TABLE 3 Nomogram for breast cancer

Decision factors	Decision goal
DCIS	To determine whether SLNB is required by examining possible microinvasion[46]
PST	To determine the type of surgery required by examining possible pCR[47]
IBTR	To determine whether RT or re-excision is necessary[48]
SLNB	To determine whether ALND is required by predicting non-SLN metastasis[18,49–52]

re-excision.[20] For a close margin (i.e., 2–5 mm), boost irradiation can be considered.

The 20-year follow-up data from the National Surgical Breast and Bowel Project (NSABP) B-06 trial showed that 39.2% of the patients who received wide local excision without radiotherapy developed ipsilateral breast tumor recurrence (IBTR), as compared with 14.3% of those who received postoperative radiotherapy. Some believe that IBTR does not influence overall survival and that it can be considered a marker of distant metastases rather than a cause; its presence therefore cannot change the intrinsic risk of distant disease.[21] However, according to a meta-analysis performed by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), the impact of local radiation therapy (RT) to prevent local recurrence, either to the breast following BCT or to the chest wall after mastectomy, exhibited overall survival benefit in patients with greater than 10% risk of local recurrence, but it did not show any benefit in patients with less than 10% risk of local recurrence.[22] An analysis of hazard ratios for distant metastases in patients who had undergone breast-conservation surgery with or without postoperative radiotherapy indicated that local recurrence might be a cause of distant metastases.[23] These results suggested that the group with a high risk for locoregional recurrence gained a survival benefit from local radiotherapy. In addition, local relapse could be a crucial psychological stressor for a patient even if her long-term survival was unaffected.

PST

In the case of sequential chemotherapeutic regimens such as doxorubicin and cyclophosphamide (AC) followed by a taxane, the pathological complete remission (pCR) rates are higher in patients who responded to the preceding regimen than in nonresponders. Furthermore, combining chemotherapy with an anti-HER2 treatment such as trastuzumab resulted in even higher pCR rates in HER2-positive cases.[24] A multidisciplinary team, which included an attending surgeon, a radiologist, a medical oncologist, and a pathologist, was indispensable in making appropriate decisions regarding BCS after PST. The findings also led to the recommendation that long-term outcome data, particularly data related to local recurrence rates, and methodologies for assessing the response and success of treatment should be collected, analyzed, and clarified at each institution.[25]

The large majority of the attendees agreed that neoadjuvant endocrine treatment (NAET) is an acceptable approach for certain patients, including those with low-grade, estrogen receptor (ER)-positive breast cancers and postmenopausal patients. Recent studies have suggested that NAET provides higher breast-conservation rates. Nevertheless, because of a lack of randomized clinical trial data, especially on local recurrence, this issue remains to be studied with respect to the tailoring of treatment using biomarkers.[26] Future studies are required to investigate the factors that are predictive of a shrinkage pattern in tumors that have responded to NAET and to determine their postoperative prognosis.

Hereditary Breast Cancer

There was some consensus that patients at higher risk for local recurrence or development of breast cancer in the contralateral breast due to genetic mutations (e.g., *BRCA1* or *BRCA2*) require a more aggressive surgery than BCS. Although this is a controversial topic, the risks of IBTR and of developing contralateral breast cancer may be higher in patients with *BRCA* abnormalities. Therefore, performing a bilateral mastectomy may be preferable to BCS. In addition, performing a bilateral mastectomy would avoid use of RT in a majority of patients. Fifteen years of follow-up data from postoperative radiotherapy in *BRCA* patients suggested that there is a higher risk of radiation toxicity in these patients. Taking these data together, bilateral mastectomy for this specific subgroup could result in reducing cancer recurrence in the affected breast, decreasing new breast cancer development in the unaffected breast, and avoiding the late toxicity of radiotherapy.[27–29]

BCS for DCIS

The Van Nuys Prognostic Index (VNPI), originally proposed and validated by Silverstein et al., is a scoring system for predicting the risk of IBTR in DCIS patients undergoing BCS. Three major factors—margin status, high histological grade, and young age—were recognized as significant risk factors for IBTR after resection of DCIS. The distribution of the opinions as to the proper margin needed for DCIS was similar to that for invasive ductal carcinoma (IDC).

Several retrospective studies have suggested that RT after BCS is useful in avoiding IBTR, especially in patients with high-risk DCIS.[30,31] Tamoxifen in combination with RT has

also been reported to decrease IBTR in DCIS.[32] Prospective trials of neoadjuvant therapies for DCIS using trastuzumab or lapatinib have recently been initiated. These trials may elucidate the effect of anti-HER2 treatments on the local management of HER-2-neu-overexpressing DCIS.

Dunne et al. performed a meta-analysis of 4,660 cases identified from Medline with regard to the margins required for DCIS and RT. They found that a negative margin significantly reduced the risk of IBTR compared with a close margin, and a 2-mm margin was superior to a margin less than 2 mm. However, they observed no significant differences in the IBTR rates with margins over 2 mm.[33] Fisher et al. demonstrated the benefit of tamoxifen in the treatment of DCIS in NSABP B-24, a randomized controlled trial.[34] Because these data suggest that BCS alone is insufficient to prevent IBTR after surgery for DCIS, there was consensus at the meeting that RT and/or endocrine therapy is necessary after BCS.

RADIATION THERAPY

RT as a Component of the Local Management of Breast Cancer

Postoperative RT reduces the risk of locoregional recurrence to approximately one-third of that without RT. Although the baseline risks have varied among existing reports, depending on the method of surgery and the pathological evaluation, the relative risk reduction related to RT was consistent.[22]

For each group of patients who received BCS, there have been continual efforts to find a subgroup of patients who do not require RT.[32,35–38] Unfortunately, such a subgroup had not yet been identified in a prospective trial. However, the eligibility criteria and systemic treatment used in early clinical trials were suboptimal in comparison with today's standards.[32] A clinical trial in a selected group of patients, which included individuals over 70 years old with hormone-responsive tumors treated with a suitable resection margin and appropriate hormonal therapy, demonstrated that the absolute reduction in the risk of local recurrence due to RT, although significant, was small enough that omission of RT could be considered.[32] It is suggested that the intrinsic subtype of breast cancer might be an independent predictive factor related to the benefit of postoperative RT.[39,40] At the meeting it was indicated that these findings should be verified in prospective trials.

Trends in Postoperative Irradiation for the Conserved Breast

Both hypofractionated whole-breast RT and accelerated partial-breast irradiation (APBI) were increasingly used after BCT. Hypofractionated whole-breast RT demonstrated equivalent tumor control and cosmetic results compared with conventional fractionation.[41,42] In the consensus conference, we discussed hypofractionation as an option for certain patients, such as those who are margin free. However, APBI is still considered an experimental treatment.

Indication for Boost to the Tumor Bed after BCT

Although a large randomized clinical trial demonstrated a significant reduction of IBTR in patients with a negative margin, we were unable to reach a consensus on the indications for an RT boost. The most important issue to be resolved was the definition of a "positive" margin after BCS. This definition varied by country and region.[43] Therefore, it should be further examined whether patients with positive margins benefit from routine administration of boost irradiation after whole-breast radiation therapy. At the consensus conference, approximately half of the participants responded that boost irradiation is not necessary if the margin is greater than 5 mm.

In addition to the dose dependency of the ipsilateral tumor control, the European Organisation for Research and Treatment of Cancer (EORTC) 22881-10882 trial clearly demonstrated that younger patients receive a greater benefit from boost irradiation secondary to their greater baseline risk of IBTR. However, in this consensus conference, approximately half of the participants answered that young age alone in not a sufficient criterion for providing a boost, if the margin is widely clear. To resolve this issue, we must standardize the definition of a positive margin, clarify the relationship between the distances required for a clear margin, and understand the magnitude of the effect of boost irradiation.

Survival Benefit of Postoperative RT for Breast Cancer

Meta-analyses performed by EBCTCG demonstrated that a reduction in the risk of locoregional recurrence at 5-year postoperative follow-up could eventually lead to a reduction in death from all causes at 15-year postoperative follow-up.[30] This survival benefit was attributed to prevention of secondary dissemination from local recurrence. However, the benefit was substantial only if the absolute risk reduction of the locoregional recurrence at 5 years exceeded 10%.

Currently, patients with four or more positive lymph nodes are regarded as being at high risk for local recurrence. Postoperative RT to the supraclavicular lymph nodes and the chest wall and breast are recommended in this group after both breast-conserving surgery and mastectomy. Furthermore, meta-analyses of existing trials have

suggested that patients with one to three positive lymph nodes might also receive a survival benefit from postoperative RT, although a randomized clinical trial investigating this hypothesis is ongoing. Patients with negative axillary lymph nodes generally exhibit a low risk of local recurrence. These patients do not benefit from such RT and may have increased risks of radiation side-effects if RT is given. Of note, the number of positive axillary lymph nodes in this context is only a surrogate for the risk of isolated locoregional recurrence. The indication for postoperative RT should ultimately be based on the absolute risk of local recurrence.

Postoperative Radiation Therapy in Patients Receiving PST

Recently, PST has been offered not only to patients with advanced disease but also to patients with early-stage breast cancer. The expansion of this practice has unveiled a new clinical question: What is the optimal RT dose for patients who respond favorably to PST? Randomized trials are needed to answer this question. However, the general consensus was that, for all patients who receive PST and BCS, postoperative RT is recommended. Retrospective studies of patients who received a mastectomy after PST showed that RT significantly improved local control even in patients with pCR after PST.[44] These investigators also found that RT improved survival in patients at higher risk of locoregional recurrence after PST and mastectomy.[45] These results provide insight that the decision to offer RT should be based on both the pretreatment assessment and the final pathologic findings. Postoperative RT is recommended for patients initially diagnosed as having a high risk of locoregional recurrence, regardless of their response to PST.

ACKNOWLEDGMENT We thank Dr. Masahiro Hiraoka, Chairman of the Department of Radiation Oncology and Image-applied Therapy at Kyoto University, the executive advisor to this project. We are also grateful to Dr. Fabrizio Michelassi, Chairman of the Department of Surgery at New York Presbyterian Hospital, Weill Cornell Medical Center, for providing guidance regarding the consensus questionnaire. We thank the Kyoto University Foundation for their generous donation of funds. The conference was part of a program, "Raising Proficient Oncologists," run by the Japanese Ministry of Education, Culture, Sports, Science, and Technology. We acknowledge the kind support of the Japan Breast Cancer Society. We express sincere thanks to the doctors who completed the consensus questionnaire. We are grateful to Dr. Hiroshi Ishiguro for his valuable help and scientific advice. We also thank Ms. Aya Morotomi, Mr. David Graham, Ms. Mihoko Yamamoto, Ms. Nastajia Burke, Ms. Chisa Takano, and Ms. Nobuko Yagi for their invaluable support. Moderators: S. K. Apple (University of California, Los Angeles), J. R. Benson (Addenbrooke's Hospital and University of Cambridge, Cambridge), W. C. Chow (UNIMED Medical Institute/Hong Kong University, Hong Kong), J. F. Forbes (The University of Newcastle, Newcastle), M. Golshan (Dana-Farber Cancer Institute, Brigham & Women's Cancer Center, Boston), T. Inamoto (Kitano Hospital, Osaka), V. S. Klimberg (University of Arkansas for Medical Sciences, Arkansas), M. Mitsumori (Kyoto University, Kyoto), S. Noguchi (Osaka University, Osaka), J. F. R. Robertson (The University of Nottingham, Nottingham), D. H. Roukos (University of Ioannina, Ioannina), H. Sasano (Tohoku University, Sendai), E. A. Strom (The University of Texas M.D. Anderson Cancer Center, Houston), M. Toi (Kyoto University, Kyoto), G. Von Minckwitz (The Centre for Gynecology and Obstetrics at the University of Frankfurt, Frankfurt), E. P. Winer (Dana-Farber Cancer Institute, Brigham & Women's Cancer Center, Boston), and A. Yamauchi (Kitano Hospital, Osaka). Discussants and organizers: S. Akashi-Tanaka (National Cancer Center Hospital, Tokyo), R. Arriagada (Karolinska institute, Stockholm/Institut Gustave-Roussy, Paris), J. Chen (Cancer Hospital of Fudan University, Shanghai), S.C. Chen (Chang Gung University, Taoyuan), S.W. Ha (Seoul National University Hospital, Seoul), S. Han (Inje University Sanggye Paik Hospital, Kyungnam), C. S. Huang and T. Ikeda (Teikyo University, Tokyo), S. Imoto (Kyorin University, Tokyo), H. Inaji (Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka), T. Iwase (The Cancer Institute Hospital of JFCR, Tokyo), H. Iwata (Aichi Cancer Center, Nagoya), R. V. Jakesz (University of Vienna, Vienna), H. Jinno (Keio University, Tokyo), H. Kawabata (Toranomon Hospital, Tokyo), T. Kinoshita (National Cancer Center Hospital, Tokyo), T. Kitai (Nara Social Insurance Hospital, Nara), N. Kohno (Tokyo Medical University, Tokyo), I. E. Krop (Dana-Farber Cancer Institute, Brigham and Women's Cancer Center, Boston), I. Kunkler (University of Edinburgh, Edinburgh), J. Kurebayashi (Kawasaki Medical University, Kurashiki), K. Kuroi (Tokyo Metropolitan Cancer and Infectious Disease Center, Tokyo), E. S. Lee (Korea University Anam-Hospital, Seoul), S. J. Lee (Yeungnam University, Gyeongsan), N. Masuda (Osaka National Hospital, Osaka), Y. Mikami (Kyoto University, Kyoto), S. Nakamura (St. Luke's International Hospital, Tokyo), D. Y. Noh (Seoul National University, Seoul), S. Ohno (National Kyushu Cancer Center, Fukuoka), N. S. Paik (Konkuk University Medical Center, Seoul), M. D. Pegram (University of Miami, Miller School of Medicine, Miami), T. Saeki (Saitama Medical University, Saitama), M. Shafir (Mount Sinai Hospital and School of Medicine, New York), H. Takei (Saitama Cancer Center, Saitama), H. Sonoo (Kawasaki Medical School Hospital, Kurashiki), T. Sugie (Kyoto University, Kyoto), M. Takada (Kyoto University, Kyoto), Y. Tanaka (Kyoto University, Kyoto), T. Ueno (Kyoto University, Kyoto), T. Varughese (Lakeshore Hospital and Research Centre, Cochin), and K. Yamagami (Shinko Hospital, Kobe).

REFERENCES

1. de Mascarel I, Macgrogan G, Deblet M, et al. Distinction between isolated tumor cells and micrometastasis in breast cancer. *Cancer.* 2008;112:1672–8.
2. Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. *Lancet Oncol.* 2010;11: 174–83.
3. Clarke D, Khonji N, Mansel R. Sentinel node biopsy in breast cancer: ALMANC trial. *World J Surg.* 2001;25;819–22.
4. Boneti C, Korourian S, Bland K, et al. Axillary reverse mapping: mapping and preserving arm lymphatics may be important in preventing lymphedema during sentinel lymph node biopsy. *J Am Coll Surg.* 2008;206:1038–44.
5. Kitai T, Inomoto T, Miwa M, et al. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer.* 2005;12:211–15.
6. Yi M, Meric-Bernstam F, Ross MI, et al. How many sentinel lymph nodes are enough during sentinel lymph node dissection for breast cancer? *Cancer.* 2008;113:30–7.

7. Dabbs DJ, Johnson R. The optimal number of sentinel lymph nodes for focused pathologic examination. *Breast J.* 2004;10:186–9.
8. Zavagno G, De Salvo GL, Bozza F, et al. Number of metastatic sentinel lymph nodes as predictor of axillary involvement in patients with breast cancer. *Breast Cancer Res Treat.* 2004;86: 171–9.
9. Barranger E, Coutant C, Flahault A, et al. An axilla scoring system to predict non-sentinel lymph node status in breast cancer patients with sentinel lymph node involvement. *Breast Cancer Res Treat.* 2005;91:113–9.
10. Boileau JF, Easson A, Escallon JM, et al. Sentinel nodes in breast cancer: relevance of axillary level II nodes and optimal number of nodes that need to be removed. *Ann Surg Oncol.* 2008;15:1710–6.
11. Rubio I, Pedreira F, Roca I, et al. Removal of all radioactive sentinel nodes in breast cancer improves the detection of positive sentinel nodes. *Clin Transl Oncol.* 2008;10:347–50.
12. Veronesi U, Galimberti V, Paganelli G, et al. Axillary metastases in breast cancer patients with negative sentinel nodes: a follow-up of 3548 cases. *Eur J Cancer.* 2009;45:1381–8.
13. Layeequr Rahman R, Siegel E, Boneti C, et al. Stage migration with sentinel node biopsy in breast cancer. *Am J Surg.* 2009;197:491–6.
14. Benson JR, della Rovere GQ; Axilla Management Consensus Group. Management of the axilla in women with breast cancer. *Lancet Oncol.* 2007;8:331–48.
15. Mamounas EP, Brown A, Anderson S, et al. Sentinel node biopsy after neoadjuvant chemotherapy in breast cancer: results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol.* 2005;23:2694–702.
16. Intra M, Rotmensz N, Veronesi P, et al. Sentinel node biopsy is not a standard procedure in ductal carcinoma in situ of the breast: the experience of the European institute of oncology on 854 patients in 10 years. *Ann Surg.* 2008;247:315–9.
17. Julian TB, Land SR, Fourchotte V, et al. Is sentinel node biopsy necessary in conservatively treated DCIS? *Ann Surg Oncol.* 2007;14:2202–8.
18. Katz A, Smith BL, Golshan M, et al. Nomogram for the prediction of having four or more involved nodes for sentinel lymph node-positive breast cancer. *J Clin Oncol.* 2008;26:2093–8.
19. Morrow M. Trends in the surgical treatment of breast cancer. *Breast J.* 2010;16(Suppl 1):17–9.
20. Sabel MS. Surgical considerations in early-stage breast cancer: Lessons learned and future directions. *Semin Radiat Oncol.* 2011;21:10–9.
21. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow up of a randomized trial comparing total mastectomy, lumpectomy and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N Eng J Med.* 2002;347:1233–41.
22. Clarke M, Collins R, Darby S, et al. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005;366:2087–106.
23. Nguyen PL, TaghianAG, Katz MS, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and HER-2 is associated with local and distant recurrence after breast-conserving therapy. *J Clin Oncol.* 2008;26:2373–8.
24. Romond EH, Perez EA,, Bryant J, et al. Trastruzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med.* 2005;353:1673–84.
25. Komoike Y, Akiyama F, Iino Y, et al. Ipsilateral breast tumor recurrence (IBTR) after breast-conserving treatment for early breast cancer: risk factors and impact on distant metastases. *Cancer.* 2006;106:35–41.
26. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst.* 2009;101:736–50.
27. Fatouros M, Baltoyiannis G, Roukos DH. The predominant role of surgery in the prevention and new trends in the surgical treatment of women with BRCA1/2 mutations. *Ann Surg Oncol.* 2008;15:21–33.
28. Ziogas D, Roukos DH. Genetics and personal genomics for personalized breast cancer surgery: progress and challenges in research and clinical practice. *Ann Surg Oncol.* 2009;16:1771–82.
29. Garcia-Etienne CA, Barile M, Gentilini OD, et al. Breast-conserving surgery in BRCA1/2 mutation carriers: are we approaching an answer? *Ann Surg Oncol.* 2009;16:3380–7.
30. Bijker N, Meijnen P, Peters JL. Breast-conserving treatment with or without radiotherapy in ductal carcinoma-in-situ: ten-year results of European Organisation for Research and Treatment of Cancer randomized phase III trial 10853—a study by the EORTC Breast Cancer Cooperative Group and EORTC Radiotherapy Group. *J Clin Oncol.* 2006;24:3381–7.
31. Viani GA, Stefalo EJ, Afonso SL, et al. Breast-conserving surgery with or without radiotherapy in woman with ductal carcinoma in situ: a meta-analysis of randomized trials. *Radiation Oncol.* 2007;2:28–39.
32. Hughes KS, Schnaper LA, Berry D, et al. Lumpectomy plus tamoxifen with or without irradiation in women 70 years of age or older with early breast cancer. *N Engl J Med.* 2004;351:971–7.
33. Dunne C, Burke JP, Morrow M, et al. Effect of margin status on local recurrence after breast conservation and radiation therapy for ductal carcinoma in situ. *J Clin Oncol.* 2009;27:1615–20.
34. Fisher B, Dignam J, Wolmark N, et al. Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. *Lancet.* 1999; 353:1993–2000.
35. Fisher B, Bryant J, Dignam JJ, et al. Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. *J Clin Oncol.* 2002;20:4141–9.
36. Forrest AP, Stewart HJ, Everington D, et al. Randomised controlled trial of conservation therapy for breast cancer: 6-year analysis of the Scottish trial. Scottish Cancer Trials Breast Group. *Lancet.* 1996;348:708–13.
37. Fyles AW, McCready DR, Manchul LA, et al. Tamoxifen with or without breast irradiation in women 50 years of age or older with early breast cancer. *N Engl J Med.* 2004;351:963–70.
38. Lim M, Bellon JR, Gelman R, et al. A prospective study of conservative surgery without radiation therapy in select patients with Stage I breast cancer. *Int J Radiat Oncol Biol Phys.* 2006; 65:1149–54.
39. Kyndi M, Sorensen FB, Knudsen H, et al. Estrogen receptor, progesterone receptor, HER-2, and response to postmastectomy radiotherapy in high-risk breast cancer: the Danish Breast Cancer Cooperative Group. *J Clin Oncol.* 2008;26:1419–26.
40. Saverio SD, Catena F, Santini D, et al. 259 Patients with DCIS of the breast applying USC/Van Nuys prognostic index: a retrospective review with long term follow up. *Breast Cancer Res Treat.* 2008;109:405–16.
41. Bentzen SM, Agrawal RK, Aird EG, et al. The UK Standardisation of Breast Radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial. *Lancet.* 2008;371:1098–107.
42. Whelan T, MacKenzie R, Julian J, et al. Randomized trial of breast irradiation schedules after lumpectomy for women with lymph node-negative breast cancer. *J Natl Cancer Inst.* 2002; 94:1143–50.
43. Bartelink H, Horiot JC, Poortmans PM, et al. Impact of a higher radiation dose on local control and survival in breast-conserving therapy of early breast cancer: 10-year results of the randomized boost versus no boost EORTC 22881-10882 trial. *J Clin Oncol.* 2007;25:3259–65.

44. McGuire SE, Gonzalez-Angulo AM, Huang EH, et al. Postmastectomy radiation improves the outcome of patients with locally advanced breast cancer who achieve a pathologic complete response to neoadjuvant chemotherapy. *Int J Radiat Oncol Biol Phys.* 2007;68:1004–9.
45. Huang EH, Tucker SL, Strom EA, et al. Postmastectomy radiation improves local-regional control and survival for selected patients with locally advanced breast cancer treated with neoadjuvant chemotherapy and mastectomy. *J Clin Oncol.* 2004;22:4691–9.
46. O'Flynn EA, Morel JC, Gonzalez J, et al. Prediction of the presence of invasive disease from the measurement of extent of malignant microcalcification on mammography and ductal carcinoma in situ grade at core biopsy. *Clin Radiol.* 2009;64:178–83.
47. Colleoni M, Bagnardi V, Rotmensz N, et al. A risk score to predict disease-free survival in patients not achieving a pathological complete remission after preoperative chemotherapy for breast cancer. *Ann Oncol.* 2009;20:1178–84.
48. Bilimoria KY, Bentrem DJ, Hansen NM, et al. Comparison of sentinel lymph node biopsy alone and completion axillary lymph node dissection for node-positive breast cancer. *J Clin Oncol.* 2009;27:2946–53.
49. Van Zee KJ, Manasseh DM, Bevilacqua JL, et al. A nomogram for predicting the likelihood of additional nodal metastases in breast cancer patients with a positive sentinel node biopsy. *Ann Surg Oncol.* 2003;10:1140–51.
50. Gur AS, Unal B, Johnson R, et al. Predictive probability of four different breast cancer nomograms for nonsentinel axillary lymph node metastasis in positive sentinel node biopsy. *J Am Coll Surg.* 2009;208:229–35.
51. Amanti C, Lombardi A, Maggi S, et al. Is complete axillary dissection necessary for all patients with positive findings on sentinel lymph node biopsy? Validation of a breast cancer nomogram for predicting the likelihood of a non-sentinel lymph node. *Tumori.* 2009;95:153–5.
52. Coutant C, Olivier C, Lambaudie E, et al. Comparison of models to predict nonsentinel lymph node status in breast cancer patients with metastatic sentinel lymph nodes: a prospective multicenter study. *J Clin Oncol.* 2009;27:2800–08.

Cancer Science
The official journal of the Japanese Cancer Association JCA

Prognostic significance of tumor/stromal caveolin-1 expression in breast cancer patients

Niansong Qian,[1,2] Takayuki Ueno,[1] Nobuko Kawaguchi-Sakita,[1] Masahiro Kawashima,[1] Noriyuki Yoshida,[1] Yoshiki Mikami,[3] Tomoko Wakasa,[4] Masayuki Shintaku,[4] Shigeru Tsuyuki,[5] Takashi Inamoto[6] and Masakazu Toi[1,7]

[1]Department of Breast Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Hepatobiliary Surgery, Chinese PLA General Hospital, Beijing, China; [3]Department of Pathology, Graduate School of Medicine, Kyoto University, Kyoto; [4]Department of Pathology, Osaka Red Cross Hospital, Osaka; [5]Department of Surgery, Osaka Red Cross Hospital, Osaka; [6]Department of Breast Surgery, Tenri Hospital, Nara, Japan

(Received December 7, 2010/Revised April 11, 2011; May 7, 2011/Accepted May 10, 2011/Accepted manuscript online May 18, 2011/Article first published online June 28, 2011)

Caveolin-1 (Cav-1) has been extensively characterized in cancer biological research. However, the role of Cav-1 in the interaction between tumor and stromal cells remains unclear. In the present study, we examined Cav-1 expression in tumor cells and stromal cells in breast cancer tissue by immunohistochemical analysis and evaluated its prognostic value in a training cohort. Immunohistochemical analysis of Cav-1 expression was scored as (++), (+) or (–) according to the proportion of positively stained tumor cells (T) and stromal cells (S). Correlation analysis between tumor/stromal Cav-1 expression and clinicopathological parameters revealed that only T(++) Cav-1 status was positively associated with tumor size and histological nodal status ($P = 0.019$ and 0.021, respectively). Univariate analysis revealed that combined T(++)/S(–) status was significantly correlated with unfavorable prognostic outcomes ($P < 0.001$). Multivariate analysis demonstrated that this combined status is an independent prognostic factor for primary breast cancer ($P = 0.002$). Clinical outcomes in different subgroups of breast cancer patients were also strictly dependent on this combined status ($P < 0.05$). The prognostic value of T(++)/S(–) Cav-1 status was also validated in the testing cohort. Collectively, our data indicate that high Cav-1 expression in tumor cells and lack of this expression in stromal cells could help identify a particular subgroup of breast cancer patients with potentially poor survival. Further studies are required to understand the regulatory mechanism of Cav-1 in the tumor microenvironment. (*Cancer Sci* 2011; 102: 1590–1596)

Breast cancer is the most common female cancer. Late-onset diagnosis, axillary lymph node metastases, tumor size, pathological type and resistance to antitumor therapy indicate a poor prognosis for breast cancer patients. Although treatment strategies for breast cancer have recently made great progress, recurrence and death rates remain unacceptably high.[1] Therefore, molecular biomarkers for recurrence and progression of breast cancer must be explored to help clinicians identify new diagnostic and therapeutic techniques to detect and treat breast cancer.[2]

Caveolins (Cav) are a family of scaffolding proteins that coat 50–100 nm plasma membrane invaginations. The Cav family is composed of three isoforms: Cav-1, Cav-2 and Cav-3. The Cav-1 gene is located on chromosome 7 (locus 7q31.1) and includes three exons (30, 165 and 342 bp) and two introns (1.5 and 32 kb).[3] Cav-1 expression depends on the type of tumor and its expression is downregulated in several human cancers such as sarcoma and lung cancer and might function as a tumor suppressor.[4,5] However, upregulation of Cav-1 expression has been reported in esophageal and pancreatic cancers and is also correlated with histopathological grade and poor prognosis.[6,7]

Cav-1 is mainly involved in vesicular transport, cholesterol homeostasis and signal transduction.[8] Furthermore, it might facilitate DNA repair and stabilize the insulin receptor against degradation. Cav-1 also plays a negative role in cell movement,[9] cellular senescence[10] and cell growth.[11] Endothelial cells from Cav-1–/– mice exhibit a diminished response to angiogenic growth factors.[12] Furthermore, Cav-1 overexpression is sufficient to induce premature cellular senescence in fibroblasts.[13,14] Cancer-associated fibroblasts (CAFs), which are derived from malignant or normal epithelial cells, promote tumor growth.[15] *In vitro* studies have shown that both stromal and epithelial Cav-1 play a protective role against mammary hyperplasia and tumorigenesis in breast cancer.[11,16,17] In addition, clinical studies have indicated that stromal loss of Cav-1 is a single independent predictor of early breast cancer recurrence and progression.[18,19] However, the value of combined tumor/stromal Cav-1 expression on the outcome of breast cancer patients is largely unknown.

In the present study, we investigated the clinical significance of Cav-1 expression (including tumor and stromal expression) in a training cohort and the correlation between tumor/stromal Cav-1 expression and clinicopathological characteristics. In addition, effects of combined tumor/stromal Cav-1 expression on outcomes in breast cancer patients were investigated. In addition, the prognostic value of combined tumor/stromal Cav-1 expression was also clarified in a testing cohort. Intriguingly, our results indicated that a counter balance of Cav-1 levels in the tumor microenvironment and epithelial compartment were the most strongly influenced clinical outcomes.

Materials and Methods

Collection of tissue samples. Tissue specimens of the training cohort were collected from the Department of Breast Surgery, Kyoto University Hospital (Kyoto, Japan) between July 2000 and February 2006. Informed consent was obtained from all patients prior to specimen collection and all study protocols were approved by the Ethics Committee for Clinical Research, Kyoto University Hospital. The clinical stage was assessed by The Japanese Breast Cancer Society classification.[20] For analysis of survival and follow up, the date of surgery was used to represent the beginning of the follow-up period. All patients who died from diseases other than breast cancer or from unexpected events were excluded from the case collection. Follow ups were terminated in June 2010. The median follow up was 74 months (range, 3–119 months). Clinicopathological parameters of the training cohort are listed in Table 1. In addition, we validated the results using an independent testing cohort of 193 consecutive patients (Table 1) who underwent surgical resection of breast cancer at Osaka Red Cross Hospital (Osaka, Japan). The protocols used in the testing group were approved by the Ethics Committee of the Osaka Red Cross Hospital. Follow ups

[7]To whom correspondence should be addressed.
E-mail: toi@kuhp.kyoto-u.ac.jp

doi: 10.1111/j.1349-7006.2011.01985.x

Table 1. Clinicopathological parameters of the training and testing cohort

Parameter	Variable	*n* (100%)
Training cohort		
Age	>50	75 (72)
	≤50	29 (28)
Tumor size	≤2 cm	24 (23)
	2–5 cm	51 (49)
	>5 cm	10 (10)
	Unknown	17 (18)
Histological nodal status	Positive	46 (44)
	Negative	56 (54)
	Unknown	2 (2)
ER	Positive	80 (77)
	Negative	24 (23)
PgR	Positive	67 (64)
	Negative	37 (36)
HER2†	Positive	18 (18)
	Negative	81 (82)
ER/PgR/HER2†	Triple negative	12 (12)
	Others	92 (88)
Grade†	Grade 1	12 (12)
	Grade 2	41 (39)
	Grade 3	44 (42)
	Unknown	2 (7)
Recurrence	Yes	22 (21)
	No	72 (69)
	Unknown	10 (10)
Death	Yes	7 (7)
	No	97 (93)
Testing cohort		
Age	>50	147 (76)
	≤50	45 (23)
	Unknown	1 (1)
Tumor size	≤2 cm	85 (44)
	2–5 cm	97 (50)
	>5 cm	11 (6)
Histological nodal status	Positive	79 (41)
	Negative	109 (56)
	Unknown	5 (3)
ER	Positive	139 (72)
	Negative	50 (26)
	Unknown	4 (2)
PgR	Positive	95 (49)
	Negative	95 (49)
	Unknown	3 (2)
HER2	Positive	22 (11)
	Negative	170 (88)
	Unknown	1 (1)
ER/PgR/HER2	Triple negative	35 (18)
	Others	154 (80)
	Unknown	4 (2)
Grade	Grade 1	58 (30)
	Grade 2	50 (26)
	Grade 3	85 (44)
Recurrence	Yes	28 (14)
	No	165 (86)
Death	Yes	15 (8)
	No	178 (92)

†Patients with ductual carcinoma in situ (DCIS) were excluded. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.

in the testing group were terminated in January 2011. The median follow up in the testing cohort was 42 months (range, 1–80 months).

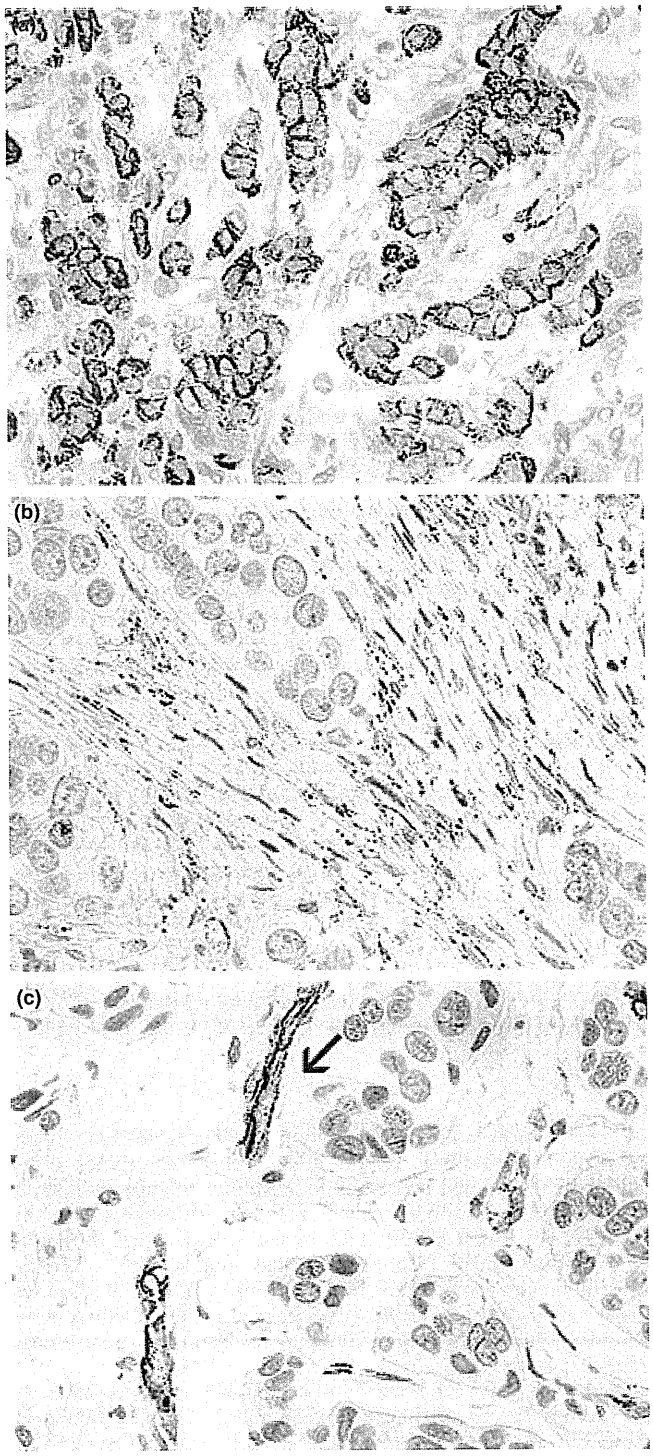

Fig. 1. Immunohistochemical analysis of caveolin-1 (Cav-1) expression (×400). (a) Tumor hot-spot expression. (b) Diffuse Cav-1 expression in stroma and (c) negative stromal Cav-1 expression. Endothelial cells indicate positive immunostaining for Cav-1 used as internal positive controls (arrows).

Immunohistochemical analysis. Immunohistochemical analysis was performed as described previously.[18,19] In brief, slides were incubated with an anti-Cav-1 monoclonal antibody (1:800; Cell Signaling Technology, Danvers, MA, USA). The

signals were detected by Envision kit (Dako, Glostrup, Denmark) and the sections were counterstained with haematoxylin. Negative control sections were incubated with phosphate-buffered saline plus 1% bovine serum albumin instead of primary antibody. Endothelial cells were used as internal positive controls because these cells commonly express Cav-1 in cancerous regions. Results of the analyses were evaluated by two pathologists, who were independent and blinded to the clinical features of the study. We determined three hot-spots at ×400 magnification, calculated the number of Cav-1-stained tumor cells (T) and stromal cells (S) and graded the cells as follows: negative expression (−), ≤5%; low expression (+), 5–50%; and high expression (++), >50%. For analyzing combined tumor/stromal Cav-1 expression, we determined tumors with a high tumor hot-spot grade that were stromal negative as T(++)/S(−).

Statistical analysis. The correlation between different types of Cav-1 expression was evaluated using Spearman's test. The correlation between Cav-1 and clinicopathological parameters was evaluated using the Kruskal–Wallis test. Disease-free survival was estimated using the Kaplan–Meier estimate and a comparison of stratified survival curves was performed using log-rank tests. Cox analysis was used to evaluate the correlation between Cav-1 and disease-free survival in the presence of various potential prognostic factors for disease-free survival. Differences were considered statistically significant at $P < 0.05$.[18,19]

Results

Immunohistochemical analysis of Cav-1 expression in the training cohort. All patients were Japanese women and their clinicopathological characteristics are listed in Table 1. Twenty-two patients developed recurrence and seven of them died as a result. Distributions of recurrence and survival parameters are also indicated in Table 1. Table S1 summarizes the number of patients in each subgroup stratified by Cav-1 grade. T(−) Cav-1 expression was observed in 16%, T(+) Cav-1 expression was observed in 67% and T(++) Cav-1 expression was observed in 17% of breast cancer patients. Strong positive staining showed a prevalent membrane pattern associated with cytoplasm positive. For total stromal Cav-1 expression, 57% were S(++/+) and 43% were S(−). In total, 72 patients were S(++/+) and 32 showed S(−) expression stratified by stromal hot-spot Cav-1 expression. For combined Cav-1 expression score grading, 5% of breast cancer patients were T(++)/S(−) and 16% were T(+)/S(−). Representative examples are illustrated in Figure 1.

Next, correlations between the different types of Cav-1 expression were analyzed. Stromal hot-spot Cav-1 expression was significantly correlated with total stromal Cav-1 expression ($R^2 = 0.517$, $P < 0.001$). However, stromal hot-spot Cav-1 expression was weakly correlated ($R^2 = 0.081$, $P = 0.003$) with tumor hot-spot expression. No significant difference was observed between total stromal and tumor hot-spot Cav-1 expression ($R^2 = 0.029$, $P = 0.083$). Because of the strong correlation between stromal hot-spot and total Cav-1 expression, we used the former as a representative for the following analysis.

Correlations between Cav-1 expression and clinicopathological parameters in the training cohort. Table 2 summarizes the correlation between Cav-1 expression and the clinicopathological parameters of breast cancer patients in the training cohort. T(++) Cav-1 expression was positively associated with tumor size and histological nodal status ($P = 0.019$ and 0.021, respectively). S(−) Cav-1 expression was independent of histological nodal and human epidermal growth factor receptor 2 (HER2) status ($P = 0.385$ and 0.055, respectively). No significant correlations were found between stromal hot-spot and tumor hot-spot Cav-1 expression and age, tumor stage, grade, estrogen receptor (ER) status or progesterone receptor (PgR)

Table 2. Associations between caveolin-1 (Cav-1) expression and clinicopathological parameters in the training and testing cohorts

Parameter	Variable	Tumor hot-spot Cav-1 expression (training cohort)			Stromal hot-spot Cav-1 expression (training cohort)			Tumor/stromal Cav-1 expression (training cohort)			Tumor/stromal Cav-1 expression (testing cohort)		
		T(+/−)	T(++)	*P*	S(−)	S(++/+)	*P*	T(++)/S(−)	Others	*P*	T(++)/S(−)	Others	*P*
Tumor size		27.0 ± 12.8	36.3 ± 17.4	0.019	30.5 ± 13.1	28.2 ± 14.6	0.498	36.6 ± 6.9	20.9 ± 3.3	<0.0001	40.9 ± 45.4	23.8 ± 12.4	<0.001
Age (%)	≤50	24 (28)	5 (28)	0.991	10 (31)	19 (26)	0.612	1 (20)	28 (28)	0.688	13 (59)	134 (79)	0.129
	>50	62 (72)	13 (72)		22 (69)	53 (74)		4 (80)	71 (72)		9 (41)	36 (21)	
T status (%)	T0/T1	24 (34)	2 (12)	0.095	5 (21)	21 (33)	0.258	0 (0)	26 (31)	0.253	5 (23)	80 (47)	0.033
	T2/T3/T4	47 (66)	14 (88)		19 (79)	42 (67)		3 (100)	58 (69)		17 (77)	91 (53)	
Histological nodal status (%)	Positive	34 (40)	12 (71)	0.021	16 (52)	30 (42)	0.385	5 (100)	41 (42)	0.012	5 (25)	104 (62)	0.002
	Negative	51 (60)	5 (29)		15 (48)	41 (58)		0 (0)	56 (58)		15 (75)	64 (38)	
Grade† (%)	Grade 1	12 (15)	0 (0)	0.140	2 (7)	10 (15)	0.264	0 (0)	12 (13)	0.208	4 (18)	54 (32)	0.113
	Grade 2	31 (39)	10 (59)		11 (37)	30 (45)		4 (80)	37 (40)		5 (23)	45 (26)	
	Grade 3	37 (46)	7 (41)		17 (56)	27 (40)		1 (20)	43 (47)		13 (59)	72 (42)	
ER (%)	Positive	69 (80)	11 (61)	0.081	21 (66)	59 (82)	0.359	3 (60)	77 (78)	0.360	13 (62)	126 (75)	0.201
	Negative	17 (20)	7 (39)		11 (34)	13 (18)		2 (40)	22 (22)		8 (38)	42 (25)	
PgR (%)	Positive	58 (67)	9 (50)	0.162	19 (59)	48 (67)	0.476	3 (60)	64 (65)	0.471	10 (48)	85 (50)	0.817
	Negative	28 (33)	9 (50)		13 (41)	24 (33)		2 (40)	35 (35)		11 (52)	84 (50)	
HER2† (%)	Positive	15 (19)	3 (17)	0.855	9 (30)	9 (13)	0.055	2 (40)	16 (17)	0.197	3 (29)	19 (33)	0.667
	Negative	66 (81)	15 (83)		21 (70)	60 (87)		3 (60)	78 (83)		18 (71)	152 (67)	

†Patients with ductual carcinoma in situ (DCIS) were excluded. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor; S, stromal hot-spot; T, tumor hot-spot.

doi: 10.1111/j.1349-7006.2011.01985.x

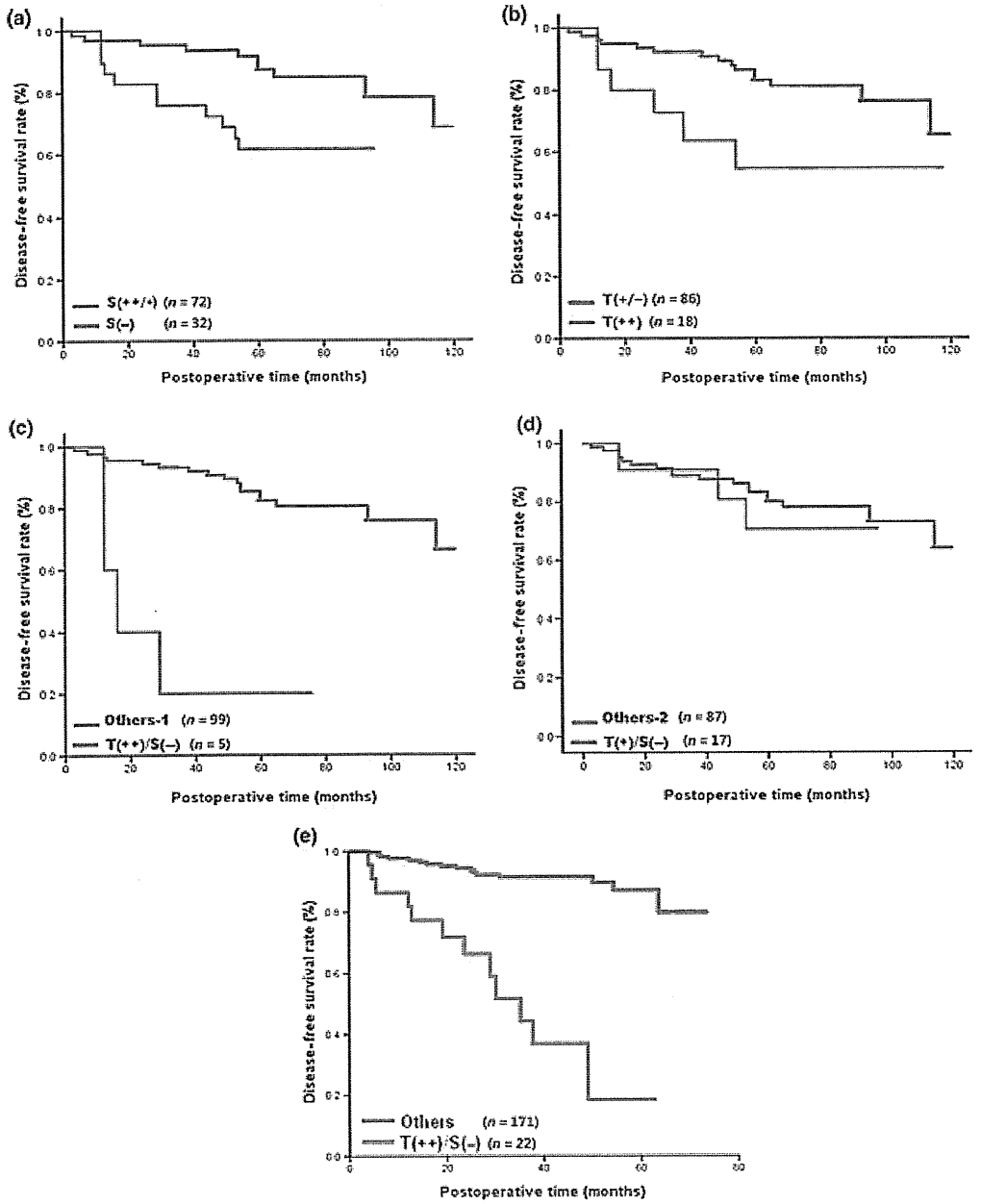

Fig. 2. Disease-free survival curves of the training cohort stratified by (a) stromal hot-spot caveolin-1 (Cav-1) expression status: S(–) *vs* S(++/+; $P = 0.009$, log-rank test); (b) tumor hot-spot Cav-1 expression status: T(++) *vs* T(+/–; $P = 0.019$, log-rank test); (c) combined Cav-1 expression: T(++)/S(–) *vs* Others 1 ($P < 0.001$, log-rank test); and (d) combined Cav-1 expression: T(+)/S(–) *vs* Others 2 ($P = 0.662$, log-rank test). Disease-free survival curves of the testing cohort stratified by (e) combined Cav-1 expression: T(++)/S(–) *vs* Others ($P < 0.001$, log-rank test).

status ($P > 0.05$). T(++)/S(–) Cav-1 expression was positively associated with tumor size and histological nodal status ($P < 0.001$ and 0.012, respectively). No significant correlations were found between T(++)/S(–) Cav-1 expression and age, tumor stage, grade, ER, PgR or HER2 status ($P > 0.05$).

Prognostic value of Cav-1 expression in the training cohort. Figure 2a–d illustrates the Kaplan–Meier curves of disease-free survival for the training cohort constructed on the basis of the Cav-1 expression level. T(++), S(–) and T(++)/S(–) status correlated closely with poor disease-free survival ($P = 0.009$, 0.019 and <0.001, respectively). No major differences were observed between S(+) and S(++) or between T(+) and T(–) Cav-1 expression on the predictive value of disease-free survival ($P > 0.05$; data not shown). T(+)/S(–) status also did not influence disease-free survival.

Kaplan–Meier analysis demonstrated a significant impact of certain clinicopathological prognostic factors such as HER2, tumor stage and ER on disease-free survival ($P = 0.015$, 0.010 and 0.010, respectively). No significant correlations were found between disease-free survival and other clinicopathological factors, including PgR, histological nodal status, grade and age ($P > 0.05$; Table 3). Cox analysis was performed to evaluate whether the correlation between Cav-1 expression and disease-free survival was related to the correlation of Cav-1

Table 3. Univariate analyses of factors associated with recurrence in the training and testing cohorts

Variable	Disease-free survival (*P*)
Training cohort	
Tumor/stromal Cav-1 expression T(++)/S(−) *vs* others	<0.001
Stromal hot-spot Cav-1 expression S(++/+) *vs* S(−)	0.009
Tumor stage (T0/T1 *vs* T2/T3/T4)	0.010
ER (positive *vs* negative)	0.010
HER2 (positive *vs* negative)	0.015
Tumor hot-spot Cav-1 expression T(++) *vs* T(+/−)	0.019
PgR (positive *vs* negative)	0.054
Histological nodal (positive *vs* negative)	0.069
Age (>50 *vs* ≤50)	0.411
Testing cohort	
Tumor/stromal Cav-1 expression T(++)/S(−) *vs* others	<0.001
Histological nodal status (positive *vs* negative)	<0.001
Grade (1 *vs* 2 *vs* 3)	<0.001
HER2 (positive *vs* negative)	<0.001
Tumor stage (T0/T1 *vs* T2/T3/T4)	0.008
ER (positive *vs* negative)	0.020
PgR (positive *vs* negative)	0.054
Age (>50 *vs* ≤50)	0.376

Cav-1, caveolin-1; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor; S, stromal hot spot; T, tumor hot spot.

Table 4. Multivariate analysis of factors that might affect disease-free survival in the training and testing cohorts

	Disease-free survival		
	HR	95% CI	*P*
Training cohort			
Stromal hot-spot Cav-1 expression	0.322	0.098–1.065	0.063
ER	1.092	0.306–3.901	0.893
HER2	3.362	1.035–10.923	0.044
Tumor stage	7.772	0.985–61.288	0.052
Histological nodal status	1.536	0.900–2.619	0.115
Grade	3.807	1.018–7.472	0.021
Tumor hot spot-Cav-1 expression	0.370	0.108–1.269	0.114
ER	0.844	0.248–2.867	0.785
HER2	4.778	1.337–17.081	0.016
Tumor stage	6.437	0.806–51.392	0.079
Histological nodal status	1.406	0.814–2.430	0.222
Grade	0.322	1.220–11.881	0.046
Tumor/stromal Cav-1 expression	0.041	0.006–0.297	0.002
ER	0.774	0.232–2.580	0.677
HER2	3.665	1.037–12.954	0.044
Tumor stage	7.234	0.900–58.129	0.063
Histological nodal status	1.327	0.753–2.341	0.328
Grade	5.868	1.523–22.614	0.010
Testing cohort			
Tumor/stromal Cav-1 expression	0.249	0.107–0.582	0.001
ER	0.846	0.362–1.982	0.701
HER2	2.141	0.875–5.238	0.096
Tumor stage	1.551	0.965–2.494	0.070
Histological nodal status	4.124	1.517–11.208	0.005
Histological grade	3.150	1.416–7.007	0.005

Cav-1, caveolin-1; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

expression with other prognostic factors. The results revealed that S(−) Cav-1 expression was not an independent prognostic factor for disease-free survival ($P = 0.063$). No significant correlation was found between T(++) Cav-1 expression and disease-free survival ($P = 0.114$). Consistent with the univariate analysis results, the multivariate analysis revealed that combined T(++)/S(−) status was strongly associated with an unfavorable prognosis ($P = 0.002$; Table 4). We conducted further subgroup analysis stratified by histological nodal, ER and HER2 status, which were associated with poor clinical outcomes. As a result, S(−) Cav-1 showed clear trends for predicting disease-free survival in histological node+ patients ($P = 0.008$). However, T(+) Cav-1 expression did not influence disease-free survival in histological node+ patients ($P = 0.088$). Notably, histological node+ patients with T(++)/S(−) Cav-1 expression exhibited lower disease-free survival ($P = 0.001$). Furthermore, T(++)/S(−) Cav-1 expression also served as an important predictor of disease-free survival for ER+ and HER2+ patients ($P = 0.001$ and 0.045, respectively). Moreover, patients with T(++)/S(−) Cav-1 expression who were in the ER−, HER2−, PgR (+ and −), tumor size (>5 and ≤5 cm), age (>50 and ≤50 years) and grade (Grade 2 and 3) subgroups had poorer disease-free survival (Table S2).

Validation of the prediction power of T(++)/S(−) Cav-1 status in the testing cohort. In the testing cohort, all patients were also Japanese women and their clinicopathological characteristics are listed in Table 1. T(++)/S(−) Cav-1 expression was observed in 11% and other expressions were observed in 89% breast cancer patients. T(++)/S(−) Cav-1 expression was significantly related to tumor size ($P < 0.001$), tumor stage ($P = 0.033$) and histological nodal status ($P = 0.002$). We could not find a statistically significant association between T(++)/S(−) Cav-1 expression and age, grade, ER, PgR or HER2 ($P > 0.05$; Table 2).

We then examined the association of T(++)/S(−) Cav-1 expression with the clinical outcome. Kaplan–Meier survival analysis showed patients with T(++)/S(−) Cav-1 expression had shorter disease-free survival than those with other expressions ($P < 0.001$, Fig. 2e). The significant impact of clinicopathological prognostic factors such as tumor stage, ER and HER2 on disease-free survival ($P = 0.008$, 0.020 and <0.001, respectively) was also validated. These results were consistent with the above findings. In addition, a significant correlation between disease-free survival and grade or histological nodal status was observed in the testing cohort ($P < 0.001$ and <0.001, respectively; Table 3).

Table 4 provides the multivariate analyses of factors related to patient disease-free survival. Cox analysis indicated that T(++)/S(−) Cav-1 status was an independent predictor of disease-free survival ($P = 0.001$), as were histological nodal status ($P = 0.005$) and grade ($P = 0.005$). Moreover, the role of T(++)/S(−) Cav-1 expression in disease-free survival in the ER (+ and −), HER2 (+ and −), PgR (+ and −), tumor size (>5 and ≤5 cm), age (>50 and ≤50 years) and grade (Grade 1 + 2 and 3) subgroups is shown in Table S2.

Discussion

The Cav-1 gene is colocalized at the D7S522 locus on human chromosome 7q31.1 and is commonly deleted in breast, colon, kidney, prostate, ovary, head and neck cancers. Thus, it seems feasible to propose that the Cav-1 gene might serve as a candidate tumor suppressor gene.[21] In the present study, we focused on breast cancer patients to determine the correlation of tumor/stromal Cav-1 expression with clinicopathological parameters and survival. Cav-1 expression was evaluated semi-quantitatively based on the proportion of positively stained tumor and stromal cells. We found that tumor Cav-1 demonstrated a prevalent membrane pattern associated with cytoplasm positive and that T(−) Cav-1 expression was noted in 16% of

doi: 10.1111/j.1349-7006.2011.01985.x

cases, which is consistent with previous reports.[22] The stromal Cav-1 expression rate and pattern in the present study are similar to a previous report.[23]

According to previous reports, total tumor Cav-1 expression has no prognostic value in primary breast cancer patients.[18,24] In the present study, we examined stromal hot-spot, total stromal and tumor hot-spot Cav-1 expression. First, we analyzed correlations between stromal hot-spot and total stromal and tumor Cav-1 expression and found a weak but significant correlation between stromal and tumor expression, indicating that Cav-1 expression could be regulated differently between tumor and stromal cells and that Cav-1 might influence different functions in those cells.[25,26] T(++) Cav-1 expression was positively associated with tumor size and histological nodal status. Previous reports revealed that tumor Cav-1expression was negatively associated with HER2 status.[27–29] Several studies have indicated that Cav-1 might function as a negative signal transduction regulator to HER2/neu and that it might play a negative regulatory role in mammary tumor development. In addition, activation of HER2/neu might downregulate Cav-1 expression *in vitro*.[30,31] However, this finding was not supported by our results. Stromal Cav-1 expression showed no significant correlation with any of the clinicopathological parameters, which was inconsistent with a previous report,[18] and therefore we focused on combined tumor/stromal Cav-1 expression. T(++)/S(−) was observed frequently in large-size tumors and histological node+ cases, indicating that tumor/stromal Cav-1 expression is involved in breast cancer progression. Interestingly, survival analyses revealed that patients with T(++)/S(−) Cav-1 expression had the shortest disease-free survival among various Cav-1 expression subgroups. Multivariate analysis confirmed an independent prognostic value of the combined status. Consistent with these results, T(++)/S(−) Cav-1 expression was significantly related to tumor size, histological nodal status and disease-free survival in the testing cohort. Besides, the positive correlation between T(++)/S(−) Cav-1 expression and tumor stage was also indicated in the testing cohort. A possible explanation for the discrepancy could be due to the difference in sample size. Furthermore, T(++)/S(−) Cav-1 expression also impacted the clinical outcomes stratified by ER status, PgR status, HER2 status, tumor size, age, histological nodal status and grade in both the training and testing cohorts. Therefore, these results indicate that combined Cav-1 status has a more potent prognostic value than either stromal or tumor hot-spot alone. We believe that these results are important when considering breast cancer biology. Given the limited number of cases, prospective studies with long-term follow-up data are warranted.[32]

Tumor Cav-1 expression with respect to tumorigenesis seems more complex than originally believed. Cav-1 loss-of-function induces ligand-independent hyperactivation of Ras-p42/44 MAPK and Smad signaling pathways as well as enhanced matrix metalloproteinase-2/9 secretion. Each of these pathways is likely to contribute to cell cycle progression, growth factor independence, cell invasiveness and epithelial–mesenchymal transition.[33] Despite extensive evidence supporting the role of Cav-1 as a tumor suppressor, several studies have suggested an alternative view of Cav-1 expression in tumors. In breast cancer, Cav-1 protects tumor cells from anoikis, promotes tumor cell survival and abrogates detachment-induced p53 activation.[34,35] Furthermore, Cav-1 expression is upregulated in multidrug-resistant MCF-7 cells.[36,37] A hypothesis has been proposed to explain the divergent roles of Cav-1; even if an initial loss of Cav-1 is observed in breast cancer, re-expression of Cav-1 at later stages might correlate with more malignant characteristics.[35,38]

Stromal Cav-1 plays a vital role in tumorigenesis. Loss of stromal Cav-1 is an independent predictor for therapeutic resistance and poor prognosis in primary breast cancers.[12,18,19,39] Woodman *et al.*[12] reported that endothelial cells from Cav-1−/− mice exhibit a disrupted response to angiogenic growth factors. Senescent human diploid fibroblasts exhibit increased levels of the Cav-1 protein.[10] In addition, loss of Cav-1 in stromal cells of various organs directly leads to disorganised stromal compartments and dysfunctional organ systems.[40]

Furthermore, recent studies have revealed a role played by Cav-1 in the interaction between tumor and stromal cells in breast cancer. During tumor formation, cancer cells and adjacent fibroblasts are metabolically coupled. A new model has been proposed in which glycolytic CAF promote tumor growth by secreting energy-rich metabolites that can be taken up by adjacent tumor cells.[41] Loss of Cav-1 *in vitro* induces metabolic coupling between CAF and tumor cells and leads to the formation of a host–parasite relationship. Martinez-Outschoorn *et al.*[42] showed that Cav-1 expression is downregulated in fibroblasts co-cultured with MCF-7 cells and that it mediates autophagic/lysosomal degradation. Furthermore, autophagy induced by loss of Cav-1 in fibroblasts provides cancer cells with essential chemical building blocks.[42,43] Loss of stromal Cav-1 fibroblasts protects adjacent cancer cells via decreased apoptosis, increased TP53-induced glycolysis and apoptosis regulator expression.[44] Furthermore, loss of Cav-1 induces oxidative stress in CAF, which is the root cause of mitochondrial dysfunction in CAF and promotes DNA damage. In the present study, the predictive value of T (++)/S(−) was demonstrated in luminal-type cancers and HER2+ cancers. Its value was stratified by an intrinsic subtype and warrants an examination with a greater number of cases.

The regulatory mechanism of Cav-1 expression in breast cancer remains to be elucidated. Pro-autophagic stimuli such as hypoxia, oxidative stress and nuclear factor κB activation might cause the loss of Cav-1.[45] Conversely, multiple factors are present during Cav-1 upregulation.

In conclusion, we provide evidence that T (++)/S(−) Cav-1 expression is closely associated with unfavorable prognostic outcomes in primary breast cancer patients. This particular subgroup seems to be engaged in rapid disease progression. Further studies involving analysis of molecular mechanisms of Cav-1 expression are required. The interaction between tumor and stromal cells and Cav-1 in the tumor microenvironment is also a key issue to investigate. Moreover, new therapies targeting Cav-1 expression might be a novel therapeutic approach, particularly for patients with T (++)/S(−) Cav-1 status.

Acknowledgment

N.S.Q. was supported by the State Scholarship Foundation of China (No. 2009659015).

Disclosure Statement

The authors have no conflict of interest.

References

1 Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N. Triple-negative breast cancer – current status and future directions. *Ann Oncol* 2010; **21**: 7–12.

2 Yamashiro H, Toi M. Update of evidence in chemotherapy for breast cancer. *Int J Clin Oncol* 2008; **13**: 3–7.

3 Shatz M, Liscovitch M. Caveolin-1: a tumor-promoting role in human cancer. *Int J Radiat Biol* 2008; **84**: 177–89.

4 Cantiani L, Manara MC, Zucchini C *et al.* Caveolin-1 reduces osteosarcoma metastases by inhibiting c-Src activity and met signaling. *Cancer Res* 2007; **67**: 7675–85.
5 Wikman H, Kettunen E, Seppänen JK *et al.* Identification of differentially expressed genes in pulmonary adenocarcinoma by using cDNA array. *Oncogene* 2002; **21**: 5804–13.
6 Kato K, Hida Y, Miyamoto M *et al.* Overexpression of caveolin-1 in esophageal squamous cell carcinoma correlates with lymph node metastasis and pathologic stage. *Cancer* 2002; **94**: 929–33.
7 Suzuoki M, Miyamoto M, Kato K *et al.* Impact of caveolin-1 expression on prognosis of pancreatic ductal adenocarcinoma. *Br J Cancer* 2002; **87**: 1140–4.
8 Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. *Physiol Rev* 2004; **84**: 1341–79.
9 Zhang W, Razani B, Altschuler Y *et al.* Caveolin-1 inhibits epidermal growth factor-stimulated lamellipod extension and cell migration in metastatic mammary adenocarcinoma cells (MTLn3). Transformation suppressor effects of adenovirus-mediated gene delivery of caveolin-1. *J Biol Chem* 2000; **275**: 20717–25.
10 Park WY, Park JS, Cho KA *et al.* Up-regulation of caveolin attenuates epidermal growth factor signaling in senescent cells. *J Biol Chem* 2000; **275**: 20847–52.
11 Sotgia F, Schubert W, Pestell RG, Lisanti MP. Genetic ablation of caveolin-1 in mammary epithelial cells increases milk production and hyper-activates STAT5a signaling. *Cancer Biol Ther* 2006; **5**: 292–7.
12 Woodman SE, Ashton AW, Schubert W *et al.* Caveolin-1 knockout mice show an impaired angiogenic response to exogenous stimuli. *Am J Pathol* 2003; **162**: 2059–68.
13 Volonte D, Zhang K, Lisanti MP, Galbiati F. Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. *Mol Biol Cell* 2002; **13**: 2502–17.
14 Chretien A, Piront N, Delaive E, Demazy C, Ninane N, Toussaint O. Increased abundance of cytoplasmic and nuclear caveolin 1 in human diploid fibroblasts in H(2)O(2)-induced premature senescence and interplay with p38alpha(MAPK). *FEBS Lett* 2008; **582**: 1685–92.
15 Ostman A, Augsten M. Cancer-associated fibroblasts and tumor growth – bystanders turning into key players. *Curr Opin Genet Dev* 2009; **19**: 67–73.
16 Qian N, Ueno T. Is dysfunction of caveolin-1 a link between systemic sclerosis and breast cancer, opening a window on both etiologies? *Arch Med Res* 2010; **41**: 297–301.
17 Williams TM, Sotgia F, Lee H *et al.* Stromal and epithelial caveolin-1 both confer a protective effect against mammary hyperplasia and tumorigenesis: caveolin-1 antagonizes cyclin D1 function in mammary epithelial cells. *Am J Pathol* 2006; **169**: 1784–801.
18 Witkiewicz AK, Dasgupta A, Sotgia F *et al.* An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. *Am J Pathol* 2009; **174**: 2023–34.
19 Witkiewicz AK, Dasgupta A, Nguyen KH *et al.* Stromal caveolin-1 levels predict early DCIS progression to invasive breast cancer. *Cancer Biol Ther* 2009; **8**: 1071–9.
20 Sakamoto G, Inaji H, Akiyama F *et al*; Japanese Breast Cancer Society. General rules for clinical and pathological recording of breast cancer 2005. *Breast Cancer* 2005; **12**(Suppl): S1–27.
21 Liu P, Rudick M, Anderson RG. Multiple functions of caveolin-1. *J Biol Chem* 2002; **277**: 41295–8.
22 Perrone G, Altomare V, Zagami M *et al.* Caveolin-1 expression in human breast lobular cancer progression. *Mod Pathol* 2009; **22**: 71–8.
23 Sloan EK, Ciocca DR, Pouliot N *et al.* Stromal cell expression of caveolin-1 predicts outcome in breast cancer. *Am J Pathol* 2009; **174**: 2035–43.
24 Liedtke C, Kersting C, Bürger H, Kiesel L, Wülfing P. Caveolin-1 expression in benign and malignant lesions of the breast. *World J Surg Oncol* 2007; **5**: 110.
25 Rønnov-Jessen L, Bissell MJ. Breast cancer by proxy: can the microenvironment be both the cause and consequence? *Trends Mol Med* 2009; **15**: 5–13.
26 Mercier I, Casimiro MC, Wang C *et al.* Human breast cancer-associated fibroblasts (CAFs) show caveolin-1 downregulation and RB tumor suppressor functional inactivation: implications for the response to hormonal therapy. *Cancer Biol Ther* 2008; **7**: 1212–25.
27 Savage K, Lambros MB, Robertson D *et al.* Caveolin 1 is overexpressed and amplified in a subset of basal-like and metaplastic breast carcinomas: a morphologic, ultrastructural, immunohistochemical, and in situ hybridization analysis. *Clin Cancer Res* 2007; **13**: 90–101.
28 Sagara Y, Mimori K, Yoshinaga K *et al.* Clinical significance of Caveolin-1, Caveolin-2 and HER2/neu mRNA expression in human breast cancer. *Br J Cancer* 2004; **91**: 959–65.
29 Park SS, Kim JE, Kim YA *et al.* Caveolin-1 is down-regulated and inversely correlated with HER2 and EGFR expression status in invasive ductal carcinoma of the breast. *Histopathology* 2005; **47**: 625–30.
30 Couet J, Sargiacomo M, Lisanti MP. Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. *J Biol Chem* 1997; **272**: 30429–38.
31 Kim YN, Wiepz GJ, Guadarrama AG, Bertics PJ. Epidermal growth factor-stimulated tyrosine phosphorylation of caveolin-1. Enhanced caveolin-1 tyrosine phosphorylation following aberrant epidermal growth factor receptor status. *J Biol Chem* 2000; **275**: 7481–91.
32 Decensi A, Guerrieri-Gonzaga A, Gandini S *et al.* Prognostic significance of Ki-67 labeling index after short-term presurgical tamoxifen in women with ER-positive breast cancer. *Ann Oncol* 2011; **22**: 582–7.
33 Sotgia F, Rui H, Bonuccelli G, Mercier I, Pestell RG, Lisanti MP. Caveolin-1, mammary stem cells, and estrogen-dependent breast cancers. *Cancer Res* 2006; **66**: 10647–51.
34 Fiucci G, Ravid D, Reich R, Liscovitch M. Caveolin-1 inhibits anchorage-independent growth, anoikis and invasiveness in MCF-7 human breast cancer cells. *Oncogene* 2002; **21**: 2365–75.
35 Ravid D, Maor S, Werner H, Liscovitch M. Caveolin-1 inhibits anoikis and promotes survival signaling in cancer cells. *Adv Enzyme Regul* 2006; **46**: 163–75.
36 Lavie Y, Fiucci G, Liscovitch M. Up-regulation of caveolae and caveolar constituents in multidrug-resistant cancer cells. *J Biol Chem* 1998; **273**: 32380–3.
37 Lavie Y, Liscovitch M. Changes in lipid and protein constituents of rafts and caveolae in multidrug resistant cancer cells and their functional consequences. *Glycoconj J* 2000; **17**: 253–9.
38 Quest AF, Gutierrez-Pajares JL, Torres VA. Caveolin-1: an ambiguous partner in cell signaling and cancer. *J Cell Mol Med* 2008; **12**: 1130–50.
39 Sotgia F, Williams TM, Schubert W *et al.* Caveolin-1 deficiency (−/−) conveys premalignant alterations in mammary epithelia, with abnormal lumen formation, growth factor independence, and cell invasiveness. *Am J Pathol* 2006; **168**: 292–309.
40 Yang G, Timme TL, Naruishi K *et al.* Mice with cav-1 gene disruption have benign stromal lesions and compromised epithelial differentiation. *Exp Mol Pathol* 2008; **84**: 131–40.
41 Pavlides S, Tsirigos A, Vera I *et al.* Transcriptional evidence for the ''Reverse Warburg Effect'' in human breast cancer tumor stroma and metastasis: similarities with oxidative stress, inflammation, Alzheimer's disease, and ''Neuron-Glia Metabolic Coupling''. *Aging (Albany NY)* 2010; **2**: 185–99.
42 Martinez-Outschoorn UE, Pavlides S, Whitaker-Menezes D *et al.* Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. *Cell Cycle* 2010; **9**: 2423–33.
43 Pavlides S, Tsirigos A, Vera I *et al.* Loss of stromal caveolin-1 leads to oxidative stress, mimics hypoxia and drives inflammation in the tumor microenvironment, conferring the ''reverse Warburg effect'': a transcriptional informatics analysis with validation. *Cell Cycle* 2010; **9**: 2201–19.
44 Martinez-Outschoorn UE, Trimmer C, Lin Z *et al.* Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFκB activation in the tumor stromal microenvironment. *Cell Cycle* 2010; **9**: 3515–33.
45 Bonuccelli G, Whitaker-Menezes D, Castello-Cros R *et al.* The reverse Warburg effect: glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts. *Cell Cycle* 2010; **9**: 1960–71.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The numbers of patients in each subgroup stratified by Cav-1 grade.

Table S2. Univariate analyses of Cav-1 expression associated with disease-free survival of the training and testing cohort.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

doi: 10.1111/j.1349-7006.2011.01985.x

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(7)867–875
doi:10.1093/jjco/hyr081

Docetaxel Followed by Fluorouracil/Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy for Patients with Primary Breast Cancer

Hiroji Iwata[1,*], Nobuaki Sato[2], Norikazu Masuda[3], Seigo Nakamura[4], Naohito Yamamoto[5], Katsumasa Kuroi[6], Masafumi Kurosumi[7], Hitoshi Tsuda[8], Futoshi Akiyama[9], Yasuo Ohashi[10] and Masakazu Toi[11]

[1]Department of Breast Oncology, Aichi Cancer Center Hospital, Aichi, [2]Department of Surgery, Niigata Cancer Center Hospital, Niigata, [3]Department of Surgery, National Hospital Organization Osaka National Hospital, Osaka, [4]Breast Surgical Oncology, St Luke's International Hospital, Tokyo, [5]Division of Breast Surgery, Chiba Cancer Center, Chiba, [6]Division of Clinical Trials and Research and Department of Surgery, Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital, Tokyo, [7]Department of Pathology, Saitama Cancer Center, Saitama, [8]Division of Clinical Laboratory, National Cancer Center Hospital, [9]Division of Clinical Pathology, Cancer Institute of the Japanese Foundation for Cancer Research, [10]Department of Biostatistics/Epidemiology and Preventive Health Science, School of Health Science and Nursing, University of Tokyo, Tokyo and [11]Department of Surgery (Breast Surgery), Graduate School of Faculty of Medicine, Kyoto University, Kyoto, Japan

*For reprints and all correspondence: Hiroji Iwata, Department of Breast Oncology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. E-mail: hiwata@aichi-cc.jp

Received January 21, 2011; accepted April 24, 2011

Objective: This multicenter, open-label, single-arm, Phase II study assessed the efficacy of a neoadjuvant chemotherapy with docetaxel (75 mg/m^2 q3w) followed by 5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2 q3w in patients with early-stage breast cancer.

Methods: Women with resectable breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. Before surgery, patients received four cycles of docetaxel followed by four cycles of 5-fluorouracil, epirubicin, and cyclophosphamide. The primary endpoint was the pathological complete response (pCR) rate defined for the breast alone, assessed by a central review committee. Secondary endpoints included clinical response and safety.

Results: One hundred and thirty-seven patients were enrolled. Of the 132 patients assessable for pathologic response, 23% (95% confidence interval, 16–31%) experienced a pathological complete response and 6% (95% confidence interval, 3–12%) had a near pathological complete response (few remaining cancer cells), resulting in a quasi-pathological complete response of 29% (95% confidence interval, 21–37%). Clinical response rate following the initial docetaxel regimen was 64%. The overall clinical response rate after completion of 5-fluorouracil, epirubicin, and cyclophosphamide was 79%; breast-conserving surgery was performed in 79% of patients. More patients with triple-negative disease (estrogen/progesterone receptors negative; human epidermal growth factor 2 negative) experienced a pathological complete response [14/29, (48%); 95% confidence interval, 29–68%] versus those with other molecular subtypes. The safety profile was acceptable.

Conclusions: Eight cycles of neoadjuvant chemotherapy—docetaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide—are tolerable and conferred high rates of pathological complete response and breast-conserving surgery. Patients with triple-negative disease were more likely to achieve pathological complete response versus other subtypes, suggesting that selecting appropriate neoadjuvant chemotherapy based on molecular subtype could be possible.

Key words: breast neoplasms – neoadjuvant therapy – FEC protocol – docetaxel

INTRODUCTION

Neoadjuvant chemotherapy has been widely used for patients with operable breast cancer to increase the chance of breast conservation (1–7). Furthermore, response to neoadjuvant treatment can provide important information on long-term survival outcomes. Pathological complete response (pCR) in the breast and axillary lymph nodes predicts a favorable prognosis, whereas a lack of pCR in the breast and node-positive status do not (6,7). This implies the possibility of tailoring subsequent treatment according to the response to initial treatment (7–12). In addition, correlative studies of tumor samples before and after treatment may provide information on markers that could predict response or resistance to treatment (13–16).

Results from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18 trial demonstrated the impact of neoadjuvant chemotherapy in patients with operable early-stage breast cancer (17). The protocol-specified anthracycline-containing regimen—four cycles of doxorubicin and cyclophosphamide (AC)—resulted in an increased likelihood of breast-conserving surgery (BCS) compared with no neoadjuvant chemotherapy. The study established pCR as a prognostic marker for long-term disease-free survival (DFS) and demonstrated that there was no difference in survival if chemotherapy was administered before or after surgery. Subsequent studies, such as the Aberdeen trial, have demonstrated the benefit of the sequential addition of taxanes to neoadjuvant anthracycline regimens (5). The NSABP Protocol B-27 trial demonstrated that, compared with neoadjuvant AC alone, the addition of sequential docetaxel doubled the pCR rate, increased the clinical complete response rate (RR) and increased the proportion of patients with negative axillary nodes (7–18).

We previously conducted a Phase II study to evaluate the clinical and pathological response and safety of the FEC regimen (5-fluorouracil, epirubicin and cyclophosphamide) followed by docetaxel as neoadjuvant chemotherapy in Japanese women with early-stage breast cancer [Japan Breast Cancer Research Group (JBCRG) 01 trial]. The results of this study have been reported previously (19). Although the pCR rate was 16% and BCS was possible for 85% of patients, there were some safety concerns, with 18% of patients experiencing febrile neutropenia and 41% of patients experiencing Grade 1/2 peripheral edema (no Grade 3/4 events observed) following the docetaxel regimen (unpublished data). Disease progression occurred in 6% of patients after the completion of all planned treatment (unpublished data).

In an effort to achieve a higher pathological RR with an improved safety profile, we decided to evaluate the efficacy and safety of docetaxel followed by FEC (JBCRG 03 trial)—the reverse of the sequence of chemotherapy used in the JBCRG 01 trial (19). The clinical and pathological effects and the toxicity profile of this regimen are presented here, and the results of predictive marker analyses are discussed.

PATIENTS AND METHODS

Patient Eligibility

This was a multicenter, open-label, single-arm, Phase II study that recruited patients via central registration. Japanese women aged 20–59 years with histologically proven early-stage breast cancer (T1c–3 N0 M0 or T1–3 N1 M0) were enrolled. No prior chemotherapy, radiotherapy, hormonal therapy or immunotherapy was allowed. Other inclusion criteria were Eastern Cooperative Oncology Group performance status 0–1; white blood cell count 4000–12 000/mm^3; neutrophil count $\geq$ 2000/mm^3; platelet count $\geq$ 100 000/mm^3; hemoglobin $\geq$ 9.5 g/dl; serum bilirubin $\leq$ 1.25 times upper limit of normal (ULN); creatinine $\leq$ 1.5 times ULN and aspartate aminotransferase and alanine aminotransferase $\leq$ 1.5 times ULN. Patients with congestive heart failure or left ventricular ejection fraction $\leq$60% were excluded. Patients were also excluded if they had confirmed infection; serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension or hemorrhagic disease; active concomitant malignancy; brain metastasis; peripheral neuropathy; history of edema with severe drug allergy; or previous long-term corticosteroid therapy. Pregnant or lactating women were excluded. Mammography, ultrasonography, magnetic resonance imaging or computed tomography was used to assess the presence of tumors. Baseline evaluations included complete blood cell and platelet count, routine blood chemistry and liver function tests, chest X-ray, bone scan, electrocardiogram and echocardiogram.

The local ethics committee or institutional review board approved the study at each institution. All patients gave written informed consent to participate. The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Treatment

Four cycles of docetaxel (75 mg/m^2) administered intravenously (i.v.) every 21 days were followed by four cycles of FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamide 500 mg/m^2) administered i.v. on Day 1 every 21 days before surgery. Premedication was administered based upon each physician's decision to prevent edema, nausea and allergic reactions (e.g. dexamethasone 12 mg i.v. and/or granisetron 4 mg i.v. on Day 1, and oral dexamethasone 8 mg on Days 2 and 3 of docetaxel treatment; dexamethasone 24 mg i.v. on Day 1 and oral dexamethasone 8 mg on Days 2–6 with the FEC regimen). Administration of granulocyte colony-stimulating factor and antibiotics was left to the judgment of each investigator.

Clinical Response Assessment

Tumor assessments were performed within 4 weeks before docetaxel treatment, after completion of docetaxel treatment

and before surgery. Tumor response was assessed using the modified Response Evaluation Criteria in Solid Tumors guidelines (in which confirmatory scans/assessments were not required due to the timing of surgery), for patients who had measurable lesions.

Central Pathologic Assessment

Hematoxylin and eosin-stained slides were prepared from core needle biopsy and surgical specimens from the primary tumor. All surgical specimens were cut in 5 mm interval and all surfaces were microscopically examined in each institution. Pathological response of chemotherapy was assessed by a central review committee consisting of three pathologists who used criteria established by the Japanese Breast Cancer Society. pCR was defined as necrosis and/or disappearance of all tumor cells, and/or the replacement of cancer cells by granulation and/or fibrosis. If only ductal components remained, the pathological response was described as a pCR. Near pCR was defined as extremely high grade marked changes approaching a complete response, with only a few remaining isolated cancer cells (19). Quasi-pCR (QpCR) was the total of both pCR and near pCR. The central review committee evaluated the pathological responses independently from local pathologists. This committee was blinded to the local pathologists' reports. Patients who did not have surgery because of disease progression were considered not to have a pCR.

Hormone Receptor and Human Epidermal Growth Factor 2 Overexpression

Estrogen receptor (ER) and progesterone receptor (PgR) status was determined by immunohistochemistry (IHC) before docetaxel treatment at each participating institute.In general, tumors with more than 10% positively stained tumor cells were classified as positive for ER and PgR. The human epidermal growth factor 2 (HER2) status of the tumor was also determined at each institute by IHC or by fluorescence *in situ* hybridization (FISH) analysis. HER2-positive tumors were defined as those scoring 3+ with IHC staining or testing positive by FISH. HER2-negative tumors were defined as those scoring 0−1+ with IHC or scoring 2+ with IHC and testing negative by FISH.

Surgery and Radiotherapy

Following chemotherapy and clinical assessment of response, patients underwent surgery. If the tumor was too large or invasive for BCS, a modified radical mastectomy was recommended. Careful pathological assessment of tumor margins was performed in accordance with the Japanese Breast Cancer Society criteria (20). Sentinel lymph node biopsy was performed to confirm disease stage or to avoid surgical axillary dissection. Autologous or heterologous reconstructive surgery was performed depending on the patient's requirements and health status. All patients who underwent BCS were given standard radiotherapy to the remaining ipsilateral breast tissue after surgical recovery. For patients diagnosed as sentinel node negative and thus not requiring axillary dissection; radiotherapy to the axilla was allowed.

Toxicity and Dose Modification

Toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3) throughout treatment with docetaxel and FEC before surgery. Treatment could be postponed for a maximum of 2 weeks only for severe toxicity. If the adverse event (AE) did not improve during this period, chemotherapy was discontinued and surgery was recommended. Dose reductions were permitted for docetaxel from 75 to 60 mg/m^2 and for epirubicin from 100 to 75 mg/m^2 in cases of febrile neutropenia or Grade 3/4 non-hematologic toxicities, except for nausea, vomiting and fatigue.

Statistical Methods

The primary endpoint was the pCR rate. Before the initiation of the current study, the pCR rate for non-taxane anthracycline regimens ranged from 12.8% (NSABP Protocol B-27) (18) to 15.4% (Aberdeen trial) (5). Previously, we had conducted JBCRG01 trial to evaluate the pCR rate defined for breast disease (19). Therefore, in order to detect improvement in the pCR rate in the same definition of our previous study, a sample of 119 patients was required according to binominal distribution, with a one-sided threshold pCR rate of 12%, an expected pCR rate of 22%, an α error of 5% and a β error of 10%. The target number of patients for recruitment was therefore 119, so assuming that 5% of patients would not be evaluable, we planned to enroll 130 patients. Secondary endpoints included safety, clinical RR, rate of BCS, DFS, overall survival and a subset analysis according to biomarkers. Pathological and clinical RRs were calculated with 95% confidence intervals (95% CIs), with each complete RR based on a binominal distribution. Pathological response was evaluated by hormone receptor status and HER2 status. A multiple logistic regression analysis was performed to examine which factors (menopausal status, tumor size, ER and PgR status, HER2 status and clinical response to docetaxel and FEC) were associated with pCR and QpCR.

RESULTS

Patients Characteristics and Treatment

Enrollment took place from October 2005 through October 2006. One hundred and thirty-seven patients were enrolled. Two patients did not receive study treatment because of early withdrawal of consent; therefore, 135 patients were evaluable for safety and clinical response. These evaluable

Table 1. Patients' characteristics

Characteristic	Value[a]
Number of evaluable[b] patients	135
Age (years)	
Median	46
Range	24–62
Performance status, *n* (%)	
0	133 (99)
1	2 (1)
Menopausal status, *n* (%)	
Premenopausal	94 (70)
Postmenopausal	41 (30)
Clinical tumor stage, *n* (%)	
T1	13 (10)
T2	98 (73)
T3	24 (18)
Clinical nodal stage, *n* (%)	
N0	62 (46)
N1	73 (54)
ER status, *n* (%)	
Positive	86 (64)
Negative	46 (34)
Unknown	3 (2)
PgR status, *n* (%)	
Positive	63 (47)
Negative	70 (52)
Unknown	2 (1)
HER2 status,[c] *n* (%)	
0	21 (16)
1+	63 (47)
2+	20 (15)
3+	31 (23)

ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PgR, progesterone receptor.
[a]Percentages may not add up to 100% because of rounding.
[b]Number of patients evaluable for safety and clinical response.
[c]Evaluated by immunohistochemistry.

patients included two patients aged 60 and 62 years (included because their age was not considered to influence the evaluation). Two patients were lost to follow-up before surgery, thus 133 patients were evaluable for surgical response. A total of 132 patients were evaluable for pathological response; one patient was excluded owing to lack of confirmation of invasive carcinoma (following the pathologic central review) due to inadequate samples from core needle biopsy before study treatment.

The patient characteristics are summarized in Table 1. Thirty patients (22%) had triple-negative disease, defined as ER-negative, PgR-negative and HER2-negative primary breast cancer, including one patient who was lost to follow-up before surgery.

Overall, 98 patients (73%) completed the planned eight cycles of treatment without dose reductions or study discontinuation. A total of 115 (85%) and 106 (82%) patients completed all four planned treatment cycles of docetaxel and FEC, respectively; dose reductions were necessary in 9 (7%) and 17 (13%) patients, respectively. The majority of the dose reductions were attributable to toxicities, particularly febrile neutropenia during treatment with FEC (10 versus 2 patients during docetaxel treatment). Dose reductions due to neutropenia were required by three patients each during the docetaxel and FEC regimens. Eleven (8%) and six patients (5%), respectively, discontinued treatment during docetaxel and FEC therapy because of toxicities (five patients discontinued during both regimens) or disease progression (six patients during docetaxel and one patient during FEC). The mean dose intensities were 24.2 and 30.3 mg/m^2/week for docetaxel and epirubicin, respectively.

Toxicities

The incidence of treatment-related AEs is summarized in Table 2. Neutropenia was the most common Grade 3/4 treatment-related AE and was observed in 44% and 60% of patients during docetaxel and FEC therapy, respectively. Overall, 67% and 15% of patients experienced at least one episode of Grade 3/4 neutropenia or febrile neutropenia, respectively. For non-hematologic toxicities of any grade, rash, sensory neuropathy, edema, muscle pain and joint pain occurred more frequently during docetaxel treatment than with FEC. Conversely, the frequency of gastrointestinal symptoms, such as nausea, vomiting and anorexia, was higher with FEC than with docetaxel. The frequency of Grade 1/2 peripheral edema was similar during exposure to docetaxel (33%) and FEC (29%); no patient had Grade 3/4 edema. Grade 3/4 non-hematologic toxicities, including gastrointestinal disturbances, were infrequent during both docetaxel and FEC. No fatal AEs were reported.

Clinical Response to Treatment

The overall clinical RR was 79% (106/135; 95% CI, 71–85%), with a clinical complete RR of 21% (29/135), a partial RR of 57% (77/135) and a disease progression rate of 5% (7/135). The clinical RR following the initial docetaxel regimen was 64%. The clinical responses to treatment with docetaxel followed by FEC according to response to initial docetaxel are shown in Table 3. Eight of the 135 patients (6%) progressed during docetaxel administration; 2 of 135 patients (1%) had disease progression during FEC. Of the 30 patients with triple-negative disease, 7 patients were observed to have disease progression following docetaxel treatment. One of the 17 patients with ER-positive, PgR-negative and HER2-negative tumors had disease

Table 2. Treatment-related adverse events

Adverse event, *n* (%)	DOC ($n = 135$)		FEC ($n = 29$)		Overall ($n = 35$)	
	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4
Non-hematologic toxicities						
Infection with neutropenia	6 (4)	2 (1)	3 (2)	2 (2)	9 (7)	4 (3)
Fever	15 (11)	0	13 (10)	1 (1)	22 (16)	1 (1)
Infection (other)	3 (2)	1 (1)	2 (2)	0	4 (3)	1 (1)
Fatigue	82 (61)	0	84 (65)	2 (2)	98 (73)	2 (1)
Nausea	52 (39)	1 (1)	102 (79)	3 (2)	108 (80)	4 (3)
Vomiting	19 (14)	1 (1)	51 (40)	3 (2)	61 (45)	4 (3)
Anorexia	53 (39)	1 (1)	86 (67)	2 (2)	91 (67)	2 (1)
Stomatitis	50 (37)	1 (1)	51 (40)	0	68 (50)	1 (1)
Diarrhea	39 (29)	1 (1)	20 (16)	0	46 (34)	1 (1)
Phlebitis	2 (1)	1 (1)	2 (2)	0	4 (3)	1 (1)
Alanine aminotransferase	36 (27)	0	50 (39)	2 (2)	57 (42)	2 (1)
Aspartate aminotransferase	19 (14)	0	34 (26)	1 (1)	40 (30)	1 (1)
Nail changes	2 (1)	0	33 (26)	1 (1)	33 (24)	1 (1)
Weight loss	5 (4)	0	6 (5)	1 (1)	8 (6)	1 (1)
Creatinine	4 (3)	1 (1)	6 (5)	0	7 (5)	1 (1)
Edema	44 (33)	0	37 (29)	0	55 (41)	0
Hematologic toxicities						
Neutropenia	60 (44)	59 (44)	91 (71)	77 (60)	100 (74)	91 (67)
Leukopenia	69 (51)	50 (37)	101 (78)	66 (51)	108 (80)	76 (56)
Thrombocytopenia	13 (10)	0	28 (22)	2 (2)	31 (23)	1 (1)
Anemia	66 (49)	0	99 (77)	1 (1)	106 (79)	1 (1)
Febrile neutropenia	9 (7)	9 (7)	15 (12)	15 (12)	20 (15)	20 (15)

DOC, docetaxel; FEC, 5-fluorouracil, epirubicin and cyclophosphamide.

Table 3. Clinical response to DOC followed by FEC according to response to initial DOC treatment ($n = 135$)

Clinical response,[a] *n* (%)	Total[b]	Responder	Non-responder
Response to DOC			
Responder	87 (64)	79 (58)	8 (6)
Non-responder	48 (36)	27 (20)	21 (16)

[a]Overall response was confirmed after completion of chemotherapy in comparison with before docetaxel treatment.
[b]Percent value of each column was calculated by dividing by the total number of the evaluable patients ($n = 135$).

progression; while of the 53 patients with ER-positive, PgR-positive, and HER2-negative tumors and of the 9 patients with ER-positive, PgR-positive, and HER2-positive tumors, no patient had disease progression during docetaxel treatment. Among those with triple-negative disease, the majority of patients with disease progression after initial docetaxel were premenopausal [6/7 patients (86%)] and had solid-tubular carcinoma which characterized by solid cluster of cancer cells with expansive growth forming sharp borders [4/7 patients (57%)], as assessed using the Japanese Breast Cancer Society histological classification of breast tumors (21) (Table 4). Excluding the differences outlined above, there were no differences between patient and tumor characteristics for those with progressive disease versus non-progressive disease.

Twenty-seven of 48 non-responders to docetaxel (56%) had a response to FEC treatment; however, 8 of 87 responders to docetaxel (9%) showed no improvement in response with FEC treatment. Following chemotherapy, BCS was performed for 105 of 133 assessable patients (79%).

Pathological Response and Predictive Factors to Treatment

The primary endpoint—pCR rate—was 23% (95% CI, 16–31%). A near pCR rate of 6% (95% CI, 3–12%) resulted

Table 4. Clinical and pathologic characteristics of triple-negative breast cancer[a] for patients with progressive disease versus patients without progressive disease, following initial docetaxel therapy

Characteristic	Without PD	PD
No. of evaluable patients	23	7
Age, years		
Median	43	46
Range	(30–62)	(29–53)
Menopausal status, *n* (%)		
Premenopausal	15 (65)	6 (86)
Postmenopausal	8 (35)	1 (14)
Tumor stage		
T1	2 (9)	0
T2	14 (61)	5 (71)
T3	7 (30)	2 (29)
Nodal stage, *n* (%)		
N0	13 (57)	3 (43)
N1	10 (43)	4 (57)
Tumor type, *n* (%)		
Solid-tubular carcinoma	6 (26)	4 (57)
Papillotubular carcinoma	5 (22)	3 (43)
Scirrhous carcinoma	3 (13)	0
Unspecified invasive carcinoma	9 (39)	0

PD, progressive disease.
[a]Triple-negative tumors were defined as ER-negative, PgR-negative and HER2-negative primary breast cancer.

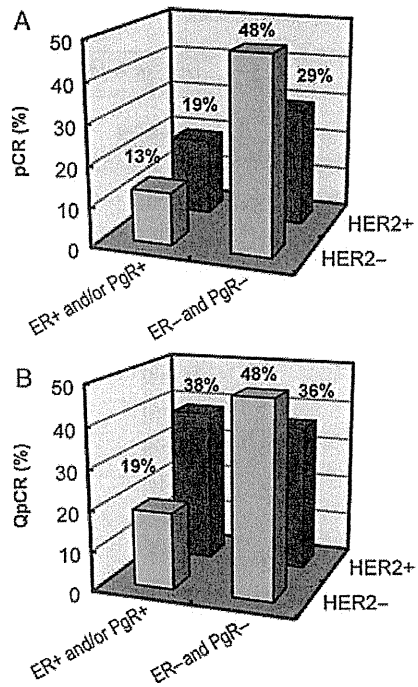

Figure 1. (A) Relationship between pCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). (B) Relationship between QpCR versus HER2 and ER/PgR status following DOC and FEC ($n = 129$). Three patients were excluded from evaluable patients for pathologic response ($n = 132$) because of their unknown hormone receptor status. There were no near pCR case observed in triple-negative (ER−, PgR− and HER2−) diseases. DOC, docetaxel; ER, estrogen receptor; FEC, 5-fluorouracil, epirubicin, and cyclophosphamide; HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response; PgR, progesterone receptor; QpCR, quasi-pathologic complete response.

in a QpCR rate of 29% (95% CI, 21–37%) when combined with the pCR. Pathological response of each subset population according to their hormone receptor and HER2 status is summarized in Fig. 1A and B. Patients with triple-negative disease had the highest pCR rate of 48% (95% CI, 29–68%). Near pCR was not observed in triple-negative disease. Patients with HER2-positive, ER-negative and PgR-negative tumors had a pCR rate of 29% (95% CI, 8–58%) and a QpCR rate of 36% (95% CI, 13–65%); patients with HER2-positive and ER-positive and/or PgR-positive tumors had a pCR rate of 19% (95% CI, 4–46%) and a QpCR rate of 38% (95% CI, 15–65%). Patients with HER2-negative and ER-positive and/or PgR-positive tumors had the lowest pCR and QpCR rates (13%; 95% CI, 6–23% and 19%; 95% CI, 10–30%, respectively). One of the seven patients who experienced clinical disease progression with initial docetaxel treatment had a QpCR following FEC.

The relationship between tumor pathological feature and pCR rate is shown in Table 5. The only variable found to be significantly associated with a pCR after docetaxel treatment was ER status.

Survival outcomes will be reported when the 5-year follow-up has been completed for this study.

DISCUSSION

This is the first report to evaluate the effectiveness of an initial docetaxel regimen for neoadjuvant therapy of Japanese patients with early-stage breast cancer. An additional component of the study was to analyze the data according to hormone receptor and HER2 status. Recently, Wildiers et al. (22) reviewed four adjuvant trials which had demonstrated the taxane-first regimens were favorable in terms of the relative drug dose intensity achieved. Also they mentioned larger non-randomized adjuvant studies for a series of 284 patients who first received three cycles of FEC followed by three cycles of docetaxel, the mean relative dose intensity was 91% for FEC and 76% for docetaxel, whereas in another series of 378 patients who received three cycles of docetaxel followed by four cycles of EC (epirubicin plus cyclophosphamide), a median docetaxel dose intensity of 100% was achieved. Therefore, they concluded such data suggest that the administration of a taxane first, followed by an anthracycline, may be preferable in line with the Norton–Simon hypothesis (23). In the JBCRG 01 study, the largest study to date to evaluate neoadjuvant chemotherapy in this patient population, the clinical and pathological responses

Table 5. Predictive variables for pCR before and following chemotherapy

Variables	Before treatment			After DOC			After FEC following DOC		
	OR	95% CI	*P* value	OR	95% CI	*P* value	OR	95% CI	*P* value
Menopausal status: pre (versus post)	1.5	0.94–2.40	0.0923	1.52	0.94–2.47	0.0867	1.42	0.87–2.31	0.1575
Tumor size: ≥3 cm (versus <3 cm)	1.51	0.94–2.41	0.0881	1.45	0.90–2.34	0.1266	1.56	0.96–2.52	0.0724
ER: negative (versus positive)	0.58	0.32–1.03	0.0650	0.51	0.28–0.95	0.0331	0.58	0.32–1.05	0.0709
PgR: negative (versus positive)	0.66	0.34–1.28	0.2211	0.72	0.37–0.95	0.3408	0.65	0.33–1.27	0.2083
HER2: 3+ (versus <3+)	1.32	0.76–2.28	0.3251	1.41	0.80–2.47	0.2360	1.39	0.80–2.41	0.2445
Clinical response to DOC									
Response (versus no response)	—	—	—	0.64	0.38–1.07	0.0875	—	—	—
Clinical response to FEC following DOC									
Response (versus no response)	—	—	—	—	—	—	0.58	0.29–1.14	0.1160

CI, confidence interval; OR, odds ratio; pCR, pathologic complete response.

and safety of FEC followed by docetaxel were investigated (19). The eligibility criteria, treatment dose and distribution of patient characteristics (menopausal status, tumor stage, hormone receptor status and HER2 status) studied in the JBCRG 01 trial were similar to those investigated in the present JBCRG 03 study (19). The incidences of Grade 3/4 neutropenia and febrile neutropenia observed in the current study were similar to those reported in the JBCRG 01 trial (19). However, the rate of Grade 1/2 edema during docetaxel treatment was lower in the present study (33%) than in the JBCRG 01 study (41%), suggesting that docetaxel might be better tolerated when given up front than when administered after completion of prior chemotherapy. Further studies are warranted to assess quality of life and the incidence of edema in order to confirm the effect of administering docetaxel as the initial therapy.

Many different neoadjuvant chemotherapy schedules and dose regimens are used in clinical practice. The NSABP Protocol B-18 trial, which compared AC treatment before and after surgery, reported no difference in DFS between the two approaches (17). However, the rate of BCS was greater with neoadjuvant AC chemotherapy, and the prognosis of patients who obtained a pCR was also better with this treatment regimen (17). Several other regimens have been evaluated in an effort to increase the pCR rate. The addition of a taxane to an anthracycline-containing regimen has been shown to improve the pCR and clinical RRs (5,18). Furthermore, excellent results have been reported by the MD Anderson Cancer Center using a regimen of paclitaxel plus trastuzumab followed by FEC plus trastuzumab in patients with operable breast cancer and HER2 overexpression (24). However, few studies have evaluated initial taxane therapy followed by an anthracycline-containing regimen in this indication (24). Thus, it was decided to evaluate such a reverse regimen and to analyze the findings according to molecular subtypes. Importantly, the primary endpoint—pCR rate—achieved in the present study was 23% (95% CI, 16–31%), far exceeding our estimate of 12% (19). Even though the pCR rate here cannot be directly compared with the results from the JBCRG 01 trial (pCR rate: 12%, QpCR rate: 25%), the pCR rate from this study is a favorable result considering the similar patient characteristics in both trials (19).

The overall clinical RR of 79% was similar to that reported in the JBCRG 01 trial (74%) (19). Furthermore, the clinical RR following the initial docetaxel regimen was 64%, similar to the clinical response following the initial FEC regimen in the JBCRG 01 trial (61%) (19). The clinical RR following the initial docetaxel regimen, however, is lower in this study than those reported in other studies (71.7–85%) (25,26). It could be hypothesized that the clinical response might be influenced by the lower dose of docetaxel used in this study (75 mg/m^2) compared with the 100 mg/m^2 dose used in previous studies (25,26).

The rate of BCS observed in our study (79%) was similar to that reported in the JBCRG 01 trial (85%) (19). Unfortunately, the overall disease progression rate (5%) was not lowered by the use of docetaxel followed by FEC in this study, and was similar to that seen in the JBCRG 01 trial (6%) (19).

Although 7 of the 29 patients with triple-negative disease had disease progression during the initial docetaxel regimen, 14 of the 22 patients without disease progression (64%) achieved a QpCR. This QpCR rate is markedly higher compared with previous findings (27).

Our results indicate that if patients with triple-negative disease who experienced disease progression following initial docetaxel therapy were excluded, the pCR rate for this group of patients would have been higher. We thus compared the clinical and pathological characteristics between patients with triple-negative disease who experienced disease progression following the initial docetaxel regimen with those who did not have disease progression. However, no

significant differences in patient or tumor characteristics were seen between these patient groups. It was noted, however, that six of seven premenopausal patients (86%) and four of seven patients (57%) with solid-tubular carcinoma had disease progression following docetaxel therapy. Given the high incidence of disease progression among patients with triple-negative disease who had solid-tubular subtype tumors, this phenotype could be used in future studies to predict which patients are more likely to experience progressive disease following docetaxel therapy. Accordingly, the identification of patients with hormone receptor-positive and HER2-negative disease would also enable the selection of patients who are more likely to benefit from neoadjuvant chemotherapy. Thus, studying patients' molecular subtypes, and selecting appropriate chemotherapy regimens accordingly, has the potential to provide superior results to those of the JBCRG 03 trial.

Recently, it has been shown that basal-like breast cancer defined by five biomarkers [epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), ER, PgR and HER2 status] provides a more specific definition of basal-like breast cancer that predicts survival better than the triple-negative phenotype (27,28). In patients treated with anthracycline-based chemotherapy, tumors found to be positive for the basal markers corresponded to a cohort of patients with a significantly worse outcome (29). Thus in future trials, it may be beneficial to assess EGFR and CK5/6 status in patients with triple-negative disease to help predict patient survival.

Interestingly, the pCR rate (27%) following neoadjuvant chemotherapy in patients with HER2-negative breast cancer was higher in this study than in the JBCRG 01 study (14%), suggesting that this subpopulation may benefit from initial docetaxel treatment. Conversely, a lower QpCR rate was observed in HER2-positive patients (37%) in this study than in the JBCRG 01 trial (52.8%). This suggests that initial anthracyclines may be required for HER2-positive disease. A study by Buzdar et al. (24) reported that a high pCR rate of 60% was observed in patients with HER2-positive disease treated with the combination of paclitaxel plus trastuzumab followed by FEC plus trastuzumab, indicating that the HER2-positive population in the current study may have benefited further from concomitant trastuzumab therapy. These findings demonstrate the benefit of selecting the most effective chemotherapy regimen according to each patient's molecular subtype and initial response to neoadjuvant treatment.

One limitation of the study was that HER2-positive patients were not treated with trastuzumab, which has been shown to improve outcomes in patients with HER2-overexpressing breast cancer (24). Further studies investigating optimal treatment regimens for different molecular subtypes should include concurrent trastuzumab for patients with the HER2-positive phenotype.

In conclusion, docetaxel followed by FEC as neoadjuvant chemotherapy is a tolerable and effective regimen for patients with early-stage breast cancer. In addition, a high pCR rate made this regimen particularly promising in patients with triple-negative breast cancer. In the future, selection of a neoadjuvant chemotherapy regimen for operable breast cancer may be possible based on molecular subtype.

Acknowledgements

Editorial support was provided by sanofi-aventis. We wish to thank the patients who participated in the JBCRG 03 clinical trial. We also thank Drs Takashi Inamoto (Department of Breast Surgery, Kitano Hospital, Osaka, Japan), Akira Yamauchi (Department of Medicine for Cell Control, Faculty of Medicine, Kagawa University, Kagawa, Japan) and Hiroshi Ishiguro (Division of Clinical Trial Design and Management, Translational Research Center, Kyoto University, Kyoto, Japan) for their helpful advice.

Funding

This work was supported by Advanced Clinical Research Organization (http://www.npo-acro.jp/). Grant number 0520.

Conflict of interest statement

Dr Yasuo Ohashi received honorarium from Sanofi Aventis for lectures.

References

1. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol* 1999;17:460–9.
2. Kaufmann M, Hortobagyi GN, Goldhirsch A, Scholl S, Makris A, Valagussa P, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update. *J Clin Oncol* 2006;24:1940–9.
3. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thurlimann B, Senn HJ. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. *Ann Oncol* 2005;16:1569–83.
4. Mauriac L, MacGrogan G, Avril A, Durand M, Floquet A, Debled M, et al. Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: a unicentre randomized trial with a 124-month median follow-up. Institut Bergonie Bordeaux Groupe Sein (IBBGS). *Ann Oncol* 1999;10:47–52.
5. Smith IC, Heys SD, Hutcheon AW, Miller ID, Payne S, Gilbert FJ, et al. Neoadjuvant chemotherapy in breast cancer: significantly enhanced response with docetaxel. *J Clin Oncol* 2002;20:1456–66.
6. Wolmark N, Wang J, Mamounas E, Bryant 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;30:96–102.
7. Bear HD, Anderson S, Smith RE, Geyer CE, Jr, Mamounas EP, Fisher B, et al. Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol* 2006;24:2019–27.