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^{111}Indium-trastuzumab visualises myocardial human epidermal growth factor receptor 2 expression shortly after anthracycline treatment but not during heart failure: A clue to uncover the mechanisms of trastuzumab-related cardiotoxicity

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ABSTRACT

Aim: Trastuzumab can induce cardiotoxicity, particularly when combined with anthracyclines. Myocardial human epidermal growth factor receptor 2 (HER2) expression may be transiently upregulated by a compensatory mechanism following cardiac stress. ^{111}In-DTPA-trastuzumab, scintigraphy can detect HER2 positive tumour lesions, however previously, we found myocardial uptake in only 1 of the 15 anthracycline-pre-treated patients with a median of 11 months after the last anthracycline administration. To evaluate whether myocardial HER2 expression is upregulated by anthracycline-induced cardiac stress or in case of heart failure by chronic pressure or volume overload, we performed ^{111}In-DTPA-trastuzumab scans in patients shortly after anthracyclines and with non-anthracycline-related heart failure.

Methods: Patients within 3 weeks after undergoing 4–6 cycles first-line anthracycline-based chemotherapy and patients with heart failure due to cardiac disease underwent gamma-camera imaging 48 and 96 h after ^{111}In-DTPA-trastuzumab intravenously.

Results: Myocardial ^{111}In-DTPA-trastuzumab uptake was observed in 5 out of 10 anthracycline-treated patients, who all were without symptomatic cardiac dysfunction. None of the 10 heart failure patients showed myocardial uptake.

Conclusion: Shortly after completion of anthracycline treatment, myocardial HER2 overexpression was detectable in 50% of the patients. ^{111}In-DTPA-trastuzumab scintigraphy

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after anthracyclines prior to adjuvant trastuzumab potentially identifies patients susceptible for trastuzumab-related cardiotoxicity and thus may facilitate the optimal timing of trastuzumab therapy.

1. Introduction

The introduction of trastuzumab, the monoclonal antibody against the human epidermal growth factor receptor 2 (HER2, also known as ErbB2), gave a new impulse to improve treatment results in HER2-positive breast cancer patients, both in the metastatic[1] as well as the adjuvant settings.[2,3] The downside of trastuzumab treatment is the increased risk of cardiac dysfunction, particularly when trastuzumab is combined with anthracyclines.

The pivotal phase III trial in metastatic breast cancer patients was the first to show this worrisome side-effect.[1] Now that trastuzumab has also proven its beneficial effects in the adjuvant setting, this issue is even more relevant. The recent adjuvant trials showed an increased incidence of cardiac dysfunction among patients who received trastuzumab after anthracycline-containing chemotherapy.[2–4] In the HERA trial, trastuzumab was started within 7 weeks from the last dose 5-fluorouracil, epirubicin and cyclophosphamide (FEC). The incidence of symptomatic cardiac dysfunction in this study was 0.5%,[2] compared to 4.1% in the combined analysis of the NSABP-B31 and NCCTG N9831 trials, in which trastuzumab was initiated 3 weeks after doxorubicin and cyclophosphamide.[5] Recently, Joensuu et al. showed that cardiotoxicity did not occur when, in the adjuvant setting, trastuzumab was combined with either docetaxel or vinorelbine, and administered before anthracyclines.[6] The lack of cardiotoxicity could be attributed to avoidance of trastuzumab administration concomitantly with or shortly after anthracyclines.[7] Anthracyclines induce myocardial oxidative stress, which can lead to myocardial damage.[8] Known endogenous defence mechanisms against oxidative stress involve enzymatic pathways, such as superoxide dismutase, glutathione peroxidase and catalase, or non-enzymatic processes, for instance ubiquinone and vitamins E and C. In addition, the HER2–neuregulin (NRG) system in the heart has the potential to modulate the response to oxidative stress.[9] It can be postulated that HER2 upregulation occurs as a result of anthracycline-induced cardiomyocyte stress and serves a compensatory mechanism. Trastuzumab may thus enhance anthracycline-induced cardiac toxicity. This HER2 upregulation may be a transient phenomenon, as is also suggested by the recent evidence regarding the reversibility of trastuzumab cardiotoxicity.[10] On the other hand, trastuzumab appears to induce another type of cardiotoxicity than anthracyclines, given the fact that trastuzumab-related cardiac dysfunction can be reversible without prior anthracycline treatment.[11] Trastuzumab cardiotoxicity may be induced by direct binding of trastuzumab to myocardium expressed HER2.

The ErbB–NRG system plays a critical role during embryogenesis. Lack of HER2 in the murine heart results in severe dilated cardiomyopathy.[12,13] In myocardial biopsies from 6 of 60 severe heart failure patients, weak positive HER2 staining was observed.[14] Chronic cardiac pressure or volume overload, as is present in heart failure patients, may also cause an upregulation of HER2 in cardiomyocytes.

We developed radiolabelled trastuzumab for clinical use and showed in a xenograft model that tumour HER2 expression can be visualised with ^{111}Indium (^{111}In)-labelled trastuzumab scintigraphy.[15] Recently, we demonstrated that ^{111}In-labelled trastuzumab single photon emission tomography (SPECT) imaging visualised myocardial ^{111}In-labelled trastuzumab uptake in 1 out of 15 patients, who received their last anthracycline administration at a median of 11 (range 5–59) months earlier.[16] In the adjuvant trials however, the interval between the last anthracycline dose and trastuzumab was shorter.[2–4] It is therefore especially of interest to know whether myocardial trastuzumab uptake is present shortly after "acute cardiac stress" by anthracycline-induced myocyte injury or during chronic pressure or volume overload in heart failure patients. The fact that HER2 expression can be detected with ^{111}In-labelled trastuzumab scintigraphy offers a unique opportunity to assess whether HER2 is expressed in the heart after several forms of stress. In this exploratory study, we therefore evaluated whether myocardial HER2 expression was present with ^{111}In-labelled trastuzumab SPECT imaging in patients shortly after anthracyclines and in patients with heart failure due to cardiac disease.

2. Patients and methods

2.1. *Patients (anthracycline group)*

Patients with HER2-negative tumours were eligible when they were aged 18 years or older, had an indication for 4–6 cycles of anthracycline-based chemotherapy, based on their underlying malignancy, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. The major exclusion criteria were prior anthracyclines, HER2 over-expressing (immunohistochemistry 3+, or fluorescence *in situ* hybridisation positive) breast cancer, trastuzumab treatment, a life expectancy of less than 3 months, uncontrolled central nervous system metastases, serious uncontrolled illness, New York Heart Association (NYHA) functional class III/IV heart failure.

2.2. *Patients (chronic heart failure group)*

Consecutive patients, aged 18 years or older, who visited the outpatient clinic of the Thorax Centre of the University Medical Centre Groningen, The Netherlands, for NYHA functional classes II–III chronic heart failure were asked to participate in the study. The major exclusion criteria were prior or concom-

itant anthracycline-containing chemotherapy, unstable cardiac disease, symptomatic intrinsic lung disease or dyspnea at rest due to any cause, or recent myocardial infarction (within the last 6 months).

The local medical ethics committee approved the study and written informed consent was obtained from all participants.

2.3. ^{111}In-DTPA-trastuzumab scintigraphy

Trastuzumab was radiolabelled with ^{111}In using diethylenetriamine penta-acetic acid anhydride (DTPA) as a chelator, as described previously.[15] The labelling procedure was validated and performed under good manufacturing practice (GMP) conditions. The mean immunoreactive fraction, verifying that the labelled product retained affinity for HER2, is 0.87 ± SD 0.06 and the radiochemical purity, determined with size exclusion high performance liquid chromatography, is always more than 95%. For the clinical study, a batch of the conjugate trastuzumab-DTPA was produced, which was stored at −20 °C. This batch was fully tested (e.g. quality control contained labelling efficiency, radiochemical purity, pH, stability and impurities, immunoreactive fraction, sterility and apyrogenicity), before labelling with ^{111}Indium, which was performed separately for each patient. All patients received the same product.

In the anthracycline group, patients received 150 MBq ^{111}In-DTPA-trastuzumab (5 mg), within 4 weeks after the last anthracycline dose. Patients with chronic heart failure underwent the scan procedure at the earliest convenience.

Planar whole body imaging, using a two-headed gamma-camera equipped with medium-energy all purpose collimators, and SPECT imaging were performed as described before,[13] 24–48 h and 96–120 h after ^{111}In-DTPA-trastuzumab administration.

2.4. Image and data analysis

SPECT reconstructions were performed with the ordered subset expectation maximisation algorithm. Myocardial uptake was assessed visually from spotviews of the cardiac region and SPECT short-axis reconstructions. Myocardial uptake was scored either as negative or positive. Images were interpreted by a nuclear medicine specialist (PLJ), blinded for clinical information and a cardiologist in training with specific expertise regarding the interpretation of the images (PJP). Images which yielded discongruent interpretations were subsequently evaluated by both observers. In case of the remaining disagreement, a third observer, a nuclear medicine specialist, was involved.

3. Results

Table 1 shows the clinical characteristics and results of the ^{111}In-DTPA-trastuzumab scans of the anthracycline treated patients. Ten patients (median age 47 (range 36–61) years) were enrolled in the anthracycline group, eight breast cancer patients received adjuvant FEC, one patient received doxorubicin, ifosfamide, etoposide for Ewing's sarcoma and one patient was treated with doxorubicin and cisplatin for metastatic osteosarcoma. Prior chest wall irradiation had been applied before chemotherapy in 2 patients. The median time interval between the last anthracycline dose and the scan was 18 (range 11–27) days. After a median follow-up of 7 (range 4–16) months, symptomatic cardiac dysfunction was not observed in any of these anthracycline treated patients. Although multigated radionuclide angiography was not routinely performed due to logistic reasons, no significant changes in left ventricular ejection fraction were observed due to anthracycline treatment (Table 1).

Seven male and three female chronic heart failure patients, median age 60 (range 41–75) years, were enrolled. Clinical characteristics are presented in Table 2. Dilated cardiomyopathy was the underlying cardiac disease in 7 patients, and ischemic heart disease in 3 patients. The median left ventricular ejection fraction was 0.34 (range 0.25–0.47). Time since the diagnosis of heart failure was median 75 (range 12–161) months. Nine patients had NYHA functional class II and one patient class III. Patients were receiving optimal medical treatment for heart failure, consisting of β-blockade and angiotensin-converting enzyme inhibition or angiotensin receptor blockade.

3.1. ^{111}In-DTPA-trastuzumab scintigraphy

After anthracycline chemotherapy, myocardial ^{111}In-DTPA-trastuzumab uptake was observed, on both scans after ^{111}In-DTPA-trastuzumab administration, in 5 of the 10 patients (Fig. 1). Comparing the patients with and without myocardial uptake, no differences were observed with regard to age, gender, chemotherapy regimen, time between the last chemotherapy dose and the scan or type of cancer. One patient without myocardial uptake on the scan, had received radiotherapy before chemotherapy. None of the patients with a positive scan had any clinical signs of heart failure or chest discomfort. No myocardial ^{111}In-DTPA-trastuzumab uptake was observed in any of the 10 heart failure patients.

4. Discussion

In this exploratory study, 50% of the patients showed myocardial HER2 expression at levels detectable with ^{111}In-DTPA-trastuzumab SPECT imaging, shortly after anthracycline-based chemotherapy. In addition, myocardial ^{111}In-DTPA-trastuzumab uptake was not observed in any of the 10 patients with heart failure due to cardiac disease. These striking observations, together with our previous findings in patients with metastatic HER2-overexpressing breast cancer, suggest that HER2 is an important signalling pathway following anthracycline-induced cardiac stress which sheds light on the mechanisms responsible for trastuzumab-related cardiotoxicity in conjunction with concurrent or sequential anthracycline treatment.

The initial phase III trial in metastatic breast cancer patients showed a 28% incidence of cardiotoxicity when trastuzumab was given concurrently with anthracyclines.[1] In the adjuvant trials, trastuzumab was administered sequentially after anthracycline-based chemotherapy. In the adjuvant

Table 1 – Clinical characteristics of the patients in the anthracycline group and scan results

Patient number:	1	2	3	4	5	6	7	8	9	10
Gender	Female	Male	Female	Female	Female	Female	Male	Female	Female	Female
Age (years)	49	38	36	61	44	55	40	55	42	50
Type of cancer	Breast	Ewing's sarcoma	Breast	Osteosarcoma	Breast	Breast	Breast	Breast	Breast	Breast
Prior chest wall irradiation	No	No	No	No	No	Left	No	No	Right	No
Chemotherapy regimen	FEC	DIME	FEC	DOX + CIS	FEC	FEC	FEC	FEC	FEC	FEC
Cumulative doxorubicin dose (mg/m^2)	–	240	–	360	–	–	–	–	–	–
Cumulative dose (mg/m^2)	450	–	450	–	450	450	450	450	450	450
LVEF before chemotherapy	–	0.62	–	0.65	–	0.64	0.63	0.53	0.63	0.60
LVEF after chemotherapy	0.55	–	–	0.68	–	0.56	–	–	–	0.55
Time between last anthracycline dose and scan (days)	27	21	17	25	14	19	11	20	14	12
Myocardial ^{111}DTPA-trastuzumab uptake	Yes	No	Yes	Yes	No	No	No	No	Yes	Yes

FEC denotes a regimen of 5 cycles of 5-fluorouracil (500 mg/m^2), epirubicin (90 mg/m^2), cyclophosphamide (500 mg/m^2), DIME a regimen of 6 cycles of doxorubicin (60 mg/m^2), ifosfamide (6 g/m^2), mesna, etoposide (450 mg/m^2), DOX + CIS cumulative dose/cycle doxorubicin (60 mg/m^2), cisplatin (80 mg/m^2), ^{111}In-DTPA-trastuzumab ^{111}Indium-diethylenetriamine penta-acetic acid anhydride-trastuzumab, and a dash implies not applicable.

NSABP and NCCTG trials, trastuzumab treatment was started 3 weeks after completion of doxorubicin, cyclophosphamide chemotherapy, whereas in the adjuvant HERA trial the interval between anthracyclines and trastuzumab was around 7 weeks.[2,5] The American/Canadian trials observed a higher incidence of trastuzumab-related cardiotoxicity than the HERA study. Also based on the results of the current study, it can be envisioned that anthracyclines induce a transient upregulation of HER2 in the heart as a compensatory mechanism, which is at its peak during anthracycline treatment. Concurrent trastuzumab may then do more harm to the heart compared to sequential administration. However, it should be noted that pre-trastuzumab LVEF values in the HERA study (55% or more) were higher than in the NSABP and NCCTG trials (50% or more). In addition, the less cardiotoxic epirubicin was used in the HERA study, whereas in the American/Canadian studies doxorubicin was given.

Based on these results, one might speculate that myocardial HER2 expression is a transient phenomenon that occurs following anthracycline treatment. The current exploratory study however lacks a pre-treatment scan. Based on our previous observation, that in 13 out of 15 metastatic breast cancer patients with HER2-positive tumours previously unknown tumour lesions were detected,[16] we included only HER2-negative patients, in order to avoid the risk of finding new tumour lesions. This design meant that the scans in these patients were only made for scientific reasons and were of no benefit for them personally. Although certainly of interest, serial

Table 2 – Characteristics of the patients in the heart failure group

Patient number:	1	2	3	4	5	6	7	8	9	10
Sex	Male	Male	Male	Male	Male	Male	Female	Female	Female	Male
Age (year)	50	43	62	75	60	61	41	57	60	67
NYHA	II	II	II	II	II	II	II	II	II	III
Cause of heart failure	DCM	DCM	DCM	IHD	DCM	IHD	DCM	DCM	DCM	IHD
Pharmacologic management										
Beta-blocker	+	+	+	+	+	+	+	+	+	+
ACE-inhibitor	+	+	+	–	+	+	+	+	–	+
Angiotensin II receptor blocker	–	–	–	–	–	–	–	–	+	–
Diuretic	+	–	+	+	+	+	+	+	+	+
Calcium antagonist	–	–	–	–	–	–	–	–	–	+
Digoxin	–	+	+	–	+	–	–	–	–	–
Nitrate	–	–	–	+	–	–	–	–	–	–
Statin	+	–	+	+	–	+	–	+	+	+
Myocardial uptake										
^{111}In-DTPA-trastuzumab	No	No	No	No	No	No	No	No	No	No

NYHA, New York Heart Association functional classification, DCM, dilated cardiomyopathy; IHD, ischemic heart disease; ACE, angiotensin converting enzyme; ^{111}In-DTPA-trastuzumab, ^{111}Indium-diethylenetriamine penta-acetic acid anhydride-trastuzumab.

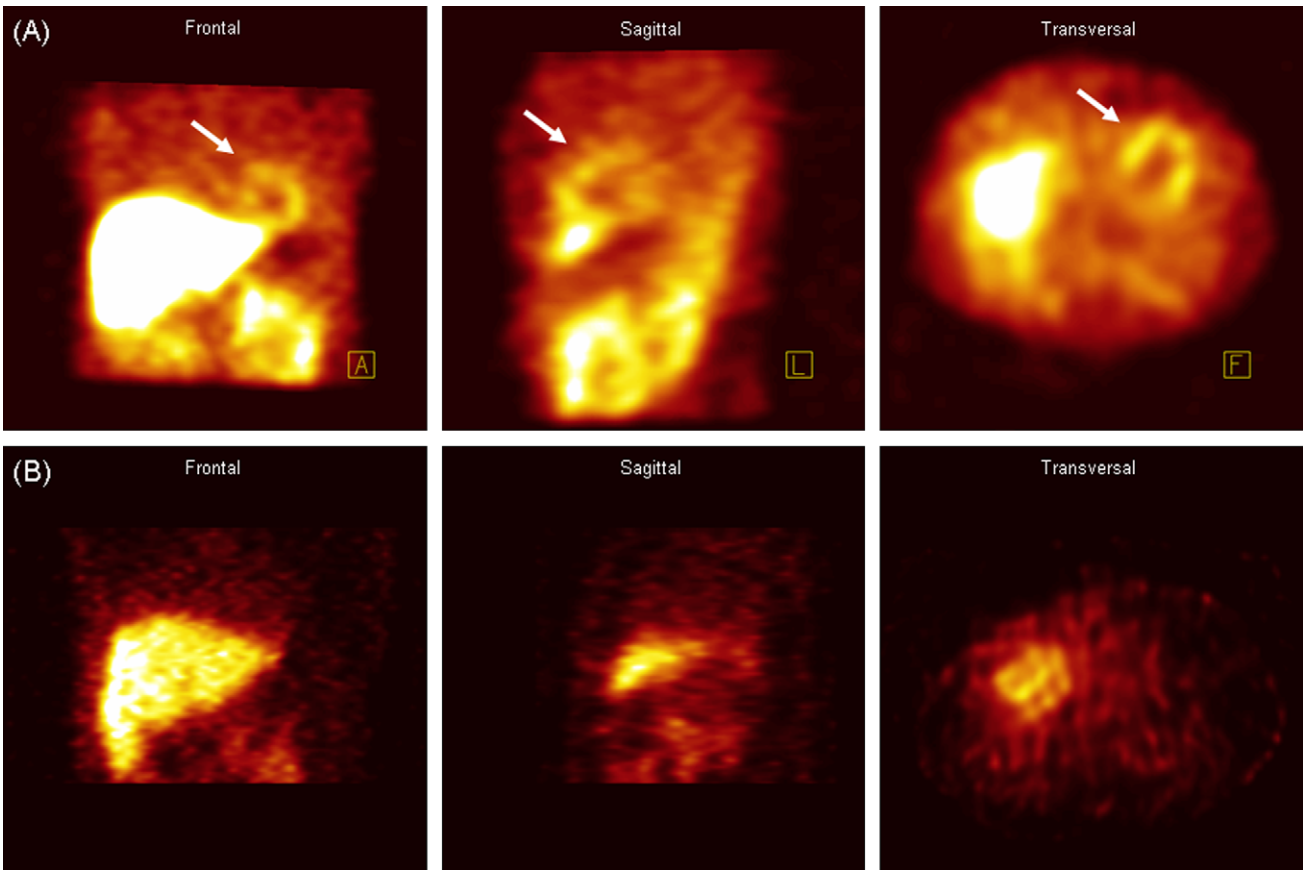

Fig. 1 – ^{111}In-DTPA-trastuzumab SPECT scan in cancer patients following anthracycline chemotherapy. Panel (A) shows patient with myocardial uptake of ^{111}In-DTPA-trastuzumab. Arrows indicate the characteristic horseshoe shape of the myocardium of the left ventricle. Panel (B) shows patient without myocardial uptake. Both patients show (normal) liver uptake.

scanning, with its radioactive burden, was considered unethical.

Trastuzumab cardiotoxicity is almost exclusively observed when trastuzumab is combined with or administered after anthracyclines. Probably anthracyclines sensitise cardiomyocytes for trastuzumab-induced injury, by upregulating HER2 expression. In the normal heart, the ErbB–NRG system is considered to function as a compensatory mechanism, counteracting cardiac stress. The crucial role of ErbB–NRG system in the heart is supported by the fact that a *HER2* gene deletion in murine hearts resulted in severe dilated cardiomyopathy.[12,13] In addition, patients who received a left ventricular assist device, as a bridge to cardiac transplantation, showed an increase in myocardial HER2 and HER4 mRNA expression.[17] Based on these data, it can be envisioned that trastuzumab-related cardiotoxicity, occurring after anthracyclines, is a two-step process. The first step, cardiac stress, is induced by anthracycline chemotherapy. This induces an increase in myocardial HER2 expression. Subsequent trastuzumab treatment represents the second step, resulting in cardiac dysfunction through inhibition of HER2-mediated signalling. Our observation that myocardial HER2 expression was present in 50% of the patients, shortly after anthracycline treatment, is well in line with this hypothesis. Although patient numbers are small, this study suggests that anthracycline chemotherapy induces HER2 expression in the myocardium. Additionally, the fact that none of the chronic heart failure patients showed myocardial ^{111}In-DTPA-trastuzumab uptake suggests that the compensatory HER2-mediated signalling is lost during heart failure. In other words, HER2 expression may be increased and detectable before left ventricular dysfunction and heart failure occurs, e.g. during the acute anthracycline-induced cardiomopathy. Different types of cardiac stress, i.e. chronic pressure or volume overload in cardiac failure on the one hand, versus from the relatively acute myocyte injury inflicted by anthracyclines on the other hand, might also be an explanation. Alternatively, myocardial oxidative stress may be much higher shortly after anthracycline treatment, compared to the setting of chronic heart failure. The fact that 50% of the patients showed myocardial ^{111}In-DTPA-trastuzumab uptake shortly after anthracyclines could also imply that these patients suffered more cardiac stress, and thus are more vulnerable to cardiac dysfunction, compared to the patients without myocardial uptake. In addition, all patients in the heart failure group received optimal medical treatment for heart failure, which might also be of influence on myocardial HER2 expression. In our previous study in patients with HER2-positive meta-

static breast cancer, 1 out of 15 patients showed myocardial uptake after a median of 11 (range 5–59) months after anthracycline-based chemotherapy. This patient differed from the other 14 patients since she suffered from pre-existent ventricular tachyarrhythmias, which may have caused an 'acute cardiac stress' resulting in upregulation of HER2. Based on these observations and the findings of the current study, we will initiate a new study exploring the time of occurrence and duration of myocardial HER2 expression in relation to anthracycline chemotherapy.

Another explanation for the fact that trastuzumab-related cardiotoxicity is particularly observed with concurrent or preceding anthracycline treatment, may be the fact that trastuzumab aggravates anthracycline-induced subclinical cardiac injury. Keeping in mind the potentially reversible nature of trastuzumab cardiotoxicity, this may also explain why cardiac function nearly returned to pre-trastuzumab levels.

The key finding of this study is that shortly after anthracycline treatment, 50% of the patients show uptake of ^{111}In-DTPA-trastuzumab in the heart. We consider this finding to be of major clinical importance, notwithstanding the fact that we do not know whether this uptake was also present before the start of therapy. From the earlier study we did not anticipate on such a result. The known and more widely observed trastuzumab associated cardiotoxicity underscores the need for more insight in its pathophysiology. The results of this exploratory study add to the current viewpoints and may also aid other investigators to rethink their research regarding trastuzumab-related cardiotoxicity.

Because of the recent proven role of trastuzumab in the adjuvant setting the number of patients treated with trastuzumab will be exponentially increasing and the issue of inherent cardiotoxicity becomes even more important.

It seems appropriate to start trastuzumab treatment after a time interval of, e.g. more than 6 weeks after the last anthracycline dose, as was applied in the HERA study and resulted in a lower incidence of cardiac dysfunction compared to the NSABP and NCCTG trials. HER2 imaging, can most likely assist in determining the optimal time for the start of trastuzumab after anthracyclines, in order to prevent cardiotoxicity. In this light, it may be of great importance to determine when myocardial HER2 expression returns back to normal in order to be able to administer trastuzumab safely. Alternatively, omitting prior anthracyclines, appears to be an effective approach to circumvent trastuzumab-related cardiotoxicity, as was suggested by Joensuu et al.[6]

Conflict of interest statement

There are no conflicts of interest.

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REFERENCES

1. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;**344**(11):783–92.
2. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;**353**(16):1659–72.
3. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;**353**(16):1673–84.
4. Slamon D, Eiermann W, Robert N, et al., Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (ACT) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (ACTH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study, Proc San Antonio Breast Cancer Symposium, 2005 [abstract #1].
5. Tan-Chiu E, Yothers G, Romond E, et al. Assessment of cardiac dysfunction in a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel, with or without trastuzumab as adjuvant therapy in node-positive, human epidermal growth factor receptor 2-overexpressing breast cancer: NSABP B-31. *J Clin Oncol* 2005;**23**(31):7811–9.
6. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006;**354**(8):809–20.
7. Chien KR. Herceptin and the heart – a molecular modifier of cardiac failure. *N Engl J Med* 2006;**354**(8):789–90.
8. Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. *N Engl J Med* 1998;**339**(13):900–5.
9. Kuramochi Y, Cote GM, Guo X, et al. Cardiac endothelial cells regulate reactive oxygen species-induced cardiomyocyte apoptosis through neuregulin-1beta/erbB4 signaling. *J Biol Chem* 2004;**279**(49):51141–7.
10. Ewer MS, Vooletich MT, Durand JB, et al. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. *J Clin Oncol* 2005;**23**(31):7820–6.
11. Ewer MS, Lippman SM. Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. *J Clin Oncol* 2005;**23**(13):2900–2.
12. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med* 2002;**8**(5):459–65.
13. Ozcelik C, Erdmann B, Pilz B, et al. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proc Natl Acad Sci USA* 2002;**99**(13):8880–5.
14. Fuchs IB, Landt S, Bueler H, et al. Analysis of HER2 and HER4 in human myocardium to clarify the cardiotoxicity of trastuzumab (Herceptin). *Breast Cancer Res Treat* 2003;**82**(1):23–8.
15. Lub-De Hooge MN, Kosterink JG, Perik PJ, et al. Preclinical characterisation of 111In-DTPA-trastuzumab. *Br J Pharmacol* 2004;**143**(1):99–106.
16. Perik PJ, Lub-De Hooge MN, Gietema JA, et al. Indium-111-labeled trastuzumab scintigraphy in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer. *J Clin Oncol* 2006;**24**(15):2276–82.
17. Uray IP, Connelly JH, Thomazy V, et al. Left ventricular unloading alters receptor tyrosine kinase expression in the failing human heart. *J Heart Lung Transpl* 2002;**21**(7):771–82.