

**Table 4 Univariate and multivariate analyses of overall survival in patients with hormone receptor-positive/HER2-negative primary breast cancer**

|                                               |                                  | Total<br>(n=142) | Univariate      |           |         | Multivariate    |          |         |
|-----------------------------------------------|----------------------------------|------------------|-----------------|-----------|---------|-----------------|----------|---------|
|                                               |                                  |                  | Hazard<br>ratio | (95% CI)  | P-value | Hazard<br>ratio | (95%CI)  | P-value |
| Ki67-p53                                      | Low Ki67 LI and Negative p53     | 88               | 1               |           |         | 1               |          |         |
|                                               | High Ki67 LI and/or Positive p53 | 54               | 8.8             | 1.9-40.4  | 0.0049  | 7.9             | 1.7-36.7 | 0.0081  |
| FOXA1                                         | Low                              | 20               | 1               |           |         |                 |          |         |
|                                               | High                             | 119              | 0.49            | 0.06-3.9  | 0.50    |                 |          |         |
| GATA3                                         | Negative                         | 30               | 1               |           |         |                 |          |         |
|                                               | Positive                         | 112              | 1.1             | 0.31-4.3  | 0.84    |                 |          |         |
| Basal phenotype marker<br>(CK5/6, CK14, EGFR) | Negative                         | 9                | 1               |           |         |                 |          |         |
|                                               | Positive                         | 133              | 1.2             | 0.15-9.3  | 0.86    |                 |          |         |
| P-cadherin                                    | Low                              | 90               | 1               |           |         |                 |          |         |
|                                               | High                             | 52               | 1.8             | 0.57-5.5  | 0.32    |                 |          |         |
| Tumor size                                    | <5.0cm                           | 17               | 1               |           |         |                 |          |         |
|                                               | ≥5.0cm                           | 122              | 3.4             | 0.90-12.8 | 0.071   |                 |          |         |
| Lymph-node metastasis                         | (-)                              | 80               | 1               |           |         |                 |          |         |
|                                               | (+)                              | 59               | 3.9             | 1.02-14.7 | 0.045   |                 |          |         |
| Nuclear grade                                 | 1, 2                             | 111              | 1               |           |         |                 |          |         |
|                                               | 3                                | 31               | 3.8             | 1.2-11.9  | 0.0020  |                 |          |         |
| Chemotherapy                                  | No                               | 93               | 1               |           |         |                 |          |         |
|                                               | Yes                              | 49               | 2.0             | 0.65-6.3  | 0.22    |                 |          |         |

Abbreviation: 95%CI 95% confidence interval, LI labeling index.

2 distinct prognostic subtypes, those with favorable-phenotype (Ki67 LI-low and p53-negative) and unfavorable-phenotype group (Ki67 LI-high and/or p53-positive) tumors. Multivariate analysis showed that the IHC panel results, tumor size and chemotherapy were independent prognostic factors for DFS and that the IHC panel results was an only independent prognostic factor for OS. Furthermore, 76 of the 78 patients (97%) with early-clinical-stage (I or II) cancers showing the favorable phenotype were alive at the end of this study. The results of a similar immunohistochemical biomarker panel for 6 markers, including p53 and Ki67, were reported by Brian et al. to be a significant prognostic factor [32]. Ross et al. also showed that the immunohistochemical detection of 5 markers, including p53, was significantly associated with clinical outcome [33]. These reports support our data, at least in part; moreover, our immunohistochemical panel using 2 easy-to-use antibodies (Ki67 and anti-p53 antibodies) was both simpler than the cited panels. Miller et al. reported the similar results to this reports [24]. They evaluated the three molecular marker (Ki67, p53 and HER2) using the whole cases with HR-positive tumors. On the other hand, we excluded the HER2-positive tumors from the whole HR-positive tumors and then evaluate the clinicopathological implication of combined Ki67-p53 status in the patients with HR-positive and HER2-negative tumors. Nowadays, the patients with HER2-

positive tumors are treated with anti-HER2 drugs and show different clinical outcome to those with HER2-negative tumors. So, our results give the more precise information and are more applicable to the dairy practice than the results of Miller et al.

The results of the immunohistochemical panel divided the HR-positive and HER2-negative breast cancer patients as follows: the 10-year DFS rates were 81% for the favorable phenotype group and 46% for the unfavorable phenotype group, while the 10-year OS rates were 97% and 65% for the favorable and unfavorable phenotype groups, respectively. To exclude the influence of adjuvant chemotherapy on the predictive value of the panel, we examined the prognostic significance of the panel separately in patients who received either pre- or post- operative chemotherapy and those who received no chemotherapy. As the immunohistochemical panel results were also identified as a significant prognostic factor in the patients who did not receive chemotherapy, we were able to exclude the influence of chemotherapy on our results. Our data indicate that patients with favorable-phenotype cancers have a clinical choice to avoid cytotoxic chemotherapy, as the baseline prognosis with adjuvant hormonal therapy alone is very good for this group.

In this report, the unfavorable phenotype-tumors exhibited significantly higher rates of positivity for

HER2, basal phenotype markers (CK5/6, CK14, and EGFR), and P-cadherin than did the favorable-phenotype tumors. P-cadherin has been previously shown to be overexpressed on basal-type tumors [14,34]. These properties suggest that unfavorable-phenotype tumors take on not only the “HER2” phenotype [7] but also the “basal” phenotype. To our knowledge, this is the first clinical study to reveal an obvious correlation between luminal subtype-B and basal-type breast tumors. Basal-type tumors exhibit more p53 mutations [9,35] and nuclear p53 protein accumulation [36] than do luminal-type or HER2-type tumors, so p53 mutation is thought to be one of the characteristics of basal-type tumors [37,38]. Our results would be consistent with this viewpoint. We suspect that some tumors should be considered “mixed intrinsic subtype” tumors, that is, tumors that exhibit characteristics of 2 or more intrinsic subtypes and therefore cannot be classified as any “pure” intrinsic subtype.

## Conclusions

In conclusion, our results revealed that the cases with Ki67 LI-high and p53 positive showed a mixed tendency towards the “HER2” and “basal” types, and that a simple immunohistochemical panel comprising Ki67 and p53 could distinguish between the cases with a favorable phenotype group and those with an unfavorable phenotype group among HR-positive and HER2-negative breast cancer patients. These suggest that our simple immunohistochemical panel comprising Ki67 and p53 is a promising tool for distinguishing between “luminal-subtype-A” and “luminal-subtype-B” breast cancers and management of patients with HR-positive breast cancer.

## Abbreviations

Ki67 LI: Ki67 labeling index; IHC panel: Immunohistochemical panel; HR: Hormone receptor; ER: Estrogen receptor; PgR: Progesterone receptor; TMA: Tissue microarray.

## Competing interests

The authors have declared no conflicts of interest.

## Authors' contributions

TK and KI conceived of the study, performed experiments, analyzed data and wrote the manuscript. TM, TY and JY provided samples, collected clinical and pathological data. HT participated in designing the study and revising the manuscript. OM participated in the overall design, study coordination and finalized the draft of the manuscript. All authors read and approved the final manuscript.

## Acknowledgment

This work was supported by the Foundation for Promotion of Defense Medicine.

## Author details

<sup>1</sup>Department of Basic Pathology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. <sup>2</sup>Department of Surgery, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359-8513, Japan. <sup>3</sup>Pathology and Clinical Laboratory Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan.

Received: 2 October 2012 Accepted: 30 January 2013  
Published: 6 February 2013

## References

1. Badve S, Nakshatri H: Oestrogen-receptor-positive breast cancer: towards bridging histopathological and molecular classifications. *J Clin Pathol* 2009, **62**(1):6–12.
2. Hortobagyi GN: Treatment of breast cancer. *N Engl J Med* 1998, **339**(14):974–984.
3. Early Breast Cancer Trialists' Collaborative Group: Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998, **351**(9114):1451–1467.
4. Mauri D, Pavlidis N, Polyzos NP, Ioannidis JP: Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. *J Natl Cancer Inst* 2006, **98**(18):1285–1291.
5. Fisher B, Jeong JH, Bryant J, Anderson S, Dignam J, Fisher ER, Wolmark N: Treatment of lymph-node-negative, oestrogen-receptor-positive breast cancer: long-term findings from National Surgical Adjuvant Breast and Bowel Project randomised clinical trials. *Lancet* 2004, **364**(9437):858–868.
6. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, et al: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004, **351**(27):2817–2826.
7. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, et al: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst* 2009, **101**(10):736–750.
8. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, et al: Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006, **24**(23):3726–3734.
9. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001, **98**(19):10869–10874.
10. Lacroix M, Toillon RA, Leclercq G: p53 and breast cancer, an update. *Endocr Relat Cancer* 2006, **13**(2):293–325.
11. Calza S, Hall P, Auer G, Bjohle J, Klaar S, Kronenwett U, Liu ET, Miller L, Ploner A, Smeds J, et al: Intrinsic molecular signature of breast cancer in a population-based cohort of 412 patients. *Breast Cancer Res* 2006, **8**(4):R34.
12. Novelli F, Milella M, Melucci E, Di Benedetto A, Sperduti I, Perrone-Donnorso R, Perracchio L, Venturo I, Nistico C, Fabi A, et al: A divergent role for estrogen receptor-beta in node-positive and node-negative breast cancer classified according to molecular subtypes: an observational prospective study. *Breast Cancer Res* 2008, **10**(5):R74.
13. Hu Z, Fan C, Oh DS, Marron JS, He X, Qaqish BF, Livasy C, Carey LA, Reynolds E, Dressler L, et al: The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics* 2006, **7**:96.
14. Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand S, Haibe-Kains B, Desmedt C, Ignatiadis M, Sengstag T, Schutz F, et al: Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res* 2008, **10**(4):R65.
15. Iwaya K, Tsuda H, Hiraide H, Tamaki K, Tamakuma S, Fukutomi T, Mukai K, Hirohashi S: Nuclear p53 immunoreaction associated with poor prognosis of breast cancer. *Jpn J Cancer Res* 1991, **82**(7):835–840.
16. Olivier M, Langerod A, Carrieri P, Bergh J, Klaar S, Eyfjord J, Theillet C, Rodriguez C, Lidereau R, Bieche I, et al: The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. *Clin Cancer Res* 2006, **12**(4):1157–1167.
17. Thorat MA, Marchio C, Morimiya A, Savage K, Nakshatri H, Reis-Filho JS, Badve S: Forkhead box A1 expression in breast cancer is associated with luminal subtype and good prognosis. *J Clin Pathol* 2008, **61**(3):327–332.
18. Jacquemier J, Charafe-Jauffret E, Monville F, Esterni B, Extra JM, Houvenaeghel G, Xerri L, Bertucci F, Birnbaum D: Association of GATA3, P53, Ki67 status and vascular peritumoral invasion are strongly prognostic in luminal breast cancer. *Breast Cancer Res* 2009, **11**(2):R23.
19. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA: Ki67 in breast cancer: prognostic and predictive potential. *Lancet Oncol* 2010, **11**(2):174–183.

20. Brown DC, Gatter KC: Ki67 protein: the immaculate deception? *Histopathology* 2002, **40**(1):2–11.
21. Viale G, Giobbie-Hurder A, Regan MM, Coates AS, Mastropasqua MG, Dell'Orto P, Maiorano E, MacGrogan G, Bray SG, Ohlschlegel C, et al: Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from Breast International Group Trial 1–98 comparing adjuvant tamoxifen with letrozole. *J Clin Oncol* 2008, **26**(34):5569–5575.
22. Kobayashi T, Tsuda H, Moriya T, Yamasaki T, Kikuchi R, Ueda S, Omata J, Yamamoto J, Matsubara O: Expression pattern of stromal cell-derived factor-1 chemokine in invasive breast cancer is correlated with estrogen receptor status and patient prognosis. *Breast Cancer Res Treat* 2010, **123**(3):733–745.
23. Sakamoto G, Inaji H, Akiyama F, Haga S, Hiraoka M, Inai K, Iwase T, Kobayashi S, Sano M, Sato T, et al: General rules for clinical and pathological recording of breast cancer 2005. *Breast Cancer* 2005, **12**(Suppl):S1–S27.
24. Millar EK, Graham PH, McNeil CM, Browne L, O'Toole SA, Boulghourjian A, Kearsley JH, Papadatos G, Delaney G, Fox C, et al: Prediction of outcome of early ER+ breast cancer is improved using a biomarker panel, which includes Ki-67 and p53. *Br J Cancer* 2011, **105**(2):272–280.
25. Bago-Horvath Z, Rudas M, Dubsky P, Jakesz R, Singer CF, Kemmerling R, Greil R, Jelen A, Bohm G, Jasarevic Z, et al: Adjuvant sequencing of tamoxifen and anastrozole is superior to tamoxifen alone in postmenopausal women with low proliferating breast cancer. *Clin Cancer Res* 2011, **17**(24):7828–7834.
26. Alba E, Calvo L, Albanell J, De la Haba JR, Arcusa Lanza A, Chacon JI, Sanchez-Rovira P, Plazaola A, Lopez Garcia-Asenjo JA, Bermejo B, et al: Chemotherapy (CT) and hormone therapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. *Ann Oncol* 2012, **23**(12):3069–3074.
27. Domagala W, Markiewski M, Harezga B, Dukowicz A, Osborn M: Prognostic significance of tumor cell proliferation rate as determined by the MIB-1 antibody in breast carcinoma: its relationship with vimentin and p53 protein. *Clin Cancer Res* 1996, **2**(1):147–154.
28. Pinto AE, Andre S, Laranjeira C, Soares J: Correlations of cell cycle regulators (p53, p21, pRb and mdm2) and c-erbB-2 with biological markers of proliferation and overall survival in breast cancer. *Pathology* 2005, **37**(1):45–50.
29. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, et al: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007, **25**(1):118–145.
30. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, Martino S, Mamounas EP, Kaufman PA, Wolmark N: Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clin Oncol* 2011, **29**(25):3366–3373.
31. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001, **344**(11):783–792.
32. Ring BZ, Seitz RS, Beck R, Shasteen WJ, Tarr SM, Cheang MC, Yoder BJ, Budd GT, Nielsen TO, Hicks DG, et al: Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. *J Clin Oncol* 2006, **24**(19):3039–3047.
33. Ross DT, Kim CY, Tang G, Bohn OL, Beck RA, Ring BZ, Seitz RS, Paik S, Costantino JP, Wolmark N: Chemosensitivity and stratification by a five monoclonal antibody immunohistochemistry test in the NSABP B14 and B20 trials. *Clin Cancer Res* 2008, **14**(20):6602–6609.
34. Matos I, Dufloth R, Alvarenga M, Zeferino LC, Schmitt F: p63, cytokeratin 5, and P-cadherin: three molecular markers to distinguish basal phenotype in breast carcinomas. *Virchows Arch* 2005, **447**(4):688–694.
35. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, et al: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006, **295**(21):2492–2502.
36. Tan DS, Marchio C, Jones RL, Savage K, Smith IE, Dowsett M, Reis-Filho JS: Triple negative breast cancer: molecular profiling and prognostic impact in adjuvant anthracycline-treated patients. *Breast Cancer Res Treat* 2008, **111**(1):27–44.
37. Brenton JD, Carey LA, Ahmed AA, Caldas C: Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 2005, **23**(29):7350–7360.
38. Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, Sledge GW, Carey LA: Triple-negative breast cancer: risk factors to potential targets. *Clin Cancer Res* 2008, **14**(24):8010–8018.

doi:10.1186/1472-6890-13-5

Cite this article as: Kobayashi et al.: A simple immunohistochemical panel comprising 2 conventional markers, Ki67 and p53, is a powerful tool for predicting patient outcome in luminal-type breast cancer. *BMC Clinical Pathology* 2013 **13**:5.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit





## Original article

## Use of the neo-adjuvant exemestane in post-menopausal estrogen receptor-positive breast cancer: A randomized phase II trial (PTEX46) to investigate the optimal duration of preoperative endocrine therapy

Takashi Hojo<sup>a,\*</sup>, Takayuki Kinoshita<sup>a</sup>, Shigeru Imoto<sup>b</sup>, Chikako Shimizu<sup>c</sup>, Hirotsugu Isaka<sup>b</sup>, Hiroki Ito<sup>b</sup>, Kentaro Imi<sup>b</sup>, Noriaki Wada<sup>d</sup>, Masashi Ando<sup>c</sup>, Yasuhiro Fujiwara<sup>c</sup>

<sup>a</sup> Department of Breast Surgery, National Cancer Center Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo, Japan

<sup>b</sup> Department of Breast Surgery, Kyorin University, Tokyo, Japan

<sup>c</sup> Department of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo, Japan

<sup>d</sup> Department of Breast Surgery, National Cancer Center Hospital East, Chiba, Japan

## ARTICLE INFO

## Article history:

Received 11 June 2012

Received in revised form

5 February 2013

Accepted 3 March 2013

## Keywords:

Neo-adjuvant endocrine therapy  
Optimal treatment duration time  
Breast cancer

## ABSTRACT

**Purpose:** The optimal treatment duration time and the causal relationship between neoadjuvant endocrine therapy and clinical response are not clear. Therefore, we conducted the present study to investigate the potential benefits of neoadjuvant exemestane therapy with the goal of identifying the optimal treatment duration.

**Methods:** This study was conducted at three hospitals, as a multicenter, randomized phase II trial (UMIN00005668) of pre-operative exemestane treatment in post-menopausal women with untreated primary breast cancer. Fifty-one post-menopausal women with ER-positive and/or PgR-positive invasive breast cancer were randomly assigned to exemestane for 4 months or 6 months. Clinical response, pathological response, and decisions regarding breast-conserving surgery were the main outcome measures.

**Results:** Of the 52 patients that enrolled, 51 patients underwent surgery. Of those, 26 and 25 patients had been treated with exemestane for 4 and 6 months, respectively. Treatments were performed at 3 hospitals in Japan between April 2008 and August 2010. The response rates as assessed by clinical examination were 42.3% and 48.0% for 4 and 6 months of treatment, respectively. Pathological responses (minimal response or better) were observed in 19.2% and 32.0% of patients, and breast-conserving surgery was performed on 50.0% and 48.0% of patients from the 4 and 6 month treatment groups, respectively.

**Conclusion:** The results of this study demonstrate that responses were equal to 4 or 6 months of exemestane treatment. Therefore, we propose that the rates of breast-conserving surgery could be maximized by 4 months of treatment. Furthermore, in addition to using exemestane as a preoperative treatment in post-menopausal women with ER-positive breast cancer, we envision administering the drug over the long term under careful clinical supervision.

© 2013 Elsevier Ltd. All rights reserved.

## Introduction

Since the 1990s, primary endocrine therapy has been considered the gold standard in the adjuvant and metastatic treatment settings for estrogen (ER) and/or progesterone (PR) receptor-positive breast cancer. The NSABP B-18 clinical trial<sup>1</sup> in 1988 demonstrated that neoadjuvant chemotherapy yielded the same survival rate as

adjuvant chemotherapy, with an improved rate of breast-conserving surgery, indicating that neoadjuvant therapy could have important clinical ramifications. With that in mind, neoadjuvant endocrine therapy for hormone receptor-positive breast cancer was also assessed, and was shown to be effective in a number of clinical trials (Table 1). Recently, clinical interest has shifted from tamoxifen to third-generation aromatase inhibitors. A few trials<sup>2–8</sup> have indicated that anastrozole led to improved response rates as compared to tamoxifen, but the results were not statistically significant. The PROACT trial reported that anastrozole treatment allowed for breast-conserving surgery in significantly

\* Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.  
E-mail address: [tahojo@ncc.go.jp](mailto:tahojo@ncc.go.jp) (T. Hojo).

**Table 1**  
Neoadjuvant endocrine trials.

| Author or trial name         | Number of patients | Design                                               | Duration (month) | Clinical ORR <sup>e</sup> |
|------------------------------|--------------------|------------------------------------------------------|------------------|---------------------------|
| IMPACT <sup>2</sup>          | 330                | ANA <sup>a</sup> vs TAM <sup>b</sup><br>vs ANA + TAM | 3                | 37%, 36%, 39%             |
| PROACT <sup>3</sup>          | 451                | ANA vs TAM                                           | 3                | 49.7%, 39.7%              |
| PO24 Trial <sup>4</sup>      | 337                | LET <sup>c</sup> vs TAM                              | 4                | 55%, 36%                  |
| GENARI Trial <sup>5</sup>    | 29                 | EXE <sup>d</sup>                                     | 4                | 37.0%                     |
| French study <sup>6</sup>    | 45                 | EXE                                                  | 14–27 weeks      | 70.6%                     |
| Gil Gil (Spain) <sup>7</sup> | 55                 | EXE                                                  | 6                | 50%                       |
| Mustacchi <sup>8</sup>       | 44                 | EXE                                                  | 6                | 66%                       |

<sup>a</sup> ANA = Anastrozole.

<sup>b</sup> TAM = Tamoxifen.

<sup>c</sup> LET = Letrozole.

<sup>d</sup> EXE = Exemestane.

<sup>e</sup> ORR = objective response rates.

more patients than did tamoxifen. The neoadjuvant drug, exemestane, has been evaluated in several small studies. The results have been promising and warrant further evaluation to determine the optimal therapeutic conditions for hormone receptor-positive patients. Specifically, the optimal treatment duration time and the causal relationship between neoadjuvant endocrine therapy and clinical response are not clear (Table 1). In addition, there are studies that have reviewed the optimal duration time of hormone treatments. Here, we investigated the benefits of 4 and 6 month long neoadjuvant exemestane therapy.

## Materials and methods

### Patients

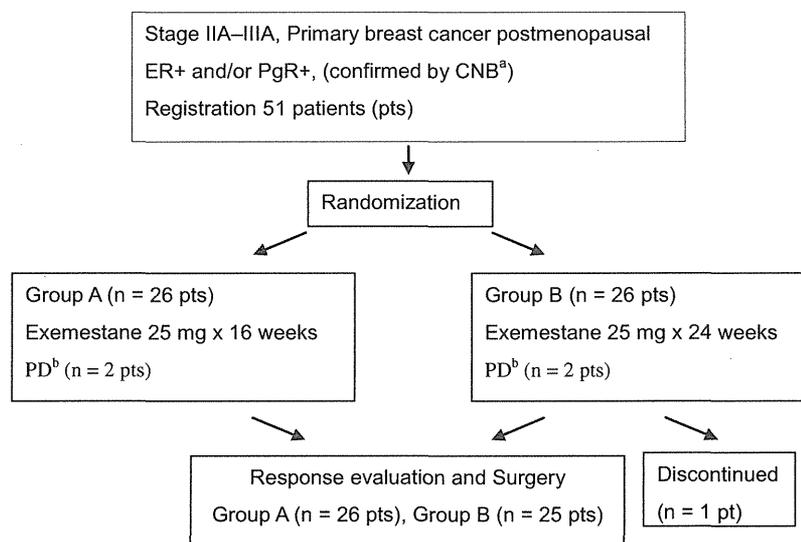
We enrolled  $\geq 55$ -year-old post-menopausal women (defined as: no spontaneous menses for  $> 1$  year; LH levels  $> 30$  IU/L; or bilateral oophorectomy prior to breast cancer diagnosis) with stage IIA–IIIA invasive ER- and/or PgR-positive breast carcinoma, as

confirmed by immunohistochemical examination of core-needle biopsies (defined as:  $> 10\%$  endocrine receptor + nuclear staining). We further required that tumors be measurable by clinical palpation. Written informed consent was obtained from each patient.

Patients were ineligible if they had any severe coincident medical disease that would prevent them from receiving surgery, place them at unusual risk, or confound the study results; were unwilling or unable to discontinue using drugs affecting sex hormones (including hormone replacement therapy); had suffered from any invasive malignancy within the previous 5 years (other than carcinoma of the skin or carcinoma in situ of the cervix, adequately cone biopsied); had received any previous breast cancer treatment or tamoxifen as part of a breast cancer prevention study; or, had received treatment with non-approved drugs during the 3 months prior to randomization. Criteria for withdrawal from the study included patients who had completed the 5-year treatment course; did not begin randomized therapy; withdrew informed consent; had confirmed clinically significant disease before surgery or confirmed recurrence after surgery; had an adverse event; or, were withdrawn at the investigator's discretion.

### Study design and setting

This study was conducted at three hospitals in Japan as a multicenter, open-label, double-arm, randomized, phase II clinical trial of pre-operative exemestane treatment in post-menopausal women with primary breast cancer. In order to optimally balance the patients in the two treatment arms with respect to prognostic factors, the patients were stratified by tumor factor, node factor, and age. The neoadjuvant endocrine treatment regimen consisted of one 25 mg exemestane tablet daily for 4 or 6 months. Fifty-one post-menopausal women with ER-positive and/or PgR-positive invasive breast cancer were randomly assigned to exemestane (25 mg/day) for 4 months (Group A) or exemestane



Setting: Multicenter study involving 3 hospitals in Japan

<sup>a</sup>CNB = core needle biopsy

<sup>b</sup>PD = Progressive Disease

The patient with PD canceled treatment and underwent immediate surgery.

**Fig. 1.** Study design.

(25 mg/day) for 6 months (Group B; Fig. 1). When antitumor effects were observed with progressive disease (PD), the treatment was canceled and patients underwent surgery immediately. All patient data was collected by UMIN (UMIN00005668) and analyzed at the National Cancer Center in Japan. Tumors were measured by caliper before exemestane treatment began, and again in the eighth week of therapy. Tumor regression by clinical examination, pathological response, decisions regarding breast-conserving surgery, and safety assessments were the main outcome measures. All patients provided written informed consent. This investigational registration period was planned three years from May 2008. The trial was conducted in accordance with the principles of Good Clinical Practice as specified in the Declaration of Helsinki (Edinburgh, 2000). The study protocol was guided by the current regulations governing clinical trials, and was approved by the Ethics Committees of the individual hospitals involved. All patients gave written informed consent before study enrollment.

#### Study endpoints

The primary endpoints were objective response rates (ORR) by caliper at 4 and 6 months of treatment using an intention to treat analysis. Secondary endpoints were the rates of breast-conserving surgery or mastectomy, and the pathological response rates.

#### Clinical assessments

The primary study objective was to compare the differences between exemestane treatment for 4 and 6 months, using objective complete responses (CRs) and partial responses (PRs) as defined by the Response Evaluation Criteria in Solid Tumors (RECIST),<sup>9</sup> which is based on caliper measurements of tumor size. Clinical response was assessed by comparing the longest diameter of the target lesions with the baseline measurement based on RECIST criteria. Every 4 weeks, patients underwent a physical examination, toxicity assessment, and tumor assessment using WHO criteria. If tumor progression was suspected, the tumor was further assessed by ultrasound or mammography. At baseline and immediately before surgery, the investigator recorded the extent of the least invasive feasible breast surgery option at that particular time: whether breast-conserving surgery or mastectomy was needed, or whether the tumor was inoperable.

#### Histological assessments

Histopathological therapeutic response was classified according to the General Rules for the Clinical and Pathological Recording of Breast Cancer 2005.<sup>10</sup> For Grade 0, no response was observed; Grade 1a comprised those tumors with mild changes in cancer cells regardless of the area, or marked changes seen in less than one-third of cancer cells; Grade 1b comprised tumors with marked changes seen in more than one-third but less than two-thirds of tumor cells; Grade 2 tumors contained marked changes in more than two-thirds of tumor cells; and Grade 3 tumors demonstrated a complete response, with no cancerous cells remaining. Mild changes included slight degenerative changes in cancer cells not suggestive of cell death (including cancer cells with vacuolation of the cytoplasm, eosinophilic cytoplasm, swelling of the nucleus, etc.). Marked changes include noticeable degenerative changes in cancer cells suggestive of cell death (including liquefaction, necrosis, and disappearance). The pathological response group was defined as tumors with Grade 1b and 2 responses. The non-response group was defined as tumors with Grade 0 and 1a responses.

#### Statistics

Based upon previous results, we assumed the response rate to be 40% and 60% after 4 and 6 months of exemestane, respectively (Table 1). To achieve an 80% statistical power, 46 examples were required to detect differences in both response rates with a 5% level of significance.<sup>1</sup> To account for attrition, we enrolled 50 patients.<sup>11</sup> Analysis was on an intention to treat (ITT) basis. The chi-squared test was used to compare tumor characteristics and responses, and rates of breast-conserving surgery between groups. Results with  $p < 0.05$  were considered to be significant.

#### Results

##### Patient baseline characteristics

The study enrolled 52 post-menopausal women at 3 hospitals in Japan between April 25, 2008 and August 12, 2010. Of these, 26 patients were allocated to Group A, and 26 to Group B. One patient withdrew and did not complete the study (Group B). The main characteristics of the eligible patients are described in Table 2. The baseline characteristics were well balanced between the two treatment arms (Table 2).

##### Efficacy results

Evaluation of the primary efficacy endpoint (overall objective response as determined by clinical palpation) revealed that there was no statistically significant difference in the overall objective response (CR + PR) between the two treatment groups: Group A, 42.3%; Group B, 48.0%;  $p = 0.89$  (Table 3). Clinically, 7.7% of Group A and 8.0% of Group B patients progressed while 50.0% and 44.0% of Group A and B patients, respectively, remained stable (not significant). As for the anti-tumor effect assessed by caliper at the eighth week, there were no differences between the two cohorts (Table 3). The pathological response rates of Groups A and B were 19.2% and 32.0%, respectively, a difference that was not statistically significant (Table 4,  $p = 0.47$ ). Pathological CR in the primary breast lesion was only observed in one patient in Group B. Withdrawals from the trial due to side effects did not occur in either Group.

**Table 2**  
Patients' baseline characteristics.

|                                                     | Group A<br>(4 months) | Group B<br>(6 months) |
|-----------------------------------------------------|-----------------------|-----------------------|
| Age, median (range)                                 | 66 (51–80)            | 64 (57–80)            |
| Tumor stage, number (%)                             |                       |                       |
| T2                                                  | 24 (92.3%)            | 24 (92.0%)            |
| T3                                                  | 2 (7.7%)              | 2 (8.0%)              |
| Nodal stage, number (%)                             |                       |                       |
| N0                                                  | 21 (80.8%)            | 24 (92.0%)            |
| N1                                                  | 5 (19.2%)             | 2 (8.0%)              |
| Clinical stage, number (%)                          |                       |                       |
| IIA                                                 | 19 (73.1%)            | 22 (84.0%)            |
| IIB                                                 | 7 (26.9%)             | 4 (16.0%)             |
| BMI <sup>a</sup>                                    | 23.9 (18.5–31.5)      | 24.5 (17.5–32.3)      |
| Tumor diameter (caliper)                            | 30.5 (20–60)          | 30.0 (13–55)          |
| Median (range) mm                                   |                       |                       |
| Receptor status                                     |                       |                       |
| ER <sup>b</sup> positive/HER2 <sup>c</sup> negative | 25                    | 22                    |
| ER <sup>b</sup> positive/HER2 positive              | 1                     | 3                     |
| PgR <sup>d</sup>                                    |                       |                       |
| Positive                                            | 20                    | 18                    |
| Negative                                            | 6                     | 8                     |

There were no differences between Groups A and B in these characteristics.

<sup>a</sup> BMI = body mass index.

<sup>b</sup> ER = estrogen receptor.

<sup>c</sup> HER2 = human epidermal growth factor receptor type 2.

<sup>d</sup> PgR = progesterone receptor.

**Table 3**  
Clinical response (caliper).

| Response <sup>a</sup>   | Group A (4 months)<br>number (26) |          | Group B (6 months)<br>number (25) |          |
|-------------------------|-----------------------------------|----------|-----------------------------------|----------|
|                         | 8 weeks                           | 16 weeks | 8 weeks                           | 24 weeks |
| CR                      | 0                                 | 1        | 0                                 | 2        |
| PR                      | 7                                 | 10       | 5                                 | 10       |
| SD                      | 17                                | 13       | 20                                | 11       |
| PD                      | 2                                 | 2        | 0                                 | 2        |
| Clinical ORR (CR or PR) | 26.9%                             | 42.3%    | 20.0%                             | 48.0%    |

$p = 0.89$ .

Complete Response: CR, Partial Response: PR, Stable Disease: SD, Progressive Disease: PD.

ORR: objective response rates.

<sup>a</sup> The RECIST methodology was used to assess response (Therasse et al., 2000).

**Table 4**  
Clinical response (pathological response).

| Pathological response <sup>a</sup> | Group A (4 months)<br>number | Group B (6 months)<br>number |
|------------------------------------|------------------------------|------------------------------|
| 3                                  | 0                            | 1                            |
| 2                                  | 0                            | 1                            |
| 1b                                 | 5                            | 6                            |
| 1a                                 | 15                           | 13                           |
| 0                                  | 6                            | 4                            |
| Response rate (1b or 2 or 3)       | 19.2%                        | 32.0%                        |

$p = 0.47$ .

0 no response, 1a mild response, 1b moderate response, 2 marked response, 3 complete response.

<sup>a</sup> Pathological response was defined as a Grade 1b, 2, or 3 lesion according to the following criteria.

### Breast conservation

Of the 52 randomized patients, 32 would have required a mastectomy at baseline (17 in Group A and 15 in Group B; Table 5). For one of these patients, an operation was not performed. Surgery outcomes with respect to breast conservation improved in 4 of 26 patients in Group A (15.4%), as compared to 1 of 25 patients in Group B (4.0%). As compared to the intent-to-treat population, the increase in patients eligible for breast conserving surgery was numerically higher in Group A than Group B, although this difference did not reach statistical significance.

### Discussion

ER-positive tumors are generally less sensitive to chemotherapy than ER-negative tumors.<sup>12,13</sup> Some trials have shown that tamoxifen is an effective primary endocrine agent for the treatment of locally advanced<sup>14</sup> and operable ER-positive breast cancers, especially in the elderly population.<sup>15,16</sup> A combined analysis of the IMPACT and PROACT clinical trials showed a trend toward better objective response rates when patients received aromatase inhibitors, but no statistically significant difference was observed between treatments with aromatase inhibitors or tamoxifen.<sup>2,3</sup>

**Table 5**  
Rate of breast-conserving surgery.

|                  | Group A (4 months)            |                   | Group B (6 months)            |                   |
|------------------|-------------------------------|-------------------|-------------------------------|-------------------|
|                  | Estimation<br>(pre treatment) | Post<br>treatment | Estimation<br>(pre treatment) | Post<br>treatment |
| Mastectomy       | 17                            | 13                | 14                            | 13                |
| BCS <sup>a</sup> | 9                             | 13                | 11                            | 12                |
| Rate of BCS      | 34.6%                         | 50%               | 44.0%                         | 48.0%             |

<sup>a</sup> BCS = Breast-conserving surgery.

However, in the P024 trial, the objective response rate for treatment with aromatase inhibitors was significantly greater than that for tamoxifen.<sup>4</sup> At present, the optimum duration of treatment for neoadjuvant endocrine treatment is not known. Ideally, the timing would be based on individual patient response. Clinical trials report a common duration period of preoperative endocrine therapy as 4–6 months. Likewise, the duration of many neoadjuvant chemotherapy treatments is 6 months. Therefore, we carried out this study to compare the use of exemestane for 4 and 6 months prior to surgery. We found no significant differences in outcomes between patients who received the drug for 4 or 6 months; however, the latter group tended to have higher anti-tumor responses. It is thought that this observation did not reach statistical significance because we set the significant difference of both groups at 20%. Our study results show that the maximum response to neoadjuvant hormone therapy by exemestane is around four months. These data are consistent with the study by Antonio Llombart-Cussac et al.,<sup>18</sup> in which the maximum response to therapy with letrozole was at 4.2 months. In addition, a randomized phase II study<sup>17</sup> compared 4–8 months of letrozole in a single arm; there tended to have higher anti-tumor responses. We think that these results indicate that the maximum response to neoadjuvant hormone therapy is also around four months. ER- and/or PgR-positive tumors are biologically heterogeneous. It is thought that biologically heterogeneous groups require detailed statistical adjustment. Krainick-Strobel UE et al.<sup>17</sup> found that 4 months of neoadjuvant exemestane therapy improved the rate of breast-conserving surgery. There was not a large difference in response rates for treatments of 3–6 month duration; however, the anti-tumor effects tended to be greater after 6 months of treatment as compared to shorter time points. In our study, neither treatment group experienced severe side effects as a result of the therapy. However, Group B tended to have a higher pathological response rate. It seems that the maximum anti-tumor effect may be reached at different time points for each patient over the course of 24 weeks of treatment. Therefore, we cannot expect a large antitumor effect by treating for longer than 4 months; however, we could extend the treatment period until the time of operation. Furthermore, in addition to using exemestane as preoperative treatment in post-menopausal women with ER-positive breast cancer, due to the mild side effects observed during the 6 month course of treatment, we envision administering the drug over the long term under careful clinical supervision.

### Ethical approval

The present work has been approved by the ethical committee of each institutional.

### Funding source

No funding source.

### Conflict of interest statement

All authors declare that they have no conflict of interest.

### Acknowledgments

None.

### References

1. Wolmark N, Wang J, Mamounas E, Byant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from

- National Surgical Adjuvant Breast and Bowel Project B-18. *J Natl Cancer Inst Monogr* 2001;96:102.
2. Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Walsh G, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol* 2005;23:5108–16.
  3. Cataliotti L, Buzdar AU, Noguchi S, Bines J, Takatsuka Y, Oliveira CT, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. *Cancer* 2006;106:2095–103.
  4. Eiermann W, Paepke S, Appfelstaedt J, Llombart-Cussac A, Eremin J, Borgs M, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: a randomized double-blind multicenter study. *Ann Oncol* 2001;12:1527–32.
  5. Krainick U, Astner A, Jonat W, Wallwiener D. Phase II study to define safety and efficacy of exemestane as preoperative therapy for postmenopausal patients with primary breast cancer – final results of the German Neoadjuvant Aromasin Initiative (GENARI). San Antonio 2003. [Abstract 239].
  6. Tubiana-Hulin M, Becette V, Bieche I, Mauriac L, Romieu G, Bibeau F, et al. Exemestane as neoadjuvant hormone therapy for locally advanced breast cancer: results of a phase II trial. *Anticancer Res* 2007;27:2689–96.
  7. Gil Gil MJ, Barnadas A, Cirera L, Tusquets I, Munoz M, Margeli M, et al. Primary hormonal therapy with exemestane in patients with breast tumours >3 cm in diameter: results of a Spanish multicenter phase II trial. *Proc Am Soc Clin Oncol* 2004;23:14S. (Abs603).
  8. Mustacchi G, Mansutti M, Barni S, Cazzaniga ME, Farris A, Dellach C, et al. Exemestane(EXE)as primary treatment in hormone-sensitive early breast cancer of the elderly :preliminary results of a phase II multicenter open Italian study. *Proc Am Soc Clin Oncol* 2006;24:18S. (Abs10689).
  9. RECIST.
  10. Kurosumi M, Akashi-Tanaka S, Akiyama F, Komoike, Mukai H, Tsuda H, et al. Histopathological criteria for assessment of therapeutic response in breast cancer (2007 version). *Breast Cancer* 2008;15:5–7.
  11. Shimon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials*; 10: 1–10.
  12. Colleoni M, Gelber S, Coates AS, Castiglione-Gertsch M, Gelber RD, Goldhirsch A, et al. Influence of endocrine-related factors on response to perioperative chemotherapy for patients with node-negative breast cancer. *J Clin Oncol* 2001;19:4141–9.
  13. Gianni L, Baselga J, Eiermann W, Guillem Porta V, Semiglazov V, Bonadonna G, et al. Feasibility and tolerability of sequential doxorubicin/paclitaxel followed by cyclophosphamide, methotrexate, and fluorouracil and its effects on tumor response as preoperative therapy. *Clin Cancer Res* 2005;11:8715–21.
  14. Tan SM, Cheung KL, Willsher PC, Blamey RW, Chan SY, Robertson JF, et al. Locally advanced primary breast cancer: medium-term results of a randomised trial of multimodal therapy versus initial hormone therapy. *Eur J Cancer* 2001;37:2331–8.
  15. Robertson JF, Ellis IO, Elston CW, Blamey RW. Mastectomy or tamoxifen as initial therapy for operable breast cancer in elderly patients: 5-year follow-up. *Eur J Cancer* 1992;28A:908–10.
  16. Mustacchi G, Ceccherini R, Milani S, Pluchinotta A, De Matteis A, Sasso F, et al. Tamoxifen alone versus adjuvant tamoxifen for operable breast cancer of the elderly: long-term results of the phase III randomized controlled multicenter GRETA trial. *Ann Oncol* 2003;14:414–20.
  17. Krainick-Strobel UE, Lichtenegger W, Wallwiener D, Tulusan AH, Janicke F, Paepke S, et al. Neoadjuvant letrozole in postmenopausal estrogen and/or progesterone receptor positive breast cancer: a phase IIb/III trial to investigate optimal duration of preoperative endocrine therapy. *BMC Cancer* 2008;8:62.
  18. Llombart-Cussac A, Guerrero A, Galán A, Carañana V, Buch E, Guillem Porta V, et al. Phase II trial with letrozole to maximum response as primary systemic therapy in postmenopausal patients with ER/PgR[+] operable breast cancer. *Clin Transl Oncol* 2012;14(2).

## Retrospective Analysis of Risk Factors for Central Nervous System Metastases in Operable Breast Cancer: Effects of Biologic Subtype and Ki67 Overexpression on Survival

Mikiya Ishihara<sup>a,d</sup> Hirofumi Mukai<sup>a</sup> Shunji Nagai<sup>a</sup> Masakatsu Onozawa<sup>b</sup>  
Keiji Nihei<sup>b</sup> Toshiyuki Shimada<sup>c</sup> Noriaki Wada<sup>c</sup>

Divisions of <sup>a</sup>Oncology/Hematology, <sup>b</sup>Radiation Oncology, and <sup>c</sup>Breast Surgery, National Cancer Center Hospital East, Kashiwa, and <sup>d</sup>Department of Immuno-Gene Therapy, Mie University Graduate School of Medicine, Tsu, Japan

### Key Words

Breast cancer · Central nervous system · Ki67 · Triple negative subtype

### Abstract

**Objective:** Identifying factors that predispose patients to central nervous system (CNS) metastases may hasten disease detection and improve treatment outcomes. **Methods:** We reviewed the records of patients who were diagnosed with clinical stage I–III primary breast cancer at the National Cancer Center Hospital East from 2003 to 2005. Cox proportional hazard models were fitted to reveal risk factors for CNS metastases. **Results:** The median follow-up period after the operation was 53.5 months. Among the 591 identified patients with breast cancer, 76 experienced a relapse. Seventeen patients developed CNS metastases. Multivariate analysis indicated that the triple negative (TN) subtype (hazard ratio = 5.5) and a high Ki67 labeling index (LI; hazard ratio = 3.9) were associated with a higher risk for CNS metastases. At 4 years, the TN subtype was associated with significantly worse overall and disease-free survival rates and a higher cumulative incidence of CNS metastases compared with hormone receptor-positive/human epidermal growth factor receptor-2-negative tumors. Breast cancers with a Ki67 LI

≥30% were also associated with lower overall and disease-free survival rates and a higher cumulative incidence of CNS metastases compared with cancers with a Ki67 LI <30%. **Conclusion:** TN or Ki67-overexpressing breast cancer produced earlier CNS metastases and lower disease-free and overall survival rates. Copyright © 2012 S. Karger AG, Basel

### Introduction

Breast cancer often metastasizes to the central nervous system (CNS) [1, 2]. Indeed, approximately 5% of patients with breast cancer will experience a recurrence in the CNS [3, 4]. Various neoadjuvant or adjuvant chemotherapeutic agents, including anthracyclines, taxanes and trastuzumab, have improved systemic disease control but do not cross the blood-brain barrier [5]. Thus, CNS metastases remain a significant problem in breast cancer. Identifying factors that predispose patients to CNS metastases may help clarify appropriate follow-up protocols and uncover populations that should be treated with drugs that penetrate the blood-brain barrier in neoadjuvant or adjuvant settings.

### KARGER

Fax +41 61 306 12 34  
E-Mail [karger@karger.ch](mailto:karger@karger.ch)  
[www.karger.com](http://www.karger.com)

© 2012 S. Karger AG, Basel  
0030-2414/13/0843-0135\$38.00/0

Accessible online at:  
[www.karger.com/oc](http://www.karger.com/oc)

Mikiya Ishihara  
Department of Immuno-Gene Therapy  
Mie University Graduate School of Medicine  
2-174 Edobashi, Tsu, Mie 514-8507 (Japan)  
E-Mail [mishihara@clin.medic.mie-u.ac.jp](mailto:mishihara@clin.medic.mie-u.ac.jp)

Several studies have shown that risk factors for CNS metastases among women with breast cancer include younger age, high T stage, high histologic grade and tumors that are negative for hormone receptor and/or positive for human epidermal growth factor receptor 2 (HER2) [3, 6–8]. Recently, several reports suggested that the triple negative (TN) subtype, which is defined as negative for estrogen receptor, progesterone receptor and HER2, is associated with a higher incidence of brain metastases [9, 10].

Ki67, a marker of proliferation in early breast cancer [11–13], can be detected using the specific monoclonal antibody MIB-1. High expression levels of Ki67 in breast cancer are associated with a high risk of relapse and decreased survival rates. However, the correlation between Ki67 expression and CNS metastases remains unclear. In this study, we re-examined reported risk factors and assessed Ki67 expression as a new potential predictor of CNS metastases.

## Patients and Methods

### Patients

We reviewed the records of female patients who were diagnosed with clinical stage I–III primary breast cancer at the National Cancer Center Hospital East from January 2003 to December 2005. Patients with bilateral breast cancer or another uncontrolled primary malignancy were excluded. All patients with CNS metastases were symptomatic and identified using computed tomography or magnetic resonance imaging. Time of CNS metastases was defined as the day identified by the imaging test. This retrospective review was approved by the institutional review board of the National Cancer Center Hospital East.

### Pathology

Pathologic diagnoses of the primary tumors were obtained by examining paraffin-embedded sections stained with hematoxylin-eosin. Histologic grade was determined based on the number of mitoses and architectural and cytologic atypia according to the grading system proposed by Tsuda et al. [14], with some modifications. The disease status for estrogen receptor and progesterone receptor was immunohistochemically determined. The HER2 status was evaluated using HercepTest (DakoCytomation, Carpinteria, Calif., USA), with 3+ defined as HER2 positive. Based on the immunohistochemical assessment, biologic subtypes were split into three groups: TN, HER2 positive and others. Hormone receptor negative/HER2 negative was defined as the TN subtype. Hormone receptor positive/HER2 positive and hormone receptor negative/HER2 positive were defined as the HER2-positive subtype. Hormone receptor positive/HER2 negative was defined as other subtype.

Ki67 measurement was done by immunohistochemistry with mouse anti-human Ki67 monoclonal antibody, MIB-1 clone (Dako, Glostrup, Denmark). The Ki67 labeling index (LI) was calculated by counting the number of brown-stained tumor

**Table 1.** Patient and tumor characteristics

|                                  | Patients | %    |
|----------------------------------|----------|------|
| Age                              |          |      |
| ≤49 years                        | 176      | 29.8 |
| ≥50 years                        | 415      | 70.2 |
| Menopausal status                |          |      |
| Before                           | 219      | 37.1 |
| After                            | 372      | 62.9 |
| Clinical stage                   |          |      |
| I                                | 241      | 40.8 |
| II                               | 261      | 44.2 |
| III                              | 89       | 15.1 |
| T stage                          |          |      |
| T1–2                             | 505      | 85.4 |
| T3–4                             | 86       | 14.6 |
| Axillary lymph node metastases   |          |      |
| 0                                | 337      | 57.0 |
| 1–3                              | 80       | 13.5 |
| ≥4                               | 31       | 5.2  |
| N/A                              | 143      | 24.2 |
| Histologic subtype               |          |      |
| IDC                              | 497      | 84.1 |
| ILC                              | 46       | 7.8  |
| Others                           | 48       | 8.1  |
| Hormone receptor and HER2 status |          |      |
| TN                               | 93       | 15.7 |
| HER2 positive                    | 107      | 18.1 |
| Others                           | 391      | 66.2 |
| HG                               |          |      |
| 1–2                              | 368      | 62.3 |
| 3                                | 212      | 35.9 |
| N/A                              | 11       | 1.9  |
| LVI                              |          |      |
| Negative                         | 257      | 43.5 |
| Positive                         | 328      | 55.5 |
| N/A                              | 6        | 1.0  |
| Ki67 LI                          |          |      |
| <30%                             | 446      | 75.5 |
| ≥30%                             | 140      | 23.7 |
| N/A                              | 5        | 0.8  |

N/A = Not assessed; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; HG = histologic grade; LVI = lymphovascular invasion.

cell nuclei among the total number of tumor cells in the most highly immunoreactive area. If the staining was homogenous, pathologists counted at least three randomly selected high-power (×40 objective) fields. When hot spots were present, defined as areas in which Ki67 staining was particularly prevalent, pathologists assessed the whole section and recorded the overall average score. Mostly, between 500 and 1,000 tumor cells were scored. The threshold for overexpression was defined as a Ki67 LI of at least 30%.

**Table 2.** Multivariate model for CNS metastases

|                                  | HR  | 95% CI   | p value |
|----------------------------------|-----|----------|---------|
| Hormone receptor and HER2 status |     |          |         |
| Others vs. HER2 positive         | 2.2 | 0.5–9.6  | 0.284   |
| Others vs. TN                    | 5.5 | 1.4–21.2 | 0.013   |
| Age                              |     |          |         |
| ≤49 vs. ≥50 years                | 1.8 | 0.4–8.6  | 0.453   |
| Menopausal status                |     |          |         |
| Before vs. after                 | 0.6 | 0.2–2.5  | 0.528   |
| Clinical stage                   |     |          |         |
| I vs. II                         | 4.1 | 0.5–35.1 | 0.192   |
| I vs. III                        | 6.5 | 0.5–90.4 | 0.166   |
| T stage                          |     |          |         |
| T1–2 vs. T3–4                    | 3.8 | 0.6–23.1 | 0.142   |
| HG                               |     |          |         |
| 1–2 vs. 3                        | 1.2 | 0.4–3.7  | 0.778   |
| LVI                              |     |          |         |
| Negative vs. positive            | 2.3 | 0.7–7.2  | 0.168   |
| Ki67 LI                          |     |          |         |
| <30 vs. ≥30%                     | 3.9 | 1.2–12.9 | 0.026   |

HG = Histologic grade; LVI = lymphovascular invasion.

#### Statistical Analysis

The follow-up period was defined as the time from primary operation. Curves reflecting overall survival, disease-free survival and the cumulative incidence of CNS metastases were calculated using the Kaplan-Meier method. Cox proportional hazard models were fitted to identify risk factors for CNS metastases. Examination included reported factors, such as age, menopausal status, clinical stage, T stage, histologic grade, lymphovascular invasion, and hormone receptor and HER2 status. Ki67 expression was added based on clinical significance. Analyses were performed using Dr. SPSS II for Windows (SPSS Japan, Tokyo, Japan).

## Results

#### Patient Characteristics

We identified 591 patients (table 1) with a median age of 56.5 years (range 24.7–87.3) and a median follow-up time of 53.5 months (range 1.1–75.1). We defined the number of axillary lymph node metastases of 143 patients (24.2%) who received primary systemic therapy as not assessed. Among the 107 patients with HER2-positive breast cancer, 31 received adjuvant trastuzumab.

Among the 591 patients with breast cancer, 76 (12.9%) had a record of relapse. Seventeen patients (2.9%) developed CNS metastases. One patient had meningeal metastases only, whereas the others had brain metastases.

**Table 3.** Incidence and duration from operation to CNS metastases

|               | Patients, %     | Median duration months |
|---------------|-----------------|------------------------|
| TN            | 9.7<br>(9/93)   | 11.3<br>[1.6–50.7]     |
| HER2 positive | 3.7<br>(4/107)  | 20.3<br>[9.1–45.0]     |
| Others        | 1.0<br>(4/391)  | 24.1<br>[6.5–57.8]     |
| Ki67          | 8.6<br>(12/140) | 12.3<br>[1.6–45.0]     |
| LI ≥30%       |                 |                        |
| Ki67          | 1.1<br>(5/446)  | 23.9<br>[6.5–57.8]     |
| LI <30%       |                 |                        |

Analysis based on biologic subtype and Ki67 overexpression. Figures in parentheses are numbers of patients; figures in brackets are ranges.

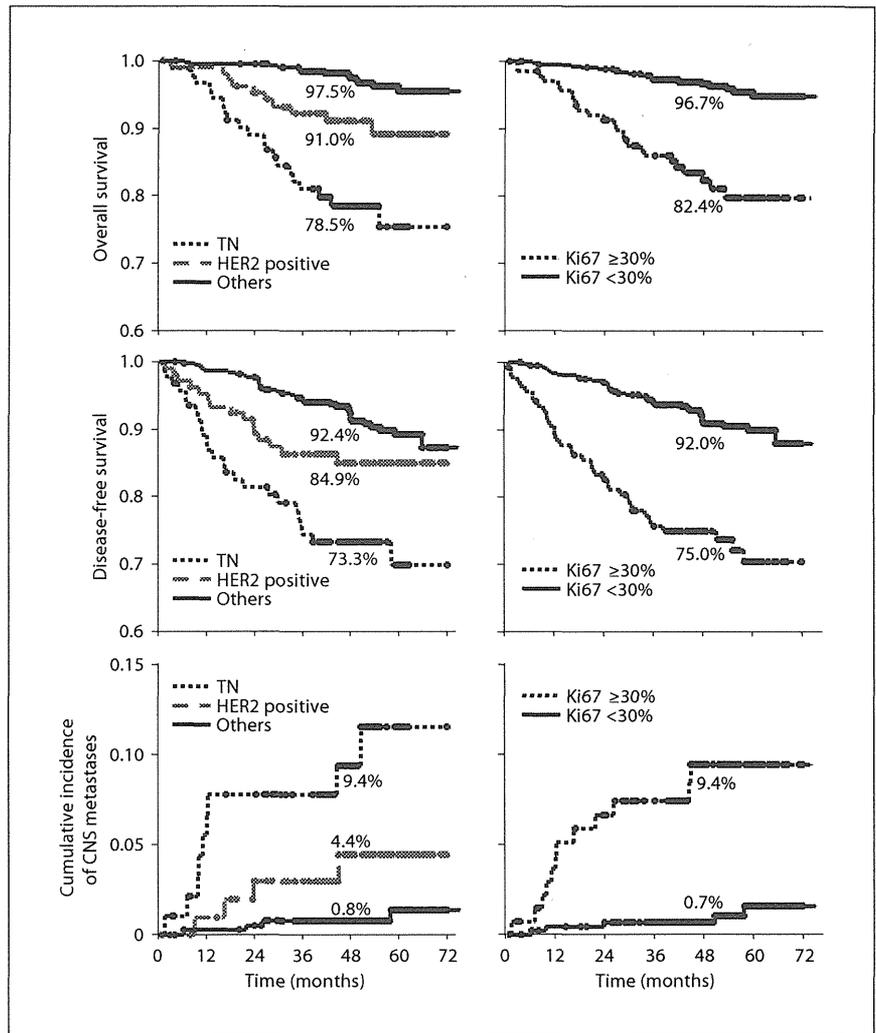
#### Multivariate Analysis

We included a number of factors in this multivariate analysis, except for the number of axillary lymph node metastases. Multivariate analysis indicated that a higher risk for CNS metastases was associated with the TN subtype [hazard ratio (HR) = 5.5, 95% confidence interval (CI) 1.4–21.2;  $p = 0.013$ ] and Ki67 overexpression (HR = 3.9, 95% CI 1.2–12.9;  $p = 0.026$ ) (table 2).

The incidence of CNS metastases and the time from the operation to detection of a CNS metastasis based on tumor subtype and Ki67 expression are shown in table 3. Among the 17 patients with CNS metastases, 9 were of the TN subtype, 4 of the HER2-positive subtype and 4 had other subtypes. Five patients developed CNS metastases as the first-known site of recurrence (3 TN subtypes and 2 other subtypes). Fifteen patients died. The median survival of patients after CNS metastases was 4.0 months (range 1.3–23.6). The TN subtype and cancers associated with overexpression of Ki67 resulted in more frequent and rapid detection of CNS metastases.

#### Overall Survival, Disease-Free Survival and Cumulative Incidence of CNS Metastases

We estimated the overall survival, disease-free survival and cumulative incidence of CNS metastases based on biologic subtype and expression of Ki67 (fig. 1). At 4



**Fig. 1.** Kaplan-Meier estimates of the overall survival, disease-free survival and cumulative incidence of CNS metastases. Analysis based on biologic subtype and Ki67 overexpression.

years after surgery, the TN subtype was associated with significantly worse overall and disease-free survival rates and a higher cumulative incidence of CNS metastases compared with the other subtypes (78.5 vs. 97.5%,  $p < 0.001$ ; 73.3 vs. 92.4%,  $p < 0.001$ ; 9.4 vs. 0.8%,  $p < 0.001$ , respectively). At 4 years, breast cancers characterized by high levels of Ki67 expression (LI  $\geq 30\%$ ) were also associated with lower overall and disease-free survival rates as well as with a higher cumulative incidence of CNS metastases compared with cancers showing less frequent Ki67 expression (LI  $< 30\%$ ; 82.4 vs. 96.7%,  $p < 0.001$ ; 75.0 vs. 92.0%,  $p < 0.001$ ; 9.4 vs. 0.7%,  $p < 0.001$ , respectively).

## Discussion

Patients with breast cancer who develop CNS metastases generally do not survive for long periods, with most dying of CNS progression. Small molecular agents may better cross the blood-brain barrier compared with other cytotoxic agents, providing a potential breakthrough in the treatment of CNS malignancy. Better knowledge of factors predictive of CNS spread would likely lead to more effective strategies for patients with breast cancer.

Approximately 15% of breast cancers are of the TN subtype, which is known to be associated with higher rates of recurrence and lower survival rates. Gene expression profiling has revealed that most TN breast cancers

are basal-like tumors [15, 16]. Heitz et al. [9] analyzed 3,193 patients treated between 1989 and 2006. Their study included 338 patients with TN breast cancer, among whom 5.6% had cerebral metastases after a median follow-up period of 51 months. The median interval between primary diagnosis of TN tumor and detection of cerebral metastases was 22 months, which was shorter than in all other subtypes (51 months). Dawood et al. [10] reviewed the records of 679 patients who were diagnosed with TN breast cancer between 1989 and 2006 at the MD Anderson Cancer Center; after a median follow-up period of 26.9 months, 6.2% of the patients had developed brain metastases, with cumulative incidence estimates of 5.6% at 2 years and 9.6% at 5 years. Although fewer patients with TN breast cancer were included in our study, we also noted more rapid and higher rates of CNS metastases in these patients.

Many studies have shown that Ki67 expression is significantly correlated with overall and disease-free survival [11, 12], although the appropriate cutoff value for the Ki67 LI has not been established. Jung et al. [17] determined that Ki67 expression in  $\geq 10\%$  of tumor cells can be used as the prognostic threshold for overall and disease-free survival in early breast cancer. Ahlin et al. [18] suggested that the optimal cutoff value for Ki67 was 15%, with a maximum value of 22%. Jalava et al. [19] divided patients into three groups based on Ki67 expression status:  $\leq 15$ , 15–30 and  $>30\%$ . Indeed, the St. Gallen International Expert Consensus 2009 supported the grouping strategy proposed by Jalava et al. [19] as relative indications for chemoendocrine therapy [20]. We defined the threshold of Ki67 LI as  $\geq 30\%$ , based on the report from Jalava and colleagues [19], but we also analyzed the cases in this study population using a threshold Ki67 LI of  $\geq 20\%$ . Multivariate analysis indicated that the TN subtype was still a significant risk factor for CNS metastases (HR = 6.2, 95% CI 1.7–22.8;  $p = 0.006$ ). Breast cancers with a Ki67 LI  $\geq 20\%$  tended to confer a higher risk for CNS metastases, although the relationship was not statistically significant (HR = 3.5, 95% CI 0.9–13.7;  $p = 0.071$ ).

This study has some limitations. First, the study was performed at only one institution. Second, we determined the HER2 status based only on immunohistochemical staining, whereas approximately 25% of patients with weakly positive HER2 breast cancer (2+ based on immunohistochemistry) have been shown to be HER2 positive using fluorescent in situ hybridization [21, 22]. In this study, fluorescent in situ hybridization was not routinely used to examine patients with operable HER2 2+

breast cancer, because adjuvant trastuzumab treatment was not approved by the Ministry of Health, Labour and Welfare of Japan. Among the 107 patients with HER2-positive breast cancer, 31 received adjuvant trastuzumab because of the doctor's decision or entry into the HERA study. It may result in relatively good disease-free and overall survival of the HER2-positive subtype. Third, tumors belonging to hormone receptor-positive/HER2-positive breast cancer and hormone receptor-negative/HER2-positive breast cancer were analyzed together. Fourth, because the median follow-up time was not long enough in our study (53.5 months), our analysis should be interpreted as revealing risk factors for earlier CNS metastases. Kennecke et al. [23] reported that brain metastases were less frequent for hormone receptor-positive/HER2-positive breast cancer than for hormone receptor-negative/HER2-positive breast cancer. These authors also showed that relapses in patients with hormone receptor-negative/HER2-positive breast cancer occurred mainly within 5 years, whereas relapses in patients with hormone receptor-positive/HER2-positive breast cancer often occurred after 5 years. Increasing the number of patients and/or length of follow-up may clarify the relationship between the HER2 status and the risk of CNS metastases.

In conclusion, our analysis indicates a higher risk for CNS metastases in patients with TN or Ki67-overexpressing breast cancer. TN or Ki67-overexpressing breast cancer was associated with earlier CNS metastases and lower rates of disease-free and overall survival. Ki67 overexpression would be a risk factor for CNS metastases in women with breast cancer.

#### Disclosure Statement

The authors declare that they have no conflicts of interest.

#### References

- 1 Schouten LJ, Rutten J, Huvneers HA, Twijnstra A: Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma. *Cancer* 2002;94:2698–2705.
- 2 Barnholtz-Sloan JS, Sloan AE, Davis FG, Vignneau FD, Lai P, Sawaya RE: Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. *J Clin Oncol* 2004;22:2865–2872.

- 3 Pestalozzi BC, Zahrieh D, Price KN, Holmberg SB, Lindtner J, Collins J, Crivellari D, Fey MF, Murray E, Pagani O, Simoncini E, Castiglione-Gertsch M, Gelber RD, Coates AS, Goldhirsch A: Identifying breast cancer patients at risk for central nervous system (CNS) metastases in trials of the International Breast Cancer Study Group (IBCSG). *Ann Oncol* 2006;17:935-944.
- 4 Tham YL, Sexton K, Kramer R, Hilsenbeck S, Elledge R: Primary breast cancer phenotypes associated with propensity for central nervous system metastases. *Cancer* 2006;107:696-704.
- 5 Muldoon LL, Soussain C, Jahnke K, Johanson C, Siegal T, Smith QR, Hall WA, Hynynen K, Senter PD, Peereboom DM, Neuwelt EA: Chemotherapy delivery issues in central nervous system malignancy: a reality check. *J Clin Oncol* 2007;25:2295-2305.
- 6 Altundag K, Bondy ML, Mirza NQ, Kau SW, Broglio K, Hortobagyi GN, Rivera E: Clinicopathologic characteristics and prognostic factors in 420 metastatic breast cancer patients with central nervous system metastasis. *Cancer* 2007;110:2640-2647.
- 7 Lin NU, Bellon JR, Winer EP: CNS metastases in breast cancer. *J Clin Oncol* 2004;22:3608-3617.
- 8 Palmieri D, Smith QR, Lockman PR, Brönder J, Gril B, Chambers AF, Weil RJ, Steeg PS: Brain metastases of breast cancer. *Breast Dis* 2006;26:139-147.
- 9 Heitz F, Harter P, Traut A, Lueck HJ, Beutel B, du Bois A: Cerebral metastases (CM) in breast cancer (BC) with focus on triple-negative tumors. *J Clin Oncol (Meeting Abstracts)* 2008;26:1010.
- 10 Dawood S, Broglio K, Esteva FJ, Yang W, Kau SW, Islam R, Albarracin C, Yu TK, Green M, Hortobagyi GN, Gonzalez-Angulo AM: Survival among women with triple receptor-negative breast cancer and brain metastases. *Ann Oncol* 2009;20:621-627.
- 11 Colozza M, Azambuja E, Cardoso F, Sotiriou C, Larsimont D, Piccart MJ: Proliferative markers as prognostic and predictive tools in early breast cancer: where are we now? *Ann Oncol* 2005;16:1723-1739.
- 12 de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ, Paesmans M: Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. *Br J Cancer* 2007;96:1504-1513.
- 13 Stuart-Harris R, Caldas C, Pinder SE, Pharoah P: Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. *Breast* 2008;17:323-334.
- 14 Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Watanabe S, Terada M, Yamamoto H: Correlation between histologic grade of malignancy and copy number of c-erbB-2 gene in breast carcinoma. A retrospective analysis of 176 cases. *Cancer* 1990;65:1794-1800.
- 15 Cleator S, Heller W, Coombes RC: Triple-negative breast cancer: therapeutic options. *Lancet Oncol* 2007;8:235-244.
- 16 Schneider BP, Winer EP, Foulkes WD, Garber J, Perou CM, Richardson A, Sledge GW, Carey LA: Triple-negative breast cancer: risk factors to potential targets. *Clin Cancer Res* 2008;14:8010-8018.
- 17 Jung SY, Han W, Lee JW, Ko E, Kim E, Yu JH, Moon HG, Park IA, Oh DY, Im SA, Kim TY, Hwang KT, Kim SW, Noh DY: Ki-67 expression gives additional prognostic information on St. Gallen 2007 and Adjuvant! Online risk categories in early breast cancer. *Ann Surg Oncol* 2009;16:1112-1121.
- 18 Ahlin C, Aaltonen K, Amini RM, Nevanlinna H, Fjallskog ML, Blomqvist C: Ki67 and cyclin A as prognostic factors in early breast cancer. What are the optimal cut-off values? *Histopathology* 2007;51:491-498.
- 19 Jalava P, Kuopio T, Juntti-Patinen L, Kotkansalo T, Kronqvist P, Collan Y: Ki67 immunohistochemistry: a valuable marker in prognostication but with a risk of misclassification: proliferation subgroups formed based on Ki67 immunoreactivity and standardized mitotic index. *Histopathology* 2006;48:674-682.
- 20 Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann B, Senn HJ: Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. *Ann Oncol* 2009;20:1319-1329.
- 21 Lebeau A, Deimling D, Kaltz C, Sendelhofert A, Iff A, Luthardt B, Untch M, Lohrs U: Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol* 2001;19:354-363.
- 22 Owens MA, Horten BC, Da Silva MM: HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 2004;5:63-69.
- 23 Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO, Gelmon K: Metastatic behavior of breast cancer subtypes. *J Clin Oncol* 2010;28:3271-3277.

## HER2陽性乳癌におけるトラスツズマブ耐性機序についての検討

山内 稚佐子\*<sup>1,2</sup> 藤井 誠志\*<sup>1</sup> 和田 徳昭\*<sup>2</sup> 米山 公康\*<sup>2</sup>  
落合 淳志\*<sup>1</sup>

## はじめに

HER2の過剰発現は乳癌患者の約15~20%で認められ、HER2陽性乳癌とされる。トラスツズマブはHER2を分子標的として開発されたヒト化抗体医薬である。しかし、HER2陽性乳癌であってもトラスツズマブの奏効率は単剤投与の場合約30%、化学療法と併用しても60~80%と報告され、現在はトラスツズマブと化学療法の併用が標準治療となっている。これまでトラスツズマブ耐性の機序についてはさまざまな検討がなされているが、基礎と臨床のコンセンサスを得るに十分ではないことから、トラスツズマブ耐性の機序についてとくに作用機序の1つである抗体依存性細胞傷害(antibody-dependent cell cytotoxicity: ADCC)に着目し、治療効果予測因子と治療耐性を克服する方法を開発することを目的として検討を行った。

## 対象と方法および結果

## 臨床検体の検討

**対象と方法:** 2003年~2006年までの間に国立がんセンター東病院において転移・再発治療としてトラスツズマブが投与されたHER2陽性乳癌22例を対象として、原発巣手術検体を用いて免疫組織化学検査にて種々のタンパクの発現について検討した。

原則として1年以上病状の増悪なくトラスツズマブ投与が継続された症例を有効群とした。

**結果:** 奏効群では非奏効群に比し有意に腫瘍細胞のE-カドヘリン発現が減弱していた(表1)。

また、E-カドヘリン陰性例では陽性例に比し、全生存期間が有意に延長された(P=0.01)。

## 基礎的検討

**対象と方法:** E-カドヘリンの発現状況が異なる2種類のヒトHER2陽性乳癌細胞株(SKBR3, HCC1569)を用いて末梢血単核球存在の有無下でトラスツズマブを投与し細胞傷害の状況を検討した。

**結果:** 末梢血単核球がない状況ではトラスツズマブの濃度にかかわらず有意な細胞傷害を認めなかったが、末梢血単核球存在下ではE-カドヘリン陰性の腫瘍細胞(SKBR3)ではトラスツズマブによる細胞傷害を認めた(図1)。

## 考察

E-カドヘリンは免疫担当細胞であるNK細胞に発現する抑制性レセプターKLRG1(killer cell lectin-like receptor G1)のリガンドであることが2006年に報告された<sup>1,2)</sup>。今回の検討において腫瘍細胞におけるE-カドヘリンの発現が治療効果予測因子となる可能性が示唆されたが、これはトラスツズマブの作用機序の1つであるADCCにおいて、ADCCの主たる免疫担当細胞であるNK細胞上のKLRG1が腫瘍細胞上のE-カドヘリンを認識することによりNK細胞内に抑制性のシグナルが伝達され細胞傷害活性が抑制されるためと考えられる(図2)<sup>3)</sup>。この機序を裏付けるものとして、HCC1569(E-カドヘリン陽性)担癌マウスモデルにおいてトラスツズマブの投与のみでは腫瘍増大は抑制できないが、トラスツズマブと抗KLRG1抗体を同時投与すると腫瘍の増大は抑制されるという実験結果がある。

現在はトラスツズマブと化学療法の併用が標準治療となっているが、乳癌治療に使用する化学療

\*1 国立がん研究センター東病院 臨床開発センター  
臨床腫瘍病理部

\*2 国立がん研究センター東病院 乳腺外科

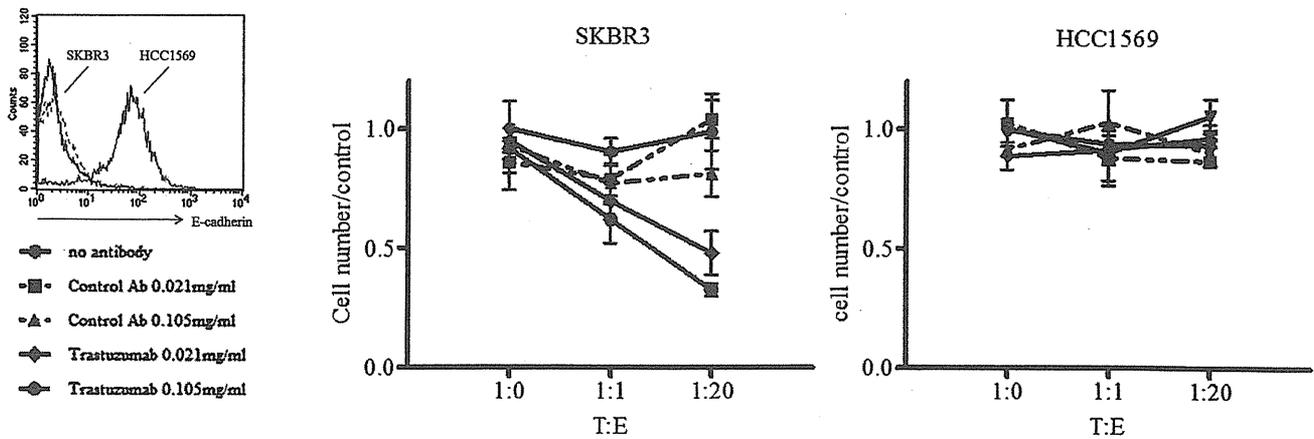


図1 基礎的検討：細胞株に対するトラスツズマブの細胞傷害についての検討

表1 免疫組織化学検査結果

| 抗体                  | 奏効       | 非奏効 | P-value |
|---------------------|----------|-----|---------|
| E-cadherin          | negative | 8   | 0.01    |
|                     | positive | 3   |         |
| N-cadherin          | negative | 11  | N.S     |
|                     | positive | 0   |         |
| Foxp3               | <15      | 10  | 0.02    |
|                     | >15      | 1   |         |
| PTEN                | negative | 7   | N.S     |
|                     | positive | 4   |         |
| p27 <sup>Kip1</sup> | negative | 7   | N.S     |
|                     | positive | 4   |         |
| IGFR                | negative | 5   | N.S     |
|                     | positive | 6   |         |
| HLA-I               | negative | 8   | N.S     |
|                     | positive | 3   |         |

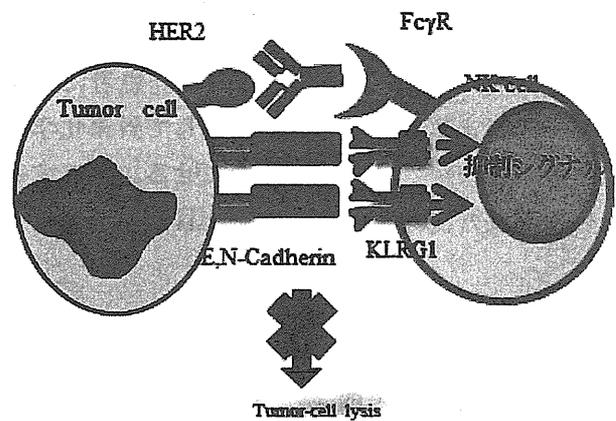


図2 トラスツズマブの作用機序と耐性について  
腫瘍細胞にE-カドヘリンが発現していることにより抗体依存性細胞傷害が抑制されると考えられる。

法薬剤は脱毛など患者にとっては辛い副作用が必発である。今回の検討結果からE-カドヘリン陰性HER2陽性乳癌症例ではトラスツズマブ単剤治療が有効である可能性が示唆される。

結語

腫瘍細胞におけるE-カドヘリンの発現がトラスツズマブ治療耐性の予測因子となることを見出した。

文献

- 1) Ito M, Maruyama T, Matsumoto N, et al : Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity. *J Exp Med* 203 (2) : 289-295, 2006
- 2) Gründemann C, Bauer M, Pircher H, et al : Cutting edge : identification of E-cadherin as a ligand for the murine killer cell lectin-like receptor G1. *J Immunol* 176 (3) : 1311-1315, 2006
- 3) Yamauchi C, Fujii S, et al : E-cadherin expression on human carcinoma cell affects trastuzumab-mediated antibody-dependent cellular cytoxicity through killer cell lectin-like receptor G1 on natural killer cells. *Int J Cancer* 128 (9) : 2125-2137, 2011

Original Article

## Association between Mammographic Breast Density and Lifestyle in Japanese Women

Setsuko Ishihara<sup>a,f</sup>, Naruto Taira<sup>b\*</sup>, Kensuke Kawasaki<sup>c</sup>, Youichi Ishibe<sup>d</sup>,  
Taeko Mizoo<sup>b</sup>, Keiko Nishiyama<sup>b</sup>, Takayuki Iwamoto<sup>b</sup>, Tomohiro Nogami<sup>b</sup>,  
Takayuki Motoki<sup>b</sup>, Tadahiko Shien<sup>b</sup>, Junji Matsuoka<sup>b</sup>, Hiroyoshi Doihara<sup>b</sup>,  
Yoshifumi Komoike<sup>e</sup>, Shuhei Sato<sup>f</sup>, and Susumu Kanazawa<sup>f</sup>

<sup>a</sup>Department of Radiology, Okayama Saiseikai General Hospital, Okayama 700-8511, Japan,

<sup>b</sup>Department of Breast and Endocrine Surgery, Okayama University Hospital, and <sup>f</sup>Department of Radiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan,

<sup>c</sup>Department of Surgery, Kagawa Prefectural Cancer Detection Center, Takamatsu 761-8031, Japan,

<sup>d</sup>Department of Surgery, Mizushima Kyodo Hospital, Kurashiki, Okayama 712-8567, Japan,

<sup>e</sup>Department of Surgery, Kinki University Hospital, Osakasayama, Osaka 589-8511, Japan

A high mammographic breast density is considered to be a risk factor for breast cancer. However, only a small number of studies on the association between breast density and lifestyle have been performed. A cross-sectional study was performed using a survey with 29 questions on life history and lifestyle. The breast density on mammography was classified into 4 categories following the BI-RADS criteria. The subjects were 522 women with no medical history of breast cancer. The mean age was 53.3 years old. On multivariate analysis, only BMI was a significant factor determining breast density in premenopausal women (parameter estimate,  $-0.403$ ;  $p$  value,  $0.0005$ ), and the density decreased as BMI rose. In postmenopausal women, BMI (parameter estimate,  $-0.196$ ;  $p$  value,  $0.0143$ ) and number of deliveries (parameter estimate,  $-0.388$ ;  $p$  value,  $0.0186$ ) were significant factors determining breast density; breast density decreased as BMI and number of deliveries increased. Only BMI and number of deliveries were identified as factors significantly influencing breast density. BMI was inversely correlated with breast density before and after menopause, whereas the influence of number of deliveries on breast density was significant only in postmenopausal women in their 50 and 60s.

**Key words:** breast cancer, mammographic breast density, life style, body mass index

Previous studies have shown an association between lifestyle and the risk of breast cancer. An expert report of the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) suggested that alcohol intake increases

the risk of breast cancer in premenopausal women. Tall stature in adulthood and high birth weight may also be risk factors. In contrast, breast feeding reduces the risk of breast cancer and high levels of body fat may also decrease the risk in premenopausal women. In postmenopausal women, the risk of breast cancer is also increased by alcohol intake, and tall stature in adulthood, but in contrast to premenopausal women, the risk of breast cancer appears to increase

Received October 25, 2012; accepted December 5, 2012.

\*Corresponding author. Phone:+81-86-225-9657; Fax:+81-86-225-9659

E-mail: ntaira@md.okayama-u.ac.jp (N. Taira)

as the amounts of abdominal fat and weight increase. Similarly to premenopausal women, breast feeding and exercise decrease the risk of breast cancer in postmenopausal women (<http://www.dietandcancerreport.org/> accessed 10/10/2012).

Higher breast density on mammography is also associated with an increased risk of breast cancer. In a meta-analysis of 42 studies, the risk in women with the highest breast density was 4.64 times greater than that in women with the lowest breast density [1]. Thus, evaluation of breast density on mammography may be important as a predictor of breast cancer risk, since it is relatively easy to perform and may serve as an objective reproducible index. If the risk of breast cancer can be evaluated accurately based on mammographic breast density, this may serve as a criterion to perform tests and advise changes in lifestyle to reduce the risk. Breast density may also serve as a surrogate parameter for interventions for prevention of breast cancer. Judgments about the final outcomes of breast cancer prevention must be based on the prevalence of breast cancer, but it takes a long time for such results to manifest. If changes in breast density induced by preventive methods can be established as a surrogate parameter of the long-term risk of breast cancer, this may reduce the cost and time of clinical studies.

Previous findings show that breast density is an important factor in breast cancer risk, but only a few studies of the relationship between breast density and lifestyle have been performed. The factors determining breast density are important to clarify if breast density is to be used as an index of breast cancer risk. In this study, we investigated the relationship between lifestyle and mammographic breast density, with the goal of identifying these factors.

## Materials and Methods

**Subjects.** The study was performed as a cross-sectional study aimed at clarifying the association between breast density and lifestyle. The subjects were healthy women who visited Kagawa Prefectural Cancer Detection Center, Mizushima Kyodo Hospital, and Okayama Saiseikai General Hospital for mammographic screening. Subjects with a medical history of breast cancer and those in whom any abnormality was found on mammographic screening were excluded.

This study was approved by the institutional ethics committee on human research. All subjects gave written consent to participation in the study.

**Survey of lifestyle.** Lifestyles were surveyed using a self-rating questionnaire survey. After obtaining consent, the survey was distributed and the subjects completed the form and sent it back by mail. The survey comprised the following items: current height and body weight and those at 18 years of age; 29 questions concerning cigarette smoking, alcohol drinking, and dietary habits (types and intakes of foods); 4 questions concerning physical activity in leisure time (type and frequency of current physical activity and those at 18 years of age); menstruation status and ages at first menstruation and menopause; history and number of deliveries and age at first delivery; history of breast feeding; familial history of breast cancer; presence or absence of hormone replacement therapy; working or not working; work hours; sleeping hours; and educational background. Body mass index (BMI) was calculated as the body weight (kg)/square of height (m). The Brinkman index for cigarette smoking was calculated from the answers to the survey. Alcohol ingestion was converted to intake per day (g/day). Metabolic Equivalents (METs) per week were calculated to evaluate physical activity. Family histories of breast cancer were limited to relatives of the first and second degree.

The types of food surveyed were cooked rice, bread, noodles, meat, fish, eggs, soybean products (tofu, fermented soybeans (natto), and miso soup), cow milk, dairy products (cheese and yogurt), green and yellow vegetables (carrot, pumpkin, spinach), fruits, mushrooms, cakes, ice cream, and instant foods. The frequencies of ingestion per week (4 categories: almost no ingestion, and ingested on one day, 2-4 days, and almost every day per week) were investigated for all of these foods.

**Evaluation of breast density.** Breast cancer screening was performed using digital mammography at all participant institutions (Kagawa Prefectural Cancer Detection Center, Mizushima Kyodo Hospital, and Okayama Saiseikai General Hospital). Digital mammography images of the subjects were exported to media and the breast density was measured on a monitor display. Two experts in reading mammographic images (S.I. and Y.I.) who had been certified by the Central Committee on Quality Control of Mammo-

graphic Screening independently judged the breast density in the mediolateral-oblique views. Breast density was classified into 4 categories following the Breast Imaging Reporting and Data System (BI-RADS) [2]: 1, breast is almost entirely fat (< 25% glandular); 2, scattered fibroglandular densities (25–50%); 3, heterogeneously dense breast tissue (51–75%); and 4, extremely dense breast tissue (> 75% glandular). The 2 examiners collated their judgments, re-evaluated mammograms in inconsistent cases, and made the final judgment after a discussion. When the density of the 2 breasts differed, the higher value was used as the final breast density.

**Statistical analysis.** Concordance between breast density judgments made by the two examiners was tested and a kappa coefficient was calculated. Associations between breast density and other factors were analyzed using ordinal logistic regression analysis with statistical significance set at 5%. All analyses were performed using JMP version 9.0.3 (SAS Institute).

## Results

**Background factors.** A total of 600 subjects gave consent to participation in the study at Kagawa Prefectural Cancer Detection Center, Mizushima Kyodo Hospital, and Okayama Saiseikai General Hospital from December 2010 to December 2011. The lifestyle survey was completed by 528 subjects, yielding a response rate of 88%. Collection and analysis of mammograms were possible in 522 of the 528 subjects, and these 522 subjects were included in the analysis. The 522 subjects had a mean age of 53.3 years, a mean height of 156 cm, a mean body weight of 54.1 kg, and a mean BMI of 22.2; at the time of the survey. 219 and 303 subjects were pre- and post-menopausal, respectively. Data for cigarette smoking and alcohol drinking habits; exercise in leisure time; ages at first menstruation, menopause, and first delivery; number of deliveries; history of breast feeding, familial history of breast cancer, occupation, educational background, sleeping hours, and hormone replacement therapy are shown in Table 1.

**Breast density evaluation.** Breast density in the 522 subjects was evaluated in bilateral mammograms (1044 images). The results and concordance rate of the evaluations by S.I. and Y.I. are shown in

Table 2. The kappa coefficient was 0.9521 (standard error: 0.008, 95% confidence interval: 0.936–0.968), showing high concordance. Breast density classifications are shown in Table 3. Of the 522 subjects, 95 (18%) were placed in category 1 (breast is almost entirely fat, < 25% glandular), 202 (18%) in category 2 (scattered fibroglandular densities, 25–50%), 192 (37%) in category 3 (heterogeneously dense breast tissue, 51–75%), and 33 (6%) in category 4 (extremely dense, > 75% glandular).

**Factors influencing breast density.** The results of breast density evaluation by age are shown in Fig. 1. In ordinal logistic regression analysis, the parameter estimate of age for breast density was  $-0.0895$ , the standard error was 0.0086, and the  $p$  value was  $< 0.0001$ , indicating that breast density decreases with age. There was no significant association of breast density with cigarette smoking (past and current cigarette smoking and Brinkman index) or alcohol drinking habits (type of alcoholic beverage and alcohol intake). Similarly, breast density showed no significant associations with dietary habits, including ingestion frequencies of rice, bread, noodles, meat, fish, eggs, soybean products, dairy products, green and yellow vegetables, fruits, mushrooms, sweets, and teas. METs calculated as an index of physical activity were not significantly correlated with breast density. Sleeping hours, the presence or absence of hormone replacement therapy, working or not working, work hours, and educational background also showed no significant association with breast density.

In age-adjusted univariate and ordinal logistic regression analyses, body weight, BMI, number of deliveries, history of breast feeding, age at first menstruation, and familial history of breast cancer were all significantly associated with breast density. The parameter estimates, standard errors, and  $p$  values for these factors are shown in Table 4. Breast density significantly decreased as body weight, BMI, and number of deliveries increased, and with an earlier age at first menstruation. The density was also significantly lower in subjects with a history of breast feeding and those with a familial history of breast cancer.

**Influence of BMI and number of deliveries on breast density.** In multivariate analysis using the significant factors in univariate analysis as explanatory variables and breast density as a response vari-

Table 1 Background factors of the subjects

|                                   |                              | Frequency (%) or mean value $\pm$ SD |                          |                           |
|-----------------------------------|------------------------------|--------------------------------------|--------------------------|---------------------------|
|                                   |                              | All<br>(N=522)                       | Premenopausal<br>(N=219) | Postmenopausal<br>(N=303) |
| Age                               |                              | 53.3                                 | 43.8                     | 60.2                      |
| Height (cm)                       |                              | 156 $\pm$ 5.6                        | 158.1 $\pm$ 5.2          | 154.5 $\pm$ 5.4           |
| Body weight (kg)                  |                              | 54.1 $\pm$ 8.4                       | 54.3 $\pm$ 8.5           | 54 $\pm$ 8.4              |
| BMI                               |                              | 22.2 $\pm$ 3.3                       | 21.7 $\pm$ 3.0           | 22.6 $\pm$ 3.4            |
| Cigarette smoking                 | Never                        | 431 (83)                             | 175 (80)                 | 256 (84)                  |
|                                   | Ever                         | 53 (10)                              | 25 (11)                  | 28 (9)                    |
|                                   | Current                      | 32 (6)                               | 19 (9)                   | 13 (4)                    |
|                                   | Unclear                      | 6 (1)                                |                          | 6 (2)                     |
| Alcohol intake (g/day)            | 0                            | 239 (46)                             | 79 (36)                  | 160 (53)                  |
|                                   | $\leq$ 10                    | 146 (28)                             | 71 (32)                  | 75 (25)                   |
|                                   | 11-50                        | 21 (4)                               | 9 (4.1)                  | 12 (4)                    |
|                                   | $\geq$ 51                    | 105 (20)                             | 56 (26)                  | 49 (16)                   |
|                                   | Unclear                      | 11 (2)                               | 4 (1.8)                  | 7 (2)                     |
| MET/week                          |                              | 10.32 $\pm$ 20.6                     | 7.5 $\pm$ 15.2           | 12.4 $\pm$ 23.6           |
| Age at the first menstruation     |                              | 12.9 $\pm$ 1.5                       | 12.5 $\pm$ 1.2           | 13.3 $\pm$ 1.6            |
| Age at menopause                  |                              |                                      |                          | 49.9 $\pm$ 4.6            |
| Age at the first delivery         |                              | 26.1 $\pm$ 3.5                       | 27.3 $\pm$ 3.7           | 25.3 $\pm$ 3.1            |
| Number of deliveries              | 0                            | 87 (17)                              | 57 (26)                  | 30 (9.9)                  |
|                                   | 1                            | 41 (7.9)                             | 27 (12)                  | 14 (4.6)                  |
|                                   | 2                            | 261 (50)                             | 102 (47)                 | 159 (52)                  |
|                                   | 3                            | 98 (19)                              | 26 (12)                  | 72 (24)                   |
|                                   | 4                            | 16 (3.1)                             | 4 (1.8)                  | 12 (4)                    |
|                                   | 5                            | 1 (0.2)                              | 1 (0.5)                  |                           |
|                                   | Unclear                      | 18 (3.4)                             | 2 (0.9)                  | 16 (5.3)                  |
| Experience of breast feeding      | Absent                       | 117 (22)                             | 60 (27)                  | 57 (19)                   |
|                                   | Present                      | 400 (77)                             | 157 (72)                 | 243 (80)                  |
|                                   | Unclear                      | 5 (1)                                | 2 (0.9)                  | 3 (1)                     |
| Familial history of breast cancer | Absent                       | 444 (85)                             | 191 (87)                 | 253 (83)                  |
|                                   | Present                      | 57 (11)                              | 18 (8.2)                 | 39 (13)                   |
|                                   | Unclear                      | 21 (4)                               | 10 (4.6)                 | 11 (3.6)                  |
| Educational background            | Junior or senior high school | 222 (43)                             | 62 (28)                  | 160 (53)                  |
|                                   | Junior college               | 163 (31)                             | 84 (38)                  | 79 (26)                   |
|                                   | University or higher         | 134 (26)                             | 72 (33)                  | 62 (20)                   |
|                                   | Unclear                      | 3 (0.6)                              | 1 (0.5)                  | 2 (0.7)                   |
| Sleeping hours                    | 3-4h                         | 8 (1.5)                              | 3 (1.4)                  | 5 (1.7)                   |
|                                   | 4-6h                         | 181 (35)                             | 77 (35)                  | 104 (34)                  |
|                                   | 6-8h                         | 316 (61)                             | 136 (62)                 | 180 (59)                  |
|                                   | 8-10h                        | 15 (2.9)                             | 3 (1.4)                  | 12 (4)                    |
|                                   | Unclear                      | 2 (0.4)                              | 0 (0)                    | 2 (0.7)                   |
| Hormone replacement therapy       | Present                      | 46 (8.8)                             | 17 (7.8)                 | 29 (9.6)                  |
|                                   | Absent                       | 470 (90)                             | 202 (92)                 | 268 (88)                  |
|                                   | Unclear                      | 6 (1.1)                              | 0 (0)                    | 6 (2)                     |