resection margins, residual microcalcification, treatment by local excision alone, age less than 35 years, tumor size, histological grading, and microinvasion are associated with high risks of local recurrence of DCIS. According to the review by Sakorafas et al. (Sakorafas et al. 2008), high expression of COX2 and/or Ki67, P53 mutation, c-erB2 amplification, presence of necrosis, high nuclear grade and EP/PgR negative can predict biological behavior of DCIS. A variety of different definitions have been used for DCISM, including DCIS showing focal microinvasion below the basement membrane or limited microscopic stromal invasion but not invading more than 10% of the surface of the histological sections examined, the maximal extent of invasion is not more than 2mm or comprising < 10% of the tumor with 90% of DCIS and a few single infiltrating tumor cells (from 1 to 15) (DCISM type 1) or a few infiltrating tumor cell clusters (DCISM type 2) (Bianchi and Vezzosi 2008). In addition, risk factors mentioned above may be related to an increased chance of axillary involvement.

Sentinel lymph node biopsy (SLNB)

The SLN is defined as the first regional lymph node to receive lymphatic fluid from a malignant tumor (Yeung et al. 2001). As a result, it seems possible to assess the complete nodal status with SLNB. In addition, the significant morbidity of ALND makes this less invasive approach more attractive and reliable (Berveiller et al. 2010). Gipponi et al. (2004) performed histopathologic validation of the SLNB in 334 early-stage breast cancer patients and showed that the prediction of ALN status was remarkably satisfying (93-95% sensitivity and 100% specificity). These findings suggested that the SLNB could accurately predict ALN status. Nowadays, SLNB is well accepted and recommended as the standard method for early-stage breast cancer patients.

Importantly, the false-negative rate of evaluation of SLN status for metastatic spread is very small. Veronesi et al. observe 953 patients who did not undergo ALND after negative SLNB for the appearance of overt axillary metastases with a median follow-up of 38 months. Only 3 cases of overt axillary metastases were found among these patients and the 5-year overall survival rate of the whole series was 98% (Veronesi et al. 2005). However, the role of repeat SLNB is not clear yet in patients with a local recurrence after a negative SLNB. It could also be related to the level of experience of the various surgeons from different hospitals or to different techniques used for injection (Derkx et al. 2010).

The role of SLNB in DCIS

As mentioned above, SLNB is indicated to patients with invasive breast cancer to determine prognosis and to guide adjuvant treatment decisions. However, its role in DCIS is still controversial. Theoretically, SLNB is not recommended for patients with DCIS patients because DCIS does not cause axillary metastasis. However, about 15% of patients who are preoperatively diagnosed with DCIS on core needle biopsy are found to have a co-existing invasive carcinoma. Thus, these patients may benefit from axillary staging (Virnig et al. 2010). Instead of ALND, SLNB might be an option in cases of widespread DCIS and the clinical suspicion of occult microinvasion and/or micrometastasis.

Experience from published retrospective studies

Retrospective studies have been widely used for investigating the role of SLNB in DCIS (Table 1). Sakr et al. (2006) reported a series of 128 patients; 4 of the 128 patients upstaged to invasive carcinoma had metastatic SLN, 10% of the pure DCIS patients with SLNB and 7% of the DCISM patients with SLNB had axillary micrometastasis. So they indicated that SLNB could not only help to avoid under or over treatment of patients with diffuse or palpable DCIS, DCISM, or casting-type calcifications programmed for mastectomy, but also be recommended for DCIS harboring a potential of upstaging to invasive carcinoma. Next, the same group analyzed the data of 195 patients who were initially diagnosed with DCIS. Of the 195 patients with pure DCIS, 31 patients were found to have invasive disease upon final histology and sixteen patients had a positive SLNB (7 in pure DCIS, 2 in DCISM, and 7 in invasive cancer, respectively). Noticeably, all 7 positive SLNB patients in pure DCIS had a tumor size of more than 30 mm. Univariate analysis results indicated that DCISM or large DCIS were one of the high risks of invasive carcinoma after final histologic examination. They further confirmed that SLNB should be performed in order to detect underlying invasive disease and to spare patients a second operating time (Sakr et al. 2008). Another retrospective study indicated that DCIS patients who were planned for mastectomy or who had DCIS size more than 5 cm should perform SLN dissection (Yi et al. 2008). van la Parra la et al. also showed that SLNB had to be considered in the case of a preoperative diagnosis of grade III DCIS or a grade II DCIS with comedo necrosis and DCISM (van la Parra et al. 2008). Similar results were also reported by Moore et al. (2007). Murphy et al. (2008) recommended SLNB in all patients undergoing mastectomy for DCIS or DCISM, although their results showed that positive SLNB in patients with DCIS or DCISM was not associated with higher risk of local or distant recurrence. In a recent retrospective study, similar results were found that SLNB appeared justified in DCIS due to the high rate of underestimation of invasive carcinoma by core needle biopsy diagnosis in their study (Doyle et al. 2009). However, Tada et al. (2010) and Takács et al. (2009) believed SLNB could safely be omitted due to the low rate of SLN metastasis in patients with pure DCIS. Polom et al. (2009) analyzed 183 patients that underwent SLNB from 2000 to 2005. They also agreed that SLNB as a diagnostic tool in DCIS remained controversial as the number of ALN micrometas-

Table 1. Retrospective studies on the role of SLNB in DCIS.

Autor	Years	Cases include in the study	SLN biopsy	Patients with positive SN	Mapping	detected by
Sakr et al	2006	128	53	5	radiotracer	IHC
Sakr et al	2008	80	80	9	radiocolloids	HE, IHC
Yi et al	2008	624	624	40	radiotracer, vital dye	HE, IHC
van la Parra et al	2008	51	51	10	radiotracer, vital dye	HE, IHC
Moore et al	2007	2,136	470	43	blue dye	HE, IHC
Murphy et al	2008	322	322	29	N/A	HE, IHC
Doyle et al	2009	145	145	7	radiotracer, vital dye	HE, IHC
Tada et al	2010	255	255	1	radiotracer	HE
Takács T et al	2009	57	48	0	radiotracer	HE, IHC
Polom et al	2009	261	183	10	radiotracer	HE

Table 2. Prospective studies on the role of SLNB in DCIS.

Autor	Years	Cases include in the study	SLN biopsy	Patients with positive SN	Mapping	detected by
Collado et al	2008	43	N/A	3	N/A	HE
Wilkie et al	2005	675	675	49	radiotracer, vital dye	HE, IHC
Intra et al	2008	854	854	12	radiotracer	HE, IHC
Moran et al	2007	470	470	43	radiotracer, vital dye	HE
Intra et al	2003	223	223	7	radiotracer	HE, IHC
Mittendorf et al	2005	85	85	9	radiotracer	HE, IHC

tases cases is minuscule (Polom et al. 2009). A recently meta-analysis showed a higher incidence of SLN metastases in patients with a preoperative diagnosis of DCIS when compared with a postoperative diagnosis (7.4% versus 3.7%). This significant difference indicated that patients with a preoperative diagnosis of DCIS should be considered for SLNB. However, further efforts are still required (Ansari et al. 2008).

Experience from published prospective studies

Several prospective studies have also been performed to evaluate the role of SLNB on DCIS (Table 2). Collado et al. (2010) reported their experiment on 65 DCIS patients. Definitive histologic study of the resected breast tumor revealed 43 cases of DCIS, 15 of ductal invasive carcinoma and 7 microinvasive tumors. In confirmed DCIS, only 6.9% of SLN were positive, 28.5% in microinvasive carcinoma and 40% in invasive carcinoma were positive (Collado et al. 2010). One of the largest prospective comparative series (675 patients) was presorted by Wilkie et al. (2005). Of all, 613 patients were with DCIS and 62 patients were with DCISM. Among these patients, 55 patients with DCIS and 11 patients with DCISM were upstaged. Forty-nine of 675 patients had positive SLN and 22 had invasive carcinoma or DCISM on final histology. Of notice, microinvasion predicted patients at higher risk for invasive carcinoma. So they recommend SLNB should be performed in selective patients who are undergoing mastectomy for DCIS, patients who have DCISM, patients who have high-grade DCIS at the time of biopsy, and patients who have a mass by mammography. Intra et al. (2003) suggested that SLNB should not be considered a standard procedure in all patients with DCIS because of the low prevalence of metastases. It could be considered in patients with DCIS undergoing mastectomy, in whom there exists a higher risk of harboring an invasive component using definitive histologic features, like large solid tumors or diffuse or multicentric microcalcifications. Similar results were observed in other studies (Moran et al. 2007; Intra et al. 2008). They also indicated that SLNB should be performed on selected high-risk DCIS patients and not be performed routinely for all patients with an initial diagnosis of DCIS. However, there are still different opinions. Mittendorf et al. (2005) studied the role of SLNB in their prospective series of 85 patients who were initially diagnosed with DCIS by biopsy diagnosis. In their investigation, 20% of patients with a core biopsy diagnosis of DCIS were upstaged to invasive disease. So they advocated performing SLNB on all patients with a core biopsy diagnosis of DCIS due to the high rate of underestimation.

Identification of subgroup of DCIS patients who may benefit from SLNB

The relationship between DCIS and invasive breast cancer remains unclear. This relationship can be found in the similarity of risk factors for both the incidence of the diseases and their similar responses to treatment. In fact, 2% to 20% of patients with a core biopsy diagnosis of DCIS were upstaged to invasive disease, depending on the

selection criteria and analytical methods. Clearly, such differences must lead to a reexamination for whom with a preoperative diagnosis of DCIS. SNLB, therefore, is indicated at least in selected DCIS patients (Intra et al. 2003, 2008; Moran et al. 2007; Sakorafas et al. 2008).

The rate of positive SLN ranged from 10% to 30% in patients with DCISM. So these patients should undergo SLNB. In addition, if the patients have DCIS of sufficient extent on mammography or MRI that a mastectomy will be advised then simultaneous SLNB should also be performed. Moreover, if the final histologic examination indicates an invasive or microinvasive focus, SLNB should be recommended as a second step (Takács et al. 2009).

In some instances, metastases could be found in pure DCIS (range from 0% to 13%). It is due to the underdiagnosis of DCIS for which the pathology sections simply missed the invasive area. In that case, it is not "true" pure DCIS. Another possibility is that iatrogenic displacement and physiologically drains into SLN during the healing process. Lots of factors, including benign transport and iatrogenic displacement, could create the situation whereby an initial needling procedure can cause epithelial cells to be displaced into a healing biopsy site from which they are physiologically transported to SLN. The potential for such cells to be overinterpreted as evidence of metastatic carcinoma is very real (Bleiweiss et al. 2005). In addition, the risk of additional metastases in patients with isolated tumor cells in SLN is very low. Moore et al. showed that the likelihood of finding isolated tumor cells in the SLN was related to the invasiveness of biopsy rather than the aggressiveness of the tumor (Moore et al. 2004). No adjuvant treatment is indicated in this group now.

The prognostic significance of SLN micrometastases is far from being understood. Although some studies have found no association between SLN micrometastases and prognosis, others have shown strong support for the prognostic power of SLN micrometastases. Reed et al. (2009) found a significantly shorter disease-free survival for 82 patients with retrospectively found SLN micrometastases out of 1259 patients enrolled in an adjuvant therapy trial. Cox et al. (2008) confirmed similar findings. In addition, Susnik et al. (2004) indicated that the presence of SLN micrometastases was significantly associated with the development of distant metastases in low-risk patients. So we recommend that further treatment is indicated in DCIS patients with SLN micrometastases.

Conclusions

Despite the widespread use of SLNB in breast cancer patients, controversies remain in DCIS. DCIS is associated with increased risk of invasive breast cancer. The potential benefit of accurately upstaging patients with DCIS and the minimal invasiveness of SLNB justify use of SLNB in selected high-risk DCIS patients. At lease patients with DCISM, have DCIS of sufficient extent on mammography or MRI, or indicated invasive or microinvasive focus by final histologic examination, are recommended for SLNB. Moreover, large randomized trials to evaluate the usefulness of SLNB in DCIS patients after long-term follow-up on local control and survival are required for further evaluation.

Acknowledgments

We thank Dr. Yonghui Liao, Dr. Fujisawa Noriyoshi and Dr. vikram Raut for their critical comments and advice. This work was supported by Chinese Government Scholarship no. 2009659015.

Conflict of Interest

All authors have no conflict of interest.

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Journal of Steroid Biochemistry & Molecular Biology xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Bone metabolism and quality-of-life of postmenopausal women with invasive breast cancer receiving neoadjuvant hormonal therapy: Sub-analyses from celecoxib anti-aromatase neoadjuvant (CAAN) trial☆

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ARTICLE INFO

Article history:
Received 30 April 2010
Received in revised form
28 December 2010
Accepted 31 December 2010

Keywords:
Breast cancer
Neoadjuvant
Aromatase inhibitor
Cyclooxygenase-2 inhibitors
Bone metabolism
Quality-of-life

ABSTRACT

Objective: Anti-aromatase therapy is important in the treatment of breast cancer in postmenopausal women but they have effects on the bone mineral density (BMD) and osteoporosis. Cyclooxygenase-2 (COX-2) inhibitors have been shown to be effective in chemoprevention in animal and clinical studies. A proof of principle study was performed to investigate the efficacy of combing anti-aromatase therapy (exemestane) and COX-2 inhibitors neoadjuvantly. The changes in the BMD, bone turnover proteins and quality-of-life (QoL) were analyzed and presented here.

Method: 82 postmenopausal patients with histologically confirmed invasive hormone-sensitive breast cancers were included for the neoadjuvant therapy (NHT). 30 patients received exemestane (EXE) 25 mg daily and celecoxib (CXB) 400 mg twice daily (group A), 24 patients received EXE 25 mg daily (group B) and 28 patients received letrozole (LET) 2.5 mg daily (group C). The same assigned treatment was intended to continue for 2 years to study the changes in the bone metabolism. BMD of 48 patients were analyzed; 23 belongs to group A, 10 to group B and 15 to group C. The serum bone turnover proteins bone-specific alkaline phosphatase (BAP) and carboxyterminal crosslinked telopeptide of type I collagen (ICTP), were measured with commercially available test kits before treatment, 3 months and 15 months after treatment. Functional Assessment of Cancer Therapy core questionnaire (FACT-G) with its additional breast cancer subscale were performed at baseline, 4, 8, and 12 weeks after NHT.

Result: Difference between groups ($p = 0.007$) for BMD at femur was significant. The changes of BMD in group B patients were significantly greater than patients in group A ($p = 0.011$, CI = 0.063–0.437), and group C ($p = 0.003$, CI = 0.146–0.620). The mean BAP increased from baseline in group B patients but decreased from baseline in group C patients at 3 months and 15 months. No statistical significance was found in the FACT-G scores and FACT-B scores among different groups at baseline, week 4, week 8 and week 12 after NHT. The Breast Cancer Subscale scores in group A patients were significantly higher than that of group C patients ($p = 0.021$). After 4 weeks of NHT, negative changes of FACT-B and FACT-G scores were found in group B and C patients, but there were positive changes in group A patients. Significant differences of FACT-B score ($p = 0.008$) and FACT-G score ($p = 0.019$) were observed at that time point.

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1. Introduction

Breast cancer is the most common cancer among women worldwide, which accounts for about 26% of all female cancers [1,2]. The global cancer incidence was estimated at 1.15 million new cases in 2002 [1]. Regular and early screening and therapeutic developments have played an important role in increasing the survival rate, and that more patients are now receiving long-term adjuvant treatments.

Many breast cancer cases are associated with female hormones exposure and the relationship between hormone and breast cancer

Abbreviations: PWB, physical well-being; SWB, social well-being; EWB, emotional well-being; FWB, functional well-being; BCS, breast cancer subscale; SD, standard deviation.

☆ Article from the Special issue on Targeted Inhibhitors.

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doi:10.1016/j.jsbmb.2010.12.018

Please cite this article in press as: L.W.C. Chow, et al., Bone metabolism and quality-of-life of postmenopausal women with invasive breast cancer receiving neoadjuvant hormonal therapy: Sub-analyses from celecoxib anti-aromatase neoadjuvant (CAAN) trial, J. Steroid Biochem. Mol. Biol. (2011), doi:10.1016/j.jsbmb.2010.12.018

has been discussed since 1896 [3]. Menarche at an early age and a late menopause may increase the breast cancer risk, while an early menopause may decrease the risk [4–6]. The breast epithelium proliferation due to the hormone fluctuations has been associated with increased chances of cancer initiation [7]. Our previous study showed that about 55% of patients possessed hormonal receptors and the frequency of hormonal receptor positivity increased with advancing age [8]. These suggest that the steroid receptor plays an important role in breast tumorigenesis and that tumor cells and normal breast cells may have different steroid receptor signaling. It is therefore of interest for researchers to investigate the effectiveness of steroid inhibitors on breast cancer.

Aromatase is an enzyme complex which belongs to the cytochrome P450 (CYP) 19 family [9–11]. It is expressed in many human tissues, but its level is highest in ovaries of premenopausal women, and in the peripheral adipose tissues of postmenopausal women [12–14]. Aromatase converts androgen into estrogen, which then circulates and binds to the estrogen receptor (ER), by which they promote the growth of epithelial cells. The ERs then bind to gene promoters in the nucleus, thus activating cell division and inhibit apoptosis. In premenopausal women, most of the estrogen is produced in the ovaries and are sensitive to luteinizing hormone (LH) changes; however, in postmenopausal women, most estrogen is produced from the conversion of androgens in peripheral tissue [15]. Therefore, the inhibition of the ER expression has become a useful target in estrogen-dependent diseases, such as breast cancer.

The role of aminoglutethimide [16], a non-selective inhibitor blocking the cholesterol side-chain cleavage enzymes and C-21, C-11, and C-18 steroid hydroxylases [17,18], is able to reduce estrogen production by over 90% [19,20]. Its success led to the research and development of the second generation AIs such as formestane and fadrozole with improved potency. However, the dosage was limited by either metabolic or symptomatic side effects, such as fatigue, dizziness, nausea and vomiting. The third generation drugs are therefore further developed to inhibit the activity of aromatase at usable dosages associated with fewer side effects, and with a higher specificity.

The third-generation AIs are classified according to their chemical structures as steroidal (type I inhibitors), for example exemestane; or nonsteroidal (type II inhibitors), such as letrozole and anastrozole. All the AIs block the aromatase activity by inhibiting the estrogen synthesis. But they differ in the aromatase binding mechanism, and the androgenic properties.

The type 1 steroidal AI acts as a competitive inhibitor against androstenedione and as an enzyme inactivator. As enzyme inactivators they function as "suicide inhibitors" in which aromatase converts the AI into a chemically reactive intermediate which can be bound covalently to the substrate binding site of the aromatase. As a result, the enzyme is irreversibly inactivated and the AI inactivator is unable to bind to other enzymes permanently [21]. These AIs have selectivity for the enzyme target. The recovery of enzyme activity is dependent on the enzyme re-synthesis and the drug pharmacokinetics. Therefore, the type I AI has got a long-term effectiveness.

The type II AIs can interact noncovalently with the iron atom of the heme prosthetic group of the enzyme due to the presence of a basic nitrogen atom [22]. They occupy the substrate-binding site of the enzyme and thus prevent the androgen substrate from binding to the catalytic site [23]. But this mechanism is reversible, and the AIs can be competitively displaced by the endogenous substrates. The structural aspects of the drugs determine the inhibition specificity to the aromatase enzyme, thus creating a high-affinity binding and limits the AIs from binding to other enzymes. Many AIs have been developed in the past 20 years, and current researches are now focusing on the use of AIs and the combination with other drugs for better efficacy and tolerability. Despite the fact that the efficacy of AI for the treatment of breast cancer in post-menopausal women has been supported by randomized clinical trials [24,25], these patients may be prone to long-term side effects such as osteoporosis.

Beside aromatase, prostaglandin E2 can stimulate estrogen biosynthesis as well [26]. The cyclooxygenase (COX) enzymes catalyze the conversion of arachidonic acid to prostaglandins. Its inducible isoform, COX-2, which is commonly overexpressed in breast cancer, was found to induce the CYP-19 [26,27]. In addition, its high level was associated with angiogenesis and bone and lymph node metastasis [28–30]. The therapeutic possibilities of COX-2 inhibition has been investigated since epidemiological studies suggested the inverse association between regular intake of nonsteroidal anti-inflammatory drugs (NSAIDs) and the breast cancer risk [31–33]. COX-2 inhibitors were found to be able to inhibit the carcinogenesis of mammary tumors in rodent models [34–37]. Celecoxib (CXB), a promising selective COX-2 inhibitor, demonstrated its chemopreventive ability in rodent models with breast cancer. The combined use of COX-2 inhibitors and AI is being studied and they showed promising results as well [38–42].

Randomized clinical trials have shown the effectiveness of using AIs in breast cancer patients, but these drugs may increase adverse events associated with bone health [43,44]. Breast cancer patients receiving cancer chemotherapy may have a higher bone loss chance and a higher potential risk for developing osteoporosis, especially in postmenopausal women, which is probably due to the decreased estrogen concentration [45–47]; whereas in premenopausal women, premature menopause and bone loss may be induced by ovarian damage by chemotherapy [48]. The rate of treatment-associated bone loss may be higher than that in normal postmenopausal women. Breast cancer patients who receive AIs have an estimated bone loss rate of 2.6% per year [49]; whereas normal women have an estimated annual rate of 2% during the first years of menopause, and about 1% per year afterwards [50]. Osteoporotic patients might suffer from bone fractures, pain, disability and even mortality [51]. Therefore, a better understanding of how these drugs affect bone density is necessary.

The selective estrogen-receptor modulator, tamoxifen (TAM), has been the standard endocrine adjuvant therapy of early breast cancer [52]. It interferes with the estrogen from binding to its receptor. 5 years of adjuvant TAM therapy has been proven as an efficient treatment, it may reduce the disease recurrence by about 50% and mortality by 28% in estrogen-receptor-positive (ER+) tumors [53]. It also has a positive effect on bone health in postmenopausal breast cancer patients. However, the clinical use of TAM against osteoporosis is limited due to its toxicity [54,55]. Although TAM has been the gold standard treatment, it has now been challenged by the AIs which have got fewer side effects. The adverse events experienced by patients receiving TAM such as hot flashes, vaginal bleeding, endometrial cancer, thromboembolic events have been associated with long-term TAM treatment [56–59] and these would be reduced by the substitution of AIs. It is also not recommended to receive TAM therapy beyond 5 years because there is no further benefit [54].

Raloxifene hydrochloride is pharmacologically related to TAM, which has been shown to prevent osteoporosis and breast cancer [60,61]. It is a unique selective estrogen receptor modulator (SERM) due to its role of estrogen antagonist in the uterus [62]. It also has antiresorptive effects on bones but less major adverse events had been found in experimental animals and humans than TAM. In Black et al.'s study, a prevention of bone loss and reduced serum cholesterol had been found in ovariectomized rats after receiving raloxifene [63]. Similar results were also reported in Draper et al.'s study, they found that raloxifene (200 mg/day or 600 mg/day) and

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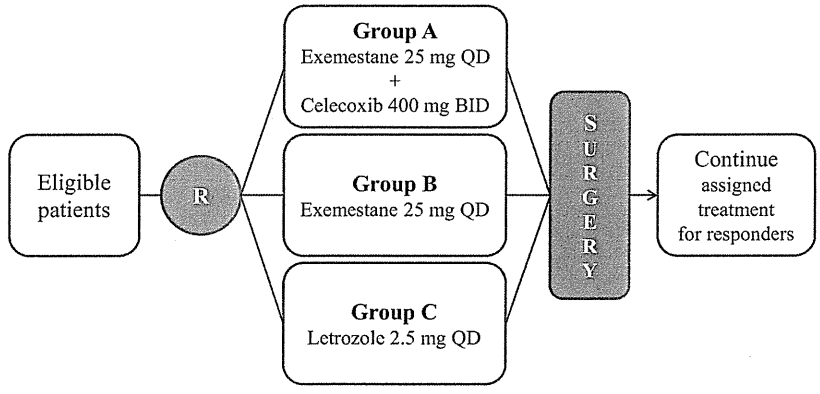

Fig. 1. Flow diagram of the process through the phases of the randomized trial. *Abbreviations*: R: randomization; QD: once daily; BID: twice a day.

conjugated equine estrogen both reduced biochemical markers of bone turnover versus placebo [64].

Bone mineral density (BMD), a good indicator of bone loss, is being used to measure the amount of calcium in bone (bone density) and determines the fracture risk. It is strongly recommended for patients especially those with a high risk of getting osteoporosis to determine their BMD before receiving any treatments. BMD is lower in breast cancer patients than normal population group [65] which might be attributed to the long-term estrogen deprivation. While TAM is beneficial on bone health [48], an increased bone loss has been observed with the use of EXE and LET [44,48]. EXE induces bone resorption and formation [66], which increases bone loss at a rate of 2–3% per year [67]. Similarly, LET also has an increased bone loss rate at 2–3% per year [68]. Thus the BMD has to be carefully monitored during the treatment.

Quality-of-life (QoL) is another important key for considering the long-term use of therapy, but is rarely performed in studies of neoadjuvant hormonal therapy (NHT) for breast cancer. This substudy was also conducted to compare the effects of the group taking steroidal AI in combination with COX-2 inhibitor, with the group taking steroidal AI alone, and the group with nonsteroidal AI on changes in BMD, bone turnover proteins and QoL during NHT in postmenopausal women.

2. Materials and methods

2.1. Patient population

The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. All patients were postmenopausal with invasive breast cancer which expressed positive estrogen receptor (ER) and/or progesterone receptor (PgR) status. Other major eligibility criteria included an ECOG performance status ≤ 3 or a Karnofsky performance status ≥ 70, ability of the patient to give written consent and follow instruction well, clinical size of tumor ≥ 3 cm. Exclusion criteria included negative hormonal receptor status, known sensitivity to anti-aromatase drugs or celecoxib, major cardiac disease or LVEF less than 50%, coronary artery disease, active liver disease, renal impairment, and prior history of other malignancy within 5 years of study entry except for basal cell carcinoma or the skin or carcinoma-in situ of the uterine cervix. The nature and purpose of the trial was explained to the patients and informed consent was obtained for inclusion in the trial.

2.2. Study design

In this randomized study, patients were randomly assigned to receive EXE 25 mg daily and CXB 400 mg twice a day (group A), EXE 25 mg daily (group B) and LET 2.5 mg daily (group C) for 3 months before surgery. Changes in the bone metabolism were determined as a sub-study in patients responding to the preoperative treatments and receiving the same assigned treatment for at least 2 years after surgery (Fig. 1). Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DEXA) scan at 12 and 24 months after surgery and the serum bone turnover proteins were measured with commercially available test kits before treatment, 3 months and 15 months after treatment.

Assessment of BMD was done by DEXA scan in lumbar spine (L1–L4) and in the femoral neck. BMD's t-score (the standard deviation from the mean value in normal adult) was obtained. To ensure consistency, all DEXA scans were standardized and performed at Hong Kong Sanatorium & Hospital, HKSAR. Assessment of bone metabolism based on measurements of the bone formation marker and bone resportion marker levels in serum: bone-specific alkaline phosphatase (BAP) and carboxyterminal crosslinked telopeptide of type I collagen (ICTP), respectively.

2.3. Quality-of-life (QoL)

The Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire has been used to assess the QoL. The FACT-B has 27 questions which measure the general QoL that are associated with cancer; and 9 questions which are breast cancer subscale (BCS). The FACT-G has five subscales assessing physical well being (PWB), social/family well-being (SWB), emotional well-being (EWB), and functional well-being (FWB). 34 out of 79 evaluable patients completed the FACT-G with its BCS at baseline, 4, 8, and 12 weeks after NHT. Incomplete questionnaires were included for cross-sectional analysis. The patients have to indicate how true the statement has been for them during the last 7 days using a five-point scale (from 0 [not at all], 1 [a little bit], 2 [somewhat], 3 [quite a bit], to 4 [very much]). A high score equate with a good QoL, whereas a low score equate with a poorer QoL. Some items have been negatively framed, so they are reversed for further analysis.

2.4. Statistical analysis

Parameters were compared using the SPSS for windows release 11.0 (SPSS Inc., USA). One-way ANOVA tests were used to compare means between all groups and post hoc tests were performed to compare means between each group. p-Values of less than 0.05 were considered as statistically significant.

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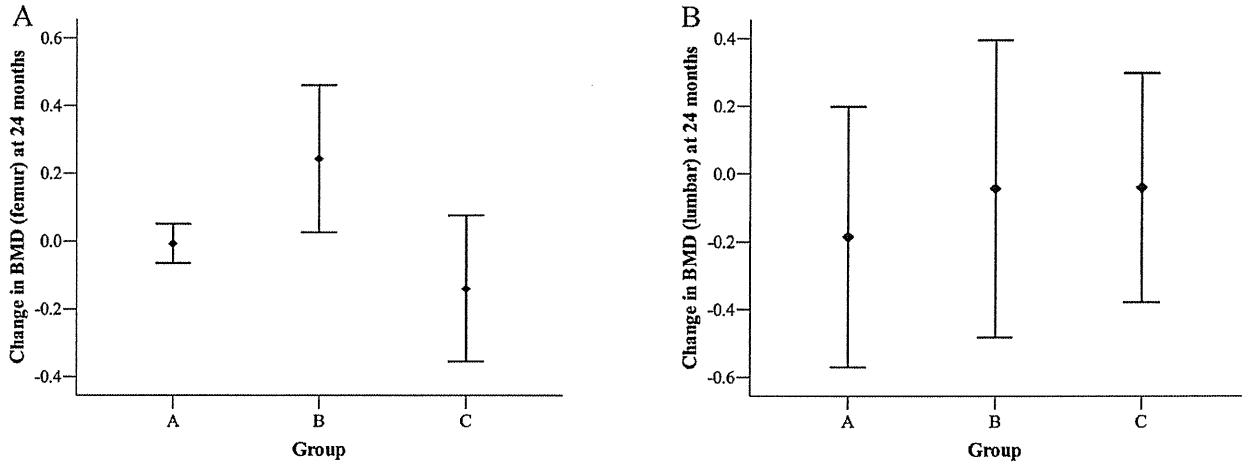

Fig. 2. Change in BMD of femur and lumbar spine at 24 months from 12 months after surgery in group A, B and C patients. *Abbreviations*: BMD: bone mineral density

3. Results

3.1. BMD

Changes in BMD at femur and lumbar were compared at 24 months from 12 months after surgery between groups. A significant difference was observed between groups ($p = 0.007$) for BMD at femur (Fig. 2A), but not significant at spine (Fig. 2B). At 24 months after surgery, there were changes in the BMD at the femur for groups B and C patients, except for group A patients which remained stable. The changes of BMD at femur in group B patients were significantly greater than patients in group A ($p = 0.011$, CI = 0.063–0.437), and group C ($p = 0.003$, CI = 0.146–0.620).

3.2. Serum bone turnover proteins

Taking into account of all patients, no significant changes were found in BAP at 3 months (Fig. 3A), and ICTP at 3 and 15 months after the treatment when compared to baseline (Fig. 3B). Although the mean BAP mildly increased from baseline in group B patients but decreased from baseline in group C patients, no significant difference was observed in the percentage change in BAP and ICTP at 3 months and 15 months from baseline between groups.

3.3. Quality-of-life (QoL)

No statistical significance was found in the FACT-G scores (PWB, SWB, EWB, and FWB) and FACT-B scores (sum of FACT-G and BCS scores) among different groups at baseline, week 4, week 8 and week 12 after NHT (Table 1). The BCS scores were similar among three groups, but the BCS scores in group A patients were significantly higher than that of group C patients ($p = 0.021$). At 4 weeks after the NHT, the SWB score in group A patients was the highest, whereas group C patients had the lowest score, and the difference was significant ($p = 0.05$). Similarly, there were significant differences in EWB scores ($p = 0.032$) across the three groups after 12 weeks of NHT, however this time group C patients had the highest score, followed by group A patients and group B patients (Table 1). In addition, after 4 weeks of NHT, negative changes of FACT-B (Fig. 4) and FACT-G (Fig. 5) scores were found in group B and C patients, but there were positive changes in group A patients. Significant differences of FACT-B score ($p = 0.008$) and FACT-G score ($p = 0.019$) were observed at that time point.

4. Discussion

The survival rate of breast cancer has largely increased due to the improved therapies [69]. A good example is TAM, which has long been the gold standard of treatment against hormone-

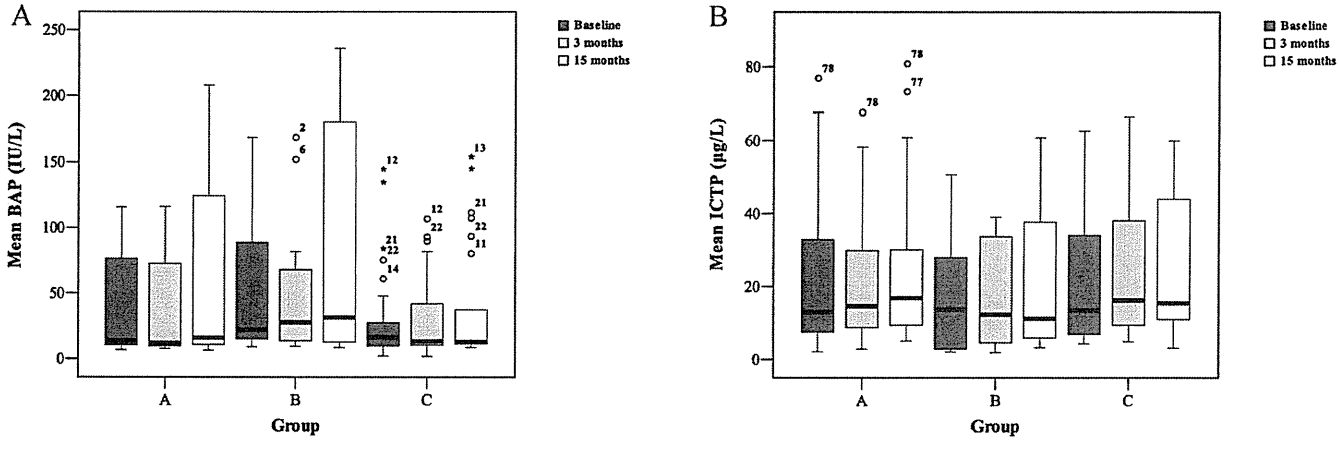

Fig. 3. Change in bone turnover proteins BAP (A) and ICTP (B) at baseline, at 3 months, and 15 months after treatment. *Abbreviations*: BAP: bone-specific alkaline phosphatase; ICTP: carboxyterminal crosslinked telopeptide of type I collagen.

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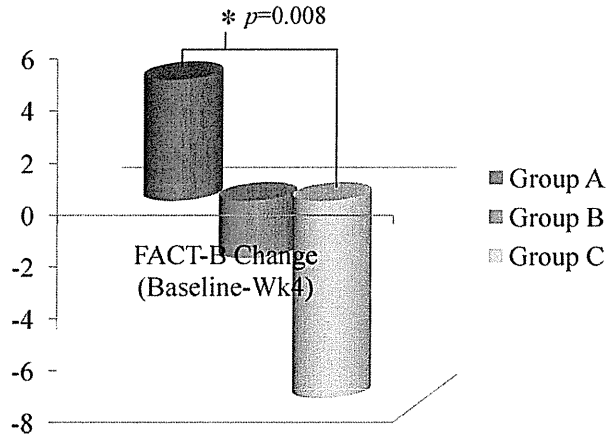

Fig. 4. FACT-B score changes in group A, B, and C patients after 4 weeks of neoadjuvant hormonal therapy.

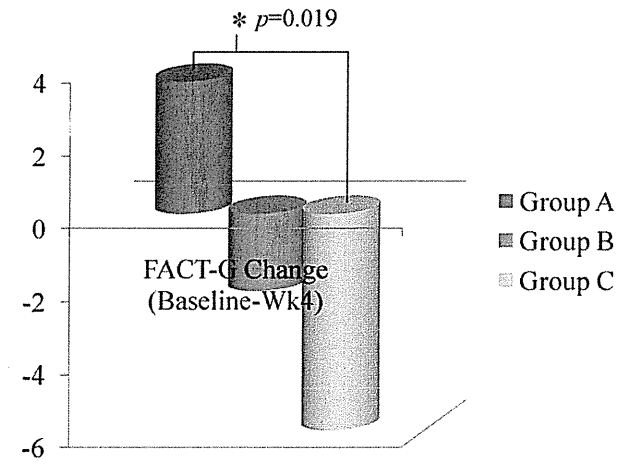

Fig. 5. FACT-G score changes in group A, B, and C patients after 4 weeks of neoadjuvant hormonal therapy.

sensitive breast cancer. Unfortunately, drug resistance develops in some tumors [70] leading to the development of AI. Nevertheless, the long-term use of AI might have adverse impact on bone health. Osteoporosis has become an important public health issue in many developed countries. Therefore, people are now more concerned about preventing bone mineral loss and bone fracture. However, it is a complicated disease because many factors are involved and estrogen deficiency may play an important role. The use of AI for treatment of hormone-sensitive breast cancer patients becomes an important clinical issue due to its adverse effect despite its clinical effectiveness.

In postmenopausal women, EXE and LET lower the estrogen levels in serum by 52–72% and 88–98%, respectively [71]. In our study we have found surprising results that the BMD at 24 months and the bone formation protein BAP at 12 months were raised in group B patients who took oral EXE 25 mg daily. This indicates that patients taking EXE might have a lower chance of suffering from osteoporosis or other skeletal problems. A study had found that bone formation markers were significantly but negatively correlated with BMD in femur in placebo group [72]. A preclinical study found that ovariectomised rats had reduced bone formation and resorption markers after taking EXE [73]. Similarly in Coleman et al.'s study, they found that the fracture risk was significantly higher in the patient group taking EXE [74]. However, our results are consistent with Martinetti et al.'s study, which they

Table 1
Comparison of mean scores of QoL between groups at baseline, week 4, week 8, and week 12 of neoadjuvant hormonal therapy.

	Baseline		Week 4		Week 8		Week 12	
	Mean(SD)	p^c	Mean(SD)	p^c	Mean(SD)	p^c	Mean(SD)	p^c
PWB								
A	24.43(3.34)		25.19(2.23)		24.19(2.80)		24.24(2.40)	
B	24.27(3.24)		25.00(2.67)		23.89(2.71)		23.89(3.37)	
C	26.44(3.39)	0.122	26.15(2.92)	0.257	25.55(3.32)	0.157	25.39(3.73)	0.320
SWB								
A	20.13(3.96)		20.77(3.59)		20.04(3.77)		19.88(3.40)	
B	18.57(4.05)		19.90(5.05)		17.94(6.58)		17.67(6.87)	
C	20.19(5.02)	0.507	17.50(8.01)	0.127	19.76(5.34)	0.388	17.17(9.93)	0.386
EWB								
A	17.30(3.01)		18.30(2.72)		18.27(2.81)		18.22(2.64)	
B	17.00(5.36)		19.05(2.99)		19.20(2.73)		17.79(1.93)	
C	19.33(3.09)	0.183	19.16(2.51)	0.470	18.75(2.41)	0.509	19.68(1.60)	0.032*
FWB								
A	21.91(4.18)		23.46(4.38)		21.42(5.11)		21.68(4.43)	
B	20.15(4.97)		20.19(7.19)		18.44(8.02)		19.50(7.25)	
C	22.19(6.22)	0.512	20.58(8.03)	0.177	19.14(6.66)	0.278	17.78(9.63)	0.209
FACT-G[a]								
A	83.78(10.59)		88.96(9.57)		85.00(10.39)		84.68(8.77)	
B	81.50(10.18)		84.33(13.97)		77.80(14.81)		78.64(14.85)	
C	87.53(12.56)	0.362	81.43(15.57)	0.160	81.91(8.48)	0.184	81.82(19.37)	0.483
BCS								
A	25.30(3.44)		26.35(3.05)		26.00(2.81)		26.80(2.08)	
B	25.08(2.43)		25.81(2.42)		25.44(2.68)		25.33(3.43)	
C	26.81(1.28)	0.159	25.50(3.91)	0.637	25.09(3.93)	0.614	23.89(6.01)	0.067
FACT-B[b]								
A	109.09(12.95)		115.26(11.29)		110.74(11.57)		111.32(9.07)	
B	106.00(12.16)		110.17(14.33)		103.20(14.69)		103.36(13.97)	
C	114.40(12.86)	0.235	107.30(16.65)	0.169	106.27(10.96)	0.191	107.91(20.14)	0.299

[a] FACT-G scores equates to the sum of PWB, SWB, EWB and FWB scores.
[b] FACT-B scores equates to the sum of FACT-G and BCS scores.
[c] p-Value for difference between groups.
* Statistically significant.

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also observed an increase in bone turnover proteins in EXE treatment patient group [75]. And recently Subar et al. suggested that the bone turnover markers in healthy women were increased after taking EXE [76]. Moreover, Goss et al. found that BAP was reduced by 20.1% in patients taking LET [77]. Some other studies also suggested that LET increases bone loss and fracture risk [78–82]. This also indicates that EXE, as a steroidal AI, has got different effect on BMD and bone turnover proteins as no-steroidal LET. However, a study with longer duration and larger sample size is needed to confirm this phenomenon.

In order to explain the difference of AIs, the basic bone physiology has to be understood first. Both androgen and estrogen regulate the normal bone turnover [83]. The importance of estrogen in bone metabolism has been suggested for long; however its underlying mechanism is still not fully understood [84]. Generally, the bone metabolism is regulated by the expression of intracellular and cell surface estrogen receptors by osteoblasts and osteoclasts. Estrogen induces the bone formation activity by osteoblasts. In addition, estrogen reduces bone resorption by decreasing the cytokine production such as interleukin-1 [85]. Besides, estrogen also increases the production of a cytokine called osteoprotegerin, which triggers more osteoclastic apoptosis [86,87]. As a result, estrogen loss increases the bone turnover, which makes the normal bone resorption and formation lose control [84], and eventually causes osteoporosis. The reason which caused the difference on bone loss between EXE and LET could be the different steroidal structure. 17-hydroexemestane, the principal metabolite of EXE plays an important role. It is androgenic, and thus it protects the bone from losing. In contrast, LET is lack of such androgenic activity [88–90]. A previous study showed [67] a possible loss of BMD in patients receiving adjuvant exemestane than that receiving tamoxifen. Patients receiving exemestane had relatively lower mean baseline T-scores of -0.44 ± 1.46 and -0.48 ± 1.31 at spine and hip respectively than those receiving tamoxifen with T-scores of -0.10 ± 1.22 and -0.23 ± 1.11, respectively. The true adverse effect on the change in one-year BMD was barely comparable between groups and that the change indeed did not contribute to osteoporosis. In our study, we did not compare the change in BMD between EXE and tamoxifen, but between different AIs at 24 months from 12 months after surgery and that a positive change in BMD was observed in EXE alone group. The true impact on BMD might be more clearly observed after prolonged adjuvant exemestane.

Apart from the concerned adverse effects of breast cancer therapy, good QoL is also essential for breast cancer patients during and after treatment. In this sub-study, patients receiving exemestane and celecoxib had better QoL as illustrated by positive change in FACT-G and FACT-B scores. The QoL was relatively worsened in patients receiving AI only. Obviously, patients given letrozole suffered from more side effects predominantly by mood alteration, bone and/or muscle aches and hot flashes [38] although, in general, side effects by AI were tolerable. It coincides with relatively worse QoL demonstrated in this sub-study. In view of the positive improvement in QoL in the group with celecoxib, further investigation on the possibility of adding COX-2 inhibitor to AI in adjuvant or neoadjuvant setting is deserved.

Higher level of COX-2 in cancer cells has been associated with poor programmed cell death and was associated with poor prognosis. Therefore, COX-2 has become a therapeutic target. Many studies have been investigating the combination use of chemotherapy drugs and COX-2 inhibitors, and some of them have suggested a better response [91–93]. The efficacy of adding COX-2 inhibitor to AI was comparable to AI alone [38], this sub-study has however demonstrated that patients receiving a combination of EXE and CXB had a better QoL and a stable bone metabolism in general. Therefore, further studies are needed to observe the effect of this combination.

Long-term treatment with AI may have different impacts on BMD, bone turnover proteins and QoL for breast cancer patients. Except BMD, bone turnover proteins have been useful as biochemical markers in assessing metabolic bone diseases. They are relatively safe and cheap than other imaging techniques. They are better in detecting small changes in bone formation and resorption than imaging techniques [94]. Our results have suggested that it is necessary to monitor the bone density in patients over time during the entire treatment, so that treatment against osteoporosis can be done immediately once a reduction in BMD has been detected.

In recent years, estrogen deprivation is regarded as one of chemopreventive strategies for breast cancer. The National Surgical Adjuvant Breast and Bowel Project has launched a clinical trial since 1999 with the use of selective estrogen receptor modulators in a population of health postmenopausal women who are at risk of breast cancer [95,96]. Results are controversial between the use of TAM and raloxifene, but it demonstrated an important milestone for the application of anti-estrogen for breast cancer chemoprevention. As for the use of AI, it is still controversial whether EXE can be used as a preventive agent or not notwithstanding an improvement in BMD. Further investigation is needed to identify the underlying mechanism of how EXE might increase the BMD and bone turnover proteins. It is also notable that patients receiving both EXE and CXB had a relatively stable change in BMD and bone turnover proteins. Although the cardiotoxicity was concerned with the use of NSAID, the combination use of celecoxib and exemestane could be further explored for appropriate dosage and duration in chemoprevention of breast cancer. More studies with larger sample size and longer investigation time can be done to observe the clinical significance of combination use of AI and COX-2 inhibitor.

5. Conclusion

Patients receiving both EXE and CXB had a relatively stable change in BMD and bone turnover proteins and relatively better QoL.

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VOLUME 28 · NUMBER 21 · JULY 20 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Longer-Term Assessment of Trastuzumab-Related Cardiac Adverse Events in the Herceptin Adjuvant (HERA) Trial

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See accompanying editorial on page 3407 and articles on pages 3416 and 3429

A B S T R A C T

Purpose

We investigated the incidence of cardiac adverse events in patients with early breast cancer in the Herceptin Adjuvant (HERA) trial who were treated with 1 year of trastuzumab after completion of (neo)adjuvant chemotherapy.

Patients and Methods

The HERA trial is a three-group, randomized trial that compared 1 year or 2 years of trastuzumab with observation in women with human epidermal growth factor receptor-2 (HER2) –positive early breast cancer. Eligible patients had normal left ventricular ejection fraction (LVEF; ≥ 55%) after completion of (neo)adjuvant chemotherapy with or without radiotherapy. Cardiac function was monitored throughout the trial. This analysis considers patients randomly assigned to 1 year of trastuzumab treatment or observation.

Results

There were 1,698 patients randomly assigned to observation and 1,703 randomly assigned to 1 year of trastuzumab treatment; 94.1% of patients had been treated with anthracyclines. The incidence of discontinuation of trastuzumab because of cardiac disorders was low (5.1%). At a median follow-up of 3.6 years, the incidence of cardiac end points remained low, though it was higher in the trastuzumab group than in the observation group (severe CHF, 0.8% *v* 0.0%; confirmed significant LVEF decreases, 3.6% *v* 0.6%) In the trastuzumab group, 59 of 73 patients with a cardiac end point reached acute recovery; of these 59 patients, 52 were considered by the cardiac advisory board (CAB) to have a favorable outcome from the cardiac end point.

Conclusion

The incidence of cardiac end points remains low even after longer-term follow-up. The cumulative incidence of any type of cardiac end point increases during the scheduled treatment period of 1 year, but it remains relatively constant thereafter.

J Clin Oncol 28:3422-3428. © 2010 by American Society of Clinical Oncology

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Submitted September 9, 2009; accepted March 15, 2010; published online ahead of print at www.jco.org on June 7, 2010.

Written on behalf of the Herceptin Adjuvant (HERA) trial study team.

Presented in part at the 45th Annual Meeting of the American Society of Clinical Oncology, May 29-June 2, 2009, Orlando, FL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

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0732-183X/10/2821-3422/$20.00

DOI: 10.1200/JCO.2009.26.0463

INTRODUCTION

Trastuzumab benefits patients with metastatic breast cancer and improves disease-free and overall survival in the adjuvant setting.[1-4] However, trastuzumab treatment is also associated with cardiac dysfunction and congestive heart failure (CHF),[5-6] likely because the human epidermal growth factor receptor-2 (HER2/ERB2) is expressed in the adult myocardium and is believed to modulate cardiac function and anthracycline cardiotoxicity.[7-9] In the Herceptin Adjuvant (HERA) trial, we therefore prospectively monitored cardiac function and found a low incidence of severe and symptomatic CHF in the trastuzumab group at a median follow-up time of 1 year.[10] The results suggested that trastuzumab-associated cardiac dysfunction has a high rate of reversibility, a characteristic that is fundamentally different from anthracycline-associated cardiac dysfunction. After a cardiac end point, questions of clinical importance are whether cardiac function recovers and, if so, whether the patient is at risk of a subsequent left ventricular ejection fraction (LVEF) decrease. Therefore, this article describes acute recovery and the cardiac advisory board (CAB) assessment of whether the patient had a favorable outcome from the cardiac end

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point or not. This manuscript reports cardiac safety during a median follow-up of 3.6 years.

PATIENTS AND METHODS

Study Design

The HERA trial was a three-group, multicenter, open-label, phase III, randomized trial involving women with HER2-positive early breast cancer. The patients were randomly assigned to observation only, 1 year of trastuzumab treatment, or 2 years of trastuzumab treatment. The primary end point was disease-free survival. The visit schedule was the same for all patients.

The results of the 1 year-trastuzumab group compared with the observation group were released and published after an interim analysis that showed a highly significant improvement in disease-free survival.[11] An article detailing cardiac safety at a median of 1 year of follow-up was published.[10] No data for the 2-year trastuzumab group has been released by the independent data monitoring committee.

A protocol amendment was made after enrollment had been completed (except for the last five patients) to allow patients in the observation group the option of switching to trastuzumab, irrespective of the time since random assignment. For patients originally randomly assigned to observation who switched to trastuzumab after the release of the trial results, only information during the observation period before the patient started trastuzumab was considered.

Eligibility Criteria

The eligibility criteria, including cardiac eligibility criteria, have been described elsewhere.[11]

Cardiac Monitoring

A cardiac questionnaire, physical examination, ECG, and assessment of LVEF by echocardiography or multiple-gated acquisition scan were performed in all three groups at baseline; at 3, 6, 12, 18, 24, 30, 36, and 48 months; and annually between year 5 and year 10 after random assignment.

Definitions of Cardiac End Points and Acute Recovery

Cardiac safety and tolerability of trastuzumab were assessed on the basis of prespecified cardiac end points, which must take place between random assignment and the start date of new therapy for recurrent disease. Cardiac death was defined as death definitely as a result of heart failure, myocardial infarction, or documented arrhythmia or as probable cardiac death within 24 hours of a cardiac event. A significant LVEF decrease was defined as an absolute decline of at least 10 percentage points from baseline LVEF and to less than 50%. Severe CHF was defined as New York Heart Association (NYHA) class III or IV, confirmed by a cardiologist, and a significant LVEF decrease. Symptomatic CHF was defined as symptomatic CHF confirmed by a cardiologist and a significant LVEF decrease. Confirmed significant LVEF decrease was defined as an asymptomatic (NYHA class I) or mildly symptomatic (NYHA class II) significant LVEF decrease, unless the next subsequent assessment of LVEF indicated a return to levels that did not meet the definition of significant LVEF decrease; or as identified by the treatment-unblinded CAB. The primary cardiac end point of the trial was cardiac death or severe CHF. The secondary cardiac end point of the trial was confirmed significant LVEF decrease.

A patient was considered to have reached acute recovery from a cardiac end point if she had two or more sequential LVEF assessments of 50% or greater after the date of the cardiac end point. The date of acute recovery was the date of the first LVEF assessment showing an LVEF $\geq$ 50%, which was part of a sequence of two or more LVEF assessments $\geq$ 50% after the date of the cardiac end point.

Assessment of Outcome of Cardiac End Point by CAB

The CAB reviewed LVEF assessments for the patients with cardiac end points and assessed if the patients had favorable outcomes from the cardiac end points or not on the basis of trends from the patients' LVEF measurements. If the trend was that the LVEF remained constant at greater than 50% or if it improved, the CAB assessment was a favorable outcome. If the trend was that the LVEF decreased, the CAB assessment was that the outcome was not favorable. The CAB did not consider trastuzumab treatment when assessing the outcome of patients.

Safety Analysis Population and Analysis Database

The database used for this analysis contains data as of January 3, 2008. Between December 2001 and June 2005, there were 1,698 patients randomly assigned to observation and 1,703 randomly assigned to 1 year of trastuzumab treatment. The median time between finishing any type of chemotherapy and start of trastuzumab treatment was 90 days.

Twenty-one patients originally randomly assigned to 1 year of trastuzumab treatment did not receive any trastuzumab before disease recurrence and were counted in the observation safety analysis population group. Therefore, there were 1,682 patients in the trastuzumab safety analysis population arm and 1,719 patients in the observation safety analysis population arm. Four patients originally randomly assigned to observation received commercial trastuzumab. For these four patients, only information before the patient started trastuzumab was considered.

There were two patients who suffered cardiac death after the start of new therapy for recurrent disease. These two patients were not considered to have any type of cardiac end point.

Discontinuation of Trastuzumab and Dose Modification

As previously described,[11] trastuzumab had to be permanently discontinued in patients who experienced severe CHF (a primary cardiac end point), and treatment for congestive heart failure was recommended. If the patient had a confirmed significant LVEF decrease (a secondary cardiac end point) trastuzumab had to be permanently discontinued. Reasons for premature discontinuation of trastuzumab are listed in Table 1.

By June 2006, all patients in the trastuzumab group had reached the end of the scheduled 1 year of trastuzumab. A total of 172 patients (10.2%) discontinued trastuzumab for reasons other than recurrence of disease.

Statistical Analysis

The difference in incidence of cardiac adverse events between the safety analysis population groups was estimated by using an approximate 95% CI with the Hauck-Anderson correction. Time to acute recovery was defined for patients with any type of cardiac end point. For patients who reached acute recovery, time to acute recovery was the number of days between the date of cardiac end point and the date of acute recovery. For patients who had not reached acute recovery, time to acute recovery was censored at the date of the last LVEF assessment. Time to LVEF decrease after acute recovery was defined for patients who reached acute recovery after a cardiac end point.

For patients who had at least one LVEF value of less than 50% after the date of acute recovery, the time to LVEF decrease after acute recovery was the number of days between the date of acute recovery and the date of the first LVEF value less than 50% after reaching acute recovery. For patients who had no LVEF value less than 50% and, therefore, no LVEF decrease after acute recovery, time to LVEF decrease after acute recovery was censored at the date of last LVEF assessment.

Table 1. Summary of Reasons for Discontinuation of Trastuzumab

Reason for Discontinuation	Trastuzumab for 1 Year (N = 1,682)	
	No.	%
Cardiac disorder	86	5.1
Other adverse event	32	1.9
Death	3	0.2
Recurrence of disease	90	5.4
Refused treatment	42	2.5
Other reason	9	0.5
Total	262	15.6

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Table 2. Summary of Cardiac End Points for Safety Analysis Populations

End Point	Analysis by Population: Observation Only (n = 1,719) No.	%	Trastuzumab for 1 Year (n = 1,682) No.	%	Incidence in Trastuzumab *v* Observation: Difference	95% CI
Cardiac death	1	0.1	0	0.0	−0.1	−0.2 to 0.1
Severe CHF	0	0	13	0.8	0.8	0.3 to 1.2
Symptomatic CHF	2	0.1	32	1.9	1.8	1.1 to 2.5
Confirmed significant LVEF decrease	11	0.6	60	3.6	2.9	1.9 to 3.9
Any type of cardiac end point	12	0.7	73	4.3	3.6	2.6 to 4.7
At least one significant LVEF decrease	49	2.9	164	9.8	6.9	5.2 to 8.6

Abbreviations: CHF, congestive heart failure; LVEF, left ventricular ejection fraction.

RESULTS

Cardiac End Points

The incidence of cardiac end points is listed in Table 2. One patient in the observation group suffered cardiac death. As expected, the incidence of severe CHF (0.8% *v* 0.0%; 95% CI for the difference, 0.3% to 1.2%), symptomatic CHF (1.9% *v* 0.1%; 95% CI for the difference, 1.1% to 2.5%), and confirmed significant LVEF decrease (3.6% *v* 0.6%; 95% CI for the difference, 1.9% to 3.9%) was significantly higher in the trastuzumab group compared with observation. The 73 patients with cardiac end points have been observed for a median of 25.1 months (range, 0.0 to 33.1 months) after the cardiac end point.

Cumulative Incidence of Cardiac End Points

The cumulative incidence of cardiac death or severe CHF (Fig 1A), cardiac death, severe CHF or symptomatic CHF (Fig 1B), and any type of cardiac end point (Fig 1C) were calculated by original randomly assigned group with a competing risk of a disease-free survival event. The patients originally randomly assigned to observation had an additional competing risk of switching to trastuzumab, which noticeably reduced the number of patients at risk of a cardiac end point. The cumulative incidence of any type of cardiac end point among patients randomly assigned to 1 year of trastuzumab treatment increased during the scheduled trastuzumab treatment period of 1 year, but it remained approximately constant thereafter (Fig 1C).

Acute Recovery After a Cardiac End Point

Acute recovery after a cardiac end point for the trastuzumab group is summarized in Table 3. The cumulative proportion of patients with a cardiac end point who reached acute recovery by time from cardiac end point is shown in Figure 2A. It should be noted that Figure 2A is based on the small number of patients with a cardiac end point.

Among the 73 patients with a cardiac end point, 59 (80.8%) reached acute recovery. The median time to acute recovery was 6.4 months (range, 0 to 33.1 months). The 59 patients who reached acute recovery have been observed for a median of 20.9 months (range, 2.5 to 51.6 months) after reaching acute recovery.

There were three patients in the trastuzumab group who did not have two or more LVEF assessments after the date of the cardiac end point. These patients lacked sufficient LVEF information to determine if the patient reached acute recovery and were considered among the 14 patients who did not reach acute recovery.

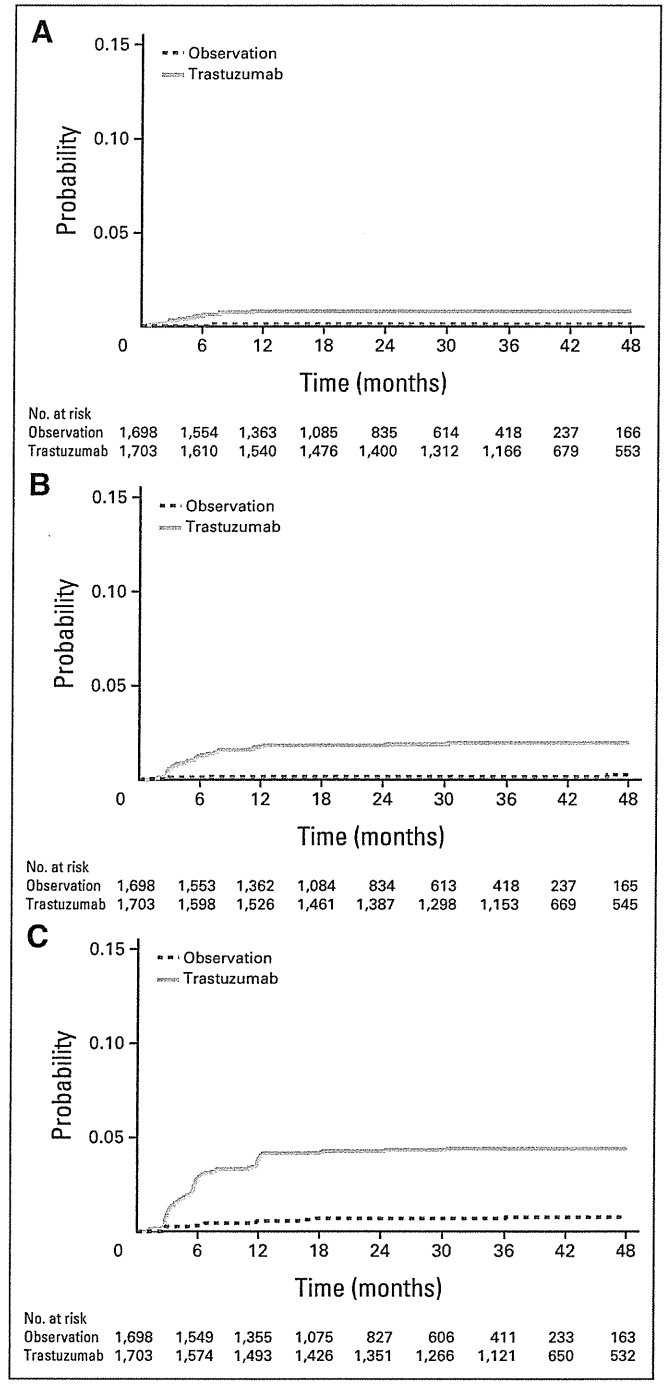

Fig 1. The cumulative incidence of competing risks by randomized group showing the risk of (A) cardiac death or severe congestive heart failure (CHF), (B) cardiac death or severe CHF or symptomatic CHF, or (C) any cardiac end point.

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Table 3. Summary of Acute Recovery After Cardiac End Point Trastuzumab Safety Analysis Population Group

Cardiac End Point	No.	%	Median (months)*	Range (months)†
Severe CHF (n = 13)				
Reached acute recovery	9	69.2		
Time to acute recovery			11.6	1.3-28.7
Occurrence of LVEF drop to < 50% after acute recovery	3	33.3		
Time to LVEF drop to < 50% after acute recovery			25.8	3.0-25.8
Symptomatic CHF (n = 32)				
Reached acute recovery	25	78.1		
Time to acute recovery			5.5	0.0-28.7
Occurrence of LVEF drop to < 50% after acute recovery	8	32.0		
Time to LVEF drop to < 50% after acute recovery			27.7	3.0-34.7
Confirmed significant LVEF drop (n = 60)				
Reached acute recovery	50	83.3		
Time to acute recovery			6.3	0.0-33.1
Occurrence of LVEF drop to < 50% after acute recovery	14	28.0		
Time to LVEF drop to < 50% after acute recovery			—	2.5-51.6
Any type of cardiac end point (n = 73)				
Reached acute recovery	59	80.8		
Time to acute recovery			6.4	0.0-33.1
Occurrence of LVEF drop to < 50% after acute recovery	17	28.8		
Time to LVEF drop to < 50% after acute recovery			—	2.5-51.6

Abbreviations: CHF, congestive heart failure; LVEF, left ventricular ejection fraction.

Subsequent LVEF Decrease to Less Than 50% After Acute Recovery and Evidence of Progressive Cardiac Disease

The cumulative proportion of patients who had a subsequent LVEF decrease to less than 50% after reaching acute recovery by time from reaching acute recovery is shown in Figure 2B. It should be noted that Figure 2B is based on the small number of patients with a cardiac end point who reached acute recovery. Among the 59 patients in the trastuzumab group who reached acute recovery, 42 patients had all subsequent LVEF assessments ≥ 50%, and 17 patients had at least one subsequent LVEF decrease to less than 50%. The CAB reviewed the 17 patients with at least one subsequent LVEF decrease and found evidence of progressive cardiac disease in only six patients. The remaining 11 of 17 patients were assessed by the CAB as having a favorable outcome.

The CAB assessment of one patient who reached acute recovery and who had all subsequent LVEF assessments ≥ 50% was undetermined. Fifty-two (88.1%) of the 59 patients who reached acute recovery were assessed by the CAB as having a favorable outcome from the cardiac end point.

CAB Assessment of Outcome From the Cardiac End Point

A flow chart of the CAB assessment for the 73 patients in the trastuzumab group is shown in Figure 3. Among these 73 patients, the CAB assessment was that 57 (78.1%) had a favorable outcome, including five patients who did not reach acute recovery, and that 14 (19.2%) did not have a favorable outcome, including six patients who reached acute recovery. The CAB assessment was undetermined for two patients.

Among the 14 patients who did not reach acute recovery, the CAB assessment was that five had a favorable outcome, eight did not have a favorable outcome, and one was undetermined. For patients in the trastuzumab group, Appendix Figure A1 (online only) illustrates the proportion of patients who had any type of cardiac end point, the proportion with a cardiac end point who reached acute recovery, and the proportion who reached acute recovery assessed by the CAB as having a favorable outcome.

Description of Patient-Related Predictive Factors

We investigated if there was a pattern of chemotherapy treatment or cardiac medication in patients who were assessed by the CAB as not having a favorable outcome from the cardiac end point.

Previous Anthracyclines

Nearly all (94.1%) of the patients enrolled had been treated with anthracyclines. Of the 73 patients in the trastuzumab group with a cardiac end point, 70 had been treated with anthracyclines. Of the 12 patients in the observation group with a cardiac end point, 11 had been treated with anthracyclines.

Patients Who Did Not Have a Favorable Outcome to the Cardiac End Point

Of the 14 patients in the trastuzumab group assessed by the CAB as not having a favorable outcome from the cardiac end point, 13 had been treated with anthracyclines. Cardiac medication was reported for seven of these 14 patients; however, there was no consistent use of a particular type of cardiac medication.

DISCUSSION

The predominant cardiovascular adverse effect of trastuzumab is the induction of cardiac contractile dysfunction, a complication that previously has been associated mainly with anthracycline treatment. In the HERA trial, the incidence of cardiac dysfunction in the

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trastuzumab arm at a median follow-up time of 1 year was 0.6% for severe CHF and 7.0% for left ventricular (LV) dysfunction.[10] Results from the HERA trial suggest that trastuzumab-associated cardiac dysfunction has a high rate of reversibility,[10] a characteristic that is fundamentally different from anthracycline-associated cardiac dysfunction. However, several questions remained unanswered: Does the incidence of cardiac end points increase with longer follow-up time? When do the cardiac events predominantly occur? What is the cardiac prognosis of a patient after a cardiac end point? What are the risk factors and outcomes of patients with progressive cardiac dysfunction after trastuzumab treatment? Does trastuzumab treatment worsen anthracycline-associated cardiac dysfunction?

We now show that, after a median follow-up time of 3.6 years, the incidence of severe CHF and LV dysfunction in the trastuzumab group remained low at 0.8% and 9.8%, respectively. Similarly, the rate of discontinuation of trastuzumab as a result of cardiac disorders was low (5.1%). Despite these reassuring results, and because anthracycline cardiac adverse effects typically become manifest 5 to 10 years after the initial exposure, longer follow-up of cardiac safety is still required. Preclinical data suggests that trastuzumab could worsen anthracycline-associated cardiotoxicity.[9]

All occurrences of severe symptomatic CHF failure and 51 (85%) of 60 confirmed significant LVEF decreases in the trastuzumab group occurred during the scheduled trastuzumab treatment period. After a cardiac end point, questions of clinical importance are whether cardiac function recovers and, if so, whether the patient is at risk of a subsequent LVEF decrease. Therefore, we defined acute recovery, and the CAB assessed if the patient had a favorable outcome from the cardiac end point or not on the basis of the patients' LVEF trends. Approximately 80% of patients (57 of 73 patients) in the trastuzumab group with cardiac end points were assessed by the CAB as having a favorable outcome. Among the patients in the trastuzumab group who reached acute recovery from a cardiac end point (59 of 73 patients; 80.8%), most were assessed by the CAB as having a favorable outcome (52 of 59 patients; 88.1%). Among the 59 patients who reached acute recovery, 15 patients in the absence of any additional trastuzumab treatment had a subsequent LVEF drop to 50%, though we do not know the cause of the subsequent LVEF decrease; nine of these 15 patients were assessed by the CAB as having a favorable outcome. Given that the majority of cardiac end points in the trastuzumab group occurred during the scheduled treatment period, reaching acute recovery after a cardiac end point may influence treatment decisions outside clinical trials. In the HERA trial, patients who had a confirmed significant LVEF decrease discontinued trastuzumab treatment.

The relatively good prognosis after a cardiac end point also sheds light on the pathophysiology of trastuzumab-associated cardiac dysfunction. Preclinical data indicate that inhibition of myocardial HER2/ERB2 leads to changes in the tertiary structure of the cardiac contractile apparatus (likely a reversible condition) but does not induce myocardial cell death (likely a progressive condition).[12] This may explain why cardiac contractile dysfunction is predominantly seen during trastuzumab treatment and appears to have a high rate of reversibility. In contrast, anthracyclines can induce myocardial cell death that leads to a maladaptive cardiac remodeling with progressive cardiac dysfunction and heart failure.[13] This suggests that the approximately 80% of patients (57 of 73 patients) in the trastuzumab group with a cardiac end point assessed by the CAB as having a favorable outcome may have primarily trastuzumab-associated cardiac dysfunction. Among the approximately 20% of patients (14 of 73 patients) in the trastuzumab group assessed by the CAB as not having a favorable outcome from the cardiac end point, almost all (13 of 14) had been treated with anthracyclines. In these 13 patients, it is possible that trastuzumab treatment worsened anthracycline-associated cardiac dysfunction.

In the HERA trial, nearly all of the patients enrolled had been treated with anthracyclines. We have insufficient information to assess the influence of cardiac medication on the prognosis of patients in the trastuzumab group with a cardiac end point.

In conclusion, given the benefit in disease-free and overall survivals of 1 year adjuvant trastuzumab in patients with HER2-positive early breast cancer, the low incidence of cardiac end points with longer term follow-up, and the suggestion that trastuzumab-induced cardiac dysfunction may be reversible, adjuvant trastuzumab should be considered as a standard treatment option for patients who fulfill the HERA trial eligibility criteria.

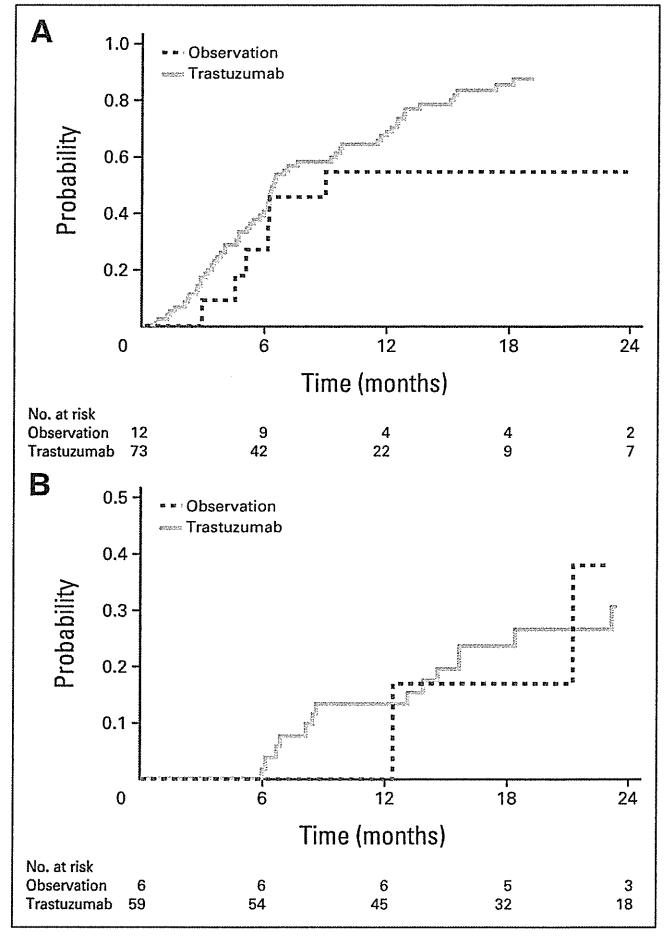

Fig 2. The cumulative incidence by safety analysis population group among patients with any type of cardiac end point of (A) reaching acute recovery and (B) subsequent left ventricular ejection fraction decrease to less than 50% after reaching acute recovery.

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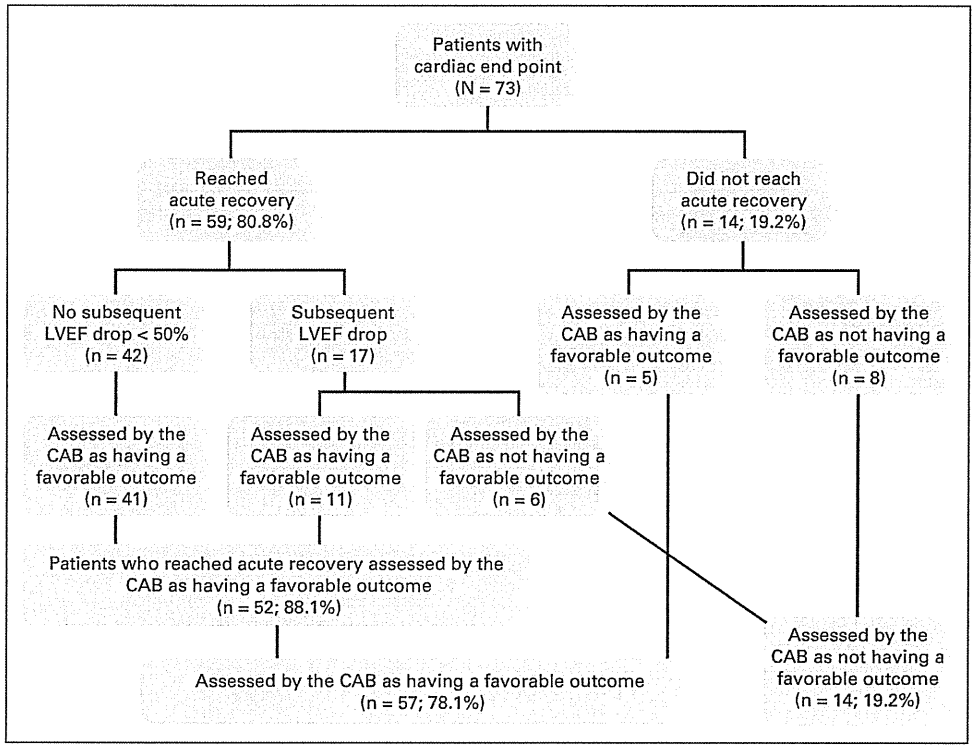

Fig 3. Flow chart of the cardiac advisory board (CAB) assessment for patients with any type of cardiac end point in the trastuzumab group. The CAB assessment was undetermined for two patients. LVEF, left ventricular ejection fraction.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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J Med Dent Sci 2010; 57: 155–163

Original Article

HER-2/neu cytoplasmic staining is correlated with neuroendocrine differentiation in breast carcinoma

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HER2 oncoprotein plays an essential role in breast cancer growth and differentiation. Determination of HER2 status contributes not only to predicting survival but also to selecting the patients for anti-HER2 therapy. HER2 protein expressed in human cancer cells often contains variant forms as well as the full-length wild-type form. In the present study, we investigated the subcellular localization of HER2 protein in 1053 primary breast cancer tissues. HER2 protein was stained by various immunohistochemical methods and studied by immunoelectron microscopy to confirm the intracellular localization.

Thirty-four of 1053 specimens showed cytoplasmic staining of the intracellular domain of HER2 protein by the HercepTest® and CB-11. In contrast, no immunoreactivity to the antibodies against the extracellular domain was observed. None of the 34 specimens showed amplification of the HER2 protein by fluorescence *in situ* hybridization. Subsequently, we studied the association of the cytoplasmic expression of HER2 with neuroendocrine differentiation. Interestingly, all 34 specimens had some positive signals of neuroendocrine markers such as synaptophysin, chromogranin A, neuron-specific enolase, and CD56. Although the result is preliminary, it warrants further study on the role of the cytoplasmic variant form of HER2 in breast cancer growth, particularly in the aspect of neuroendocrine differentiation.

Key words: HER2/neu, cytoplasmic staining, neuroendocrine differentiation, breast cancer, immunohistochemistry.

INTRODUCTION

The HER2/neu (HER2) proto-oncogene, located on chromosome 17, encodes a 185-kDa glycoprotein that acts as a growth factor receptor on the cell surface.[1] In the breast, oncogenic overexpression of the HER2 protein is both a marker for poor prognosis and a target for trastuzumab (Herceptin™; Genentech, Inc., South San Francisco, CA). HER2 gene amplification or protein overexpression can be determined by various reagents and techniques, including fluorescence *in situ* hybridization (FISH), immunohistochemistry (IHC), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA).[2-5] The most frequently employed method is immunohistochemical detection using antibodies against the HER2 protein in paraffin sections; the IHC staining procedure is performed using standard equipment.[4,5]

HER2 status can be classified into four categories based on the degree of positivity in the cell membrane and the percentage of positive tumor cells.[4,5] Cytoplasmic staining for antibodies against HER2/

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Received February 1 ; Accepted March 12, 2010