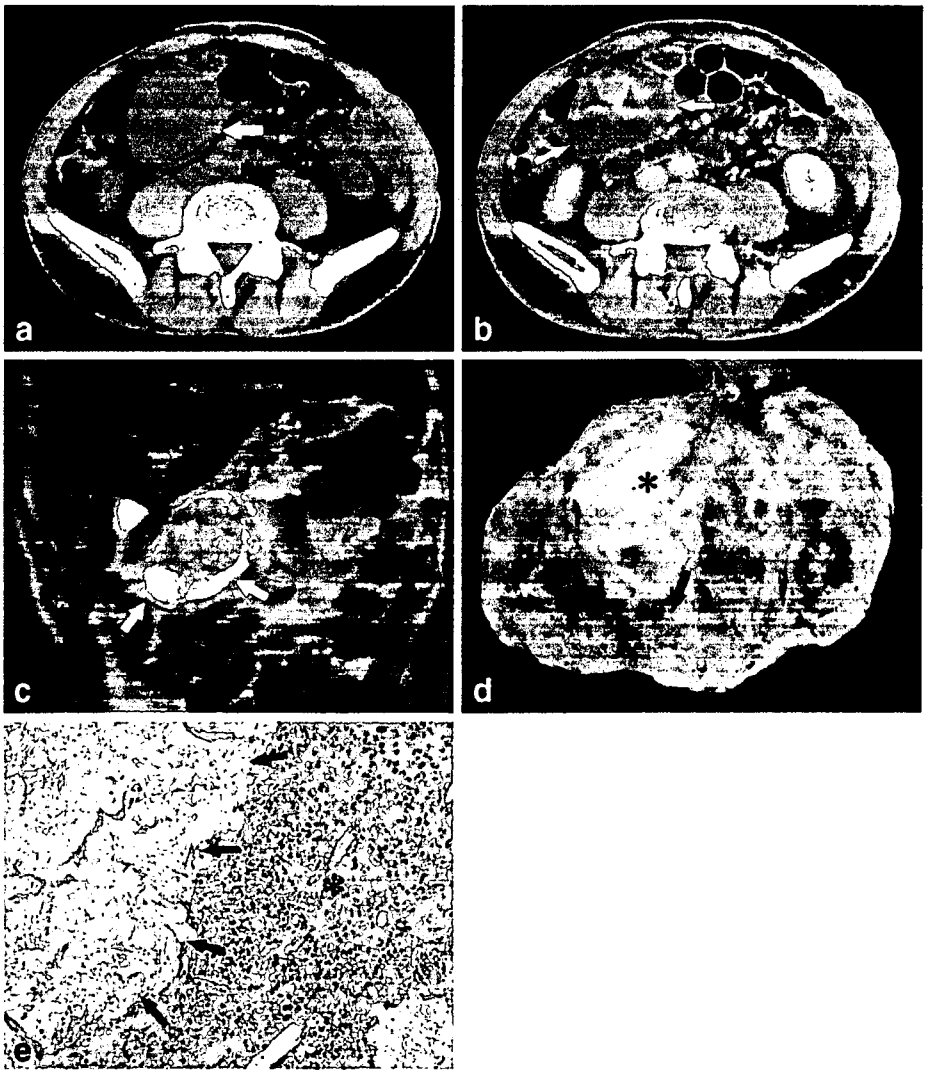

Fig. 1 A 69-year-old man with epigastric discomfort. **a** Unenhanced CT shows heterogeneous antral mass (*arrow*). **b** Contrast-enhanced CT reveals a mass in antrum with heterogeneous enhancement (*arrow*). **c** Coronal T2-weighted fast spin echo MRI (TR/TE_{eff}, 12,000 ms/80 ms) shows a cystic components with significantly high signal intensity (*arrows*). **d** Photograph of the gross specimen shows cystic components (*arrows*) and soft tissue elements (*asterisk*) in an antral mass. **e** Photomicrograph of the tumor shows oval epithelioid cells containing eosinophilic cytoplasm and peripherally placed nuclei (asterisk) with an abundant myxoid background (*arrows*). Hematoxylin and eosin stain; original magnification ×100

cases and inadequate in one case. In the patient with inadequate surgical margins wide resection was performed subsequently. One patient who developed peritoneal dissemination received oral imatinib mesylate therapy (400 mg per day) because the pathological examination of the resected specimens revealed weak-positive immunostaining for KIT.

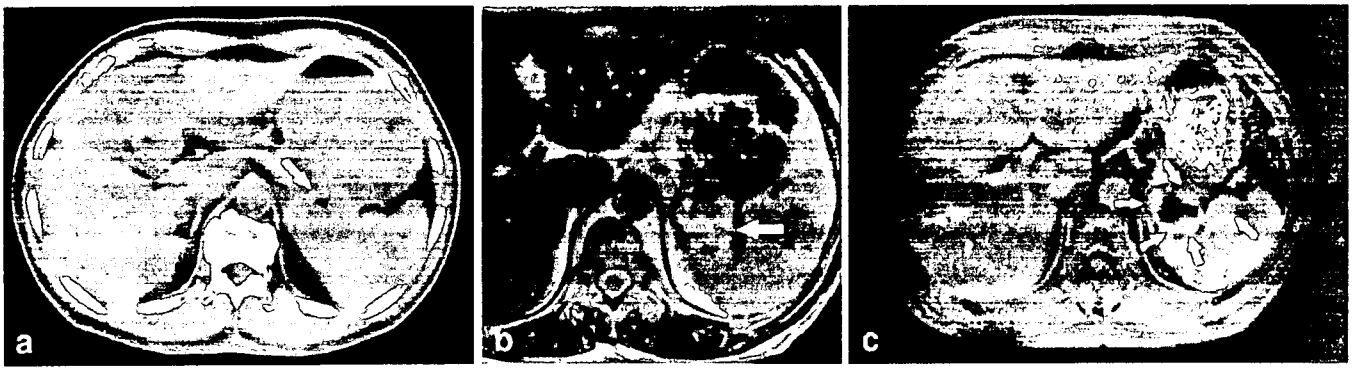

Fig. 2 A 72-year-old man with abdominal pain. **a** Unenhanced CT shows a heterogeneous mass in the gastric body that contains central gas (*arrow*). **b** Transverse T2-weghted fast spin echo MRI (2550/120) shows central cystic regions of high signal intensity interspersed with septumlike structures of low signal intensity (*arrow*). **c** Transverse fat saturated contrast-enhanced T1-weighted SE MRI (400/8.9) shows heterogeneously mild to moderate enhancement of soft tissue elements (*arrows*) and lack of obvious enhancement of the cystic region

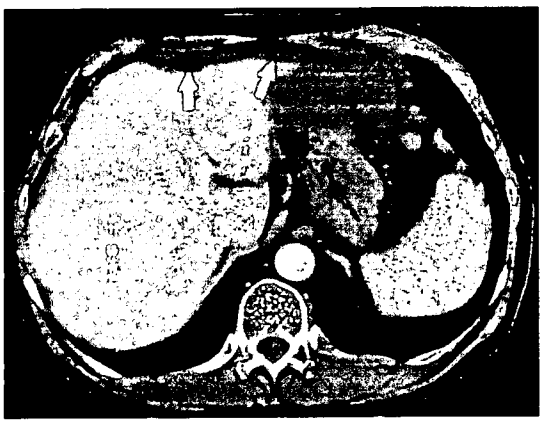

Fig. 3 A 52-year-old man with abdominal pain. Contrast-enhanced CT scan shows masses with significantly decreased density in the gastric body and hepatic surface consistent with peritoneal dissemination (*arrows*)

In this patient mild remission was observed for 16 months, but tumor regrowth was identified 18 months after the initial administration of imatinib mesylate. Tumor showed enlarged cystic mass and soft tissue elements with pronounced enhancement on CT. The median follow-up period of all cases was 65 months. Two other patients developed peritoneal dissemination after initial resection, but did not received imatinib mesylate because the pathological examination showed negative immunostaining for KIT. Recurrent tumors of these two patients also showed cystic mass and soft tissue elements with enhancement on CT. None of the cases developed visceral metastases. One patient with peritoneal disemination has died of the disease, and nine patients are alive with disease and seven with no evidence of disease.

Discussion

GISTs are generally characterized by genetic mutations of c-kit and highly sensitive to immunostaining of KIT which can be distinguished from other mesenchymal tumors of gastrointestinal tract. Recent advances in molecular targeting therapy reveals that imatinib mesylate (Gleevec, formerly known as STI571), which is a KIT-selective tyrosine kinase inhibitor, has a significant efficacy against metastatic or unresectable lesions in patients with KIT-positive GISTs [4, 5]. On the other hand, recent pathological studies have shown that the existence of KIT-weak or KIT-negative GISTs [12–16]. While making a correct pathological diagnosis is not usually a problem in patients with KIT-positive GISTs, it might be more difficult to establish a definite diagnosis in patients with KIT-weak or KIT-negative tumors.

Most atypical GISTs occur in the stomach, omentum, or mesentery [12–16]. Tumors arising from the duodenum and small intestine are less frequent than conventional GISTs. In our study seven tumors were extraluminal masses arising from the gastric body. The gastrohepatic ligament or gastrosplenic ligament was involved in many of them.

The imaging features of atypical GISTs reflect the underlying pathological findings. CT and MRI findings included a large extraluminal mass with heterogeneous lesion containing cystic regions and soft tissue elements. Cross-sectional imaging clearly depicted the gastric origin, extraluminal epicenter, and intrinsic characteristics of the tumors, and they corresponded to the pathological composition of the lesion. CT and MR images demonstrated cystic regions in all tumors of our series, with various degrees of soft tissue elements. Conventional GISTs appear an ill-defined mass with extrinsic or extraluminal epicenter, and internal areas of hemorrhage, necrosis, and cystic change are frequent causes of density or signal changes on CT and MRI [6–10]. Sandrasegaran and colleagues [6] reported that central hypoattenuation due to necrosis or apoptosis was identified in 33% of conventional gastric GISTs. Larger tumors may undergo massive liquefactive necrosis and cystic change leaving a rim of viable tissue.

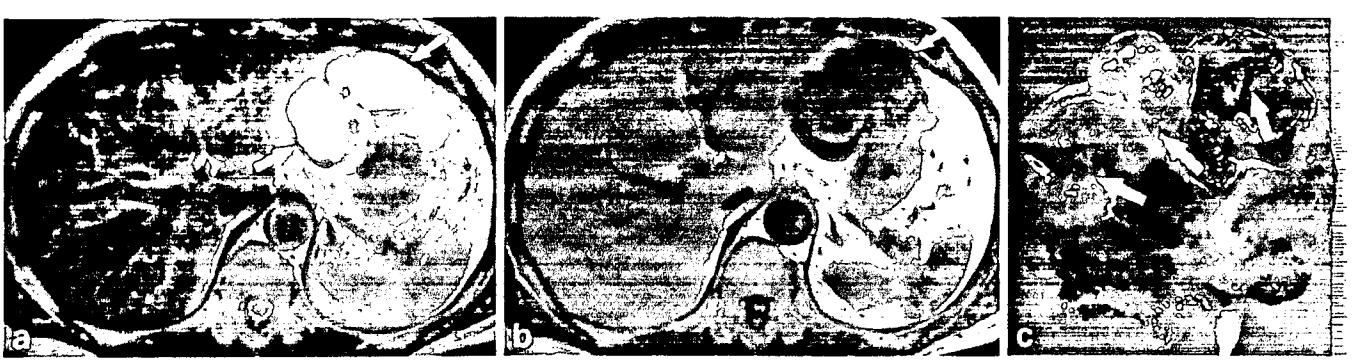

Fig. 4 A 60-year-old man with nausea and vomiting. **a** Transverse T2-weighted fast spin echo MRI (4000/112) shows a predominantly cystic mass (*arrows*) in the body of stomach with no evidence of infiltration. **b** Transverse T1-weighted SE MR image (550/15) shows soft tissue elements with homogeneously low signal intensity (*arrow*). **c** Photograph of gross pathology specimen shows cystic regions (*arrows*)

Therefore it is impossible to diagnose atypical GISTs from conventional GISTs based on CT and MRI findings. Imaging features of cystic regions and soft tissue elements are not specific but characteristic in atypical GISTs.

Myxoid degeneration or cystic change in tumors can occur after the treatment with imatinib mesylate which is one of the molecular-targeted chemotherapeutic agent and tyrosine kinase inhibitor [21–23]. Active metabolites of imatinib mesylate block the adenosine triphosphate binding site of tyrosine kinase. Morphological response to imatinib mesylate therapy has been reported in a tendency toward liquefaction before the tumor diminishes in size [21]. Choi and colleagues [22] described that the mean tumor density on CT decreased significantly after 2 months of treatment with imatinib mesylate compared with baseline. Pathological examination revealed a significant reduction in number of tumor cells and a hypocellular myxohyaline stroma. Myxoid or cystic change after treatment of imatinib mesylate often accompanied by a rim of soft tissue attenuation elements on CT and MRI. It is usually not possible to radiologically distinguish atypical GISTs from myxoid or cystic tumors treated by imatinib mesylate.

It may be important to make the distinction of atypical GIST from conventional GIST because of the difference in clinical behavior and patient prognosis. Sakurai and colleagues [16] reported 30 patients with atypical GISTs and 24 alive patients with mean follow-up period of 62 months. In our study only one patient died in a long period of follow-up and nondeveloped distant metastases except peritoneal dissemination. This evidence suggests that atypical GISTs have better prognosis than conventional GISTs. A recent study has revealed that imatinib mesylate can also bind and inhibit PDGFRA similar to KIT [14]. However, response to this drug in atypical GISTs is unclear and further clinical trials are needed.

Since KIT-weak and KIT-negative GISTs are rare, the number of cases in our retrospective study was rather small. While our limited data do not allow precise conclusions as to whether CT and MRI findings are helpful in diagnosing KIT-weak or KIT-negative GISTs, they do suggest that patients with a gastric submucosal tumor containing cystic regions and soft tissue elements should be cytogenetically tested for a PDGFRA mutation even if immunohistochemical survey shows that the lesion is weak or negative for KIT. More prospective studies are needed to define the value of CT and MRI as diagnostic tools in patients with an atypical GIST.

In conclusion, our review of data from ten patients with confirmed atypical GIST revealed characteristic findings. Tumors have the appearance of a submucosal tumor containing cystic regions and soft tissue elements on CT and MRI. These tumors are considered to be a variant of GIST which may represent mutation of KIT but PDGFRA by cytogenetic analysis.

Acknowledgements This work was supported in part by grants for Scientific Research Expenses for Health and Welfare Programs, No. 17-12 and BMS Freedom to Discovery Grant.

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ORIGINAL ARTICLE

Diagnosis of Complete Response to Neoadjuvant Chemotherapy Using Diagnostic Imaging in Primary Breast Cancer Patients

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■ **Abstract:** Advances in the therapeutic agents used for neoadjuvant chemotherapy (NAC) have recently achieved higher response rates and induced a greater number of pathologic complete responses (pCR) than ever before. The aim of this study is the diagnosis of pCR after NAC by diagnostic imaging of clinical complete response (cCR) patients. This study included 35 breast cancer patients who demonstrated cCR after receiving NAC with a combination of anthracycline and taxane from May 1998 to August 2003. Surgical treatment included breast-conserving therapy followed by radiotherapy or mastectomy. The identity of post-NAC lesions as either a complete response (CR) or partial response (PR) were made by mammography, ultrasonography, and contrast-enhanced computed tomography (CT). Among the 35 patients, 11 achieved pCR, including the disappearance of both invasive and intraductal components. Of the patients achieving pCR, eight were defined as CR and three were determined to be PR by CT. There was a significant relationship between the pCR and the determination of CR by CT. The determination of CR by ultrasonography was indicative of the disappearance of pathologic invasive components. While mammography appeared to reflect the observed histologic results, we did not observe any statistical differences. A subset of cases exhibited discrepancies between the imaging and pathologic results, likely due to the replacement of destroyed tumor cells by fibrosis and granulomatous tissue. The evaluation of CR by CT was significantly indicative of pCR. The positive predictive value, however, was not large enough to avoid surgical treatment. Further studies will be needed to establish a diagnosis of pCR. ■

Key Words: breast cancer, complete response, computed tomography, diagnostic images, neoadjuvant chemotherapy

With advances in the therapeutic agents and combinations used for neoadjuvant chemotherapy (NAC), we have recently achieved higher response rates and greater numbers of pathologic complete responses (pCR). Until now, the highest rate of pCR reported was 66% (1). The accurate evaluation of the existence of residual disease after NAC would facilitate more effective strategies for local treatment after NAC.

We previously reported the efficacy of contrast-enhanced computed tomography (CT) as a method to determine the extent of residual breast cancer following NAC (2). Multiple reports have also demonstrated the accuracy of magnetic resonance imaging (MRI) in the detection of residual breast cancer after NAC (3,4). However, these studies utilized only a small number of pCR cases and did not describe the specific findings of the pCR images. To our knowledge, only a few reports detailing the diagnostic findings for patients with clinical complete responses (cCR) have been published (5,6); these suggest the accuracy of breast CT or MRI for precise evaluation of pCR. This study sought to diagnose pCR precisely after NAC by diagnostic imaging of cCR patients.

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 1075-122X/05
The Breast Journal, Volume 11 Number 5, 2005 311–316

MATERIALS AND METHODS

Patients

A total of 202 women with pathologically confirmed breast carcinomas measuring more than 3 cm in diameter were eligible for NAC at the National Cancer Center Hospital (NCCH), Tokyo, Japan, from May 1998 to August 2003. This study examined 35 patients who obtained cCR

Table 1. Chemotherapy Regimen

Regimen	Treatment period	Number of patients
ADM (50 mg/m^2), DTX (60 mg/m^2) × 4	98.5–2001.8	26
ADM (60 mg/m^2), CPA (600 mg/m^2) × 4 PTX (80 mg/m^2) × 12 weeks[a]	2002.3–2005	4
5FU (500 mg/m^2), EPI (100 mg/m^2), CPA (500 mg/m^2) × 4, PTX (80 mg/m^2) × 12 weeks[a]	2002.10–2003.8	5

ADM, adriamycin; CPA, cyclophosphamide; DTX, docetaxel; EPI, epirubicin; 5FU, 5-fluorouracil; PTX, paclitaxel.
[a]Trastuzumab was added when the tumor showed overexpression of HER-2.

after NAC followed by local treatment. Inflammatory breast carcinomas were excluded from this study. All patients gave informed consent for study participation as approved by the institutional review board of NCCH. Of the 167 non-cCR patients, pCR was achieved in 7 cases. These cases were not examined in this study. Responses were assessed before and after NAC by clinical measurement by palpation of the primary tumor. The response was determined according to the criteria of the International Union Against Cancer (7). cCR was defined as total resolution of the breast mass by physical examination without considering the result of diagnostic imaging.

Treatment

The presence of a breast carcinoma was confirmed pathologically by core needle biopsy (CNB). The NAC regimen employed a combination of anthracycline and taxane (Table 1). Patients underwent surgical treatment, including modified radical mastectomy or breast-conserving therapy (BCT) approximately 4 weeks after the last NAC cycle. BCT was followed by radiotherapy to achieve a total dose of 50 Gy. When margin was involved by cancer cells, we added a radiation boost to the tumor bed.

Imaging Examinations

Mammography, ultrasonography, and CT were examined before and after NAC, as reported previously (8). CT scans were performed using a helical CT scanner (X-vigor, Toshiba Medical Systems, Tokyo, Japan) before 2002 and a multislice helical CT scanner (Aquilion 4, Toshiba Medical Systems) at a current of 200 mA after 2002. Patients underwent a single spiral acquisition during inspiratory apnea for 30 seconds in the supine position. An enhanced CT scan was performed for the whole breast using a slice thickness of 1 mm with 100 ml of nonionic contrast material injected into the patient at a rate of 3 ml/second. After 40 seconds of bolus (2) administration of contrast material, we began early phase scanning. The late-phase scan was performed 3 minutes after administration. Using a Mammomat 3000 (Siemens, Malvern, PA), craniocaudal and mediolateral mammography views were obtained without magnification. Breast ultrasound images were obtained using a SSA340A (Toshiba Medical Systems). We measured the diameter of tumor in the transverse plane with all modalities.

The responses to NAC in the obtained images were classified as complete response (CR) and partial response (PR) (Fig. 1). By mammography, we defined CR as the absence of a mass and spiculation, while PR was when an obvious mass was observed. In the absence of tumor shadows, microcalcifications were classified as PR (Iwamoto E, et al., personal communication). Three cases could not

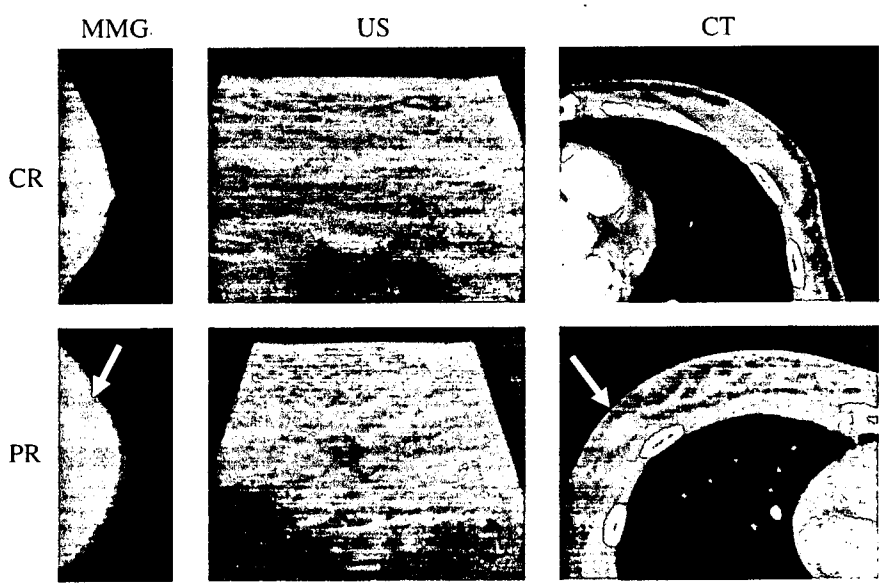

Figure 1. Typical CR and PR determined by each imaging modality.

be evaluated because of the density of the breast. By ultrasonography, a diagnosis of CR was made when the ultrasound findings were normal and PR when images exhibited low echoic lesions with lower echoes than fat tissue or obvious masses. By CT, we defined CR as the complete absence of marks or small and faint enhanced lesions, which were diagnosed as mastopathy when the original tumor location was unknown. CT findings were classified as PR when the images exhibited a highly enhanced mass, regardless of size, or a well-recognized mass, regardless of the enhancement. We classified the tumors into localized and diffuse types by diagnostic imaging, as reported previously (8).

Images were evaluated independently by at least two doctors. Cases without coincident interpretation were mutually agreed upon following discussion.

Histopathologic Examinations

After sectioning in 7–10 mm slices along the transverse axis, all specimens were analyzed by breast pathologists. The response to NAC was classified as either pCR or pathologic partial response (pPR). When neither invasive nor intraductal cancer cells could be observed pathologically, samples were classified as pCR. When residual invasive or noninvasive components were observed, specimens were classified as pPR.

Statistical Analysis

The chi-square test was used for the comparison of CR and PR classifications. Differences of $p < 0.05$ were considered to be significant. Fisher's exact test was used for the comparison when zero was included.

RESULTS

The characteristics of the 35 CR patients are detailed in Table 2. The mean age of the patients obtaining cCR was 48.3 years (range 26–67 years). BCT was performed in 25 cases (71%). Eleven cases (31%) achieved pCR. Twenty-four cases (69%) demonstrated pPR. The determination of histologic type after surgical treatment (pCR cases were diagnosed before NAC by CNB) revealed that 28 cases were diagnosed as invasive ductal carcinoma and 7 were determined to be intraductal carcinoma. Of the seven intraductal carcinomas, six were diagnosed as invasive ductal carcinoma in CNB before NAC. The invasive components were likely diminished by NAC administration.

Tumor sizes were measured before and after NAC on each image (Table 3). The mean tumor size before treatment ranged from 3 cm to 4 cm, diminishing in size to 1–2 cm following NAC. We did not observe a significant difference between the pathologic responses and the sizes obtained at each examination. The mean pathologic sizes were 2.2 cm in pPR patients and 0 cm in pCR patients.

The responses to NAC were evaluated by each imaging method (Table 4). By CT, eight cases were defined as CR and three were determined to be PR in the pCR group. We observed a significant correlation between pCR and the determination of CR by CT ($p < 0.05$). The positive predictive value of CT diagnosis of pCR was 53%. Responses evaluated by ultrasonography, however, did not reflect the pathologic results. In the pPR group, the invasive tumor components disappeared in two of three patients with CR determined by ultrasonography. In these patients, residual intraductal carcinomas were revealed pathologically. The diagnosis of CR by ultrasonography predicted the absence of residual invasive components ($p = 0.1$). Both

Table 2. Patient's Characteristics

Age (years)	48.3 (26–67)
Menopausal status	
Premenopausal	17 (49%)
Postmenopausal	18 (51%)
Tumor size (before NAC)	4.7 cm (3.2–6.5 cm)
TNM category	
T2	29 (83%)
T3	4 (12%)
T4	2 (6%)
Stage before NAC	
IIA	18 (51%)
IIB	13 (37%)
IIIA	2 (6%)
IIIB	2 (6%)
Local treatment	
Mastectomy	10 (29%)
BCT	25 (71%)
Pathologic response to NAC	
Pathologic PR	24 (69%)
Pathologic CR	11 (31%)

Table 3. Mean Tumor Size (cm) before and after NAC and Pathologic Response

	Pathologic PR	Pathologic CR
Mammogram		
Before NAC	3.7	3.6
After NAC	1.5	1.5
Ultrasound		
Before NAC	3.8	3.5
After NAC	1.0	1.2
CT		
Before NAC	4.5	4.2
After NAC	1.4	1.6
Pathology	2.2	0

After NAC, all 35 cases were confirmed total resolution of the breast mass by physical examination and defined as cCR.

Table 4. Response to NAC by Each Imaging Modality and Pathologic Response

	Pathologic PR	Pathologic CR	*P*-Value
CT			< 0.05
CR	7	8	
PR	17	3	
Ultrasound			N.S
CR	3	3	
PR	21	8	
Mammogram			0.07
CR	0	2	
PR	23	7	
Not evaluated	1	2	

of the patients evaluated as CR by mammography also exhibited pCR.

Of the 35 cCR subjects, 15, 6, and 2 patients were diagnosed as CR by CT, ultrasonography, and mammography, respectively. In each imaging modality, the complete absence of marks was seen in only two patients. With the exception of these patients, in 13 and 4 patients classified as CR by CT and ultrasonography, respectively, the tumor location was barely identifiable, displaying small lesions recognized only when compared to the pre-NAC images, demonstrating that tumor localization could be identified in all patients using previous images in combination with these three imaging modalities.

One case was defined as CR by all three modalities (Fig. 2). Two cases classified as PR by all three imaging techniques achieved pCR. Pathologically, the observed masses consisted primarily of hyline fibrosis and foamy cells (Fig. 3). One case evaluated as CR by two independent images was shown to possess residual invasive components pathologically (Fig. 4).

The relationships between morphologic tumor type determined by CT before NAC and pathologic response are shown in Table 5. In the pCR group, eight tumors were of the localized type, while three were of the diffuse type. The localized type was more likely to achieve pCR than diffuse type (8).

Table 5. Morphologic Tumor Types on CT Before NAC and Pathologic Response

	Pathological PR[a]	Pathologic CR
Tumor type (pretreatment)		
Localized	10	8
Diffuse	12	3

[a]Two cases were excluded because a CT was not performed before chemotherapy.

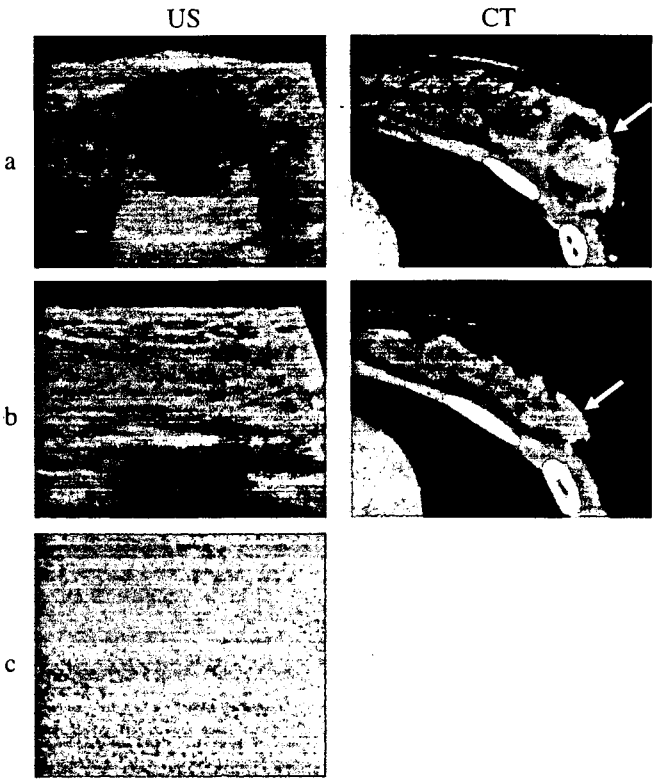

Figure 2. Typical imaging findings for a 30-year-old female with CR. (a) A 5.5 cm × 5.0 cm circumscribed tumor was observed by ultrasonography and CT prior to NAC. (b) After NAC, complete reduction of the tumor was observed by each imaging modality. (c) Subsequent histologic analysis revealed that this case achieved pCR.

DISCUSSION

The determination of CR by CT significantly correlated with pCR. While all tumors or marks could be identified by at least one diagnostic modality to facilitate local excision, tumor size prior to NAC determined by all modalities did not predict pCR, similar to our previous report (2).

This study sought to define CR by diagnostic imaging. Even after the disappearance of all tumor cells, replacement by granuloma-like and/or fibrous tissue could be observed histopathologically. These types of lesions can be identified as low-echoic lesions by ultrasonography and weak-enhanced areas by CT, possibly resulting in false-positive detection of pCR by imaging examination. While these marks provide the important benefit of easy identification of tumor localization when local excision is necessary, the absence of disease signs did not always predict pCR. Of the two tumors lacking any faint shadows or marks identified by ultrasonography, only one achieved pCR. In the false-negative case, we could not distinguish the low-echoic mass surrounded by the aggressive mottled

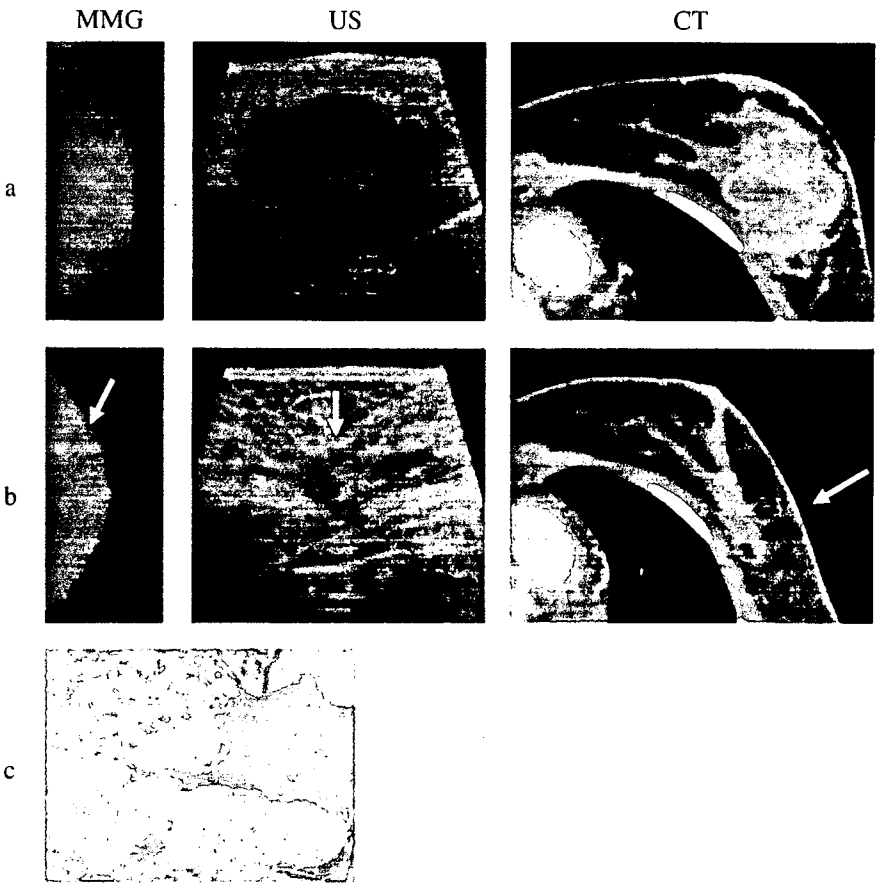

Figure 3. A patient, determined to exhibit PR by imaging, achieved pCR. (a) The localized tumor measured 6.5 cm in size before NAC. (b) After NAC, despite the absence of a palpable tumor, a 1.5 cm localized mass was observed by ultrasonography, CT, and mammography. (c) By histologic analysis, no malignant cells were observed, but hyaline degeneration was seen. This case achieved pCR.

pattern that resulted from fibrocystic changes. Of the two tumors without any enhancement or spiculated marks on CT, neither achieved pCR, instead exhibiting residual intraductal carcinoma. In patients not receiving NAC, the intraductal components of the noncomedo type or low histologic grade demonstrated weaker enhancement than that seen in comedo or high-grade tumors (9,10). These results suggest that low-grade intraductal components may not be well enhanced after NAC, explaining the decreased accuracy of imaging techniques evaluated in this study.

The rate of pCR reported is approximately one-third of the cCR cases (11). Of the few reports examining the role of surgery in patients achieving cCR after NAC, retrospective analysis demonstrated that in patients who achieved cCR after NAC, radiotherapy alone exhibited higher local recurrence rates than surgery (12). These studies suggest that CR defined by residual mass or parenchymal distortion on ultrasonography exhibits better local control in comparison with those lacking ultrasound-detected residual masses. Unfortunately, these patients were not evaluated by mammography, CT, or MRI. While this was not a randomized trial, there were no significant differences in the survival rates following radiotherapy alone or surgery after cCR.

The classification of tumors into either localized or diffuse types using CT prior to NAC administration accurately predicts tumor shrinkage patterns and those tumors that are suitable candidates for BCT following NAC (8). This classification also predicts good pathologic responses (the disappearance of more than two-thirds of tumor cells). Essermann et al. (13) reported similar results using MRI. Of the five predominant MRI patterns, a circumscribed mass pattern significantly predicted good clinical responses to NAC. In this study, localized tumors more frequently achieved pCR. These results were not significant ($p = 0.14$), perhaps because the classification of tumors by diagnostic imaging is a predictor of good pathologic response rather than pCR. The limited number of cases evaluated in these studies, however, requires further evaluation of these imaging techniques as predictors of CR.

In conclusion, CR determined by CT was a significant predictor of pCR. The positive predictive value, however, was not large enough to avoid the necessity of surgical treatment. Further study is required to establish accurately the diagnosis of pCR.

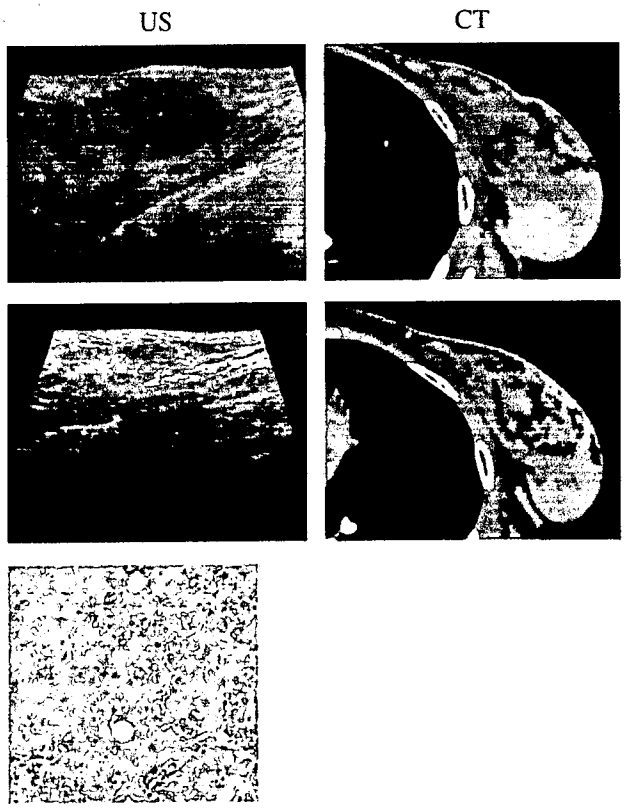

Figure 4. Despite classification as CR by CT and ultrasonography, residual invasive components remained in the specimen by histologic examination. (a) The localized tumor measured 7.5 cm × 3.5 cm in diameter. (b) After NAC, no enhanced lesions were observed by CT. Only a low-echoic area, as low as fat tissue, could be observed by ultrasonography. (c) Invasive components could still be observed histologically.

Acknowledgments

This study was supported in part by a grant-in-aid for cancer research from the Ministry of Health, Labor and Welfare, and a Health and Labor Sciences research grant for research on advanced medical technology (toxicogenomics), Japan.

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Breast Cancer Research and Treatment (2005) 89: 237–241

Report

Efficacy of weekly paclitaxel in patients with docetaxel-resistant metastatic breast cancer

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Key words: docetaxel, metastatic breast cancer, paclitaxel, predictive factor, resistance, taxane

Summary

Background. Partial cross-resistance to paclitaxel and docetaxel has been demonstrated in pre-clinical studies.

Patients and methods. We retrospectively evaluated the efficacy of weekly paclitaxel 80 mg/m^2 in 82 patients with docetaxel-resisitant metastatic breast cancer. Docetaxel resistance was classified into primary resistance, defined as progressive disease while receiving docetaxel, and secondary resistance, defined as progression after achievement of a documented clinical response to docetaxel. Secondary resistance was subclassified according to the interval between the final infusion of docetaxel and the start of weekly paclitaxel into: (1) short interval, $\leqq$120 days, and (2) long interval, >120 days.

Results. The response rate of the 82 patients was 19.5% (95% confidence interval, 10.8–27.9%). The response rate according to the docetaxel resistance category was: primary resistance ($n=24$), 8.3%; secondary resistance ($n=58$), 24.1% (short interval [$n=39$], 17.9%, and long interval, [$n=19$], 36.8%). The differences in response rates among the three categories were statistically significant ($p=0.0247$, Cochran–Mantel–Haenszel test). The interval between from the final docetaxel infusion and disease progression were predictors for response of weekly paclitaxel.

Conclusion. Weekly paclitaxel is modestly effective and safe in docetaxel-resistant metastatic breast cancer patients. However, weekly paclitaxel should not be recommended for primary resistance patients with docetaxel.

Abbreviations: MBC: metastatic breast cancer

Introduction

Paclitaxel and docetaxel are currently two of the most effective anticancer drugs in breast cancer chemotherapy [1, 2]. Paclitaxel and docetaxel are the first members of a class of microtubule-stabilizing anticancer agents. They bind to the β-tubulin subunit of the tubulin hetero-dimer, accelerate the polymerization of tubulin, and stabilize the resultant microtubules to inhibit their polymerization. This inhibition results in the arrest of the cell division cycle, mainly at the G2/M2 stage, which triggers the cell signaling cascade, leading to apoptosis of the cancer cells [3–6]. Although the mechanism of action of paclitaxel and docetaxel is similar, there are several notable differences in the way they form stable, non-functional microtubule bundles, and in the affinity of the two compounds for binding sites [7]. Pre-clinical studies have demonstrated docetaxel to be 100-fold more potent than paclitaxel in achieving bcl-2 phosphorylation and apoptotic cell death, and the cellular uptake of docetaxel is greater than that of paclitaxel, both of which lead to greater cytotoxic activity [8, 9]. *In vivo* evidence has suggested the existence of partial cross-resistance between the two drugs despite the fact they share a similar antitumor mechanism [10].

Paclitaxel and docetaxel have shown similar clinical efficacy in patients with anthracyline-resistant metastatic breast cancer (MBC) [1], and the response rate to both was almost the same: 21.5–53% to weekly paclitaxel, and 22.9–57% to docetaxel [10–16].

In retrospective study of Lin et al. observed a response rate of 25% in patients treated with docetaxel at a dose of 75 mg/m^2, who had pre-treated with anthracycline and paclitaxel [17]. In a phase II study Valero et al. observed a response rate of 18.1% in patients with paclitaxel-resistant MBC treated with deocetaxel at a

dose of 100 mg/m^2 infused over 1 h every 3 weeks [18]. These studies suggested partial cross-resistance between paclitaxel and docetaxel [17, 18].

The taxanes, i.e., docetaxel and paclitaxel, are widely used to treat breast cancer, but docetaxel is more frequently used than paclitaxel, particularly in Japan. As far as we have been able to determine, there have been only two case reports describing the effectiveness of weekly paclitaxel therapy in patients, previously treated with docetaxel [19, 20]. And the objective of this study was to evaluate the efficacy, toxicity, and predictive factors for success of weekly paclitaxel therapy in MBC patients previously treated with docetaxel.

Patients and methods

A total of 308 patients with MBC were treated with weekly paclitaxel as salvage chemotherapy between January 1999 and October 2002 at the National Cancer Center Hospital. We retrospectively selected patients who fulfilled the following selection criteria as subjects for the present study: (1) docetaxel administered during prior chemotherapy for MBC; (2) adequate bone marrow and organ function (neutrophils >1500 μ^{-1}, AST <100 IU/l, ALT <100 IU/l, serum creatinine <2.0 mg/dl); (3) written informed consent before treatment. Patient treated with weekly paclitaxel plus trastuzumab combination was excluded.

Patients were intravenously (i.v.) infused with chorpheniramine maleate 10 mg and dexamethazsone 8 mg 30 min before the paclitaxel infusion. Paclitaxel 80 mg/m^2 was administered over a 1-h period weekly. Each 8-week cycle consisted of six consecutive weekly courses of treatment followed by a 2 week rest. Paclitaxel adminstration was repeated until there was evidence of disease progression or until unacceptable toxicity occurred. In the event of serious toxicity, treatment was withheld until recovery.

Patients with no bidimensionally measurable lesions were not eligible for objective response evaluation. Objective responses were evaluated according to WHO criteria [21]. Patients without measurable lesions were classified as not assessable (NA). Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) ver 2.0.

Statistical analysis

The primary statistical analysis was performed to assess the effect of prior docetaxel response ('CR, PR, and NC' or 'PD') and interval between from the final infusion of docetaxel and disease progression. Since these two factors were highly correlated, we combined them and created a categorical variable (DTX profile) that has three levels: 'primary resistance,' 'secondary resistance' (short interval), and 'secondary resistance (long interval)', and the frequencies of response and non-response to weekly paclitaxel therapy were counted for each of these three levels of the DTX profile. The Cochran–Mantel–Haensxel test was performed for the 3×2 contingency table on the assumption that the DTX profile is an ordered categorical variable.

The secondary analysis consisted of a multivariate logistic regression to assess the effect of the following other factors on the response to paclitaxel therapy: DTX profile, performance status, number of organs involved, disease site, the number of prior regimens for MBC.

Time to progression was measured from the first day of treatment until disease progression or the final day of the follow-up period without disease progression, and overall survival time was measured from the first day of treatment until death or the final day of the follow-up period. Median time to progression and median overall survival were estimated by the Kaplan–Meier method. The statistical analysis was performed with SAS version 8.2 software (SAS Institute, Cary NC), and the significance level of the results was set at 0.05 level (two-sided).

Results

Patient characteristics

Of the 308 patients treated with weekly paclitaxel in our hospital, 96 patients had received prior docetaxel chemotherapy, and 14 patients of them were excluded based on the selection criteria described above: two patients on the basis of neutrophill count; 11 patients on the basis of liver function; one patient on the basis of serum creatinine value. Ultimately 82 of the 98 patients were included in the analysis. The patient characteristics are listed in Table 1. Median age was 54 years. Forty-one patients had received a regimen as adjuvant chemotherapy. The median number of organs involved was 2 (range: 1–5). The majority of the patients (67.1%) had visceral-dominant disease. Most of the patients (91.5%) had received two or more chemotherapy regimens for MBC. Seventy-five patients had received prior anthracycline-containing chemotherapy for MBC, and their median cumulative anthracycline exposure was 240 mg/m^2 (range: 80–480 mg/m^2). The median number of prior docetaxel cycles was 6 (range: 1–16). Most of the 82 patients (85.4%) had received docetaxel at a dose of 60 mg/m^2. The median cumulative docetaxel exposure in the study was 360 mg/m^2 (range: 120–960 mg/m^2). The median interval between the final infusion of docetaxel and the start of weekly paclitaxel therapy was 2.9 months (range: 0.5–23 months). Median follow-up time was 9.5 months, and the follow-up times ranged from 0.5–39 months.

Response

The total number of courses of weekly paclitaxel therapy was 909, and the median number of courses was 10

Table 1. Patient characteristics

	No. of patients (%)
Number	82
Age	
Median	54
ECOG performance status	
0	31
1	36
2	6
≧3	9
No. of organs involved	
1	20
2	31
3	19
≧4	12
Disease sites	
Primary lesion	6
Soft tissue metastasis	32
Lymph node metastasis	36
Liver metastasis	29
Lung metastasis	28
Pleural effusion	23
Bone metastasis	35
Brain metastasis	7
Disease pattern	
Visceral-dominant	54
Non-visceral dominant	28
No. of previous chemotherapy regimens	
1	7
2	57
≧3	18
Prior docetaxel chemotherapy	
Median number of courses	6
Range	1–16
Hormonal status (ER or PgR)	
Positive	38
Negative	31
Unknown	13

Abbreviations: ECOG: Eastern Cooperative Oncology Group; HER2: Human Epidermal Growth Factor Recepter type 2.

(range: 2–45). The response rate among all 82 patients was 19.5% (Table 2; 4 CR and 12 PR, 95% confidence interval (CI): 10.9–28.1%). Objective response rates according to previous docetaxel treatment profile are listed in Table 2. The differences in response rates between docetaxel treatment profiles (primary resistance, secondary resistance [Short interval], secondary resistance [Long interval]) were statistically significant ($p = 0.0247$, Cochran–Mantel–Haenszel test). The results of the multivariate analyses did not suggested that any other factors affected the response to weekly paclitaxel treatment (Table 3). The median time to progression was 3.7 months (Figure 1; 95% CI: 2.75–4.72 months). Median overall survival was 9.4 months (Figure 1; 95% CI: 7.25–11.55 months).

Toxicity

A total of 909 courses in the 82 patients were assessable for toxic effects. The median cumulative dose of paclitaxel was 800 mg/m^2 (range: 160–3600 mg/m^2). The paclitaxel dosage was reduced in five patients due to toxicities: Grade 4 neutropenia in 2; Grade 3 fatigue in 1; Grade 3 diarrhea in 1; and Grade 3 neuropathy in 1. The toxicity profiles are listed in Table 4. Weekly paclitaxel treatment was generally well tolerated and manageable in an outpatient setting. Although grade 3 or 4 netropenia occurred in 10 patients (12.2%), no febrile neutropenia was observed. Neurosensory toxicity was observed in 51 patients (62.2%). No grade 4 non-hematological toxicity was reported, and there were no unexpected adverse reactions or treatment-related deaths.

Discussion

This study evaluated the efficacy and safety profile of weekly paclitaxel in docetaxel resistant MBC patients.

The definition of resistance to docetaxel referred to various definitions of drug resistance had been used in previous reports [12, 14, 18, 22]. The overall objective

Table 2. Objective response rate to weekly paclitaxel according to DTX profile

DTX profile	No. of patients	CR	PR	NC	PD	NA	RR (95% CI)
Primary resistance	24	0	2	10	10	2	8.3% (0–19.4%)
Secondary resistance	58	4	10	29	13	2	24.1% (13.1–35.1%)
Short interval	39	2	5	20	10	2	17.9% (5.9–30.0%)
Long interval	19	2	5	9	3	0	36.8% (15.1–58.5%)
Total no. of patients	82	4	12	39	23	4	19.5% (10.9–28.1%)

Cochran–Mantel–Haenszel test: $p = 0.027$ (primary resistance, short interval, long interval).

Abbreviations: CR: complete response; PR: partial response; NC: no change; PD: progressive disease; NA: not assessable; RR: response rate; CI: confidence interval; Short interval means ≦120 days between the final docetaxel infusion and disease progression. Long interval means > 120 days between the final docetaxel infusion and disease progression. All cases classified as 'primary resistance' experienced disease progression within 120 days of the final docetaxel infusion.

Table 3. Multivariate analyses of weekly paclitaxel response according to variables before weekly paclitaxel therapy (logistic regression model)

Variables before WPTX therapy	Odds ratio	95%CIs	*p* value
DTX profiles			
'Primary resistance':'Long interval'	0.131	0.022–0.773	0.0248
'Short interval':'Long interval'	0.368	0.101–1.339	0.1292
Performance status			
0–2:3–4	0.755	0.113–5.038	0.7716
Number of organs involved			
≧3:1–2	0.481	0.130–1.776	0.2723
Disease pattern			
Visceral:Non-visceral	1.276	0.345–4.720	0.7152
Number of prior regimens for MBC			
≧3:1–2	0.845	0.196–3.643	0.8212

Abbreviations: WPTX: weekly paclitaxel therapy.

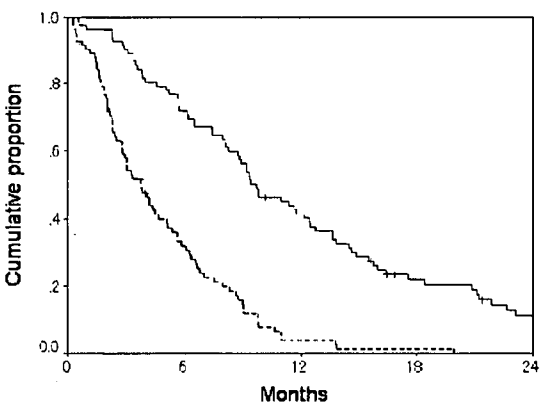

Figure 1. Kaplan–Meier analysis of time to progression (dots line) and overall survival (solid line). Vertical bars indicate censored cases.

Table 4. Maximum grade toxicity (% of patients)

	Maximum grade (NCI-CTC ver 2.0) % of patients			
	1	2	3	4
Leukopenia	36.6	30.5	8.5	0
Neutropenia	28	25.6	9.8	2.4
Anemia	36.6	14.6	4.9	0
Thrombocytopenia	1.2	0	0	0
Fatigue	23.1	3.7	1.2	0
Appetite loss	18.3	3.7	0	0
Nausea	23.2	0	1.2	0
Vomiting	14.6	0	1.2	0
Stomatitis	1.2	1.2	0	0
Diarrhea	3.7	0	1.2	0
Arthalgia/myalgia	4.9	2.4	0	0
HSR	7.3	3.7	0	0
Neurosensory	52.4	9.8	0	0

Abbreviations: HSR: hypersensitivity reactions.

response rate was 19.5%, and the response rate was higher in the secondary-resistance patients than in the primary-resistance patients (24.1 versus 8.3%), but the difference did not reach statistical significance. On the other hand, the interval between the final infusion of docetaxel and disease progression was a statistically significant predictor of response to the weekly paclitaxel. Previous studies on breast, ovarian and small-cell lung cancer described sensitive relapse were defined patients who relapse more than 3–6 months following completion of primary chemotherapy, and can be effectively retreated with same regimen or second-line chemotherapy [12, 22, 23]. Our result was attributable to the tumor biology of chemo-resistant as sensitive or refractory recurrence.

The results of study showed that weekly paclitaxel is modestly active in patients with docetaxel-resistant MBC and showed definite partial cross-resistance between paclitaxel and docetaxel, as reported previously in pre-clinical and clinical studies [9, 10, 17, 18]. Our study may be criticized for not a prospective study, but the overall objective response rate of 19.5% was almost the same as the overall response rates to docetaxel treatment in paclitaxel-resistant populations (18.1, 25%) [17, 18]. The response rate to weekly paclitaxel treatment in the primary docetaxel-resistance patients was poor than docetaxel treatment in the primary paclitaxel-resistance patients (8.3 versus 17.6, 20%) [17, 18]. In pre-clinical study, docetaxel exhibited greator cytotoxicity in paclitaxel-resistant cells [24]. Docetaxel has reported to be more active than paclitaxel against multi-drug resistance protein-expressing tumor [25]. Considering these findings it is reasonable that, there might be difference in the response in each primary resistant patient. We think that paclitaxel might not be useful in patients with primary docetaxel resistance.

In the present study, most patients were heavily treated MBC patients, and as a result the incidence of neutropenia (of any grade) was slightly higher than in previous studies of weekly paclitaxel in patients with anthracycline-refractory disease, however, the incidence of severe neutropenia (grade 3 or more) was comparable [15, 16]. By contrast, the incidence of paclitaxel-associated neurosensory toxicity was similar to its incidence in the previous studies [15, 16]. Therefore, weekly paclit-

axel was almost feasible treatment in outpatient setting, even if heavily treated MBC patients.

In conclusion, weekly paclitaxel therapy (80 mg/m^2) was modest effecacy in patient with docetaxel resistant MBC. However, the response rate of weekly paclitaxel therapy in primary resistance was clearly lower than that of patients with short and long interval. Therefore, weekly paclitaxel therapy should not be recommended for primary resistance patients with docetaxel.

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Cancer Chemother Pharmacol (2005) 56 (Suppl 1): s90–s93
DOI 10.1007/s00280-005-0104-6

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Translational studies for target-based drugs

Published online: 5 November 2005

Abstract The biological background for the clinical and prognostic heterogeneity among tumors within the same histological subgroup is due to individual variations in the biology of tumors. The number of investigations looking at the application of novel technologies within the setting of clinical trials is increasing. The most promising way to improve cancer treatment is to build clinical research strategies on intricate biological evidence. New genomic technologies have been developed over recent years. These techniques are able to analyze thousands of genes and their expression profiles simultaneously. The purpose of this approach is to discover new cancer biomarkers, to improve diagnosis, predict clinical outcomes of disease and response to treatment, and to select new targets for novel agents with innovative mechanisms of action. Gene expression profiles are also used to assist in selecting biomarkers of pharmacodynamic effects of drugs in the clinical setting. Biomarker monitoring in surrogate tissues may allow researchers to assess "proof of principle" of new treatments. Clinical studies of biomarkers monitoring toxicity profiles have also been done. Such pharmacodynamic markers usually respond to treatment earlier than clinical response, and as such may be useful predictors of efficacy. Epidermal growth factor receptor (EGFR) mutation in lung cancer tissues is a strong predictive biomarker for EGFR-targeted protein tyrosine kinase inhibitors. Monitoring of EGFR mutation has been broadly performed in retrospective and prospective clinical studies. However, global standardization for the assay system is essential for such molecular correlative studies. A more sensitive assay for EGFR mutation is now under evaluation for small biopsy samples. Microdissection for tumor samples is also useful for the sensitive detection of EGFR mutation. Novel approaches for the detection of EGFR mutation in other clinical samples such as cytology, pleural effusion and circulating tumor cells are ongoing.

Keywords Biomarker · Proof of principle · Pharmacodynamic marker · EGFR mutation

This work was presented at the 20th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "New Concepts of Treatment Strategies for Hormone-Related Cancer", 11–12 March 2005, Nagoya, Japan.

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Correlative studies at the National Cancer Center Hospital

Molecular correlative studies are essential for the development of anticancer molecular-targeted drugs. One of the major purposes of a correlative study is "proof of principle" (POP). However, clinical POP studies for small molecules are often more difficult to complete than those for antibodies.

Since 2001, the National Cancer Center Hospital (Tokyo, Japan) has been operating as a laboratory for translational studies to develop molecular correlative studies. The laboratory members include medical oncologists, basic researchers, CRC research fellows, invited researchers from abroad, technicians and statisticians. The laboratory is located next to the phase I wards in the hospital, enabling more than ten molecular correlative studies to be simultaneously performed. New clinical samples can be quickly obtained from patients (including outpatients), prepared for storage and stored in the laboratory. The medical doctors

Table 1 Classification of biomarkers and their goals

Biomarker	Goal
Diagnostic markers	
Prognostic markers	
Predictive markers (patient selection)	Selection of patients most likely to benefit from given treatment
Pharmacodynamic markers	Dose finding and schedule
Response and efficacy markers	To measure or infer patient benefit/relate patient benefit to target inhibition
Toxicity prediction markers	

working in the laboratory are often research fellows supported by government grants as these individuals are often interested in this kind of research.

The location of the laboratory also gives frequent opportunities to medical oncologists to communicate with researchers. The significance of study endpoints, study design, technical and statistical information and feasibility are often discussed, especially among young medical oncologists and researchers. As a result, young oncologists and researchers often collaborate in the proposal of new molecular correlative studies.

The major activities of the laboratory are pharmacokinetics and pharmacodynamics studies for early clinical trials (phase I–II) and reverse translational studies. Essentially, "biomarker monitoring" using various biological technologies in these clinical studies are preformed. The selection and validation of biomarkers is a major endpoint for molecular correlative studies. Biomarkers are defined as described in Table 1. Tissue banking and quality control are two of the most important activities. Part of clinical sample testing is performed in collaboration with the Contract Research Organization (CRO) (Fig. 1).

Gene expression profiles

Gene expression array (DNA chips) has been widely used in clinical studies to predict response and in POP studies [3]. Many kinds of DNA chip are now available. Oligonucleotide arrays containing >40,000 genes have recently become popular. These chips can be used differentially depending on the requirements. Before the clinical use, however, an array's quality (linearity and reproducibility) should be determined in preclinical studies. At the National Cancer Center Hospital, the quality of each array is evaluated and expressed as the Pearson's product-moment coefficient of correlation. Based on the validated quality of the cDNA, protocols based on "experienced designs" are then established.

In clinical settings, sample quality and protocol feasibility are often major limitations in the design of new studies. To maintain the quality of clinical samples, a system for sample flow has been established. First, purity of the nucleotides must be carefully examined. Purification methods largely depend on the tumor types. For example, brain tumors contain large amounts of carbohydrate chains, lung cancer samples are sometimes very hard, and breast cancer biopsy samples are lipid rich. These sample characteristics influence the purification quality and efficiency.

After the gene expression profiles have been obtained for each sample, the data are analyzed by standardization, clustering, statistical analysis and validation methods. Statistical and biological validation are essential. Ideally, clinical cross-validation studies should be performed for independent clinical studies. On the other

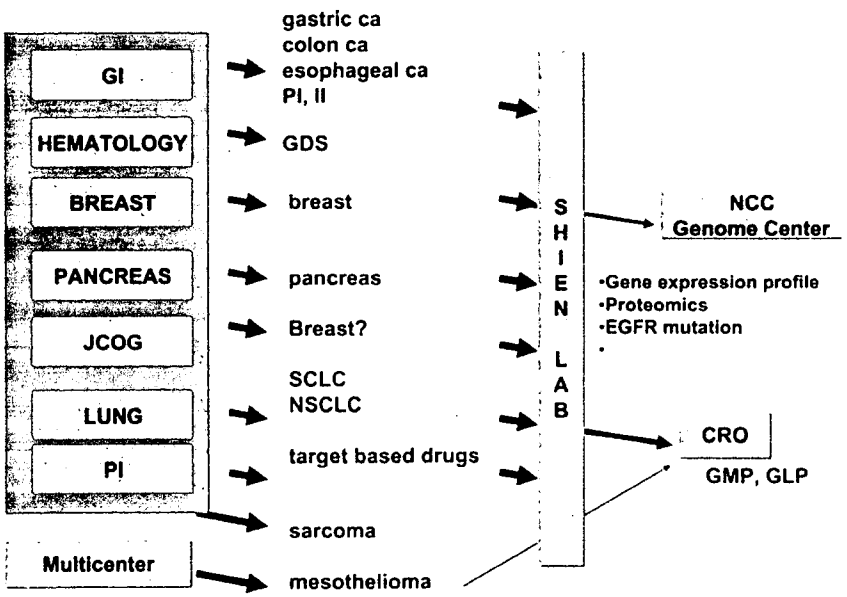


Fig. 1 Flow of clinical samples in molecular correlative studies at the National Cancer Center Hospital. (*GI* gastrointestinal, *JCOG* Japan Clinical Oncology Group, *PI* clinical phase I study, *PII* clinical phase II study, *GDS* gene delivery system, *SCLC* small cell lung cancer, *NSCLC* non-small cell lung cancer, *NCC* National Cancer Center, *CRO* Contact Research Organization, *GMP* Good Manufacturing Practice)

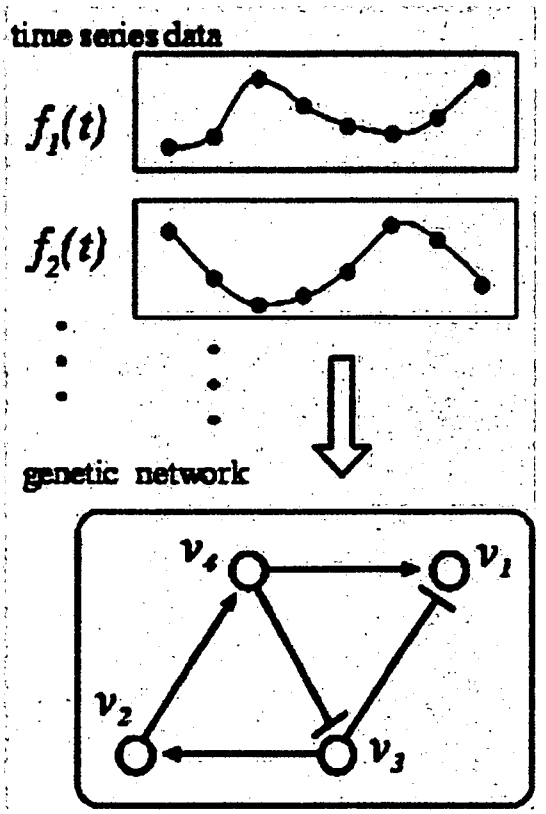


Fig. 2 Network analysis to determine transcriptional pathway and signal transduction pathway modulated by transcriptional regulators and multitarget tyrosine kinase inhibitors using gene expression profiling dataset

hand, biomarkers can be validated in the same clinical study by the "leave-one-out" method. The endpoint of these correlative studies is usually the selection of biomarkers for predicting response or toxicity. For such endpoints, the quality of the clinical study itself is also very important.

We have also used other endpoints in early clinical studies, such as comparing clinical samples obtained before and after the treatment. Analysis of gene alterations after treatment can be utilized to reveal pharmacodynamic effects. We have completed such correlative studies as part of a clinical assessment of multitarget tyrosine kinase inhibitors (TKI), farnesyl transferase inhibitor, and cytotoxic drugs [7].

For biological confirmation, we usually perform real-time RT-PCR and immunostaining. However, we recently discovered that "pathway analysis" is a powerful method for improving our understanding of the alteration of genes related to biological signal transduction pathways. To analyze transcription factors, "network analysis" can be used to identify their signaling pathways (Fig. 2).

Toxicogenomic project for breast cancer

As an approach of gene expression profiling in clinical samples, we monitored gene expression in breast cancer

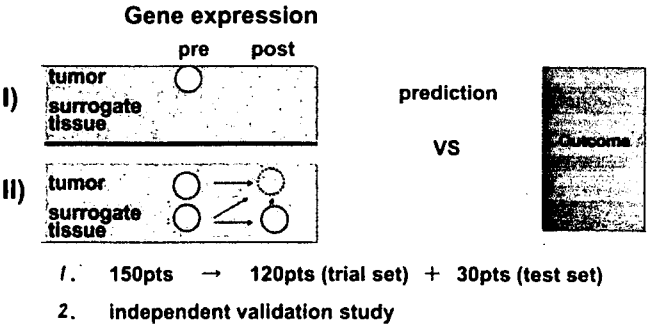


Fig. 3 Gene expression monitoring to distinguish the outcome of treatment for breast cancer patients

patients during treatment with FEC followed by weekly paclitaxel ± trastuzumab in the adjuvant setting. The purpose of this approach was to predict outcomes as well as to study the pharmacodynamic effects of each treatment. Gene expression profiles of peripheral blood mononuclear cells obtained pre- and posttreatment and of tumor biopsy samples obtained pretreatment were determined (Fig. 3). An algorithm to distinguish outcomes using the dataset of these three sampling points was created and expected to be more powerful than conventional outcome assessment techniques.

It seems quite an unusual approach to use normal cells in gene expression profiling in oncology; however, this has proved to be a useful way to monitor drug pharmacodynamic effects and to select biomarkers. Using this approach, we selected biomarkers to capture adverse effects of the treatments. Such "biomarker monitoring" is a rapidly growing field of research.

Biomarker monitoring for tyrosine kinase inhibitors

Recently, EGFR mutation has become an exciting topic in research on TKI [4, 6]. Mutation analysis is now essential for any correlative studies for TKI. Patients with tumors containing the EGFR mutation in different exons are thought to have different responses to TKI. A short, in-frame deletional mutant (E746-A750del) is one of the major mutant forms of EGFR in Japanese populations, and a determinant for EGFR-specific TKI such as gefitinib and ZD6474 (Fig. 4) [1, 8]. We investigated the biological and pharmacological functions of this mutated EGFR to determine whether tumors with deletional-EGFR status are responsive to ligand stimulation, whether mutated EGFR is constitutively active, and whether the downstream intracellular signaling pathway is altered. We concluded that deletional EGFR is constitutively active and that its downstream events are shifted to the AKT pathway (Fig. 5). In addition, a cell-free kinetic assay using mutant EGFR proteins demonstrated differential affinity to TKI among different EGFR mutants. Additional mutations after treatment are also generating interest with regard to their role in acquired resistance to TKI [2]. Thus, the mutation

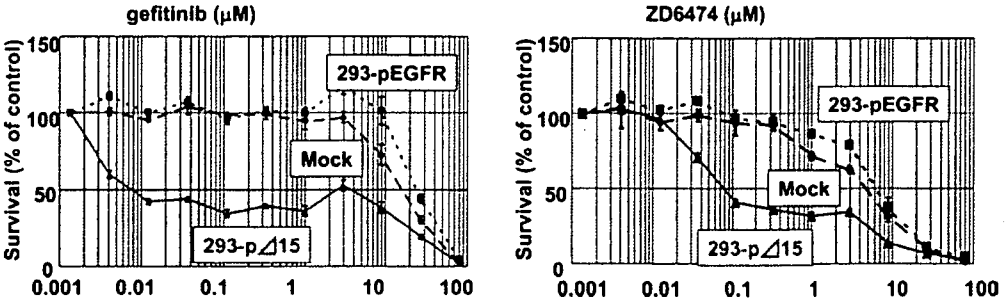
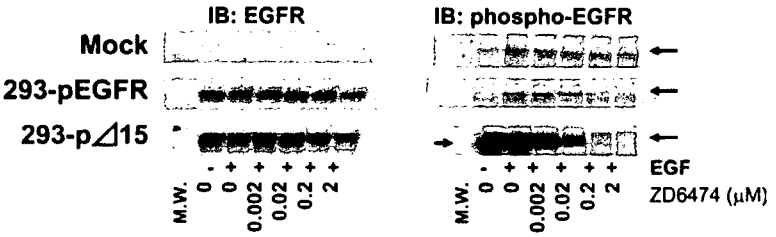


Fig. 4 In vitro sensitivity of 293 cells transfected with a deletional epidermal growth factor receptor (*EGFR*) gene (E746-A750) to tyrosine kinase inhibitors (gefitinib and ZD6474) determined by MTT assay. EGFR mutation (E746-A750 type deletion) increases sensitivity to tyrosine kinase inhibitors (gefitinib and ZD6474). HEK293 cells were transfected with empty vector (293-mock), wild-type EGFR (293 p-EGFR), and deletional EGFR (293-pΔ15). Reprinted with permission of the American Association for Cancer Research Inc., from Arao et al. [1]



Simple Δ15 *vs* Del L747-P753insS ?

Fig. 5 Constitutive phosphorylation of mutant EGFR. Phosphorylation of EGFR was determined by immunoblotting in 293 cells transfected with Mock, wild-type EGFR, and deletional EGFR cDNA. Increased phosphorylation was observed in the 293-pΔ15 cells under no ligand stimulation. Reprinted with permission of the American Association for Cancer Research Inc., from Arao et al. [1]. (*EGF* epidermal growth factor receptor, *IB* immunoblotting)

status of EGFR is one of the determinants for the prediction of tumor response to EGFR-targeted TKI. On the other hand, the clinical impact of EGFR mutation on survival in patients treated with these TKI remains unclear. Therefore, molecular correlative study including EGFR mutation analysis is quite important for prospective studies. Various technologies for EGFR mutation assay have been developed and some of these assays have been validated in the clinical situation [5]. Gene mutation analysis in prospective studies of TKI using standardized technologies is very important.

Protein arrays

Proteomics technology has been developed and successfully used to identify biomarkers for target-based drugs in a few clinical studies. Additional approaches such as antibody arrays and "PowerBlots®", especially those using phospho-specific antibodies, should enable us to perform "kinome" analyses. Hence, these protein analysis technologies are now powerful tools for research on TKI.

Acknowledgements This work was supported by funds for the Third Term Comprehensive 10-Year Strategy for Cancer Control and a Grant-in-Aid for Scientific Research and for Health and Labour Science Research Grants, Research on Advanced Medical Technology, H14-Toxico-007.

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0The Role of DNA-Microarray in Translational Cancer Research

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FINAL

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Abstract: The overall prognosis for the majority of cancer patients remains poor. Current conventional strategies in clinical cancer research are unable to adequately answer a large number of important unsolved questions. Although some patients achieve substantial benefits from classical cytotoxic chemotherapy, others will not. The mechanisms behind this phenomenon are still not identified in detail. Furthermore, the activity of promising novel molecular targeting anticancer agents like tyrosinekinase inhibitors is currently not predictable within the individual patient. The biological background for this clinical and prognostic heterogeneity in behavior is more or less the large individual variation in the biological nature of tumors within the same classified histological subgroup. The overall usefulness of conventional histopathological classifications to adequately predict patient prognosis or response to chemotherapy is limited. The most promising way to solve this issue is to found clinical research strategies on basic biological evidence. New genomic technologies have been developed within recent years. These techniques are able to analyze thousands of genes and their expression profiles simultaneously. An increasing number of investigations has reported applications of these novel technologies within clinical trials settings. The aim of this approach is to identify new subsets of cancer patients, to improve prediction of their clinical outcome or response to treatment and select new targets for innovative therapeutic drugs based on the findings from gene expression profiles. Results of these gene expression profile studies could potentially lead to more individually tailored systemic cancer therapy. In the recent years, a remarkable number of studies based on these techniques have already been reported. Although the published results are clearly impressive and highly promising, a lot of work remains to be done. Moreover, there is a strong need for an increase in reliability and reproducibility of such gene expression profiling techniques and thus introduction of reproducible quality control in the performance of these assays. Although a large number of issues remain to be clarified prior to a more general application of genomic profiling techniques in clinical cancer research, this strategy will eventually turn out as a promising approach to improve successful management of cancer patients.

INTRODUCTION

The overall prognosis for the majority of cancer patients is still unsatisfactory. Hardly any stage IV lung cancer patient will be alive five years following initial diagnosis [Mountain 1997]. Even new generation cytotoxic agents with higher efficacy and more favorable toxicity profiles like paclitaxel, docetaxel and gemcitabine have not brought an identifiable breakthrough in cancer therapy [Schiller 2002]. A large group of tumor entities is primarily resistant or will develop secondary resistance to cytotoxic chemotherapy. On the other hand, there is a definite subset of patients with proven benefit from cancer chemotherapy. The basic mechanisms behind this clinical phenomenon are not clearly identified. Adjuvant chemotherapy following definitive local treatment of early disease (e.g complete resection) represents another important issue. In earlier stages there is currently no reliable method to predict those patients who will gain significant benefit from adjuvant treatment. The current situation regarding the use of novel molecular targeting drugs is of striking parallelity. Activity of these agents is at the moment not predictable in the individual patient. The background of this lies in the remarkable individual variety of biological nature and clinical behavior of tumors even within the same pathological entity. Thus, the impact of classical histological subclassifications to adequately predict patient prognosis or response to chemotherapy is limited. In contrast, more information on molecular tumor biology may improve cancer treatment strategies in the future. This strategy could be one important step to individualize cancer management. New genomic technologies have been developed within the recent years. These techniques have the capability to analyze the expression and activity of thousands of different genes simultaneously. An increasing number of investigations has applied these genomic techniques as an adjunct to clinical studies with the purpose to discover new sub-classes of tumors or predict outcome of therapy on the basis of these gene expression profiles. Although a number of studies have been published during the last years with impressive and clinically relevant results, a lot of work remains to be performed. One major challenge will be to find the appropriate statistical method for correctly analyzing the large data sets to get valid and reliable scientific results. Currently, another major problem is the lack of comparability between results from different investigations. Several different genomic techniques (cDNA-microarray, filter-array, short and long oligonucleotide arrays) and statistical methods (supervised and unsupervised analysis) have been used in recent studies. International standardizations of gene profiling based studies are needed for a proper interpretation of results in the future. Despite several remaining issues in applying genomic techniques to clinical cancer research, these methods still belong to the most promising tools for improving treatment results in the

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