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Short report

Level of HER2/neu amplification in primary tumours and metastases in HER2-positive breast cancer and survival after trastuzumab therapy☆

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ABSTRACT

Background: The level of HER2/neu amplification may vary widely in breast cancers with HER2/neu alteration. The clinical significance of this phenomenon is still unclear. This study was aimed to explore the level of HER2/neu amplification in primary tumours and metastases in HER2-positive metastatic breast cancer (MBC) and its potential impact on survival after a trastuzumab-containing therapy.
Methods: We retrospectively identified MBC patients treated with a trastuzumab-containing therapy and performed dual-colour FISH on tumour samples from either primary tumour and/or metastasis in a central laboratory.
Results: We retrieved 110 tumour samples from 91 patients and included 79 tumour samples (primary = 56; metastasis = 23) from 63 patients in the final analysis. We found higher level of HER2/neu amplification in the metastases than in the primary tumours (median HER2/CEP17 ratio: 10.5 vs 7.0, respectively). In 69% of patients ($n = 16$) with two tumour samples, the level of HER2/neu amplification was higher in the metastasis than in the paired primary tumour (median HER2/CEP17 ratio: 10.9 vs 8.3, respectively, $p = 0.004$). The incremental gain in level of HER2/neu amplification was associated with significantly shorter OS after trastuzumab-containing therapy ($p = 0.023$, HR 1.014, CI95%: 1.002–1.025).
Conclusions: The level of HER2/neu amplification tends to increase from the primary tumour to the paired metastases in a significant proportion of patients with HER2-positive MBC. This phenomenon, although still not completely understood, could lead to a shorter OS after trastuzumab therapy.

Introduction

The presence of amplification of the HER2/neu gene in metastatic breast cancer (MBC) is a strong predictor of response to therapy with monoclonal antibody trastuzumab.[1] The guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) indicate that HER2/neu has to be regarded as amplified when the HER2/centromeric region of chromosome 17 (CEP17) ratio assessed by dual-colour FISH is >2.2.[2] It is well known that the level of HER2/neu amplification may present wide variations in HER2-positive breast cancers. In a previous study we found HER2/CEP17 ratios ranging from 2.5 to 21 in HER2-positive MBC and observed that tumours with HER2/CEP17 ratio >6 had a shorter progression-free survival (PFS) when treated with trastuzumab.[3] However the clinical significance of such phenomenon remains unclear and is still a matter of debate.[4,5]

Moreover, there is a lack of data regarding the level of HER2/neu amplification in primary breast tumours as compared with metastases and its potential impact on tumour biology and response to anti-HER2 therapies, especially trastuzumab.

☆ Results of this study were presented in part at the 35th Congress of the European Society of Medical Oncology, Milan (Italy), in October 2010.

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Following on to our previous study, we conducted this preliminary study to explore the role of level of HER2/neu amplification as a potential prognostic or predictive marker in patients with MBC treated with a trastuzumab-containing therapy.

Materials and methods

Patients were included in the study if they had metastatic or locally advanced (not amenable of curative surgery) HER2-positive breast cancer (defined as score 3+ by immunohistochemisty [IHC] or HER2/CEP17 ratio >2 by FISH), had been treated with a trastuzumab-containing therapy for advanced disease, had at least one formalin-fixed paraffin-embedded (FFPE) sample from either primary tumour and/or a metastasis available for FISH testing, measurable or evaluable disease, adequate follow up information. Patients previously treated with trastuzumab in the (neo)adjuvant setting were excluded. Eligible patients were identified by systematical cross-match of the datasets of Medical Oncology, Pathology and Pharmacy Departments. The study protocol was approved by the local Ethics Committee from each participating institution.

All FISH assays were performed specifically for this study at the Laboratory of Cytogenetics of Humanitas Clinical and Research Center, Rozzano (Milano, Italy) using the PathVysion kit (Abbott-Vysis, FDA approved). Assays were performed according to the manufacturer's instruction and in strict adherence to the ASCO/CAP guidelines.[2] In the event of FISH results near the cutoff point (HER2/CEP17 ratio 1.8–2.2), particularly if there also appeared to be variability of the counts from nucleus to nucleus, 20 additional nuclei were assessed and the specimen slide was re-enumerated by a second technician to verify the results. If still in doubt, the assay was repeated with a fresh specimen slide. Details on the FISH assays have been described in full in a previously published article.[3] The cytogeneticists worked on anonymous material without information on the patients medical history.

Overall survival (OS) was measured from the first infusion of trastuzumab to death for any cause or until patient was last contacted. The Wilcoxon Matched-Pairs Signed-Ranks Test was calculated to compare the HER2/CEP17 ratio in primary and metastatic tumour specimens. OS was evaluated with the Kaplan–Meier method and groups were compared with the log-rank test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed with the R software package.

Results

We identified and retrieved a total of 110 FFPE tumour samples from 91 patients. Twenty-eight patients were excluded from the final analysis because of failure of FISH (18 patients) or lack of HER2/neu amplification (10 patients). Eight out of 18 cases (44%) with failure of FISH came from one single Institution and we suppose that a fixative not compatible with the hybridization probe was used when the tumour specimens were originally collected. With respect to the cases with lack of HER2/neu amplification, they all had a score 2+/3+ on IHC according to the local histopathology report but resulted to have a HER2/CEP17 ratio <2.0 on central FISH testing. These cases were regarded as HER2-negative for this study purpose and therefore excluded from the final analysis. Of note, one patient with HER2/CEP17 ratio = 2.12 was included in the analysis as the ratio was at the upper limit of the "equivocal" area for HER2 amplification (ratio >1.8 and <2.2). The degree of concordance between the two FISH technicians of the study was 100%. The remaining 79 tumour samples (primary tumours = 56; metastases = 23) from 63 patients form the core group of the study. Patients characteristics are summarized in Table 1. Median follow up period for the patients included in the analysis is 19.2 months (range 1.2–94.6).

Table 1
Patients characteristics.

	No (%)
Total patients	63
Median age (range)	55.6 (27.3–76.8)
Stage at initial diagnosis	
M0	36 (57)
M1	25 (40)
Unknown	2 (3)
Histology	
Ductal	57 (90)
Lobular	4 (7)
Unknown	2 (3)
ER or PGR positive	27 (43)
ER and PGR negative	36 (57)
Sites of metastatic disease	
Visceral	40 (63)
Non-visceral	23 (37)
CNS involvement	19 (30)
Line of trastuzumab	
First	41 (65)
Second or over	21 (33)
Unknown	1 (2)
Regimen of trastuzumab	
Taxane	38 (60)
Vinorelbine	10 (16)
Monotherapy	12 (19)
Other	1 (3)
Unknown	2 (2)

ER: oestrogen receptor; PGR: progesterone receptor; CNS: central nervous system.

We found an absolute higher level of HER2/neu amplification in metastases (median HER2/CEP17 ratio: 10.5) than in primary tumours (median HER2/CEP17 ratio: 7.0). In keeping with this observation, in 69% of patients ($n = 16$) who had the primary tumour and a paired metastasis tested, the level of amplification of HER2/neu resulted higher in the metastasis than in the primary

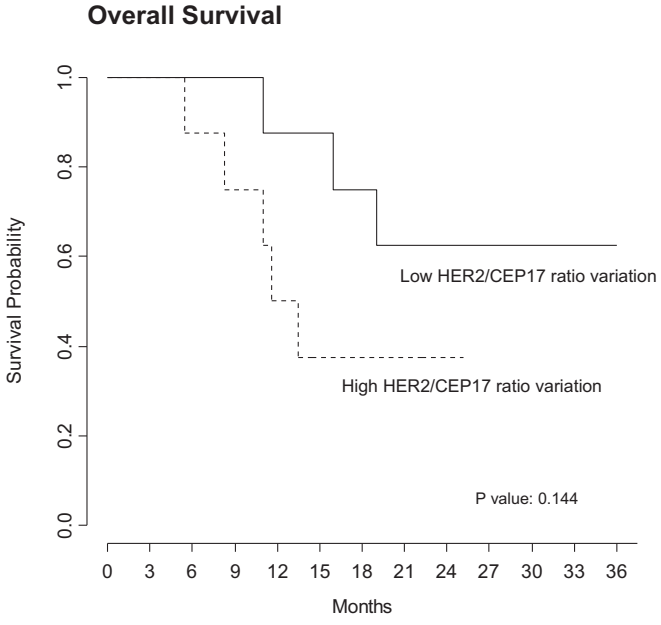

Fig. 1. Kaplan–Meier curves of OS for patients with low vs high variation of HER2/CEP17 ratio. The figure represents the probability of death for patients with a normalized variation of HER2/CEP17 ratio from the primary tumour to the matched metastasis that is above (high variation) or below (low variation) the median value.

Table 2
Characteristics of patients with tumour samples from both primary (prim) and metastasis (met).

	Hist	ER/PGR	Site of biopsy	AMPL (prim)	AMPL (met)	Level of AMPL	Line of trastuzumab	Regimen	FU months (alive/deceased)
1	Ductal	neg/neg	Liver	4.7	4.9	Stable	First	Tax	95 (a)
2	Ductal	neg/neg	Liver	8.6	10.8	Increased	Third	Mono	72 (d)
3	Ductal	neg/neg	Ovary	11.4	8.6	Decreased	Second	Tax	41 (a)
4	Lobular	neg/neg	Skin	4.4	4.6	Stable	First	Mono	40 (d)
5	Ductal	neg/neg	LN	9.8	6	Decreased	First	Tax	37 (d)
6	Ductal	pos/pos	Lung	10.5	16.7	Increased	First	Tax	26 (a)
7	Ductal	neg/neg	Skin	11.6	12	Stable	First	Vnr	19 (d)
8	Ductal	pos/pos	Skin	8	11.1	Increased	Second	Mono	16 (d)
9	Ductal	pos/pos	Liver	9.6	15.3	Increased	First	Tax	15 (a)
10	Ductal	neg/neg	Liver	6.6	9.7	Increased	First	Tax	14 (d)
11	Ductal	neg/neg	Liver	11.3	20.7	Increased	Second	Mono	12 (d)
12	Ductal	neg/neg	Brain	18.4	20.8	Increased	First	Tax	11 (d)
13	Ductal	pos/neg	Skin	6.4	10.9	Increased	First	Vnr	10 (d)
14	Ductal	neg/neg	Skin	3.1	10.1	Increased	Third	Mono	10 (d)
15	Ductal	pos/pos	Pleura	4.5	10	Increased	First	Tax	8 (d)
16	Ductal	neg/neg	LN	7	11.2	Increased	First	Tax	6 (d)

Hist: histology type; ER/PGR: estrogen/progesteron receptors, AMPL: HER2/CEP17 ratio, FU: follow up, Tax: taxane (paclitaxel or docetaxel); Vnr: vinorelbine; Mono: monotherapy; LN: lymph nodes.

tumour. The median HER2/CEP17 ratio was found to increase from 8.3 (range: 3.1–18.4) in the primary tumour to 10.9 (range: 4.6–20.8) in the matched metastasis. The range of increase in the level of HER2/neu amplification in the metastases, reported as the percentage change from the paired primary tumour, was 13%–225%. Using the Wilcoxon Matched-Pairs Signed-Ranks Test the increase in HER2/CEP17 ratio was statistically significant ($p = 0.004$). The incremental gain in the level of *HER2/neu* amplification from primary tumour to the paired metastasis was associated with a significantly shorter OS (HR 1.014, CI95%: 1.002–1.025, $p = 0.023$). The OS Kaplan–Meier curves for high- vs low-HER2/CEP17 ratio variation are reported in Fig. 1. The HER2/CEP17 ratios in all the primary tumours and corresponding metastases are detailed in Table 2.

We observed no statistically significant correlation between the absolute number of HER2/neu copies due to polysomy and OS after trastuzumab-based therapy. Similarly, the HER2/neu copy number had no impact on any of the other variables we investigated, such as stage of disease at diagnosis, oestrogen/progesterone receptor status, tumour grading.

Discussion

In this study we aimed to investigate the level of HER2/neu amplification in both primary tumours and metastases in a cohort of patients with HER2-positive advanced breast cancer treated with trastuzumab. Our results indicate that the level of HER2/neu amplification in HER2-positive breast cancer tends to be significantly higher in the metastases than in the primary tumours. In those cases where we were able to assess two tumour specimens from the same patient, the level of HER2/neu amplification showed a definite tendency to increase from the primary tumour to the matched metastasis. As all of our patients had the site of metastasis biopsied prior to receiving trastuzumab, we hypothesize that this is a purely genomic phenomenon intrinsically connected with tumour progression and not influenced by anti-HER2 therapy.

Furthermore and perhaps more importantly, we observed that the incremental gain in the level of HER2/neu amplification from primary tumour to metastasis was proportionally associated with an increased risk of death after patients received a trastuzumab-containing therapy. At present we cannot say whether this is due to an inferior activity of trastuzumab or to a naturally more aggressive disease itself.[6]

The increase in the level of HER2/neu amplification in patients with HER2-positive MBC can have potentially relevant clinical implications. First, it may predict an intrinsically more aggressive disease, even when trastuzumab is added to chemotherapy. Furthermore, this observation could prompt speculations that the molecular pathology of HER2-positive breast cancer with an increase in the degree of HER2/neu amplification can be more complex that the HER2-positive breast cancer with a stable level of HER2/neu amplification.[7] In our opinion the molecular and genetic basis of this phenomenon should be addressed by future studies focussing also on the HER2/neu amplicon and the numerous other genes that are often co-amplified with HER2/neu.[8,9] These patients could benefit from a dual-blockade of HER2 or from the addition of molecular therapeutics targeting other pathways than HER2 to standard trastuzumab-containing chemotherapy.

We are aware that our study has some important limitations that include its retrospective design, the small number of patients investigated and some heterogeneity in systemic therapies that patients received. Nonetheless, the observation that in a substantial number of patients with HER2-positive MBC the level of HER2/neu amplification is higher in the metastasis than in the primary is new and may lead a better understanding of biology of HER2-positive breast cancer. Ultimately, a deeper knowledge of intrinsic biology of HER2-positive breast cancer is the only condition for a better clinical use of the emerging new classes of therapeutics targeting the HER2-pathway.

At present we are conducting a larger study in which the level of HER2/neu amplification in the primary tumour and matched metastases is being investigated together with other molecular predictors of response to anti-HER2 therapeutics in HER2-positive breast cancer.

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Conflict of interest statement

The authors declare that they have no competing interests.

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