5]Lebanon VA Medical Center, Lebanon, PA; [6]Laboratory Corporation of America, Research Triangle Park, NC.

Background

Accurate assessment of HER2 status is critical in determining appropriate therapy for patients with invasive breast cancer. HER2 status of primary breast cancer (PBC) is routinely used to determine systemic treatment for metastatic breast cancer (MBC) patients. Discordance rates of HER2 status between PBC and MBC range from 5.5% to 29% based on published meta-analyses *(N. Houssami et al., Breast Cancer Res Treat. 2011, 129:659; S. Richter, 2011 SABCS abstract PD05-05)*. The clinical benefit of re-assessing HER2 status in MBC tissues remains controversial. In this study, we measured quantitative HER2 protein expression in matched PBC and MBC tissues and correlated changes of HER2 expression with mutations in the catalytic domain of Phosphoinositide 3-kinase (PIK3CA).

Methods

Tissue Samples

66 pairs of matched primary-metastatic breast cancer tissues were provided by Saint Barnabas Medical Center, Livingston, NJ. 34 pairs of matched primary-metastatic breast cancer tissues were provided by University of Modena, Modena, Italy. Tissue samples with inadequate amount of invasive tumor for either HERmark® or PI3KCA mutation testing were excluded. Cases of bone metastasis were also excluded due to uncertain impact of fixation of bone samples on HER2 testing. A total of 41 pairs of matched primary-metastatic, formalin-fixed, paraffin-embedded (FFPE) samples had valid results for both HERmark and PI3KCA mutation testing and were included in the final analysis (Figure 2).

The HERmark® Breast Cancer Assay

Total HER2 protein expression (H2T) was quantified using the HERmark assay (Monogram Biosciences, So. San Francisco, CA) as previously described (*Huang et al. Am J Clin Pathol 2010;134:303*). H2T was detected through the release of a fluorescent tag (*V* for "VeraTag® reporter", Figure 1a) conjugated to a monoclonal antibody directed against the cytoplasmic domain of HER2 (Ab8, LabVision, part of Thermo Fisher Scientific). The antibody is paired with a biotinylated second antibody directed against the C-terminus of HER2 (Ab15, LabVision). The photosensitizer molecule (*PM*) liberates 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. The continuous H2T results are categorized as HERmark Negative, HERmark Equivocal, and HERmark Positive with pre-defined H2T cutoff values (*Huang et al. Am J Clin Pathol 2010;134:303*).

Mutations of catalytic domain of PI3 kinase (PIK3CA)

PIK3CA mutation status in exons 9 (E545K and E542K) and 20 (H1047R) was determined using pyrosequencing method as previously described (*Cook J, 2011 ASCO Annual Meeting, abstract # 582*), and performed at the Center for Molecular Biology and Pathology (CMBP, Laboratory Corporation of America, Research Triangle Park, NC).

The HERmark® Assay

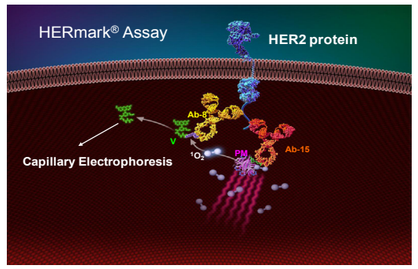


Figure 1a: The principle of HERmark assay

A monoclonal antibody specific for a unique epitope of HER2 is conjugated to a fluorescein VeraTag reporter (V) or a photosensitizer molecular (PM) by means of a cleavable tether. The photosensitizer molecule (PM) liberates singlet O_2 upon illumination with red light. The free radicals cleave all thioether bonds in close proximity (within approximately 30-100 nM), releasing the VeraTag reporter. The signal (V) can then be collected and analyzed on a capillary electrophoresis (CE) array. Each VeraTag reporter is designed with a unique charge-mass ratio and can thus be identified and quantified by comparison to assay standards. The standard unit of VeraTag measurement from tumor samples is relative peak area (RPA) x collection volume (uL)/ tumor area (mm^2).

Patient/Tissue Characteristics

Characteristic	No. (range, %)
Total patients	**41**
	53
Median age at diagnosis	**(35 – 82 yr)**
Tumor histological type	
Invasive ductal carcinoma	**17 (71%)**
Invasive lobular carcinoma	**5 (21%)**
Other tumor types	**2 (8%)**
N/A (data not available)	***17***
Tumor grade	
Grade 1 (well differentiated)	**1 (3%)**
Grade 2 (moderately differentiated)	**13 (38%)**
Grade 3: (poorly differentiated)	**20 (59%)**
N/A (data not available)	***7***
Estrogen receptor (ER)	
Negative	**8 (21%)**
Positive	**30 (79%)**
N/A (data not available)	***3***
Metastatic site	
Soft tissue	**17 (41%)**
Lymph node	**5 (12%)**
Viscera	**13 (32%)**
Brain	**6 (15%)**

Results

Figure 2: Tissue Sample Flow Chart

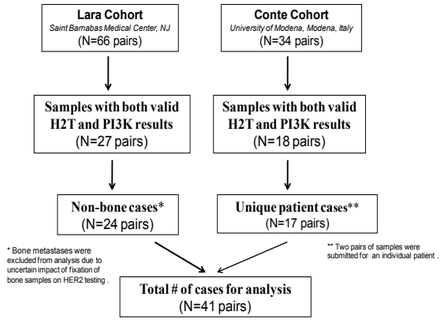


Figure 4: Conversion of HERmark HER2 status

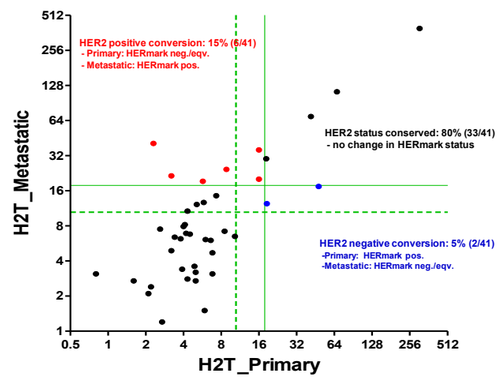
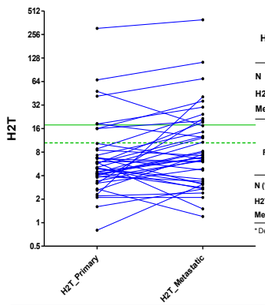


Figure 3: H2T change in paired primary-metastatic tumor tissues



H2T levels in matched primary-metastatic tissues

	Primary	Metastatic
N	41	41
H2T, range	0.8 to 307	1.2 to 395
Median H2T	5	6.9

Fold change of H2T in matched primary-metastatic tissues

	Metastatic>Primary	Primary>Metastatic
N (%)	27 (66%)	14 (34%)
H2T fold change*	1.02 to 17.57	0.25 to 0.90
Median H2T fold change	1.87	0.66

* Defined as: $H2T_{metastatic}/H2T_{primary}$

- HERmark Equivocal: defined by the two green horizontal lines
- HERmark Negative: area below the Equivocal zone
- HERmark Positive: area above the Equivocal zone

Table 1: HERmark status and PI3KCA mutation in primary and metastatic tumors

	HERmark HER2 status					PIK3CA mutation status				
	Negative/Eqvocal		Positive		Total	Negative		Positive		Total
	N	%	N	%	*HERmark*	N	%	N	%	*PI3K*
Primary tumor	35	85%	6	15%	41	28	68%	13	32%	41
Metastatic tumor	31	76%	10	24%	41	22	54%	19	46%	41
Total	66		16			50		32		

Table 2: HER2 conversion and PI3KCA mutation

	Primary breast cancer					Metastatic breast cancer				
	PIK3CA mut. Negative		PIK3CA mut. Positive		Total	PIK3CA mut. Negative		PIK3CA mut. Positive		Total
	N	%	N	%	*HERmark*	N	%	N	%	*HERmark*
HER2 conserved	23	70%	10	30%	33	18	55%	15	45%	33
HER2 negative conversion	2	100%	0	0%	2	2	100%	0	0%	2
HER2 Positive conversion	3	50%	3	50%	6	2	33%	4	67%	6
Total	28		13			22		19		

*HER2 status by HERmark: (1) negative or equivocal; and (2) positive

- HER2 conserved: no change in HER2 status by HERmark
- HER2 negative conversion: HERmark pos. in primary; neg./eqv. in metastasis
- HER2 positive conversion: HERmark neg./eqv. in primary; pos. in metastasis

Summary & Discussion

- Quantitative HER2 assessment by HERmark revealed a 20% discordance in HER2 status between matched PBC and MBC tissues, with more frequent conversion (66%) from low HER2 in PBC to high HER2 in matched MBC.
- A significant overall increase of H2T in matched MBC lesions (fold change 0.25-17.57; p=0.005, paired Wilcoxon rank sum test) was observed.
- HER2 positive conversion (HERmark negative/equivocal in PBC, but positive in matched MBC) was found in 15% cases, while HER2 negative conversion (HERmark positive in PBC, but negative/equivocal in matched MBC) was seen in 5% cases. HER2 status was unchanged in 80% cases.
- PIK3CA mutations were detected in 32% of PBC and 46% of MBC samples.
- PIK3CA mutation was observed more frequently in patients who converted from HER2 negative PBC to HER2 positive MBC. Among the HER2 positive conversion cases, PIK3CA mutation was identified in 50% PBC and 67% MBC, compared to 0% (PBC or MBC) in the HER2 negative conversion cases. Among cases with unchanged HER2 status, PIK3CA mutation was observed in 30% PBC and 45% MBC.
- MBC cases with positive HER2 conversion and PI3KCA mutation (~10% in this study) may present a significant clinical challenge because of more aggressive phenotype associated with acquired HER2 overexpression and resistant to therapy due to PIK3CA mutations.
- These results suggest that re-assessment of biomarkers in MBC tissues may better inform the selection of therapeutic options for patients with metastatic breast cancer.

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