HER2 Assessment to Define Outcomes for Breast Cancer Patients Treated with Neoadjuvant Therapy

Huan Cheng[1], Yalai Bai[1], William Sikov[3], Natalie Sinclair[3], Maysa Abu-Khalaf[2], Lyndsay N. Harris[4], David L. Rimm[1]

[1]Departments of [1]Pathology and [2]Internal Medicine, Yale School of Medicine, New Haven, CT; [3]Department of Medicine, Brown University, Providence, RI; [4]Division of Hematology and Oncology, Seidman Cancer Center, Case Western Reserve University School of Medicine, Cleveland, OH

Abstract (Updated)

Background: Assessments for HER2 protein overexpression or gene amplification are established companion diagnostic tests in breast cancer. Only patients with HER2+ cancers benefit from targeted therapies like trastuzumab, but it is not known if level of HER2 expression influences response. In this study, we quantitatively assess HER2 expression prior to neoadjuvant chemotherapy and trastuzumab in the BrUOG BR-211B (NCT00617942) trial to determine whether the level of expression of the target (HER2 protein) correlates with achievement of pathologic complete response (PathCR). We also study whether brief exposure to either trastuzumab or chemotherapy affects the level of HER2 expression or its predictive value.

Methods: We evaluated core biopsy samples from breast cancer patients enrolled in a preoperative clinical trial using trastuzumab, nab-paclitaxel, and carboplatin combination therapy. Tumor core biopsies were taken before and 9-13 days after "run-in" doses of either trastuzumab or nab-paclitaxel, after which patients received combination chemotherapy and trastuzumab for 6 cycles (18 weeks) followed by surgery. PathCR was defined as the absence of invasive cancer in the breast and axillary nodes. Automated quantitative analysis (AQUA), a fluorescent-based method for analysis of in situ protein expression, was used to assess HER2 and phospho-HER2 expression.

Results: Of 20 evaluable patients, the 10 with a PathCR showed a mean HER2 level of 10214 compared with 4766 in patients without PathCR (p = 0.0021, power = 0.938). This is notable in that a HER2 score of 2000 is equivalent to HER2 2+ (usually considered HER2-negative) in previous studies. Measurement of phospho-HER2 showed no difference between the PathCR and Non-PathCR groups. In 9 patients with repeat biopsies taken after the single "run-in" dose of trastuzumab, no significant change in HER2 or phospho-HER2 expression levels was detected.

Conclusions: High levels of HER2 are associated with an increased likelihood of achieving a PathCR with the combination of chemotherapy and trastuzumab in the neoadjuvant setting. Levels of phospho-HER2 are not associated with response. Further studies are underway to determine changes in levels of HER2 immediately after treatment.

Material & Methods

Trial Design: BrUOG BR-211B (NCT00617942) trial is a neoadjuvant trial lead by Brown University Onocology Group and Yale Cancer Center. The trial is designed to determine the clinical and pathologic response rates of treatment with q3week carboplatin, weekly nab-paclitaxel and weekly trastuzumab in resectable and unresectable locally advanced breast cancer. The eligibility of the trial includes histologically documented adenocarcinoma, female age greater than 18, stage IIA-IIIC disease, no evidence of metastatic disease, no prior systemic therapy, not pregnant or lactating, no baseline greater or equals to 2 neuropathy, HER2 positive defined by IHC 3+ or FISH ratio greater or equals to 2.0. Baseline biopsies were performed before the treatment. "Run-in" treatment was given to patients either with trastuzumab 6mg/kg alone (n=17) or nab-paclitaxel 100mg/m^2 alone (n=10). After 9-13 days, biopsy was repeated. Weekly trastuzumab 2mg/kg (patients who received nab-paclitaxel was treated 4mg/kg for the first week), weekly nab-paclitaxel and q3week carboplatin 6 AUC were given to patients for 18 weeks. Pathologic complete response was then accessed before definitive surgery within 6 weeks from the last dose of neoadjuvant therapy (**Figure 1A**).

Methods: Biopsy pairs of 27 patients were stained with HER2 (CB11, 1:1000, Biocare, Concord, MA) and phospho-HER2 (PN2A, 1:100, LabVision, Fremont, CA) and analyzed by Automated QUantitative Analysis (AQUA), a fluorescent-based method for analysis of in situ protein expression (**Figure 1B**). Fischer's PLSD test were applied to compare the level of HER2 and phospho-HER2 in pCR group and in non-pCR group. Statistic calculation was done by StatView® (SAS, Cary, NC), and significant level was tested at α= 0.05.

Figure 1. Material and Method

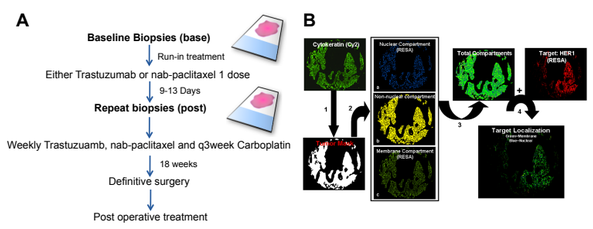


Figure 1. Material and Method. **(A)** Trial Design; **(B)** Illustration of AQUA®: AQUA uses Cytokeratin and DAPI staining to define tumor mask (step 1) and make subcellular compartments (step 2 and step 3). AQUA score is the total intensity of a biomarker under tumor mask (or in particular subcellular compartment) divided by the total area of tumor mask (or the particular subcellular compartment). (Step 4) AQUA score under tumor mask was used in this analysis.

Results

Table 1. Cohort Description

A

Variables	n	%
ER		
Negative	14	51.9%
Positive	9	33.3%
Unknown	4	14.8%
PR		
Negative	18	66.7%
Positive	5	18.5%
Unknown	4	14.8%
Run-in Treatment		
Herceptin	17	63.0%
Abraxane	10	37.0%
Response		
pCR	13	48.1%
non pCR	10	37.0%
Unknown	4	14.8%

B

	Association with pCR			
Variable	pCR	non pCR	Chi-Sq	p
ER			0.833	0.3613
Negative	7	5		
Positive	3	5		
PR			5.000	0.0253
Negative	10	6		
Positive	0	4		
Run-in Treatment			1.308	0.2528
Abraxane	7	3		
Herceptin	6	7		

Table 1. Cohort Description. (A) Cohort Description; **(B)** Association between clinical variables and pathological complete response

Figure 2. Examples of HER2 (CB11) and phospho-HER2 (PN2A) AQUA Images

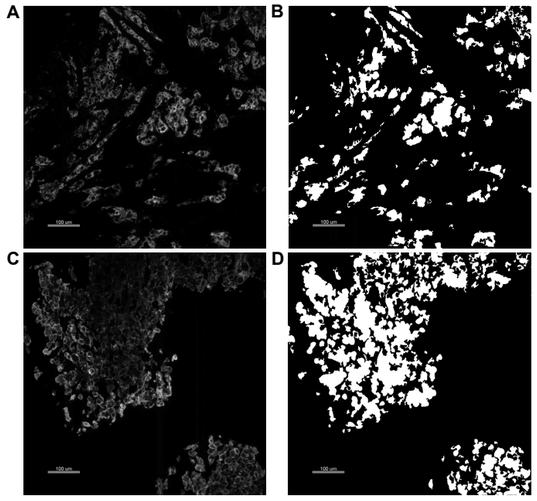


Figure 2. Examples of HER2 (CB11) and phospho-HER2 (PN2A) AQUA Images. (A) HER2 (CB11) from Patient ID 493 Base biopsy, Field of Interest (FOI) #8, AQUA=8548.4; **(B)** Tumor area defined by AQUA for image A; **(C)** phospho-HER2 (PN2A, pY1248) from Patient ID 481 Post biopsy, Field of Interest (FOI) #23, AQUA=3271.1; **(D)** Tumor area defined by AQUA for image C

Figure 3. No correlation between HER2 and phospho HER2

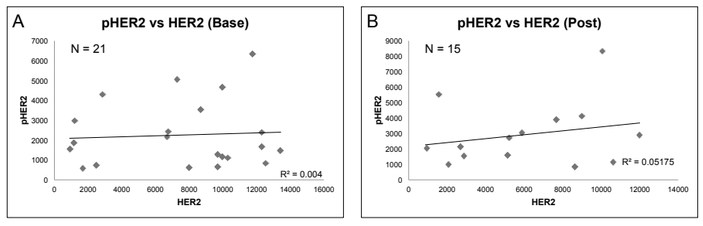


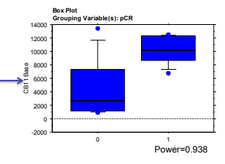
Figure 3. No correlation between HER2 and phospho-HER2. (A) Correlation between HER2 and phospho-HER2 level in baseline biopsies; **(B)** correlation between HER2 and phospho-HER2 level in post "run-in" treatment biopsies.

Table 2. Association between pathological complete response and the level of HER2 and phospho-HER2

A

	no-pCR			pCR			
	N	mean	SEM	N	mean	SEM	P value
HER2 AQUA (base)	10	4766.3	1381.1	10	10214.6	639.1	0.0021
HER2 AQUA (post)	9	4853.5	1428.0	3	8309.3	1256.0	0.2191
Post/Base AQUA ratio	9	1.09	0.203	2	0.765	0.089	0.4897

*Given the sample size, sample mean and standard deviation in pCR and non pCR groups, power calculation was performed at two-sided α= 0.05 level by StatView®. Power=0.938



B

	no-pCR			pCR			
	N	mean	SEM	N	mean	SEM	P value
pHER2 AQUA (base)	10	2183.7	472.2	11	2604.9	538.7	0.5669
pHER2 AQUA (post)	9	2482.7	507.2	7	3042.4	1005.5	0.6030
Post/Base AQUA ratio	9	1.49	0.326	6	0.919	0.145	0.1953

*Given the sample size, sample mean and standard deviation in pCR and non pCR groups, power calculation was performed at two-sided α= 0.05 level by StatView®. Power=0.084

Figure 4. No Change in HER2 and phospho HER2 level after the "run-in" treatment

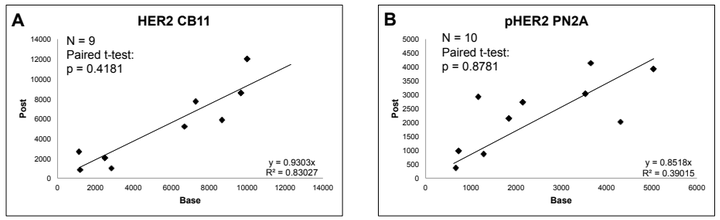


Figure 4. No Change in HER2 and phospho-HER2 level after the "run-in" treatment. (A) HER2 level change by one dose of trastuzumab (6mg/kg) for 9-13 days; **(B)** phospho-HER2 level change by one dose of trastuzumab (6mg/kg) for 9-13 days.

Conclusion

- Patients treated with chemotherapy and trastuzumab neoadjuvant therapy who achieve a pathologic complete response (PathCR) show significantly higher pre-treatment HER2 expression levels (measured by CB11) than patients who do not achieve a PathCR.
- Brief exposure to trastuzumab (6mg/kg) for 9-13 days does not change HER2 (n=9) and phospho-HER2 (n=10) level in a small set of patients.
- Patients treated with chemotherapy and trastuzumab neoadjuvant therapy who achieve a PathCR show no difference in pre-treatment phospho-HER2 pY1248 expression levels (measured by PN2A) from patients who did not achieve a PathCR.
- There does not appear to be a correlation between HER2 and phospho-HER2 levels taken before or after "run-in" treatment.

References

- BrUOG BR-211B (NCT00617942) trial. Retrieved March 23, 2012, from http://clinicaltrials.gov/ct2/show/NCT00617942
- Camp, R. L., G. G. Chung, et al. (2002). "Automated subcellular localization and quantification of protein expression in tissue microarrays." *Nat Med* **8**(11):1323-7.
- Gustavson, M.D., Bourke-Martin, B., et al. (2009). "Development of an unsupervised pixel-based clustering algorithm for compartmentalization of immunohistochemical expression using Automated QUantitative Analysis". *Appl Immunohistochem Mol Morphol* **17**(4):329-37.

Presented at 2012 AACR Annual Conference Poster Session #25 Poster #705

This Work is Supported by grant: NIH R01 CA139431-01 (PI: D.L. Rimm)