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ORIGINAL ARTICLE

Patterns of relapse and metastatic spread in HER2-overexpressing breast cancer according to estrogen receptor status

Yeon Hee Park · Soohyeon Lee · Eun Yoon Cho · Yoon La Choi · Jeong Eon Lee · Seok Jin Nam · Jung-Hyun Yang · Jin Seok Ahn · Young-Hyuck Im

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Abstract
Purpose The primary aim of this study was to compare the relapse patterns of estrogen receptor (ER)-positive and ER-negative patients with HER2-overexpressing breast cancer. A secondary aim was to distinguish the preferential primary site of metastases in HER2-overexpressing breast cancer.
Methods Out of 886 patients treated for metastatic breast cancer (MBC) between January 1995 and December 2006, 269 patients with HER2-positive tumors were identified. Of these, 198 patients with relapsed breast cancer following surgery were included in this study. Rates and patterns of relapse and metastatic spread in HER2+/ER+ and HER2+/ER− patients were analyzed. This analysis was evaluated by the validation patients' cohort of our institute prospectively.
Results Median relapse-free survival was longer in the HER2+/ER+ group than in the HER2+/ER− group (32.0 vs. 19.5 months, $p = 0.0012$). The peak of recurrence occurred at 12 months after surgery in the HER2+/ER− patients. The peak of relapse was later and the level was lower in the HER2+/ER+ patients (66 and 78 months following surgery) than in the HER2+/ER− patients (33 and 39 months following surgery, respectively). This result was reproduced by the validation cohort with great similarity. Young age [hazard ratio (HR) 1.59, $p = 0.002$], TNM stage 3 (HR 1.51, $p = 0.005$), and ER-negativity (HR 1.68, $p < 0.0001$) were identified as independent risk factors for relapse. Severe bone metastasis (HR 4.48, $p = 0.028$) and massive hepatic metastasis (HR 5.24, $p = 0.043$) were identified as independent risk factors for early relapse.
Conclusions Our study shows that HER2-overexpressing breast cancer displays characteristic patterns of relapse and metastatic spread depending on ER status.

Keywords ER status · HER2-overexpressing breast cancer · Relapse pattern · Metastasis

Introduction

It is known that breast cancer is not a single disease; gene expression profiling by microarray analysis according to the level of mRNA expression of specific genes has offered a new way to classify breast tumors into at least five distinct subtypes: luminal A, luminal B, normal breast-like, HER2, and basal-like [1, 2]. Among them, breast cancers overexpressing HER2 constitute a heterogeneous group which can be subdivided according to their ER status: HER2+/ER− and HER2+/ER+. Estrogen receptor (ER)-negative breast cancer has been known to be significantly different from ER-positive breast cancer in the pattern, type, and complexity of the genetic aberrations. A recent study suggests that specific genetic aberrations may be specific to ER status, although hormone receptor (HR) status does not

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Y. H. Park · S. Lee · J. S. Ahn · Y.-H. Im (✉)
Division of Hematology/Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, Korea
e-mail: imyh00@skku.edu

E. Y. Cho · Y. L. Choi
Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

J. E. Lee · S. J. Nam · J.-H. Yang
Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

determine the overall genetic profile of HER2-positive breast cancers [3]. However, it has been known that HER2-overexpression is an independent adverse prognostic factor regardless of the hormonal status of the tumor [4]. ER-positive luminal tumors with the poorest prognosis tend to have elevated HER2 levels [5]. In patients with over-expressed HER2 advanced breast cancer, there are some reports that an ER-negative phenotype has reverted to an ER-positive phenotype after trastuzumab-containing therapy [6]. There may be an inverse and compensatory relationship between HER2 and ER and inhibition of one pathway leads to activation of the other [7, 8]. This complexity of the co-expressions of HER2 and ER originates from the interaction between them. Moreover, whether one has a more dominant role in tumor development, invasion, and metastasis than does the other has not been fully studied and needs to be determined.

The five major molecular subtypes of breast cancer differ in their ability to metastasize to distant organs and in their preferential site of relapse [9–13]. The most prominent studies to date are primarily concerned with the biology of each metastatic organ, such as brain, lung, and bone [12, 14]. HER2-positive patients have a high incidence of relapse involving liver and brain metastases [13], and some studies indicate an association between specific metastatic sites and ER status [9–11].

The effect of ER on the clinical outcome and relapse pattern of HER2 molecular subtypes of breast cancer has rarely been evaluated, and the biological significance of ER status in HER2-overexpressing breast cancer remains unclear. Based on this biologic understanding of breast cancer, we analyzed how ER status affects the clinical course of HER2-positive breast cancer, in particular the relapse pattern. In addition, the pattern of metastatic spread in HER2-positive breast cancer was evaluated according to ER status in terms of the metastatic sites preferentially associated with biologic aggressiveness and poor long-term clinical outcome.

Materials and methods

Study population

Between January 1995 and December 2006, 886 patients with metastatic breast cancer (MBC) received palliative chemotherapy, hormonal therapy and/or targeted therapy at Samsung Medical Center (SMC). Of these, 198 patients were HER2-positive with relapsed breast cancer after receiving curative surgery and had clinical data available. We retrospectively obtained these patients' medical records, including demographic characteristics and clinical follow-up data, together with the data of 387 patients who were HER2-negative and had relapsed after curative resection of breast cancer during the same time period. All pathologic specimens were reviewed by two experienced pathologists who determined the receptor status of the ER and HER2 using immunohistochemical (IHC) staining. ER-negativity was defined as an Allred score from 0 to 2 by IHC using antibodies to the ER (Immunotech, France). ER status was defined as "positive" in cases of Allred score from 3 to 8 by IHC using the same antibody. HER2 status was evaluated by IHC staining using an antibody (DAKO, USA) and/or fluorescence in situ hybridization (FISH). Grade 3 by IHC was defined as a positive result for HER2, and the presence of amplification of HER2 was confirmed by FISH if HER2 was rated as grade 2 or higher by IHC. Each patient was followed up at 3-month intervals. Severe bone metastasis was defined as having one or more of the following clinical manifestations: multiple spine metastases requiring local treatment, $\geq$10 extensive bone involvements, bone destruction combined with soft tissue formation, or a pathologic weight-bearing bone fracture. Massive hepatic metastasis was defined as multiple metastatic nodules extending to both hepatic lobes in conjunction with greater than or equal to a fivefold elevation in serum chemistry profile. This study was approved by the Samsung Medical Center Institutional Review Board.

Statistical analysis

Relapse-free survival (RFS) was defined from the date of curative breast cancer surgery to the date of the documentation of relapse, including locoregional and/or distant metastasis. Post-relapse overall survival (PR-OS) was measured from the first day of treatment for relapsed breast cancer to the date of death or the final follow-up day. Recurrence rates were calculated as the percentage of the patients with relapse who were followed up at each 3-month interval. They were calculated as '100 multiplied the number of the relapsed patients at each time divided the number of whole relapse patients during the follow-up period (constant)'. The follow-up schedule of the patients after surgery and adjuvant chemotherapy was every 3 months for the first 2 years and then at an interval of every 6 months for the next 3–5 years; after that, follow-up was conducted every year with conventional laboratory and radiologic studies as indicated to evaluate the failure sites at relapse.

Clinicopathological variables were compared between HER2+/ER+ and HER2+/ER− patients using the Pearson Chi-square (χ^2) test and Fisher's exact test for categorical variables. Survival curves were calculated using the Kaplan–Meier method and compared with other prognostic variables using the log-rank test. A p value <0.05 was considered significant. A binary logistic regression analysis was used for multivariate analysis of each potential clinical

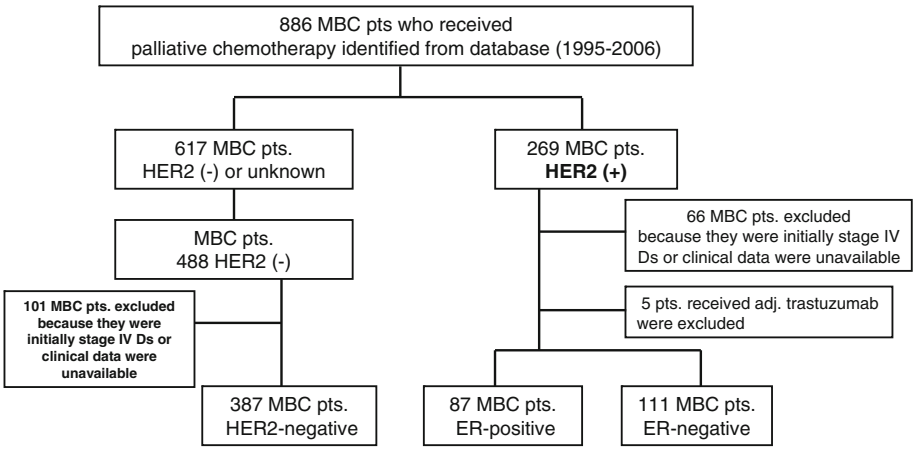

Fig. 1 Experimental patient cohort

variable for ER-negativity. Cox proportional hazards regression model was used to assess the effect of each potential prognostic variable on RFS and PR-OS.

Results

Patient population

From our hospital database, we identified 886 MBC patients who received palliative chemotherapy, hormonal therapy and/or targeted therapy at Samsung Medical Center (SMC) between January 1995 and December 2006. Among 886 MBC patients, 269 patients were shown to be HER2-positive by IHC staining or FISH. Sixty-six out of 269 HER2-overexpressing MBC patients presented metastatic diseases from the start. Trastuzumab was not used as adjuvant treatment between 2000 and 2003 because it was unavailable or not reimbursed by the Korean medical insurance system for clinical use. However, five patients who had received trastuzumab therapy as adjuvant treatment were excluded. After excluding these 71 patients, 198 patients who had relapsed after receiving curative breast cancer surgery at SMC were included in this study (Fig. 1).

Patient characteristics

Patient demographics of all 198 HER2-positive patients are described in Table 1. The median age was 48 years (range 25–77). The median follow-up durations after the primary surgery and after relapse were 90 months (range 30–143) and 64 months (range 12–125), respectively. Eighty-seven patients showed ER-positivity (43.9%), and the median RFS was 27 months (range 1.0–159.0). Ninety-seven patients (49.0%) showed initial TMN stage 3 at the time of curative surgery. Most of the patients (91.4%) were treated with adjuvant chemotherapy, and adjuvant hormonal therapy was performed for 97% of the ER-positive patients. We excluded five patients who received adjuvant trastuzumab therapy because it might influence the natural disease course and relapse pattern of the HER2-positive disease. Common first sites of distant metastasis at the time of relapse were the lymph nodes (49.0%), lung (48.5%), and bone (46.5%). Severe bone metastasis and massive hepatic metastasis were found at the time of relapse in 6.1 and 4.5% of HER2+ patients, respectively.

Patterns of relapse and metastatic spread according to ER status

There were no significant differences between HER2+/ER+ and HER2+/ER− patients in terms of median age, initial TNM staging, and histological grade (Table 1). Adjuvant treatment modalities other than hormonal treatment were not different between two groups (Table 1). There was no significant difference between the groups in terms of chemotherapy regimens. However, the median RFS was significantly longer in the HER2+/ER+ group than in the HER2+/ER− group (32.0 months, 95% CI 29.0–35.0 vs. 19.5 months, 95% CI 15.8–23.2; $p = 0.0012$ by log-rank test) (Fig. 2a). The median PR-OS was also significantly longer in the HER2+/ER+ group than in the HER2+/ER− group (37.5 months, 95% CI 29.4–45.6 vs. 27.0 months, 95% CI 19.7–34.3; $p = 0.0439$ by log-rank test) (Fig. 2b). High nuclear grade in the tumor cells was more common in the HER2+/ER− group than in the HER2+/ER+ group (61.4 vs. 33.8%, $p = 0.001$). Bone metastasis was more common in the HER2+/ER+ group than the HER2+/ER− group with marginal statistical significance (52.9 vs. 41.4%, $p = 0.072$). The incidence of pleural metastasis was greater in the HER2+/ER− group than in the HER2+/ER+ group (16.2 vs. 8.0%, $p = 0.080$), although it was not statistically significant. There was no significant difference between the two groups with respect to local failure, or lung, liver, and brain metastasis (Table 2); however, the incidence of bone-only metastasis was significantly greater

Table 1 Patient characteristics according to ER status at relapse

	HER2+ (n = 198) (%)	HER2+/ER+ group (n = 87) (%)	HER2+/ER− group (n = 111) (%)	P value (χ^2 test)
Age				
Median (year, range)	48 (25–77)	48	53	0.232
Initial stage 3	97 (49.0)	40 (46.0)	57 (51.4)	0.453
Median RFS (95% CI)	27.0 (1.0–159.0)	32.0 (28.8–35.2)	19.5 (16.4–25.6)	**0.0022** (log-rank test)
Nuclear grade high (n = 159)	78 (39.4)	24 (33.8)	54 (61.4)	**0.001**
Histologic grade high (n = 144)	68 (34.3)	28 (44.4)	38 (46.9)	0.768
Adjuvant chemotherapy	181 (91.4)	80 (92.0)	101 (91.0)	0.810
Antracycline containing	89 (49.1)	40 (50.0)	49 (48.5)	0.386
Antracyclin-taxane containing	29 (16.0)	10 (12.5)	19 (18.8)	
Others	63 (34.8)	30 (37.5)	33 (32.7)	
Adjuvant radiation therapy	125 (63.1)	56 (64.4)	69 (62.2)	0.750
Adjuvant hormonal therapy	85 (42.9)	84 (96.6)	1 (0.9)	**<0.0001**
RFS				
<24 months	87 (43.9)	25 (28.7)	62 (55.9)	**<0.0001**
<36 months	135 (68.2)	51 (58.6)	84 (75.7)	**0.011**
<48 months	165 (83.3)	67 (77.0)	98 (88.3)	**0.035**
<60 months	179 (90.4)	74 (85.1)	105 (94.6)	**0.024**
Metastatic site				
Local failure	12 (6.1)	7 (8.3)	5 (4.6)	0.285
Lymph node	97 (49.0)	38 (43.7)	59 (53.2)	0.186
Bone	92 (46.5)	46 (52.9)	46 (41.4)	*0.072*
Soft tissue	16 (8.1)	4 (4.6)	12 (10.8)	0.124
Skin	2 (1.0)	0	2 (1.8)	0.208
Lung	96 (48.5)	44 (50.6)	52 (46.8)	0.602
Pleura	25 (12.6)	7 (8.0)	18 (16.2)	*0.080*
Liver	59 (29.8)	26 (29.9)	33 (29.7)	0.981
Bone marrow	11 (5.6)	4 (4.6)	7 (6.3)	0.602
Brain	14 (7.1)	6 (6.9)	8 (7.2)	0.933
Bone-only metastasis	14 (7.1)	10 (11.5)	4 (3.6)	**0.032**
Severe bone metastasis[a]	12 (6.1)	6 (6.9)	6 (5.4)	0. 663
Massive hepatic metastasis[b]	9 (4.5)	2 (2.3)	7 (6.3)	0.303
Median PR-OS (95% CI)	32.1 (26.8–37.4)	37.5 (29.4–45.6)	27.0 (19.7–34.3)	*0.044* (log-rank test)

ER estrogen receptor, *RFS* relapse free survival, 95% CI: 95% confidence interval, PR-OS (post-relapse overall survival): overall survival after relapse

Bold values emphasize statistically significant p values, and the italics mean the p values with marginal statistical significances

[a] Severe bone metastasis was defined as having one or more of the following clinical manifestations: multiple spine metastases requiring local treatment, ≥10 extensive bone involvements, bone destruction combined with soft tissue formation, or a pathologic weight-bearing bone fracture

[b] Massive hepatic metastasis was defined as multiple metastatic nodules extending to both hepatic lobes in conjunction with ≥5-fold elevation in serum chemistry profiles

in the HER2+/ER+ group than in the HER2+/ER− group (11.5 vs. 3.6%, $p = 0.032$). We performed additional analysis for the incidence of CNS metastasis throughout the disease course. The incidence was 38.4% in all HER2+ patients. There was difference between the patients with HER2+/ER+ and HER2+/ER2- in terms of CNS metastasis throughout the disease course though it was not statistically significant (31.8% for HER2+/ER+ vs. 43.5% for HER2/ER− patients, $p = 0.091$).

Relapse rates according to ER status

Figure 2c shows rates of relapse following curative surgery in the HER2-positive breast cancer patients. The rate of relapse was calculated every 3 months. This shows that the peak of relapse occurred 12 months after surgery, irrespective of ER status. The dark-blue line in the figure shows the relapse rate of 111 HER2-positive ER-negative breast cancer patients. In these patients, the

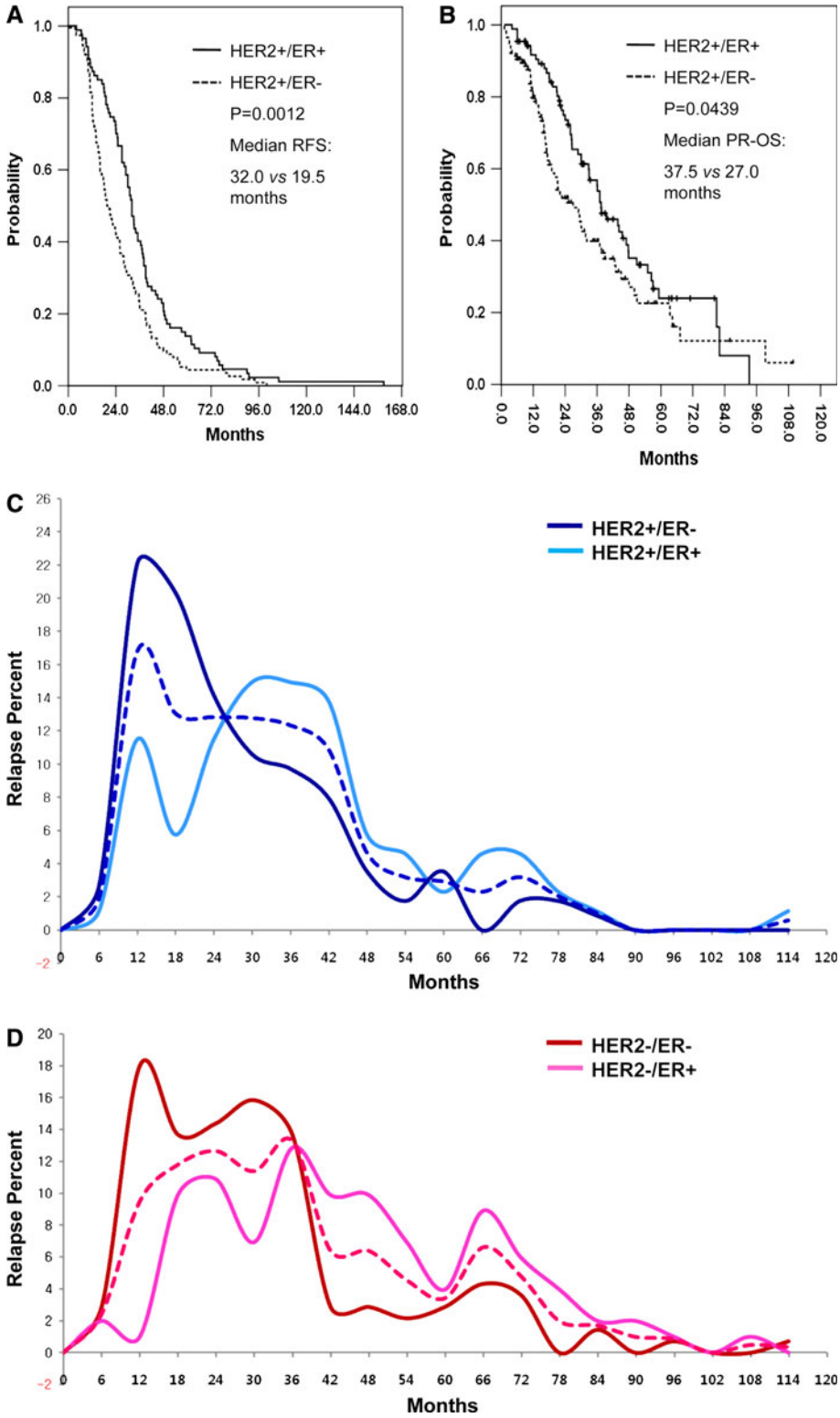

Fig. 2 RFS, PR-OS, and relapse rates according to ER status in HER-positive and negative patients. **a** The *solid line* represents the RFS of the HER2-positive and ER-positive patients (n = 87). The *dotted line* represents the RFS of the HER2-positive and ER-negative patients (n = 111). **b** The *solid line* represents the PR-OS of the HER2-positive and ER-positive patients (n = 87). The *dotted line* represents the PR-OS of the HER2-positive and ER-negative patients (n = 111). **c** The *dotted blue line* represents the relapse rates of all 198 HER2-positive patients. The *light-blue line* represents the percentage of relapsed patients among 87 HER2-positive/ER-positive patients. The *dark-blue line* represents the percentage of relapsed patients among 111 HER2-positive/ER-negative patients. Relapse rates were calculated as '100 multiplied the number of the relapsed patients at each time divided the number of whole relapse patients during the follow-up period (constant)'. **d** The *dotted red line* represents the relapse rates of all 387 HER2-negative patients. The *light-red line* represents the percentage of relapsed patients among 229 HER2-negative ER-positive patients. The *dark-red line* represents the percentage of relapsed patients among 158 HER2-negative/ER-negative patients. Relapse rates were calculated as '100 multiplied the number of the relapsed patients at each time divided the number of whole relapse patients during the follow-up period (constant)'

peak of relapse was also at 12 months after surgery; however, the peak was much higher than in all of the HER2-positive patients combined. The light-blue line represents the relapse rates of 87 HER2-positive ER-positive patients. The peak relapse rates of these patients occurred at 36 months after surgery, with additional high relapse rates seen at 12 and 68 months after surgery. The peak height was lower in the HER2-positive ER-positive group than in the HER2-positive ER-negative group.

Table 2 Logistic regression multivariate analysis on early relapse within 24 months and Cox-regression multivariate analysis on RFS and PR-OS

	Significance (p value)	Hazard ratio (HR)	95% CI
Early relapse within 24 months (logistic regression model)			
Severe bone metastasis[a]	**0.028**	4.48	1.05–17.24
Massive hepatic metastasis[b]	**0.043**	5.24	1.17–25.64
RFS (Cox-regression model)			
Young age (<48 years)	**0.002**	1.59	1.18–2.13
ER-negativity	**<0.0001**	1.68	1.26–2.25
Initial TNM stage 3	**0.005**	1.51	1.14–2.01
PR-OS (Cox-regression model)			
ER-negativity	**0.042**	1.476	1.01–2.15
DFS ≥24 months	**<0.0001**	0.389	0.26–0.57
Trastuzumab treatment[c]	**<0.0001**	0.418	0.28–0.63
Only lung metastasis	**0.001**	0.239	0.11–0.55
Hepatic metastasis	**<0.0001**	3.022	1.97–4.64
Brain metastasis	**0.001**	3.146	1.63–6.07
Initial TNM stage 3	**0.349**	1.189	0.83–1.71

ER estrogen receptor, 95% CI: 95% confidence interval, *RFS* relapse free survival; *PR-OS* post-relapse overall survival

[a] Severe bone metastasis was defined as having one or more of the following clinical manifestations: multiple spine metastases requiring local treatment, ≥10 extensive bone involvements, bone destruction combined with soft tissue formation, or a pathologic weight-bearing bone fracture

[b] Massive hepatic metastasis was defined as multiple metastatic nodules extending to both hepatic lobes in conjunction with ≥5-fold elevation of serum chemistry profiles

[c] Trastuzumab treatment was given for metastatic disease

Comparison of relapse rates of HER2-negative breast cancer patients according to ER status

Figure 2d shows the rates of relapse for the 387 HER2-negative patients. The dotted red pink line represents the relapse rate of all of the 387 HER2-negative patients combined. Although the peak of relapse occurred at 27 months after surgery, there were many additional smaller peaks. The dark-red line shows the relapse rate of the 158 HER2-negative ER-negative patients. In these patients, the major peaks of relapse were at 12 and 27 months after curative surgery, with a lower peak at 66 months. The light-red line represents the relapse rate of the 229 HER2-negative ER-positive patients. There was no definite peak of relapse in this group; instead, multiple small peaks over time were observed.

Relapse rates according to HER2 status in ER-negative and ER-positive patients

Supplementary Figs. 4A and 4B show the rates of relapse according to HER2 status in the ER-negative and ER-positive patients. As compared to the relapse curve of the HER2-negative patients, the relapse curve of the HER2-positive patients appeared earlier and was higher than that of the ER-negative patients (Fig. 4A). In the ER-positive patients, the differences in the peaks and the relapse patterns between the HER2-positive and the HER2-negative patients were relatively small, and the onsets and heights of the peaks between the HER-positive and the HER2-negative patients were not very different (Fig. 4B).

Multivariate analysis for metastatic spread at early relapse (<24 months of RFS)

Multivariate logistic regression analysis on ER-negativity and early relapse within 24 months of RFS with respect to metastatic spread identified severe bone metastasis (HR 4.48, $p = 0.028$) and massive hepatic metastasis (HR 5.24, $p = 0.043$) as independent predictive factors of early relapse within 24 months following surgery (Table 2).

Multivariate analysis of RFS and PR-OS

Cox-regression multivariate analysis revealed three independent risk factors for relapse: age less than 48 years (HR 1.59, $p = 0.002$, 95% CI 1.18–2.13), initial TNM stage 3 (HR 1.51, $p = 0.005$, 95% CI 1.14–2.01), and ER-negativity (HR 1.68, $p < 0.0001$, 95% CI 1.26–2.25). Cox-regression multivariate analysis for overall survival revealed that ER-negativity (HR 1.47, $p = 0.044$, 95% CI 1.01–2.14), RFS <24 months (HR 2.62, $p < 0.0001$, 95% CI 1.776–3.88), trastuzumab treatment (HR 0.409, $p < 0.0001$, 95% CI 0.27–0.62), lung-only metastasis (HR 0.235, $p = 0.001$, $p = 0.10$–0.54), hepatic metastasis (HR 3.07, $p < 0.0001$, 95% CI 2.00–4.73), and brain metastasis (HR 3.10,

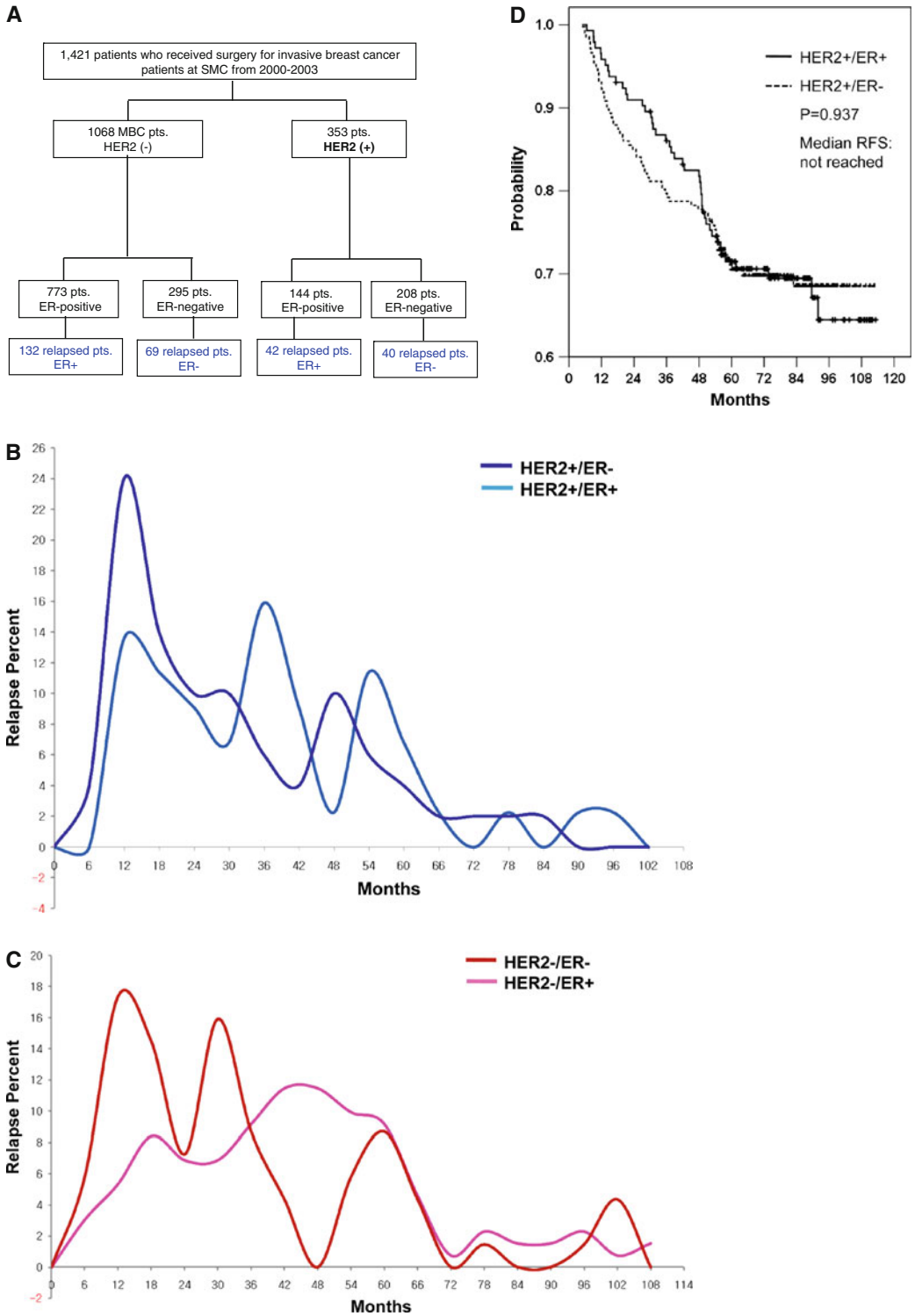

Fig. 3 Relapse rates and RFS of validation patients cohort. **a** Validation patients cohort. **b** The *light-blue line* represents the relapse rates of the HER2-positive and ER-positive patients (n = 144). The *dark-blue line* represents the relapses rate of the HER2-positive and ER-negative patients (n = 208). Relapse rates were calculated as '100 multiplied the number of the relapsed patients at each time divided the number of whole relapse patients during the follow-up period (constant)'. **c** The *light-red line* represents the relapse rates of the HER2-negative and ER-positive patients (n = 116). The *dark-red line* represents the relapse rates of the HER2-negative and ER-positive patients (n = 57). Relapse rates were calculated as '100 multiplied the number of the relapsed patients at each time divided the number of whole relapse patients during the follow-up period (constant)'. **d** The *solid line* represents the RFS of the HER2-positive and ER-positive patients (n = 144). The *dotted line* represents the RFS of the HER2-positive and ER-negative patients (n = 208)

Table 3 Patient characteristics between two cohorts

	Experimental cohort (n = 198) (%)	Validation cohort (n = 82) (%)	P value (χ^2 test)
Age			
Median (year, range)	47	45	0.094 (T test)
ER positivity	86 (43.4)	42 (51.2)	0.234
Initial stage 3	99 (50.0)	35 (42.7)	0.265
Median RFS (95% CI)	26.0 (23.0–29.0)	28.1 (23.9–32.3)	0.447 (log-rank test)
Nuclear grade high (n = 240)	79 (49.4)	44 (55.0)	0.411
Histologic grade high (n = 324)	68 (46.9)	44 (59.5)	0.079
Adjuvant chemotherapy	180 (90.9)	76 (92.7)	0.629
Adjuvant radiation therapy	124 (62.6)	55 (67.1)	0.481
Adjuvant hormonal therapy	84 (42.4)	33 (40.2)	0.514

ER estrogen receptor, *RFS* relapse free survival, 95% CI: 95% confidence interval

$p = 0.001$, 95% CI 1.60–6.00) were identified as independent prognostic factors for survival following relapse (Table 2).

Comparison of relapse patterns to those of the validation patients' cohort

Another patient cohort was used to validate the reproducibility of the results, in which patients (n = 1,421) received curative resection for breast cancer in our institution from 2000 to 2003 and had a similar follow-up duration after curative surgery. We tested our relapse rate curves to evaluate reproducibility with another patient cohort in our institute. A total of 1,421 patients who had received primary surgery from 2000 to 2003 were used to evaluate our retrospective data (Fig. 3a). Among the 1,421 patients, 353 (24.8%) patients were identified as having HER2-overexpressing breast cancer. These 353 patients were evaluated in 3-month intervals to detect relapse. With a median follow-up duration of 89.1 months (range 57.3–112.7), 82 (23.2%) patients relapsed after the primary surgery, irrespective of local and/or distant failures. There was no significant difference between the two cohorts in terms of patient characteristics (Table 3). Figure 3b–d shows the relapse rate curves and the Kaplan–Meier RFS of the HER2+ patients according to ER status. The similar peaks and trends for relapse were maintained.

Discussion

Our results showed significant differences in nuclear grade, RFS, and preferential metastatic sites between the HER2+/ER+ and HER2+/ER− patients: HER2+/ER− patients appeared to have a higher nuclear grade and a much shorter RFS than those of the HER2+/ER+ patients (Tables 2). In addition, bone-only metastasis was more common in the HER2+/ER+ group. Comparing the relapse curves of the ER-negative patients with those of the ER-positive patients according to HER2 status, the main features of the relapse curves of the HER2+/ER-negative patients were maintained. HER2-positivity seems to emphasize ER-negativity in terms of aggressiveness of the disease. Not surprisingly, the main features of the relapse curves of the ER-positive patients were also maintained under a minor influence of HER2 status. In addition, these peaks were not affected by adjuvant treatments (data not shown). These patterns were reproduced by the validation patient cohort, which had a similar follow-up duration after the primary surgery. In spite of minor differences in the relapse patterns between the two cohorts, the relapse patterns in terms of the peaks and trend for relapse were maintained throughout the period. Interestingly, the Kaplan–Meier curve of RFS for the validation cohort may reflect the characteristic relapse pattern of HER2+ breast cancer according to ER status (Fig. 3d). This figure shows a rapid decline of RFS in HER2+/ER− patients for the first 2 years after surgery. After 4 years, relapse occurred rarely. Conversely, the HER2+/ER+ patients showed continuing relapses, with the curve crossing that of the HER2+/HER2- patients, although the median RFS was not reached.

In multivariate logistic regression analysis, severe bone metastasis (HR 4.48, $p = 0.028$) and massive hepatic metastasis (HR 5.24, $p = 0.043$) were identified as independent risk factors for early relapse within 24 months following surgery. Other than ER status, Cox-regression multivariate analysis revealed that young age and initial TNM stage 3 were also independent risk factors for relapse. Characteristic metastatic patterns correlated with early relapse within 24 months after surgery were severe bone metastasis and massive hepatic metastasis (Table 2). Taken together, our results suggest that HER2-positive patients who are young, have initial TNM stage 3, and are ER-negative may relapse early, usually within 24 months, and have a much higher

incidence of severe bone metastasis and massive hepatic metastasis.

These findings suggest that ER-negativity interacts with HER2-overexpression to cause a more aggressive clinical behavior, and that the acquisition of ER-positivity in HER2-positive disease affects the disease course of HER2-positive breast cancer and ameliorates the aggressiveness of HER2+/ER− diseases. There is increasing evidence that *HER2* amplification may be the main biological driver of HER2-positive diseases, overriding the influence of HR, and that acquisition or loss of ER expression may be a late event in pathogenesis [3, 15–17]. However, maintaining ER-positivity independent of HER2 status may be more closely related to intrinsic dormancy, which is regulated by the microenvironment of the host and could be part of later events in tumorigenesis. This elucidation was supported by relapse curves according to HER2 status in the ER-positive and ER-negative patients (supplementary Figs. 4A, 4B). Apparently, favorable clinical outcomes of ER-positive patients are not only due to hormonal therapy, but also from the less aggressive biologic behavior of the tumor, irrespective of the presence or absence of HER2.

Importantly, not all of the factors showing clinical significance for relapse showed correlation with PR-OS. For example, tumor staging was not an independent risk factor for PR-OS. Despite the additional trastuzumab effect after relapse, the statistical significance of ER status and RFS was retained for PR-OS. Our interpretation of these findings is that the biologic aggressiveness of the tumor is more critical than the objective tumor burden at diagnosis. Moreover, down-regulation of HER2 by trastuzumab might contribute as a confounding variable. Considering metastatic factors, lung-only, hepatic, and brain metastases demonstrated independent prognostic significances. This may reflect preferential metastatic sites rather than overall tumor burden and may involve biological differences yet to be defined. Hepatic and brain metastases have been reported to be common characteristics of HER2-positive diseases, and HER2-overexpressing breast cancer shows greater metastatic affinities for the CNS and liver [13, 18–20]. The acquisition of ER-positivity in HER2-overexpressing breast cancer may ameliorate the aggressiveness of the tumor and switch the preference of metastatic sites such as bone, which need to be defined.

There were some limitations to this study. This was a retrospective, single-institution study with a cross-sectional cohort and a relatively small number of patients, and our patient population might not represent all HER2-positive breast cancer patients. Nevertheless, our study demonstrated that HER2-positive cancer can have remarkably heterogeneous clinical features, dependent on the ER status. It is also possible that another, currently unidentified factor is responsible for the repressive effect on ER. Considering that the final phenotype of a tumor may be the product of the interactions between the phenotype of its cell origin and the stochastic genetic and epigenetic events [17, 21–23], ER status of HER2-overexpressing breast cancer may be a reflection of the stage of differentiation of the cell of origin. Further investigation of HER2 biology and its interaction with ER should be conducted to understand the factors determining the clinical course of HER2-positive breast cancer.

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