術前化学療法終了/中止時点で継続する因果関係を否定できない有害事象

（経過を観察し、後観察期間（術前化学療法終了日あるいは中止日より 4 週間±7 日）終了時点で転帰の確認を行ってください。回復が見られない事象については、追跡調査を行ってください。）

1□ なし　2□ あり（→以下にご記入ください【CTCAE Ver.4.0 使用】）

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
1		[redacted]	2 0
	最終観察時 Grade 0□0 1□1 2□2 3□3 4□4	1■ 術前化学療法終了時より継続	1□ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1□ 後治療 2□ その他 →＊＊（理由をコメント欄へ）		

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
2		[redacted]	2 0
	最終観察時 Grade 0□0 1□1 2□2 3□3 4□4	1■ 術前化学療法終了時より継続	1□ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1□ 後治療 2□ その他 →＊＊（理由をコメント欄へ）		

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
3		[redacted]	2 0
	最終観察時 Grade 0□0 1□1 2□2 3□3 4□4	1■ 術前化学療法終了時より継続	1□ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1□ 後治療 2□ その他 →＊＊（理由をコメント欄へ）		

術前化学療法終了/中止時点で継続する因果関係を否定できない有害事象

（経過を観察し、後観察期間（術前化学療法終了日あるいは中止日より 4 週間±7 日）終了時点で転帰の確認を行ってください。回復が見られない事象については、追跡調査を行ってください。）

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
1		[redacted]	2 0
	最終観察時 Grade 0☐0 1☐1 2☐2 3☐3 4☐4	1■ 術前化学療法終了時より継続	1☐ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1☐ 後治療 2☐ その他 →＊＊（理由をコメント欄へ）		

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
2		[redacted]	2 0
	最終観察時 Grade 0☐0 1☐1 2☐2 3☐3 4☐4	1■ 術前化学療法終了時より継続	1☐ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1☐ 後治療 2☐ その他 →＊＊（理由をコメント欄へ）		

	事象名	発現日 (YYYY/MM/DD)	回復日 (YYYY/MM/DD)
3		[redacted]	2 0
	最終観察時 Grade 0☐0 1☐1 2☐2 3☐3 4☐4	1■ 術前化学療法終了時より継続	1☐ 未回復だが追跡不要（→＊） （「回復日」欄に「最終観察日」をご記入ください。）
	＊追跡不要の理由	＊＊コメント	
	1☐ 後治療 2☐ その他 →＊＊（理由をコメント欄へ）		

追跡調査実施日	YYYY 2 0	MM	DD

生存調査

1☐	生存	YYYY 2 0	MM	DD	(最終生存確認日)
2☐	死亡	YYYY 2 0	MM	DD	(死亡日)
		死因	1☐ 原病死　0☐その他(→以下に詳細をご記入下さい)		
			「その他」詳細:		
3☐	不明	YYYY 2 0	MM	DD	(最終生存確認日)

術後治療 (手術を施行した患者のみご記入ください)

既に報告済の内容以外で、術後新たに開始した治療がありますか？

1☐ なし　2☐ あり(→以下に詳細をご記入下さい)

治療内容(複数選択可)	治療開始日(YYYY/MM/DD)
1☐ 放射線療法	20____ / ____ / ____
2☐ 内分泌療法	20____ / ____ / ____
0☐ その他(　　)	20____ / ____ / ____

転移・再発確認 (手術を施行した患者のみご記入ください)

当該試験登録後、転移・再発が確認されましたか？

1☐	既に転移・再発を報告済				
2☐	いいえ	YYYY 2 0	MM	DD	(確認日)　引き続き追跡調査してください
3☐	はい	YYYY 2 0	MM	DD	(＊再発イベント診断日)
		1☐ 局所再発(乳房温存療法後の局所再発を含む) 2☐ 局所リンパ節再発 3☐ 遠隔臓器転移(部位:　　)			

＊再発イベント診断日

－再発の診断が画像診断による場合、「画像上疑い」の検査日ではなく、後日「確診」が得られた画像検査の「検査日」をもってイベントとする。

－画像診断によらず臨床的に再発と判断した場合は、担当医が再発と判断した日をもってイベントとする。

－再発の確定診断が生検病理診断による場合、臨床上再発と診断し得た場合は臨床診断日を、臨床上再発と診断し得ず生検病理診断によって再発と診断した場合は生検施行日をもってイベントとする。

NCC–IDC–007	症例番号			回収頁の確認	Page.8.01

記入が完了したページを以下よりご選択下さい。

☐	スクリーニング		全ページ
	☐	被験者背景	0.01
	☐	原発巣(乳癌)病歴	0.02
	☐	既往歴・合併症	0.03
	☐	PS・体重	0.04
	☐	臨床検査値	0.05
	☐	感染症検査・妊娠検査	0.06
	☐	臨床的効果判定	0.07
☐	Extra form 臨床検査値		9.01.___~___
☐	Extra form 臨床的効果判定		9.02.___~___

	記載者署名	記載完了日		
		YYYY	MM	DD
治験分担/責任医師		2 0		
		2 0		
治験協力者		2 0		
		2 0		

記入が完了したページを以下よりご選択下さい。

	CP/P（1コース）		全ページ		CP/P 療法（2コース）		全ページ
☐	☐	臨床所見	1.00	☐	☐	Day1 PS・体重	1.10
	☐	Day1 PS・体重	1.01		☐	Day1 臨床検査値	1.11
	☐	Day1 臨床検査値	1.02		☐	Day1 臨床的効果判定	1.12
	☐	Day1 投与状況	1.03		☐	Day1 投与状況	1.13
	☐	Day8 PS	1.04		☐	Day8 PS	1.14
	☐*	Day8 臨床検査値*	1.05*		☐*	Day8 臨床検査値*	1.15*
	☐	Day8 投与状況	1.06		☐	Day8 投与状況	1.16
	☐	Day15 PS	1.07		☐	Day15 PS	1.17
	☐*	Day15 臨床検査値*	1.08*		☐*	Day15 臨床検査値*	1.18*
	☐	Day15 投与状況	1.09		☐	Day15 投与状況	1.19

*CP 群のみ提出必須

☐	CP/P 療法 有害事象	1.40.____～____
☐	CP/P 療法 併用治療	1.41.____～____
☐	Extra form 臨床検査値	9.01.____～____
☐	Extra form 臨床的効果判定	9.02.____～____

	記載者署名	記載完了日 YYYY	MM	DD
治験分担/責任医師		2 0		
		2 0		
治験協力者		2 0		
		2 0		

記入が完了したページを以下よりご選択下さい。

	CP/P（3コース）		全ページ
☐		Day1 PS・体重	1.20
☐		Day1 臨床検査値	1.21
☐		Day1 臨床的効果判定	1.22
☐		Day1 投与状況	1.23
☐		Day8 PS	1.24
☐*		Day8 臨床検査値*	1.25*
☐		Day8 投与状況	1.26
☐		Day15 PS	1.27
☐*		Day15 臨床検査値*	1.28*
☐		Day15 投与状況	1.29

	CP/P 療法（4コース）		全ページ
☐		Day1 PS・体重	1.30
☐		Day1 臨床検査値	1.31
☐		Day1 臨床的効果判定	1.32
☐		Day1 投与状況	1.33
☐		Day8 PS	1.34
☐*		Day8 臨床検査値*	1.35*
☐		Day8 投与状況	1.36
☐		Day15 PS	1.37
☐*		Day15 臨床検査値*	1.38*
☐		Day15 投与状況	1.39

*CP 群のみ提出必須

☐	CP/P 療法 有害事象	1.40.____～____
☐	CP/P 療法 併用治療	1.41.____～____
☐	Extra form 臨床検査値	9.01.____～____
☐	Extra form 臨床的効果判定	9.02.____～____

	記載者署名	記載完了日 YYYY	MM	DD
治験分担/責任医師		2 0		
		2 0		
治験協力者		2 0		
		2 0		

記入が完了したページを以下よりご選択下さい。

☐	CEF 療法(1コース)	全ページ	☐	CEF 療法(3コース)	全ページ
	☐ PS・体重	2.01		☐ PS・体重	2.09
	☐ 臨床検査値	2.02		☐ 臨床検査値	2.10
	☐ 臨床的効果判定	2.03		☐ 臨床的効果判定	2.11
	☐ 投与状況	2.04		☐ 投与状況	2.12
☐	CEF 療法(2コース)	全ページ	☐	CEF 療法(4コース)	全ページ
	☐ PS・体重	2.05		☐ PS・体重	2.13
	☐ 臨床検査値	2.06		☐ 臨床検査値	2.14
	☐ 臨床的効果判定	2.07		☐ 臨床的効果判定	2.15
	☐ 投与状況	2.08		☐ 投与状況	2.16
☐	CEF 療法 有害事象		2.17.____~____		
☐	CEF 療法 併用治療		2.18.____~____		
☐	Extra form 臨床検査値		9.01.____~____		
☐	Extra form 臨床的効果判定		9.02.____~____		
☐					
☐					
☐					

	記載者署名	記載完了日		
		YYYY	MM	DD
治験分担/責任医師		2 0		
		2 0		
治験協力者		2 0		
		2 0		

記入が完了したページを以下よりご選択下さい。

☐	術前化学療法終了時		全ページ	☐	術前化学療法中止時	全ページ
	☐	PS・体重	3.01		☐ PS・体重	5.01
	☐	臨床検査値	3.02		☐ 臨床検査値	5.02
	☐	臨床的効果判定	3.03		☐ 臨床的効果判定	5.03
☐	手術療法:手術前		全ページ	☐	術前化学療法 完了・中止時	
	☐	検査	4.01		完了/中止報告	5.04
	☐	臨床的効果判定	4.02	☐	術前化学療法 終了/中止後	
	☐	手術結果報告(1)	4.03(1)		有害事象	6.01.____～____
	☐	手術結果報告(2)	4.03(2)	☐	追跡調査	7.01.____～____
☐	Extra form 臨床検査値			9.01.____～____		
☐	Extra form 臨床的効果判定			9.02.____～____		
☐						
☐						
☐						

	記載者署名	記載完了日		
		YYYY	MM	DD
治験分担/責任医師		2 0		
		2 0		
治験協力者		2 0		
		2 0		

本症例報告書について、

記入されたデータがすべて原資料に基づき正しいことを確認・承認致しました。

	署名	署名日			
		YYYY		MM	DD
治験責任医師		2	0		

血液学的検査

検査日	YYYY 2 0 / MM / DD	
白血球数		/mm^3
好中球数		/mm^3
ヘモグロビン		g/dL
血小板数		×10^4/mm^3

生化学的検査

検査日	☐ 血液学的検査と同日 （異なる場合は記載してください⇒）	YYYY 2 0 / MM / DD	
総ビリルビン**			mg/dL
アルブミン			g/dL
AST（GOT）			IU/L
ALT（GPT）			IU/L
クレアチニン			mg/dL
ALP			IU/L
Na			mEq/L
K			mEq/L
Ca（ｱﾙﾌﾞﾐﾝ補正値）**			mg/dL

**血清アルブミン値が 4.0(g/dL)未満の場合

血清 Ca 補正値(mg/dL)=血清 Ca 値(mg/dL) + { 4 - 血清アルブミン値(g/dL) }

臨床的効果判定

評価病変 ＊「ベースライン評価/(Page.0.07)」をご参照の上、同番号部位の長径をご記入ください。		
部位*(長径)	病変 No.1	⌊⌋⌊⌋⌊⌋ mm
	病変 No.2	⌊⌋⌊⌋⌊⌋ mm
	病変 No.3	⌊⌋⌊⌋⌊⌋ mm
	病変 No.4	⌊⌋⌊⌋⌊⌋ mm
	病変 No.5	⌊⌋⌊⌋⌊⌋ mm
長径和		⌊⌋⌊⌋⌊⌋ mm

新病変	
出現の有無	1☐ なし　2☐ あり(→以下に詳細をご記入下さい)
出現部位	
評価方法	1☐ 触診　0☐ その他(　　)

腫瘍縮小効果判定					
判定日	YYYY 2 0 ⌊⌋⌊⌋	MM ⌊⌋	DD ⌊⌋		
効果判定	1☐ CR	2☐ PR	2☐ SD	2☐ PD	2☐ NE(→以下に理由をご記入ください)
NE の理由					

修正記録用紙（*Data Clarification Form*）

症例番号			

施設名			
記入者名		記入日	20　/　/
責任医師名		確認日	20　/　/

No.	Page	項目名	【現在】の記載内容(上段) 【修正後】の記載内容(下段)	修正理由
1	__.__ __(__ __)		↓	□誤記 □追記 □その他: (　　)
2	__.__ __(__ __)		↓	□誤記 □追記 □その他: (　　)
3	__.__ __(__ __)		↓	□誤記 □追記 □その他: (　　)
4	__.__ __(__ __)		↓	□誤記 □追記 □その他: (　　)
5	__.__ __(__ __)		↓	□誤記 □追記 □その他: (　　)

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表レイアウト

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hirata T, Schimizu C, Yonemori K, Hirakawa A, Kouno T, Tamura K, Ando M, Katsumata N, Fujiwara Y	Change in the hormone receptor status following administration of neoadjuvant chemotherapy and its impact on the long-term outcome in patients with primary breast cancer	Br J Cancer	101	1529-1536	2009
Shien T, Akashi-Tanaka S, Miyakawa K, Hojo T, Shimizu, C, Seki K, Ando M, Kohno T, Taira N, Doihara H, Katsumata N, Fujiwara Y, Kinoshita T	Clinicopathological features of tumors as predictors of the efficacy of primay neoadjuvant chemotherapy for operable breast cancer	World J Sur	33	44-51	2009

Ⅳ．研究成果の刊行物・別刷

British Journal of Cancer (2009) 101, 1529–1536

www.bjcancer.com

Change in the hormone receptor status following administration of neoadjuvant chemotherapy and its impact on the long-term outcome in patients with primary breast cancer

T Hirata[1], C Shimizu*[,1], K Yonemori[1], A Hirakawa[2], T Kouno[1], K Tamura[1], M Ando[1], N Katsumata[1] and Y Fujiwara[1]

[1]Department of Breast and Medical Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo 104-0045, Japan; [2]Department of Management Science, Graduate School of Engineering, Tokyo University of Science, Shinjuku-ku, Tokyo 162-8601, Japan

BACKGROUND: To evaluate the impact of change in the hormone receptor (HR) status (HR status conversion) on the long-term outcomes of breast cancer patients treated with neoadjuvant chemotherapy (NAC).

METHODS: We investigated 368 patients for the HR status of their lesions before and after NAC. On the basis of the HR status and the use/non-use of endocrine therapy (ET), the patients were categorised into four groups: Group A, 184 ET-administered patients with HR-positive both before and after NAC; Group B, 47 ET-administered patients with HR status conversion; Group C, 12 ET-naive patients with HR status conversion; Group D, 125 patients with HR-negative both before and after NAC.

RESULTS: Disease-free survival in Group B was similar to that in Group A (hazard ratio, 1.16; $P=0.652$), but that in Group C was significantly lesser than that in Group A (hazard ratio, 6.88; $P<0.001$). A similar pattern of results was obtained for overall survival.

CONCLUSION: Our results indicate that the HR status of tumours is a predictive factor for disease-free and overall survival and that ET appears to be suitable for patients with HR status conversion. Therefore, both the CNB and surgical specimens should be monitored for HR status.

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Published online 6 October 2009

Keywords: breast cancer; endocrine therapy; hormone receptor status change; neoadjuvant chemotherapy; prognosis

Neoadjuvant chemotherapy (NAC) was introduced in the early 1980s for patients with locally advanced breast cancer, initially to improve the operability of tumours (Kaufmann *et al*, 2003). Recently, the application of this therapy has been extended to cases of operable disease. The previously reported results of a meta-analysis indicated that neoadjuvant and adjuvant chemotherapy are equivalent in terms of overall survival (OS) and disease-free survival (DFS) (Mauri *et al*, 2005). As the pertinent published reports present conflicting views, the actual indications for NAC remain controversial.

Before the initiation of NAC, core-needle biopsy (CNB) is often performed to establish the histological diagnosis and to assess certain factors considered predictive of treatment outcomes. The hormone receptor (HR) status is one such factor. Although this status is known to change after NAC (Bottini *et al*, 1996; Lee *et al*, 2003; Taucher *et al*, 2003; Colleoni *et al*, 2004; Burcombe *et al*, 2005; Shet *et al*, 2007; Tacca *et al*, 2007; Kasami *et al*, 2008; Neubauer *et al*, 2008), its impact on long-term outcomes has not been assessed. The objective of this retrospective study was to evaluate the frequency and impact of change in the HR status (HR status conversion) on the long-term outcomes in the NAC-administered breast cancer patients.

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MATERIALS AND METHODS

Patients

We selected 459 primary breast cancer patients treated at the National Cancer Center Hospital between May 1995 and July 2007. All the patients had received anthracycline- and taxane-based NAC. The clinical stages of the patients ranged from cT2N0M0 to cT4dN3M0, which includes inflammatory (T4d) carcinoma. Data were collected on the pre- and post-NAC statuses of oestrogen receptor (ER), progesterone receptor (PgR), and human epidermal receptor (HER) 2 expressions in the lesions. Patients in whom pathologic complete response (pCR) was obtained (91 patients) after surgery, including those with only residual ductal carcinoma *in situ* (DCIS), were excluded from this analysis because the HR status of the lesions of these patients could not be accurately evaluated. The remaining 368 patients were classified into four groups on the basis of the HR status of their lesions before and after NAC and the use/non-use of endocrine therapy (ET): Group A, 184 ET-administered patients with lesions that were HR-positive

both before and after NAC; Group B, 47 ET-administered patients with lesions showing HR status conversion; Group C, 12 ET-naive patients with lesions showing HR status conversion; Group D, 125 patients with lesions that were HR-negative both before and after NAC. The mean age at the time of diagnosis of breast cancer was almost the same in the four groups.

Hormone status and HER2 status determination

All the patients underwent CNB performed using an 18G needle. The ER, PgR, and HER2 statuses of all the CNB and surgical specimens were determined by immunohistochemistry (IHC). Details regarding the antibodies used, the clones used, and the time periods for which they were used, as well as the antigen retrieval and the source of antibodies for IHC studies, are listed in Table 1. Positive staining for ER/PgR was defined as nuclear staining in $\geqslant$10% of the tumour cells. HER2 protein overexpression was defined as with 3+ complete membrane staining. If HER2 staining on IHC was determined to be 2+, fluorescent *in situ* hybridisation (FISH) was used to confirm the results. FISH was performed using the PathVysion kit (Abott-Vysis Lab, Abott Park, IL, USA). HER2 gene amplification was defined as a HER2:chromosome 17 ratio of $\geqslant$2.1. HR positivity was defined as positivity for ER and/or PgR. The Allred scoring system was used to assess the degree of staining (Allred *et al*, 1998). Standard controls were prepared on a daily basis for each tumour to ensure the results of IHC.

Tumour size determination and evaluation of neoadjuvant chemotherapy response

Before each chemotherapy treatment and before surgery, the two greatest perpendicular diameters of the tumours in the breast and axillary nodes were measured, and the products of these diameters were added as a measure of total tumour size. No clinical response of palpable tumour in the breast and axillary lymph nodes was defined as a complete response (CR). Reduction in total tumour size of 50% or greater was graded as a partial response (PR). An increase in total tumour size of more than 50% or the appearance of new suspicious ipsilateral axillary adenopathy was considered as a progressive disease (PD). Tumours that did not meet the criteria for objective response or progression were considered as a stable disease (SD).

Chemotherapy

Patients receiving NAC were administered an anthracycline and a taxane, either concurrently or sequentially. Those receiving concurrent therapy were administered four cycles (doxorubicin at 50 mg m^{-2} plus docetaxel at 60 mg m^{-2}) every 21 days. Patients showing clinical CR or PR to the above treatment were administered two additional cycles of the same regimen after the surgery. However, patients who did not achieve objective clinical response to NAC were administered with four cycles of 5-fluorouracil (600 mg m^{-2}), methotrexate (40 mg m^{-2}), and cyclophosphamide (600 mg m^{-2}) after the surgery. For patients receiving the sequential regimen, four cycles of 5-fluorouracil (500 mg m^{-2}), epirubicin (100 mg m^{-2}), cyclophosphamide (500 mg m^{-2}) or doxorubicin (60 mg m^{-2}), and cyclophosphamide (600 mg m^{-2}) were administered every 21 days, followed by a taxane. As a taxane, paclitaxel was administered weekly at a dose of 80 mg m^{-2} per week for 12 weeks or at a dose of 175 mg m^{-2} every 3 weeks for four cycles, or docetaxel was administered every 3 weeks at a dose of 75 mg m^{-2} for four cycles.

Adjuvant endocrine therapy (ET) and irradiation

Adjuvant radiotherapy was administered to patients who underwent breast-conserving surgery. Adjuvant radiotherapy was recommended to those who underwent modified radical mastectomy for the disease ranging from cT3N1M0 to cT4dN3M0. The decision to administer ET was taken on the basis of the treating physician's and/or the patient's preferences. Most patients with HR-positive lesions were administered 20 mg of tamoxifen daily for 5 years. From 2005 onwards, postmenopausal women taking tamoxifen were (1) allowed to switch to an aromatase inhibitor before completing 5 years of tamoxifen, (2) allowed to begin taking an aromatase inhibitor after a 5-year course of tamoxifen or (3) recommended an aromatase inhibitor for the first 5 years.

Statistical analysis

The frequencies and descriptive statistics of the demographic and clinical variables from the four groups—A, B, C, and D—were obtained. The ER and PgR statuses of the lesions before and after NAC were compared using the consistency test. DFS was defined as the time from surgery to the detection of relapse, death from any cause, or the date of the last visit for patients without events. OS was defined as the time from surgery to death from any cause or the date of the last visit for patients without events. DFS and OS were estimated using the Kaplan–Meier method, and the survival curves were compared using the log-rank test. Multivariate Cox regression analysis with stepwise selection ($\alpha = 0.05$) was used to estimate the hazard ratio, 95% confidence interval (CI), and the effects of the clinical and pathological variables. A two-sided $P < 0.05$ was considered to be statistically significant. All the analyses were performed using the SAS (version 9.1; SAS Institute Inc., Cary, NC, USA).

Table 1 Panel of antibodies

Antigen	Period used for	Clone	Type	Antigen retrieval	Source
ER	Until Oct 2002	ID5	Mouse monoclonal	A/C citrate buffer	Dako
	From Nov 2002 to Feb 2005	ER88	Mouse monoclonal	As above	Bio Genex
	From Mar 2005	ID5	Mouse monoclonal	As above	Dako
PgR	Until Oct 2002	1A6	Mouse monoclonal	As above	Novocastra
	From Nov 2002 to Feb 2005	PR88	Mouse monoclonal	As above	Bio Genex
	From Mar 2005	PgR636	Mouse monoclonal	As above	Dako
HER2	Until Oct 2002	c-erbB-2	Rabbit polyclonal	As above	Dako
	From Nov 2002 to Feb 2005	CB11	Mouse monoclonal	As above	Bio Genex
	From Mar 2005	c-erbB-2	Rabbit polyclonal	As above	Dako

Abbreviations: A/C: autoclave for 10 min at 121°C; ER: estrogen receptor; HER2: human epidermal receptor 2; PgR: progesterone receptor; citrate buffer: 10 mM citrate buffer, pH 6.0.

Table 2 Patient and tumour characteristics

Characteristics	Group A (*N* = 184)	Group B (*N* = 47)	Group C (*N* = 12)	Group D (*N* = 125)
Mean ± StdDev age, years	48.7 ± 9.9	49.0 ± 9.5	49.5 ± 8.1	49.7 ± 8.8
Tumour stage				
T1	1 (0.5)	0 (0.0)	1 (8.3)	1 (0.8)
T2	99 (53.8)	20 (42.6)	4 (33.3)	52 (41.6)
T3	46 (25.0)	19 (40.4)	5 (41.7)	50 (40.0)
T4a–c	36 (19.6)	6 (12.8)	2 (16.7)	20 (16.0)
T4d	2 (1.1)	2 (4.3)	0 (0.0)	2 (1.6)
N stage				
N0	95 (51.6)	22 (46.8)	5 (41.7)	55 (44.0)
N1	67 (36.4)	21 (44.7)	6 (50.0)	51 (40.8)
N2	16 (8.7)	4 (8.5)	1 (8.3)	15 (12.0)
N3	6 (3.3)	0 (0.0)	0 (0.0)	4 (3.2)
Clinical stage				
IIA	59 (32.1)	12 (25.5)	2 (16.7)	26 (20.8)
IIB	49 (26.6)	15 (31.9)	5 (41.7)	39 (31.2)
IIIA	35 (19.0)	12 (25.5)	3 (25.0)	34 (27.2)
IIIB	36 (19.6)	8 (17.0)	2 (16.7)	22 (17.6)
IIIC	5 (2.7)	0 (0.0)	0 (0.0)	4 (3.2)
Histological grade				
G1	14 (7.61)	3 (6.4)	1 (8.3)	2 (1.6)
G2	111 (60.3)	26 (55.3)	4 (33.3)	40 (32.0)
G3	57 (31.0)	16 (34.0)	7 (58.3)	78 (62.4)
Unknown	2 (1.1)	2 (4.3)	0 (0.0)	5 (4.0)
HR status before NAC				
Positive	184 (100.0)	29 (61.7)	1 (8.3)	0 (0.0)
Negative	0 (0.0)	18 (38.3)	11 (91.7)	125 (100.0)
HER2 status before NAC				
Positive	34 (18.5)	17 (36.2)	4 (33.3)	57 (45.6)
Negative	150 (81.5)	30 (63.8)	8 (66.7)	68 (54.4)
HR status after NAC				
Positive	184 (100.0)	18 (38.3)	11 (91.7)	0 (0.0)
Negative	0 (0.0)	29 (61.7)	1 (8.3)	125 (100.0)
HER2 status after NAC				
Positive	26 (14.1)	18 (38.3)	4 (33.3)	55 (44.0)
Negative	158 (85.9)	29 (61.7)	8 (66.7)	70 (56.0)
NAC regimen				
AT	69 (37.5)	22 (46.8)	10 (83.3)	62 (49.6)
AC followed by T	56 (30.4)	9 (19.2)	2 (16.7)	24 (19.2)
CEF followed by T	59 (32.1)	16 (34.0)	0 (0.0)	39 (31.2)
Clinical response				
CR/PR	157 (85.3)	40 (85.1)	12 (100.0)	103 (82.4)
SD/PD	27 (14.7)	7 (14.9)	0 (0.0)	22 (17.6)
Operation				
Lumpectomy	65 (35.3)	18 (38.3)	3 (25.0)	40 (32.0)
Mastectomy	119 (64.7)	29 (61.7)	9 (75.0)	85 (68.0)
Radiotherapy				
Yes	127 (69.0)	30 (63.8)	9 (75.0)	82 (65.6)
No	57 (31.0)	17 (36.2)	3 (25.0)	43 (34.4)
Number of lymph node metastases				
0	65 (35.3)	22 (46.8)	3 (25.0)	58 (46.4)
1–3	59 (32.1)	13 (27.7)	5 (41.7)	42 (33.6)
4>	60 (32.6)	12 (25.5)	4 (33.3)	25 (20.0)
Endocrine therapy				
TAM, 5 years	112 (60.9)	30 (63.8)	0 (0.0)	0 (0.0)
TAM followed by AI	44 (23.9)	11 (23.4)	0 (0.0)	0 (0.0)
AI, 5 years	28 (15.2)	6 (12.8)	0 (0.0)	0 (0.0)
None	0 (0.0)	0 (0.0)	12 (100.0)	125 (100.0)

Abbreviations: AC = doxorubicin and cyclophosphamide; AI = aromatase inhibitor; AT = doxorubicin and docetaxel; CEF = cyclophosphamide, epirubicin and 5-fluorouracil; ER = estrogen receptor; HER2 = human epidermal receptor; *N* = number of patients; PgR = progesterone receptor; T = taxane (weekly or triweekly paclitaxel, or triweekly docetaxel); TAM = tamoxifen; Figures in parentheses are percentage of patients except for age.

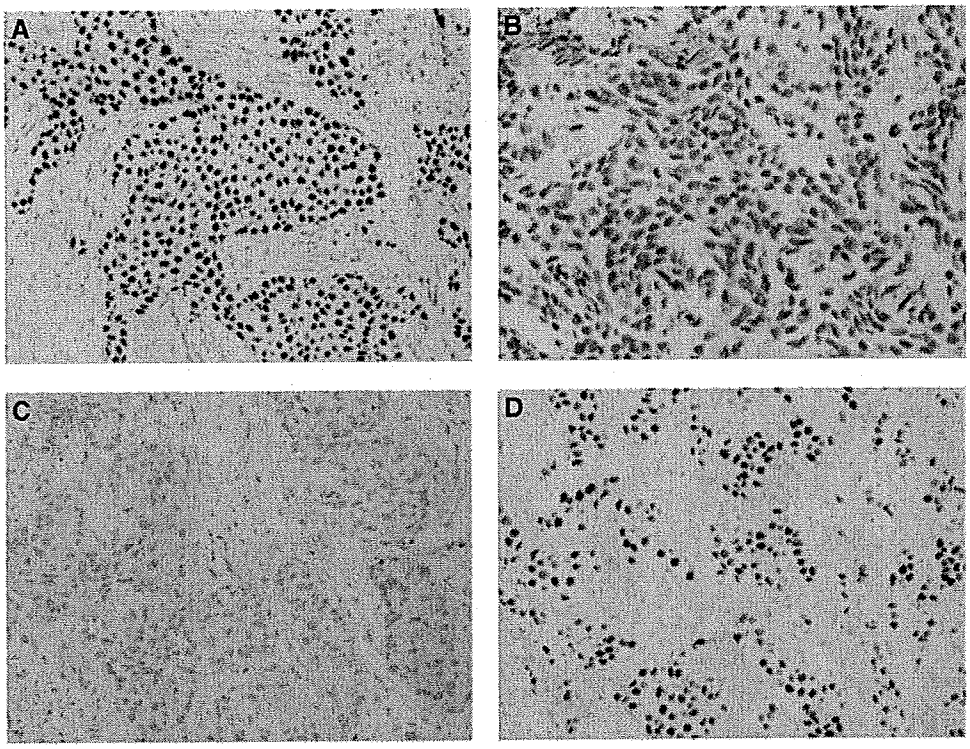

Figure 1 Immunostaining for oestrogen receptor in core needle biopsy and surgery specimens after neoadjuvant chemotherapy. (**A**) Staining of tumour cells in core-needle biopsy sample (CNB) staining positively for oestrogen receptor (ER). (**B**) Staining of tumour cells in surgical samples with ER-negative status after neoadjuvant chemotherapy (NAC). (**C**) Staining of tumour cells in CNB specimens with ER-negative status. (**D**) Staining of tumour cells in surgical samples with ER-positive status after NAC.

RESULTS

Patient characteristics

Among the 459 NAC-administered patients, pCR after surgery was achieved in 91 patients. Pathological assessment of the CNB specimens of patients with pCR revealed that 26 (28.6%) and 19 (20.9%) patients, respectively, were ER-positive and PgR-positive and that 63 (69.2%) were negative for both ER and PgR.

Examination of the surgical specimens revealed residual invasive disease in 368 patients. The distribution of these patients in the four groups was as follows: Group A, 184 (50.0%) patients; Group B, 47 (12.8%) patients; Group C, 12 (3.3%) patients; Group D, 125 (34.0%) patients.

The patient and tumour characteristics of the four groups are listed in Table 2. The postoperative performance status (PS 0 or 1) of all the patients was good. HR status conversion after NAC was observed in 59 (16.0%) patients. None of the HER2-positive patients were administered trastuzumab during neoadjuvant or adjuvant chemotherapy. Twelve (3.3%) did not receive adjuvant ET, although it was not contraindicated, but all of their lesions showed HR status conversion after NAC. All patients whose lesions showed ER status from positive to negative after chemotherapy had been administered ET.

Change in the HR status and HER2 status

The typical staining patterns of the CNB and surgical specimens are shown in Figure 1. The pre- and post-NAC ER and PgR statuses are shown in Table 3. Lesions of 23 (6.3%) patients showed a change in both the ER and PgR statuses after NAC. The HR and HER2 statuses changed from positive to negative in 30 (8.2%) and 22 (6.0%) patients, and changed from negative to positive in 29 (7.9%) and 13 (3.5%) patients, respectively.

Table 3 Number of patients classified by estrogen receptor and progesterone receptor statuses before and after neoadjuvant chemotherapy

	(ER, PgR) after NAC			
(ER, PgR) before NAC	**(+, +)**	**(+, −)**	**(−, +)**	**(−, −)**
(+, +)	69 (18.8)	41 (11.1)	3 (0.8)	10 (2.7)
(+, −)	18 (4.9)	27 (7.3)	1 (0.3)	7 (1.9)
(−, +)	11 (3.0)	6 (1.6)	8 (2.2)	13 (3.5)
(−, −)	6 (1.6)	11 (3.0)	12 (3.3)	125 (34.0)

Abbreviations: ER = estrogen receptor; HER2 = human epidermal receptor 2; PgR = progesterone receptor. Figures in parentheses are percentage of patients.

Figures 2 and 3 show the pre- and post-NAC proportion, intensity, and total scores of ER and PgR staining, determined on the basis of the Allred scoring system, for patients who underwent a change in the HR status. As shown in Figures 2 and 3, the changes in the ER and PgR statuses were observed not only in cases with borderline positive (total score 3–5) staining but also in those with strongly positive (total score 7–8) staining. The change in the HR status was not caused by a change in only the proportion or intensity scores of ER and PgR. In addition, Figure 4 shows the results of HER2 testing in the 59 patients who showed HR status conversion.

Long-term outcomes

The median duration of follow-up was 47 months. Figure 5 shows the Kaplan–Meier curves for DFS in the four groups. The differences among the four curves were statistically significant,

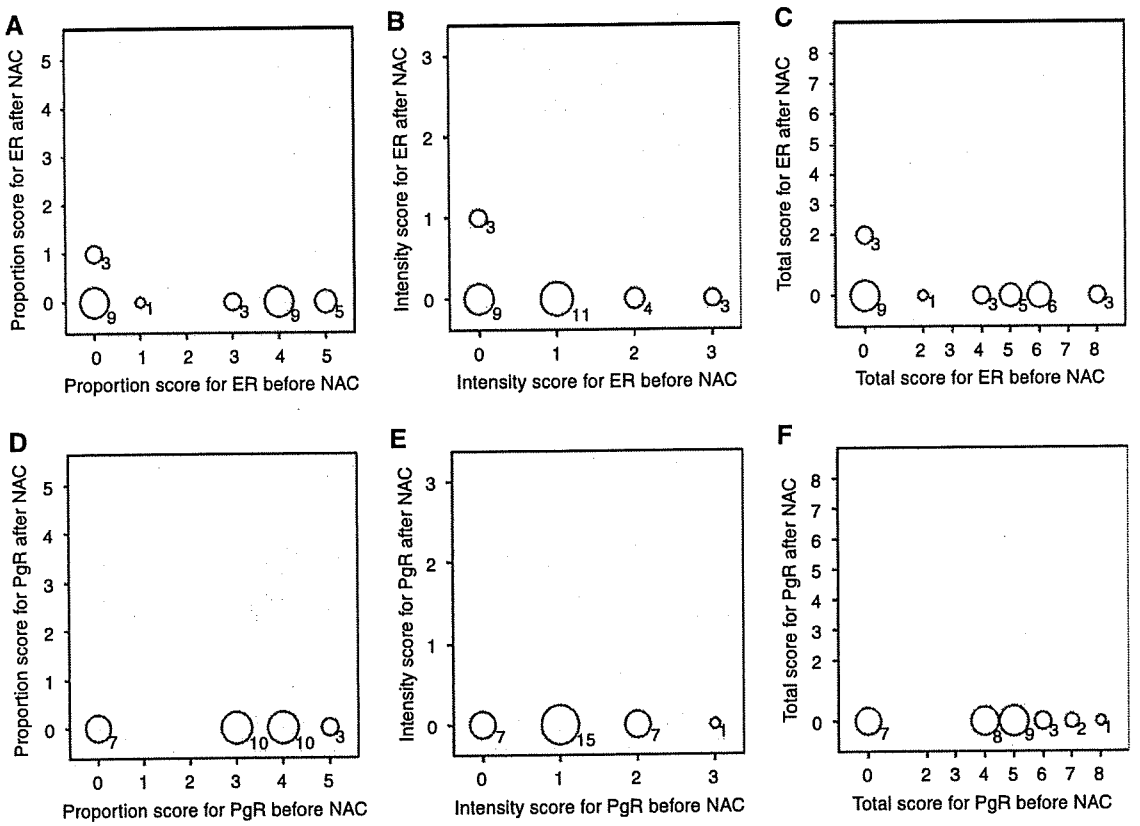

Figure 2 The distribution of scores for oestrogen receptor and progesterone receptor staining before and after neoadjuvant chemotherapy in 30 patients whose lesions changed from hormone receptor (HR)-positive status to HR-negative status. The size of the circle indicates the number of patients and the number is below the circle. (**A–C**) Proportion score, intensity score and total score of ER before and after NAC. (**D–F**) Proportion score, intensity score and total score of PgR befoe and after NAC.

as determined by the log-rank test ($P=0.008$). The 3-year DFS rates in Groups A, B, C, and D were 80.3, 78.4, 36.4, and 72.2%, respectively.

Table 4 shows the results of the multivariate Cox regression analysis of DFS with stepwise selection. The following six variables were chosen as prognostic factors for inclusion in the Cox proportional hazard model: age (<35 *vs* ⩾35 years), clinical stage at diagnosis (IIA and IIB, or IIIA *vs* IIIB or IIIC), histological grade (1 *vs* 2 and 3), HER2 status (positive *vs* negative), clinical response (CR, PR *vs* SD, PD), and the number of lymph node metastases (0 *vs* 1–3 *vs* ⩾4). Three of these variables—the HER2 status, clinical response to NAC, and the number of lymph node metastases—were identified by the stepwise selection method in the multivariate Cox regression model as the variables affecting the DFS.

The DFS of Groups B and A was similar (hazard ratio, 1.16; 95% CI, 0.61–2.19), whereas that of Group C was significantly shorter than that of Group A (hazard ratio, 6.88; 95% CI, 3.00–15.80). Table 5 summarises the results of the analysis of the efficacy of ET in the 59 patients who showed HR status conversion by using the multivariate Cox regression model. The DFS of the ET-administered patients was significantly longer than that of ET-naive patients (hazard ratio, 0.19; 95% CI, 0.06–0.60; $P<0.004$).

Figure 6 shows the Kaplan–Meier curves for OS in the four groups. The differences among the four curves were statistically significant, as determined by the log-rank test ($P=0.035$). The 5-year survival rates of Groups A, B, C, and D were 90.3, 86.3, 58.9, and 78.2%, respectively. The pattern of results of the analyses for OS in the four groups was similar to that for DFS.

DISCUSSION

This is the first report on the long-term outcomes and impact of adjuvant ET in patients with HR status conversion after NAC. In this study, the DFS and the OS of ET-administered patients with HR status-converted lesions were similar to those of ET-administered patients with lesions that were HR-positive both before and after NAC, whereas the DFS of ET-naive patients whose lesions show HR status conversion was significantly shorter than that of ET-administered patients whose lesions were HR-positive both before and after NAC. Analysis of OS yielded results similar to that pertaining to DFS. These findings indicate that the change in the status alone did not seem to influence the long-term outcome; rather, the non-administration of adjuvant ET seemed to be associated with a worse prognosis.

ER, PgR, and HER2 status changes were observed in 14.9, 29.1, and 9.5% of the patients included in our study. The overall frequency of patients with HR status conversion was 16.0%. This incidence of HR status conversion was similar to previous reports on post-NAC change in the ER, PgR, and HER2 statuses, which reported incidences of 8–28%, 6–59% (Bottini *et al*, 1996; Lee *et al*, 2003; Taucher *et al*, 2003; Colleoni *et al*, 2004; Burcombe *et al*, 2005; Shet *et al*, 2007; Kasami *et al*, 2008; Neubauer *et al*, 2008), and 0–21% (Bottini *et al*, 1996; Colleoni *et al*, 2004; Arens *et al*, 2005; Burcombe *et al*, 2005; Quddus *et al*, 2005; Adams *et al*, 2008; Kasami *et al*, 2008; Neubauer *et al*, 2008), respectively. Although the rate of cases with no change in the HR status after NAC was high, the incidence of change in the HR status is