SUSAN F. SMITH CENTER FOR WOMEN'S CANCERS

DANA-FARBER CANCER INSTITUTE

Prospective characterization of HER2-positive circulating tumor cells in patients with HER2- negative metastatic breast cancer

Krop IE[1], Macrae E[2], Galler SR[1], Guo H[1], Fauni R[3], Sales E[3], Huynh L[3], Mitchell C[3], Clarin-Tamayo T[3], Anderson M[3], Abad L[3], Bischoff FZ[3], Winer E[1]

[1]Dana-Farber Cancer Institute, Boston, MA [2]Ohio State University, Columbus, OH, [3]Biocept, Incorporated, San Diego, CA

Background

Women traditionally undergo tumor biopsies of their primary breast cancer at the time of initial diagnosis and the results from this biopsy are typically used to dictate care throughout the remainder of a patient's therapy. Importantly, repeat biopsies of growing distant metastases late into treatment are often not performed despite evidence that tumor phenotypes may change over time in a subset of patients. In the case of HER2, a minority of patients (9%) with HER2-negative primary breast cancers demonstrate evidence of HER2-positive cells in biopsies of the metastatic tumor tissue. (1). Because of the benefits of HER2-directed therapy in HER2-positive cancers, it is critical to identify the patients whose tumors have acquired HER2 overexpression during progression and ensure that these women receive an appropriate targeted regimen.

We hypothesize that circulating tumor cells (CTCs) will offer clinicians a non-invasive approach to molecularly characterize a patient's tumor. Supporting this hypothesis, we have previously shown feasibility by CLIA validation of HER2 FISH on CTCs. In prior small studies by our group and others, 32-37% of patients with HER2-negative primary tumors were found to have HER2-positive CTCs (2-4). To functionally validate the significance of HER2-positive CTC in patients with HER2-negative primary tumors we initiated a phase II single arm study of HER2-directed therapy (trastuzumab/vinorelbine) in this patient population. We present here the results of the prospectively collected CTC screening phase of the study.

Methods

Approval for this study was obtained from the Institutional Review Board of the Dana-Farber/Harvard Cancer Center. We prospectively collected CTCs on patients with HER2-negative recurrent breast cancer between January 11, 2013 and June 4, 2014. Patients were eligible for the study if they had documented HER2-negative disease, defined as fluorescent in situ hybridization (FISH) ratio < 2.0 or 0, 1+, or 2+ by immunohistochemistry (IHC) on review of pathology records of all available primary and metastatic biopsies. If IHC 2+, a negative FISH was required. CTCs were measured at Biocept Inc. in a CLIA-approved laboratory. CTCs were captured using an antibody cocktail directed to ten different tumor-associated cell surface antigens, further labeled with a biotin-conjugated secondary antibody, and captured in a proprietary streptavidin-derivatized microchannel. FISH assays for HER2 and CEP17 were performed in the microchannel using Abbott probes, and were evaluated and scored by cytotechnicians at high power under the microscope. CTCs were considered amplified if the ratio of HER2 copy number to CEP17 copy number was ≥2.0. If a sample was FISH positive by this definition, the patient was eligible for the treatment portion of the study.
Univariate logistic regression was used to test the association between HER2+ CTC status and baseline tumor characteristics. Odds ratio (OR) and 95% confidence interval (CI) are estimated.

Results

CTCs were observed in 201 out of 311 pts (65%) (Table 1). Median number of CTCs was 10 (range 1 to > 34195). 69 of the 311 pts (22%) had HER2+ CTCs, with a median number of 3 HER2+ CTCs (range 1 to 21). 36% (25/69) of these pts had cytokeratin (CK)+/HER2+ CTCs and 45% (31/69) of pts had only CK-/HER2+ CTCs. The remaining pts (13/69, 19%) had both CK+/HER2+ and CK-/HER2+ CTCs present. There was no significant association between HER2+ CTC status and histopathological characteristics of the patient's primary tumor (Table 2). This lack of association was observed when evaluating all HER2 CTC as well as within specific HER2+ CTC CK subgroups (data not shown).

Table 1. Prevalence of HER2-positive CTCs in patients with metastatic HER2-negative breast cancer

	n (%)
Total patients tested	311
Patients with CTC detected	201 (65)
Patients with no CTC detected	110 (35)
Patients with HER2+ CTC	69 (22)
Patients with detectable CTC	201
HER2- CTCs	132 (66)
HER2+ CTCs	69 (34)
CK+ and CK- HER2+ CTCs	13 (6)
CK+ HER2+ CTCs	25 (12)
CK- HER2+ CTCs (CK+ cells absent)	13 (6)
CK- HER2+ CTCs (CK+ HER2- cells present)	18 (9)

Figure 1. Schema of clinical trial testing the efficacy of trastuzumab combined with vinorelbine in patients with HER2-negative metastatic breast cancer with HER2-positive CTCs. ClinicalTrials.gov/NCT01185509

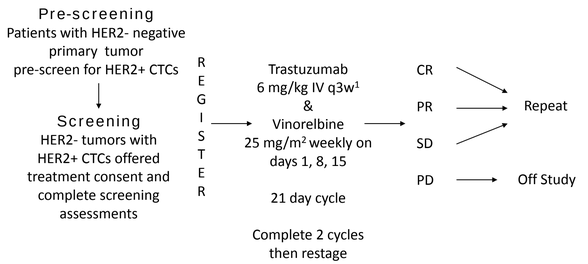

Table 2. Association between histopathological characteristics of patients' primary tumor and CTC HER2 status

	HER2+ CTC (n=69)		HER2- CTC (n=132)		OR (95% CI)	p-value
	n	%	n	%		
Ductal/Lobular Status						
Ductal	46	35	87	65	0.96 (0.42-2.18)	0.78
Ductal and Lobular	6	27	16	73	0.68 (0.21-2.25)	
Lobular	11	35	20	65	Ref.	
Unknown	6	40	9	60		
Tumor Grade						
I/III or II/III	28	31	63	69	0.74 (0.39-1.38)	0.34
III/III	32	38	53	62	Ref.	
Unknown	9	36	16	64		
Tumor Grade						
I/III	4	40	6	60	1.31 (0.36-4.83)	0.69
II/III or III/III	56	34	110	66	Ref.	
Unknown	9	36	16	64		
Hormone Receptor Status						
Positive	51	34	97	66	1.02 (0.53-1.98)	0.95
Negative	18	34	35	66	Ref.	
HER2 IHC						
0 or 1	51	39	81	61	2.20 (0.83-5.83)	0.11
2	6	22	21	78	Ref.	
Unknown	12	29	30	71		

Conclusion

- HER2 amplified CTCs are present in a subset (22%) of pts with clinically HER2-negative breast cancers.
- Histopathological characteristics of the patient's primary tumor were not able to predict the presence of HER2 amplified CTC.
- The unique multi-antibody CTC capture method used here identified a substantial subset of patients who had only CK-/HER2 amplified CTCs (31 out of 311; 10%).
  - These CK- CTC would not likely be detected with commonly used CK-based CTC capture technologies.
- The functional significance of CK-/HER2+ and CK+/HER2+ CTCs in patients with clinically HER2 negative breast cancer is currently being evaluated in a prospective study with HER2-directed therapy.

References

1. Simmons C et al. Does confirmatory tumor biopsy alter the management of breast cancer patients with distant metastases? Ann Oncol 2009;20(9):1499-504.
2. Flores LM et al. Improving the yield of circulating tumour cells facilitates molecular characterisation and recognition of discordant HER2 amplification in breast cancer. Br J Cancer 2010;102(10):1495-502.
3. Fehm T et al. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. Breast Cancer Res Treat. 2010 Nov;124(2):403-12.
4. Meng S et al. HER-2 gene amplification can be acquired as breast cancer progresses. Proc Natl Acad Sci U S A. 2004 Jun 22;101(25):9393-8.

Acknowledgements

Funding for this work was provided by:

- Susan G. Komen for the Cure
- Genentech, Inc