hazard ratios and confidence intervals were estimated using the Cox proportional hazards model. p Values <0.05 were considered statistically significant. All the statistical analyses were performed with the SAS software (Release 8.2, SAS Institute Inc., Cary, NC, USA).

Results

Of the 41 patients with phyllodes tumors included in this study, 20 (48%) were benign, 5 (12%) were borderline, and 16 (39%) were malignant. The median tumor size was 6 cm (range 2–30 cm), and the median age of the patients was 47 years (range 22–65 years). Thirty patients underwent wide excision, and the remaining patients underwent mastectomy. Five patients underwent axillary dissection, and none of them was found to have axillary node metastases. The median follow-up duration was 42 months (range 1–90 months). Although the surgical margins were adequate in all the patients as assessed at the time of wide excision, local recurrence occurred in 4 patients. Three of these patients underwent mastectomy, and the other underwent repeated wide excision by preference. Two patients with local recurrence developed systemic recurrence. The 2-year OS and RFS rates were 84% and 77%, respectively. All of the 9 instances of systemic recurrence occurred in patients with malignant phyllodes tumor, and in 6 of these 9 patients, recurrence occurred within the first year after surgery on average (median duration 11.5 months, range 1–66 months). The median number of organs involved was 3 (1–4), and all patients had multiple lung metastases.

The immunohistochemical staining profiles of the phyllodes tumors are summarized in Table 1. None of the phyllodes tumors showed positive staining for HER2/neu or CD117/c-kit. Most of them (85%, $N = 35/41$) were positive for EGFR, which was seen more frequently in the benign and borderline phyllodes tumors than in the malignant tumors. Positive p53 expression was seen in 10 phyllodes tumors (24%), and approximately half of the malignant phyllodes tumors showed positive p53 expression. The overall median MIB-1 index was 10%; however, whereas it was 5% in both the benign and borderline phyllodes tumors (range 1–30% and 1–10%, respectively), the index in the malignant phyllodes tumors was 30% (range 10–90%). In 3 of the 9 patients with systemic recurrence, we analyzed the differences in the immunohistochemical staining profiles between the primary and the metastatic phyllodes tumor (two lung metastases and one skin metastasis). There were no significant differences in the p53 expression scores or the MIB-1 index between the primary and secondary tumors in these patients.

The size of the primary tumor ($\leqslant 10$ cm vs. >10 cm) was not associated with RFS and OS ($p = 0.96$, $p = 0.62$, log-rank test). There was no correlation between EGFR expression and RFS and OS, p53 expression status, and MIB-1 index was significantly associated with RFS and OS ($p = 0.009$, $p = 0.014$, Fig. 1A. and B for p53; $p = 0.01$, 0.017, Fig. 2A and B for the MIB-1 index, log-rank test). Table 2 shows the hazard ratios as estimated by Cox regression analysis. Both p53 expression and MIB-1 index, but not EGFR expression, were found to be significant prognostic factors in terms of RFS and OS in patients with phyllodes tumors (Figs. 3–6).

Discussion

Metastatic phyllodes tumors of the breast have a poor prognosis, and the average interval from diagnosis to death in patients with metastasis has been reported to be

Table 1. Immunohistochemical findings in phyllodes tumors of the breast

	Benign ($N = 20$)	Borderline ($N = 5$)	Malignant ($n = 16$)
HER2/neu positivity[a]	0	0	0
CD117/c-kit positivity[a]	0	0	0
EGFR			
Negative	1	—	5
Positive	19	5	11
p53			
Negative	19	5	7
Positive	1	—	9
MIB-1 index			
0%	—	—	—
1–9%	15	4	—
10–29%	4	1	7
$\geqslant 30\%$	1	—	9

[a]None of the tumors showed positive staining for HER2/neu or CD117/c-kit.

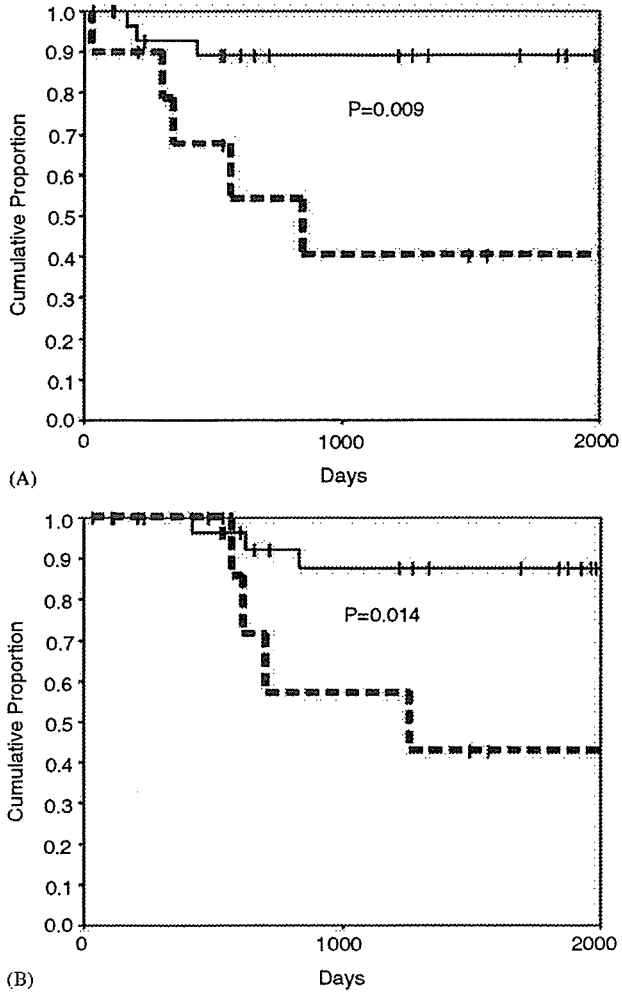

Fig. 1. (A) Correlation of p53 expression with systemic recurrence-free survival. (B) Correlation of p53 expression with overall survival. The dotted line represents patients positive for p53 expression.

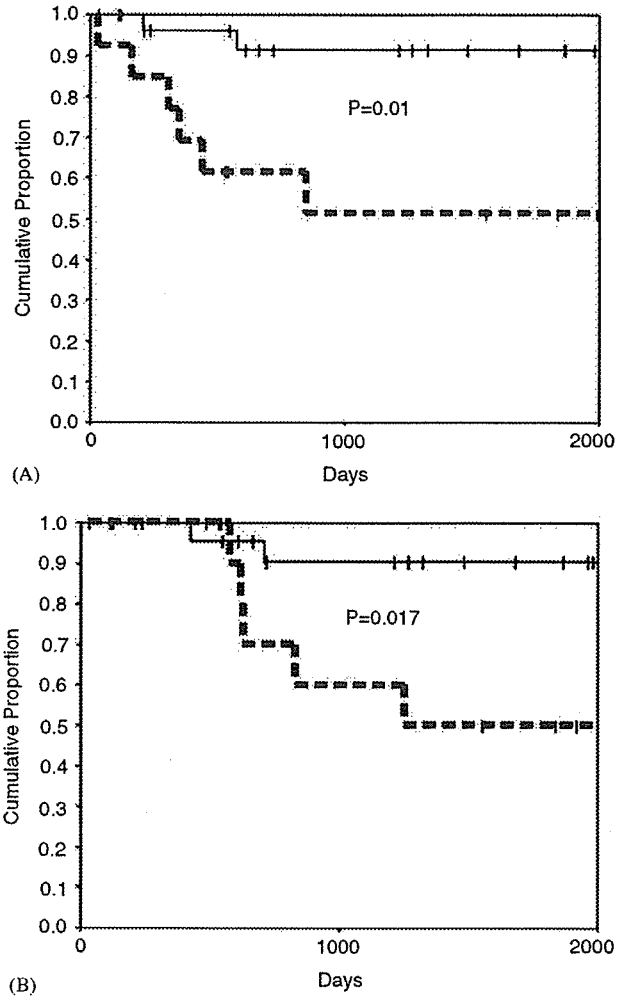

Fig. 2. (A) Correlation of MIB-1 index with systemic recurrence-free survival. (B) Correlation of MIB-1 index with overall survival. The cut-off point for value of the MIB-1 index ($>11.2\%$ vs. $\leqslant 11.2\%$) was defined based on the results of a previous study [8]. The dotted line represents patients with high MIB-1 index ($>11.2\%$).

30 months [2]. The present study demonstrated that both p53 expression and the MIB-1 index were significantly associated with RFS and OS in patients with phyllodes tumors. Numerous studies have attempted to determine whether immunohistochemical testing of phyllodes tumors might be useful to predict the clinical outcome of the patients, but without success. Many studies have suggested that p53 and MIB-1 expression status may be correlated with the histological grade of the tumors [6,11,12,19,24] but only one study investigating 118 cases has demonstrated a correlation between the expression of these two tumor markers and the rates of recurrence and survival [13]. In that study, the results of multivariate analysis suggest that p53 expression might be a prognostic factor in terms of the disease-free survival, and the MIB-1 index might be a prognostic factor in terms of OS [13]. These results are confirmed in our study, which indicates that immunohistochemical analysis might be useful for identifying patients at high risk of systemic recurrence and death from disease. The discrepancy between the results of most of the previous studies and our study might be attributable to differences in the proportions of patients with different subtypes of phyllodes tumors and in the limited rates of recurrence or death.

Although most studies define recurrence to include both local and systemic recurrence, our RFS analysis excluded local recurrence in this study. Local recurrence appears to be related to an inadequate surgical excision margin. In most patients, local recurrence was isolated

Table 2. Cox regression analysis of the immunohistochemical staining profile of PTs of breast

	Recurrent-free survival			Overall survival		
	Hazard ratio	95% CI	*p* Value	Hazard ratio	95% CI	*p* Value
EGFR						
Negative	1.0			1.0		
Positive	0.34	0.1–1.4	0.1	0.27	0.06–1.2	0.09
p53						
Negative	1.0			1.0	—	—
Positive	5.0	1.3–19	0.02	5.4	1.2–24	0.03
MIB-1 index						
⩽11.2%	1.0			1.0		
>11.2%	5.2	1.3–21	0.02	5.8	1.1–30	0.04

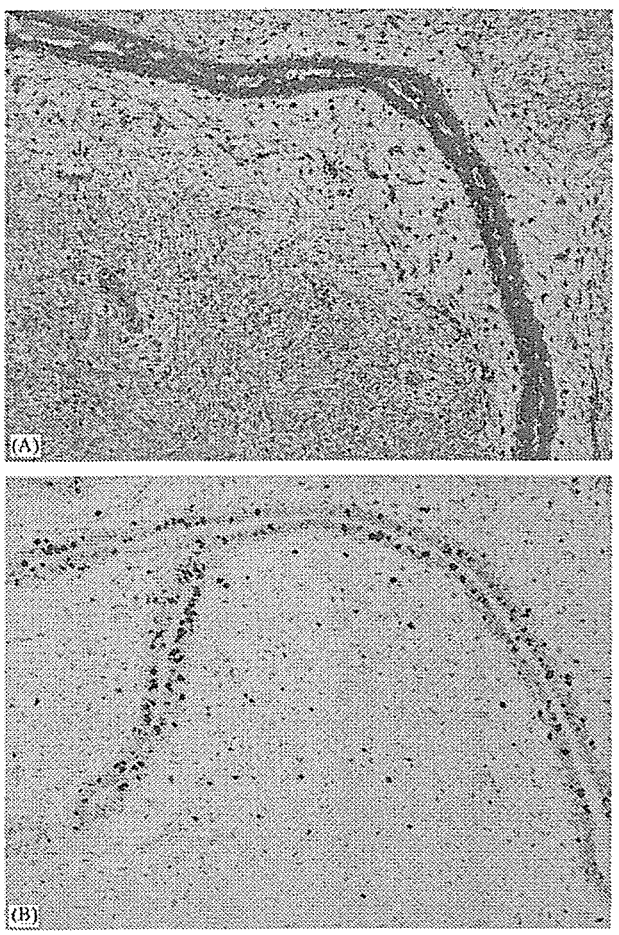

Fig. 3. Benign phyllodes tumor. Nuclei of stromal cells positive for Ki-67 antigen are rare (hematoxylin–eosin stain, original magnification × 100 (A); MIB-1 labeling index 1%, original magnification × 100 (B)).

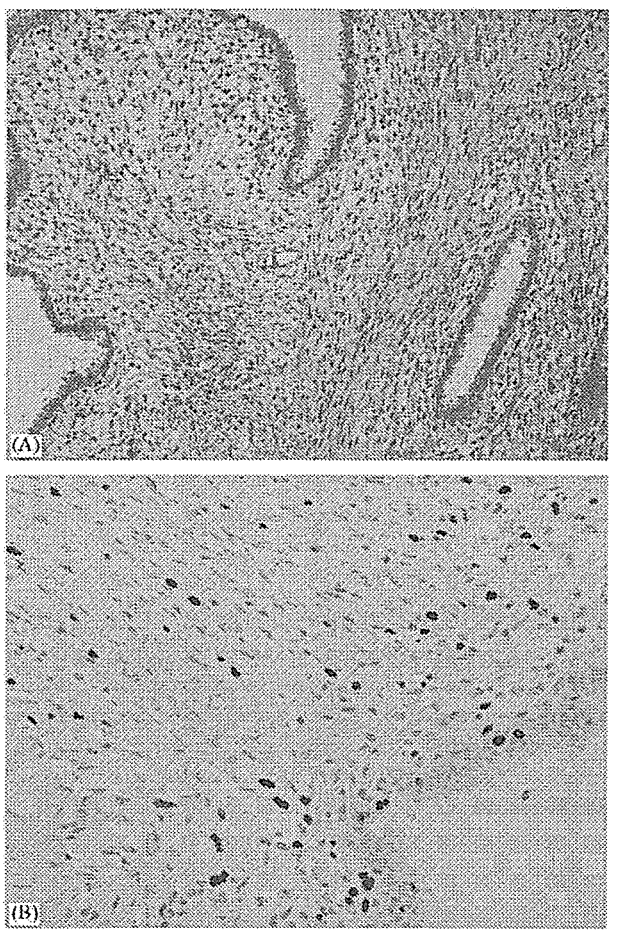

Fig. 4. Borderline phyllodes tumor. Nuclei of stromal cells positive for Ki-67 antigen are rare (hematoxylin–eosin stain, original magnification × 200 (A); MIB-1 labeling index 5%, original magnification × 200 (B)).

and not associated with distant metastases [14]. It has been reported that local recurrence can usually be controlled by repeated wide excision, without any survival disadvantage [15]. This might be the reason why most of the previous studies have failed to predict recurrence or survival.

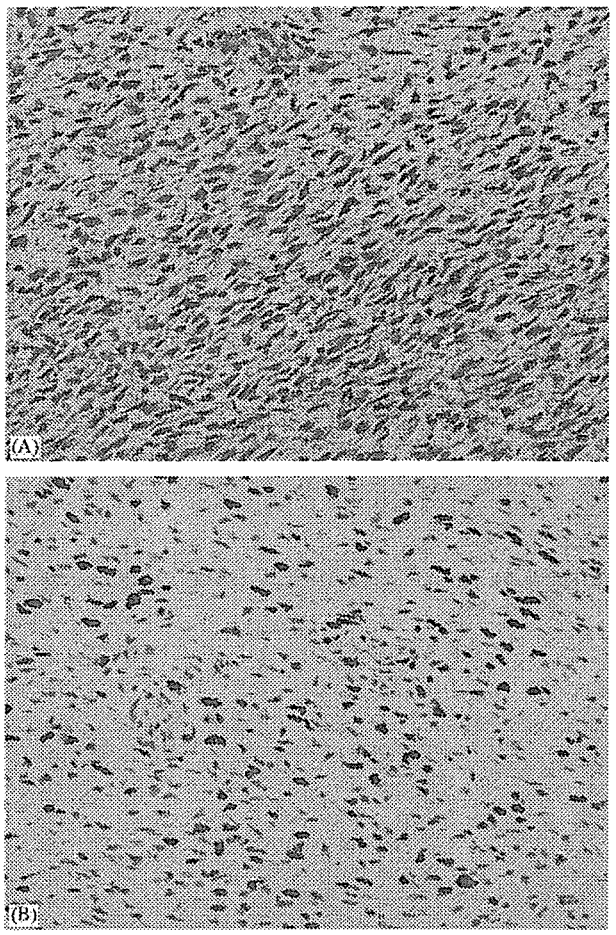

Fig. 5. Malignant phyllodes tumor. Many nuclei of stromal cells positive for Ki-67 antigen are present (hematoxylin–eosin stain, original magnification × 200 (A); MIB-1 labeling index 30%, original magnification × 200 (B)).

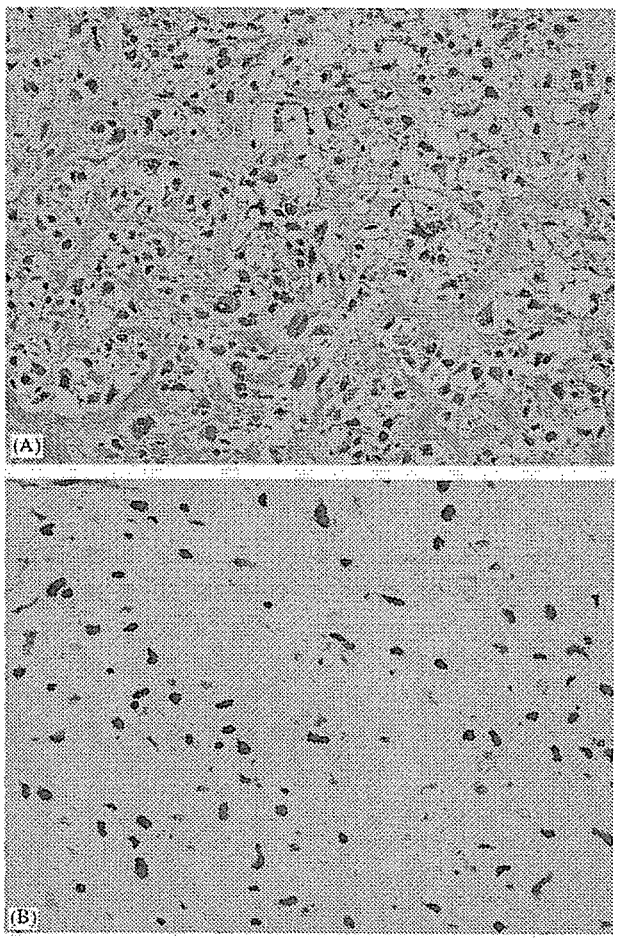

Fig. 6. Malignant phyllodes tumor. Increased nuclear atypia and mitotic activity are noted. p53 immunohistochemical staining shows strong nuclear expression (hematoxylin–eosin stain, original magnification × 200 (A); p53 immunostaining, original magnification × 200 (B)).

The frequency of systemic recurrence in cases of phyllodes tumors has been reported to range between 7% and 30% [7,13,14,16]. In the present study, systemic recurrence occurred in 22% of the patients, predominantly during the first year after surgery. Previous studies have reported that systemic recurrence mainly occurs during the first 3 years after surgery [13,16,19]. The lung is the most common site of distant metastasis in cases of phyllodes tumors [10,13,16]. August et al. suggest physical examination and breast imaging studies twice a year for the first 5 years, chest and abdominal computed tomographic scanning annually for 2–5 years in cases with high-risk lesions [1]. In addition, one study reported that three benign phyllodes tumor patients with an MIB-1 index > 10% had recurred and progressed to malignant phyllodes tumors [3]. Although the benefits of routine imaging of the lung and liver have not been proven yet, routine chest imaging might be considered in patients with positive p53 expression and/or an MIB-1 index > 11.5% for the first 3 years after surgery.

In this study, previously reported prognostic factors, such as tumor size, were not significantly associated with clinical outcome. Since this study investigated only a small number of patients regarding each histopathologic type, and recurrence was not observed in patients with borderline or benign phyllodes tumor, it is uncertain whether these immunohistochemical markers could provide independent prognostic information beyond histopathological typing, which frequently involved discordance. Although phyllodes tumor is a rare neoplasm, further investigations are necessary to resolve this question. These markers might help identify the high-risk group for a poor clinical outcome, with potential indication for adjuvant systemic therapy.

The present study investigated the expression of EGFR family members in phyllodes tumors, including EGFR and HER2/neu. While no distinct HER2/neu

expression was observed in the tumors, most of the tumors expressed EGFR. The expression of EGFR family members in phyllodes tumors has not been widely investigated. Suo et al. reported that EGFR, c-erbB-3, and c-erbB-4, but not c-erB-2 proteins, were expressed in most phyllodes tumors [21]. From these results, trastuzumab, a therapeutic monoclonal antibody used for the treatment of HER2/neu-positive breast cancer, probably would not be useful for the treatment of phyllodes tumors.

While CD117/c-kit was not expressed in the present study, previous studies have reported CD117/c-kit expression in phyllodes tumors in the range of 20–50% [4,18,23]. On the other hand, whereas in most of these studies, CD117/c-kit expression was observed only in malignant phyllodes tumors [4,18], one study reported CD117/c-kit expression in all histological types of phyllodes tumors [23]. The differences in the results of CD117/c-kit expression might be attributable to differences in the antibodies used for CD117/c-kit detection or to technical differences between the studies. The result of our immunohistochemical study in relation to CD117/c-kit expression suggests the need for further evaluation of CD117/c-kit using anti-CD117/c-kit antibodies, which have been widely used for the assessment of gastrointestinal stromal tumors. If CD117/c-kit expression was recognized at a high frequency in future studies, a trial of the new therapeutic agent, STI571 (Glivec, Novartis), for tumor recurrences should be conducted.

The present study demonstrated a correlation between the results of immunohistochemical examination and clinical outcome, and suggested that more careful postoperative follow-up may be important for patients showing expression of p53 and/or MIB-1.

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ORIGINAL PAPER

Tumor-marker analysis and verification of prognostic models in patients with cancer of unknown primary, receiving platinum-based combination chemotherapy

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Abstract
Objectives: To evaluate the usefulness of tumor-marker measurements and to identify prognostic factors in patients with cancer of unknown primary (CUP), receiving platinum-based combination chemotherapy and to verify the adjustment of previously reported prognostic models in this population.
Methods: We conducted univariate and multivariate analyses in consecutive patients with CUP receiving platinum-based combination chemotherapy. Previously reported prognostic models were then validated in this population.
Results: A total of 93 patients were analyzed and the response rate to platinum-based chemotherapeutic regimens among the 93 patients was 39.8%. The median time to progression and overall survival period were 4.1 and 12.4 months, respectively. The ST-439 level was significantly higher in patients with histologically confirmed adenocarcinoma than in patients with poorly differentiated adenocarcinoma or poorly differentiated carcinoma. A multivariate analysis indicated that performance status, the number of involved organs, and the serum lactate dehydrogenase level were the prognostic factors of the outcome. Both the previously reported prognostic models for predicting the duration of survival in this population were shown to be valid.
Conclusion: Tumor-marker measurements are not helpful in the management of patients with CUP. Previously reported prognostic models may be useful for selecting indication for chemotherapy or for stratifying the patients in clinical trial.

Keywords Tumor marker · Chemotherapy · Cancer of unknown primary · Prognostic model · Stratification

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Introduction

Cancer of unknown primary (CUP) represents a group of heterogeneous malignancies and is defined by the presence of a metastatic disease without an identifiable primary tumor site on presentation. CUP accounts for approximately 2–3% of all newly diagnosed patients with solid malignancies. Approximately half of these patients will be diagnosed as having adenocarcinoma, 30% as having poorly differentiated adenocarcinoma or carcinoma, 15% as having squamous cell carcinoma, and the remaining 5% as having undifferentiated neoplasms (Greco and Hainsworth 2005).

Serum tumor markers for human chorionic gonadotropin β subunit (β-HCG), α-fetoprotein (AFP), and prostate-specific antigen (PSA) are useful for identifying treatable germ cell tumors or metastatic prostate cancer. In female patients, carbohydrate antigen 125 (CA125) can be of some help in diagnosing peritoneal carcinoma, which is usually treated as ovarian cancer (Greco and Hainsworth 2005; Varadhachary et al. 2004). A few studies have reported that common

serum tumor markers like carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA15-3), and carbohydrate antigen 19-9 (CA19-9) were not generally useful in diagnostic or prognostic tests (Greco and Hainsworth 2005; Varadhachary et al. 2004; Pavlidis et al. 2003). However, routine measurements of various tumor markers in CUP and the role of tumor-marker measurements, besides indicating favorable subsets, have not been previously studied.

The prognosis of CUP is generally poor, with a median survival period of approximately 6–12 months. Some favorable subsets of patients with either clinical or pathologic features require specific treatment approaches and have the potential for prolonged survival (Greco and Hainsworth 2005; Pavlidis et al. 2003). However, most patients fit into an unfavorable subset that does not benefit from specific treatments and that potentially includes patients with broadly heterogeneous malignancies. In the 1980s, the use of platinum agents was shown to produce better responses and prolonged survival. With the introduction of new antineoplastic agents (i.e., paclitaxel, docetaxel, gemcitabine, and irinotecan), platinum-based combination chemotherapy provided treatment options for a large group of patients. However, since all of the studies were performed in non-randomized control settings, the benefits of the current therapy remains limited (Greco and Hainsworth 2005; Pavlidis et al. 2003). Many investigators have called for designed randomized trials in CUP patients belonging to the unfavorable subset, thereby generating the need for methods to select indication for chemotherapy or stratifying randomized trials, appropriately (van der Gaast et al. 1995; Culine et al. 2002).

Identifying prognostic models for patients with CUP is a challenge because of the vast heterogeneous nature of CUP. Previous studies used multivariate Cox regression analysis to identify prognostic factors for estimating the survival. Many prognostic factors were identified, including age, performance status, smoking history, number of metastatic sites, the presence of liver metastasis, and elevated serum alkaline phosphatase (ALP), or lactate dehydrogenase (LDH) levels (van der Gaast et al. 1995; Culine et al. 2002; Hainsworth et al. 1992; Abbruzzese et al. 1995; van de Wouw et al. 2004). Two studies described simple prognostic models for predicting survival. The previous study presented, but did not validate, a prognostic model based on the performance status and serum-ALP level (van der Gaast et al. 1995). Another study reported an externally validated prognostic model based on the performance status and serum-LDH level (Culine et al. 2002). However, these models may not be widely accepted in practical settings or clinical trials because both models were based on data from European populations and because CUP includes patients with heterogeneous cancers. Thus, these models may not be applicable to other populations or institutions. Consequently, these prognostic models should be verified in different populations; such an effort might contribute to advances in treatment strategies for CUP.

The aims of this study were as follows: (1) to evaluate the usefulness of various tumor-marker measurements for primary unknown cancer patients receiving platinum-based combination chemotherapy, (2) to identify predictive factors for response to chemotherapy and prognostic factors in this population, and (3) to verify the adjustment of previously reported simple prognostic models.

Patients and methods

This study retrospectively analyzed a total of 93 consecutive patients with CUP, who were treated with platinum-based combination chemotherapy between November 1997 and December 2005 at the National Cancer Center Hospital, Tokyo. All patients were diagnosed as having CUP if no primary tumor site could be identified after a thorough history and physical examination, complete blood cell counts and blood chemistry using routine tumor-marker measurements, chest radiography, a computed tomography scan between the neck and pelvis, upper gastrointestinal endoscopy, lower gastrointestinal endoscopy or barium enema imaging, urologist examination (male patients), mammography and gynecologist examination (female patients), and radiologic work-up for any symptomatic areas. All the pathological specimens were carefully evaluated by two or three pathologists to confirm the epithelial origin of the disease and to exclude other malignancies and specific tumor sites. All patients were examined for the presence of routine tumor markers, including AFP, β-HCG, protein induced by vitamin K absence-2, CEA, sialyl-specific embryonic antigen, cytokeratin 19 fragment (Cyfra), squamous-cell carcinoma antigen, CA19-9, CA15-3, sialyl Tn antigen, national cancer center-ST439 (ST-439), neuron-specific enolase (NSE), and progastrin-releasing peptide. In addition, the presence of PSA was examined in men and the presence of CA125 was examined in woman. Patients with squamous cell carcinoma or neuroendocrine carcinoma and patients with carcinomas belonging to any of the favorable subsets requiring well-defined treatments were excluded from the present study. All patients were required to provide written informed consent to review

medical chart and imaging, which approved by the institutional review board at the National Cancer Center.

We used the World Health Organization criteria to assess the response to treatment of patients with measurable lesions (Miller et al. 1981). We also used Response Evaluation Criteria in Solid Tumors to evaluate the response to treatment (Therasse et al. 2000).

Time to progression was measured from the first day of treatment with platinum-based combination chemotherapy until disease progression, and the overall survival time was measured from the first day of treatment until death. Event-free cases at the final day of the follow-up period were censored in time to event analyses. The median time to progression and the median overall survival period were estimated using the Kaplan–Meier method, and differences between survival curves were assessed using a log-rank test. Observed differences in proportion were tested using the Fisher exact test. A multivariate logistic regression analysis was performed to determine the predictive factors for response to chemotherapy. A Cox regression analysis using a stepwise procedure was used to evaluate prognostic factors that were significantly related to survival in the univariate analysis performed in this study as well as the previously reported factors, including age, performance status, smoking history, number of metastatic sites, the presence of liver metastasis, and elevated serum-ALP and -LDH levels (van der Gaast et al. 1995; Culine et al. 2002; Hainsworth et al. 1992; Abbruzzese et al. 1995; van de Wouw et al. 2004). Statistical analysis was performed using SPSS 12.0 J (SPSS Inc., Chicago, IL, USA), the significance level for the results was set at 0.05 (two-sided) and the multiplicity of the statistical test was not corrected.

Results

Patient characteristics

A total of 93 patients including 48 men, were included in the analysis. The median age was 60 years (range, 28–76 years), and the median performance status was 1 (range, 0–3). The histologic types consisted of 48 patients with adenocarcinoma, 21 patients with poorly differentiated adenocarcinoma, and 27 patients with poorly differentiated carcinoma. The median number of involved organs was 1 (range, 1–5). Most patients (78%) had lymph-node metastasis, but liver metastasis (n=15), lung metastasis (n=16), bone metastasis (n=18), and brain metastasis (n=4) were also seen. Almost all the patients (91 of 93 patients) exhibited an elevated serum tumor marker. The median number of elevated serum tumor markers was 5 (range, 0–11); the serum tumor marker characteristics are listed in Table 1. Elevations in Cyfra and ST-439 were significantly associated with histologically confirmed adenocarcinoma in a univariate analysis (P=0.04 and P= 0.005, respectively), and an elevation in ST-439 was associated with histologically confirmed adenocarcinoma in a multivariate analysis (P=0.006).

A total of 340 courses of platinum-based combination chemotherapy were administered and the median number of administered courses was 4 (range, 1–6). Approximately two-thirds of the patients in this study received a taxanes plus platinum regimen (37 patients received paclitaxel plus carboplatin, 36 patients received docetaxel plus cisplatin) and the remaining 20

Table 1 Characteristics of serum tumor markers

Tumor markers	Percentage of patients with elevated levels (%)	Increased levels[a] (mean ± SD)
AFP (>10 ng/ml)	5.3	3,700.8±7,178.3
β-HCG (>0.5 mIU/ml)	54.8	14.1±53.0
PIVKA-II (≥40 mAU/ml)	10.7	1,650.0±4,268.2
CEA (>5.0 ng/ml)	44.1	658.3±1,957.5
SLX (>38 U/ml)	57.0	133.7±217.6
Cyfra (>2.2 ng/ml)	69.9	55.4±144.2
SCC (>1.5 ng/ml)	7.5	8.2±10.0
CA19-9 (>37 U/ml)	38.7	4,085.5±10,303.9
CA15-3 (>28 U/ml)	28.0	288.5±734.1
Erastase (>300 ng/dl)	9.7	866.2±801.5
STN (>45 U/ml)	46.2	694.5±1,660.2
ST-439 (>4.5 U/ml)	36.6	496.9±1,594.4
NSE (>15 ng/ml)	32.3	38.7±43.8
ProGRP (≥46 pg/ml)	12.9	77.5±36.1
PSA in male (>2.7 ng/ml)	8.3	5.54±2.18
CA125 in female (>35 U/ml)	64.4	299.5±356.0

AFP α-fetoprotein, *β-HCG* human chorionic gonadotropin β subunit, *PIVKA-II* protein induced by vitamin K absence-2, *CEA* carcinoembryonic antigen, *SLX* sialyl-specific embryonic antigen, *Cyfra* cytokeratin 19 fragment, *SCC* squamous-cell carcinoma antigen, *CA19-9* carbohydrate antigen 19-9, *CA15-3* carbohydrate antigen 15-3, *STN* sialyl Tn antigen, *ST-439* national cancer center-ST439, *NSE* neuron-specific enolase, *ProGRP* progastrin-releasing peptide, *PSA* prostate-specific antigen, *CA125* carbohydrate antigen 125

[a] The mean serum tumor marker level in patients with elevated levels

patients received irinotecan plus carboplatin. The response rate of the 93 patients was 39.8% (95% confidence interval, 29.9–49.7%). No treatment-related deaths occurred in this study. The median time to progression and overall survival period were 4.1 and 12.4 months, respectively (Fig. 1). At the time of the analysis, 64 of the 93 patients had died.

Prediction of response to treatment and prognostic models

Table 2 shows the relationship between patient characteristics, including the presence of elevated tumor markers, and response to platinum-based combination chemotherapy. No significant predictive factors of response to chemotherapy were seen in the univariate and multivariate analyses. The results of the univariate analysis for prognostic factors are listed in Table 3. Poor performance status (>1), number of involved organs (>2), and elevated serum-LDH and -NSE levels were significantly associated with survival in this univariate analysis. The multivariate analysis indicated that performance status, number of involved organs, and elevated serum-LDH levels were the prognostic factors (P=0.01, P=0.033, P=0.006, respectively).

All 93 patients with a complete data set were analyzed to verify the previously reported prognostic models. The prognostic model by Culine et al. significantly divided these patients into two groups with median survival times of 21.0 and 10.1 months, respectively (P=0.003, Fig. 2). The other prognostic model by Van der Gaast et al. significantly divided these patients into three groups with median survival times of 19.6, 12.2, and 6.7 months, respectively (Fig. 3).

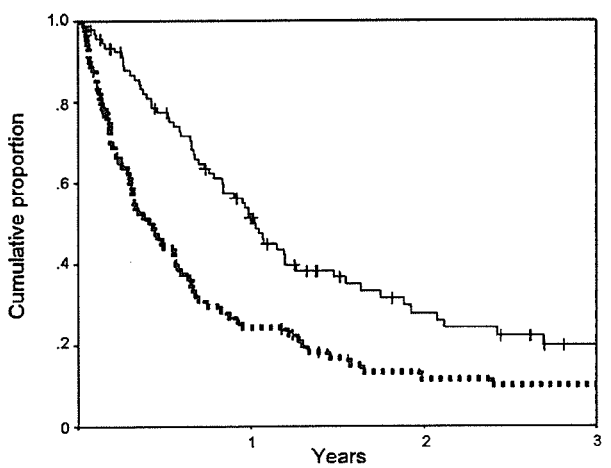

Fig. 1 Kaplan–Meier analysis of time to progression (*dotted line*) and overall survival (*solid line*). *Vertical bars* indicate censored cases

Table 2 Univariate analysis of response to chemotherapy

Variables	No. of patients	Response rate (%)	P value
Sex			
Male	48	37.5	0.68
Female	45	42.2	
Age (years)			
≥60	46	43.4	0.53
<60	47	36.2	
Performance status			
>1	18	22.2	0.11
0 or 1	75	44	
Smoking history			
Past or current smoking history	41	46.3	0.29
No smoking history	52	34.6	
Histologic type			
Adenocarcinoma	45	33.3	0.22
PDA or PDC	48	45.8	
No. of involved organs			
>2	26	38.5	0.99
1 or 2	67	40.3	
Presence of liver metastasis			
Yes	15	33.3	0.78
No	78	41.0	
ALP (U/l)			
Elevated (>359)	24	25.0	0.1
Normal (≤359)	69	44.9	
LDH (U/l)			
Elevated (>229)	43	39.5	0.99
Normal (≤229)	50	40.0	
CRP (mg/dl)			
>1.0	33	30.3	0.19
≤1.0	60	45.0	
No. of elevated tumor markers			
>5	41	36.6	0.67
≤5	52	42.3	
AFP (ng/ml)			
Elevated (>10)	5	80.0	0.08
Normal (≤10)	88	37.5	
β-HCG (mIU/ml)			
Elevated (>0.5)	51	37.3	0.67
Normal (≤0.5)	42	42.9	
PIVKA-II (mAU/ml)			
Elevated (≥40)	10	61.0	0.19
Normal (<40)	83	37.3	
CEA (ng/ml)			
Elevated (>5.0)	41	41.5	0.83
Normal (≤5.0)	52	38.5	
SLX (U/ml)			
Elevated (>38)	53	33.9	0.21
Normal (≤38)	40	47.5	
Cyfra (ng/ml)			
Elevated (>2.2)	65	40.0	0.99
Normal (≤2.2)	28	39.3	
SCC (ng/ml)			
Elevated (>1.5)	7	42.9	0.99

Table 2 Continued

Variables	No. of patients	Response rate (%)	*P* value
Normal (≤1.5)	86	38.6	
CA19-9 (U/ml)			
Elevated (>37)	36	33.3	0.39
Normal (≤37)	57	43.9	
CA15-3 (U/ml)			
Elevated (>28)	26	34.6	0.64
Normal (≤28)	67	41.8	
Erastase (ng/dl)			
Elevated (>300)	9	44.4	0.99
Normal (≤300)	84	39.3	
STN (U/ml)			
Elevated (>45)	43	39.5	0.99
Normal (≤45)	50	40.0	
ST-439 (U/ml)			
Elevated (>4.5)	34	41.2	0.99
Normal (≤4.5)	59	39.0	
NSE (ng/ml)			
Elevated (>15)	30	43.3	0.66
Normal (≤15)	63	38.1	
ProGRP (pg/ml)			
Elevated (≥46)	12	25.0	0.35
Normal (<46)	81	42.0	

PDA poorly differentiated adenocarcinoma, *PDC* poorly differentiated carcinoma, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *AFP* α-fetoprotein, *β-HCG* human chorionic gonadotropin β subunit, *PIVKA-II* protein induced by vitamin K absence-2, *CEA* carcinoembryonic antigen, *SLX* sialyl-specific embryonic antigen, *Cyfra* cytokeratin 19 fragment, *SCC* squamous-cell carcinoma antigen, *CA19-9* carbohydrate antigen 19-9, *CA15-3* carbohydrate antigen 15-3, *STN* sialyl Tn antigen, *ST-439* national cancer center-ST439, *NSE* neuron-specific enolase, *ProGRP* progastrin-releasing peptide

Discussion

Based on the results of the present study, various routine tumor-marker measurements were not useful for predicting either the response to chemotherapy or survival in patients with CUP. Poor performance status, the number of involved organs, and an elevated serum-LDH level were the prognostic factors for survival in patients receiving platinum-based combination chemotherapy, including taxanes or irinotecan. In addition, the previously reported prognostic models were validated in this population. To our knowledge, this is the first report to verify prognostic models for patients with CUP.

Serum tumor markers are substances that can be measured quantitatively using laboratory methods and that can be used to detect cancer and possibly the organ where it resides, as well as being useful for monitoring responses to therapy. Several tumor markers like PSA,

Table 3 Univariate analysis of survival period

Variables	No. of patients	Median survival (months)	*P* value
Sex			
Male	48	12.2	0.24
Female	45	14.3	
Age (years)			
≥60	46	12.6	0.86
<60	47	12.2	
Performance status			
>1	18	7.1	<0.01
0 or 1	75	14.3	
Smoking history			
Past or current smoking history	41	12.4	0.85
No smoking history	52	12.6	
Histologic type			
Adenocarcinoma	45	12.4	0.69
PDA or PDC	48	12.9	
No. of involved organs			
>2	26	7.9	0.01
1 or 2	67	14.3	
Presence of liver metastasis			
Yes	15	10.1	0.11
No	78	12.9	
ALP (U/l)			
Elevated (>359)	24	11.8	0.66
Normal (≤359)	69	12.8	
LDH (U/l)			
Elevated (>229)	43	10.1	0.01
Normal (≤229)	50	17.7	
CRP (mg/dl)			
>1.0	33	11.8	0.49
≤1.0	60	12.8	
No. of elevated tumor markers			
>5	41	11.4	0.48
≤5	52	14.3	
AFP (ng/ml)			
Elevated (>10)	5	7.1	0.18
Normal (≤10)	88	12.4	
β-HCG (mIU/ml)			
Elevated (>0.5)	51	10.2	0.62
Normal (≤0.5)	42	12.9	
PIVKA-II (mAU/ml)			
Elevated (≥40)	10	10.1	0.31
Normal (<40)	83	12.4	
CEA (ng/ml)			
Elevated (>5.0)	41	12.4	0.83
Normal (≤5.0)	52	12.6	
SLX (U/ml)			
Elevated (>38)	53	11.4	0.15
Normal (≤38)	40	15.1	
Cyfra (ng/ml)			
Elevated (>2.2)	65	11.4	0.21
Normal (≤2.2)	28	17.6	

Table 3 Continued

Variables	No. of patients	Median survival	*P* value
SCC (ng/ml)			
Elevated (>1.5)	7	7.2	0.12
Normal (≤1.5)	86	12.8	
CA19-9 (U/ml)			
Elevated (>37)	36	9.5	0.13
Normal (≤37)	57	15.1	
CA15-3 (U/ml)			
Elevated (>28)	26	12.4	0.93
Normal (≤28)	67	12.6	
Elastase (ng/dl)			
Elevated (>300)	9	10.9	0.56
Normal (≤300)	84	12.4	
STN (U/ml)			
Elevated (>45)	43	12.6	0.86
Normal (≤45)	50	11.8	
ST-439 (U/ml)			
Elevated (>4.5)	34	12.2	0.84
Normal (≤4.5)	59	12.8	
NSE (ng/ml)			
Elevated (>15)	30	8.8	0.01
Normal (≤15)	63	14.4	
ProGRP (pg/ml)			
Elevated (≥46)	12	6.4	0.09
Normal (<46)	81	12.9	

PDA poorly differentiated adenocarcinoma, *PDC* poorly differentiated carcinoma, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *AFP* α-fetoprotein, *β-HCG* human chorionic gonadotropin β subunit, *PIVKA-II* protein induced by vitamin K absence-2, *CEA* carcinoembryonic antigen, *SLX* sialyl-specific embryonic antigen, *Cyfra* cytokeratin 19 fragment, *SCC* squamous-cell carcinoma antigen, *CA19-9* carbohydrate antigen 19-9, *CA15-3* carbohydrate antigen 15-3, *STN* sialyl Tn antigen, *ST-439* national cancer center-ST439, *NSE* neuron-specific enolase, *ProGRP* progastrin-releasing peptide

CA125, AFP, and β-HCG have been useful for screening for cancer, monitoring treatment, and detecting recurrence. Although a positive correlation exists between tumor mass and the marker level, most tumor markers are elevated in various types of cancer and sometimes even in benign conditions. At present, the role of serum tumor markers in the management of various cancers might be limited (Canil and Tannock 2002).

A few studies have examined tumor markers in patients with CUP, but their implications for the management of CUP are controversial. Koch and McPherson suggested that a CEA above 10 ng/ml indicated that the tumor site was more likely to be an endodermally derived organ, like a breast or ovary, containing a mucinous carcinoma (Koch and McPherson 1981). However, a previous study reported that CEA, CA19-9, CA15-3, and CA125 levels were not correlated with histologic type, the number of involved organs, or the disease site but that an elevated CEA level was a significant prognostic factor for survival (Milovic et al. 2002). Yet another study reported that elevated CA19-9 levels were related to histologic adenocarcinoma and the presence of liver metastasis (Pavlidis et al. 1994). However, CEA, CA19-9, CA15-3, CA125, β-HCG, and AFP were not reported as predictive factors for response to chemotherapy or survival in two other reports (Pavlidis et al. 1994; Currow et al. 1996). In our study, almost all of the patients exhibited several elevated serum tumor markers, suggesting that patients with CUP exhibit a non-specific over-expression of serum tumor marker. Based on an analysis of our study and a previous one (Pavlidis et al. 1994), we concluded that the routine measurement of tumor markers does not offer any diagnostic or therapeutic assistance to patients with CUP, except for identifying some specific cancers such as germ-cell tumors, prostate cancer, and peritoneal carcinoma.

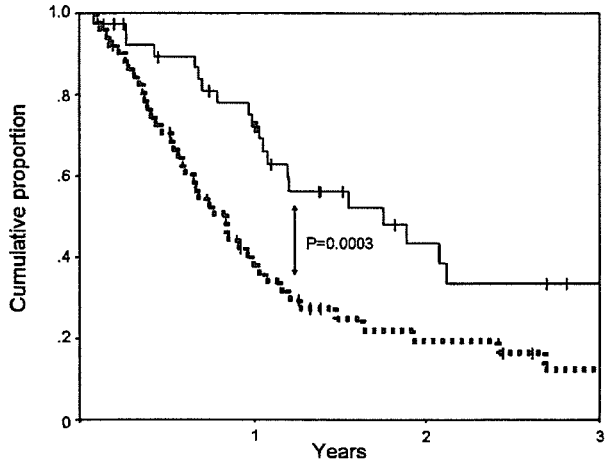

Fig. 2 Overall survival according to a previously reported prognostic model using performance status and serum LDH level (Culine et al. 2002). The *solid line* indicates good risk patients (performance status of 0 or 1 and a normal LDH level), and the *dotted line* represents poor-risk patients (performance status >1 or elevated LDH level). *Vertical bars* indicate censored cases

We retrospectively analyzed 93 consecutive patients with CUP, who had been treated with platinum-based combination chemotherapy. In this study, the response rate and the median survival period were 39.8% and 12.4 months, respectively; these results are similar to those of the previous reports on taxanes-plus-platinum-based combination chemotherapy (Pavlidis et al. 2003; Greco et al. 2000; Greco and Hainsworth 2005; Briasoulis et al. 2000). Since all the patients in this study received new-generation anticancer drugs plus platinum agents, the median survival time was longer than those of the previous studies reporting prognostic models (van der Gaast et al. 1995; Culine et al. 2002). In a previous prognostic report conducted in France, 10% of the patients only

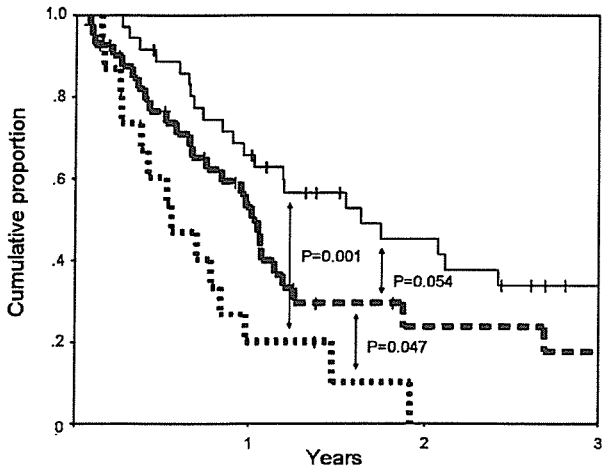

Fig. 3 Overall survival according to a previously reported prognostic model using performance status and serum-ALP level (van der Gaast et al. 1995). The *solid line* indicates patients with a performance status of 0 and an ALP level <1.25 N, the *broken line* indicates patients with a performance status ≥1, or an ALP level ≥1.25 N, and the *dotted line* represents patients with a performance status ≥1 and an ALP level ≥1.25 N. *Vertical bars* indicate censored cases

received the best supportive care and most of the patients (41%) received doxorubicin-plus-etoposide-plus-cyclophosphamide-plus-platinum agent therapy (van der Gaast et al. 1995). Patients in another study were treated with either bleomycin-plus-etoposide-plus-cisplatin therapy or etoposide-plus-cisplatin therapy (Culine et al. 2002). Although there are many differences in the patient characteristics, chemotherapy regimens, and treatment results among these previous reports and ours, it is noteworthy that the prognostic factors for survival are similar (van der Gaast et al. 1995; Culine et al. 2002; van de Wouw et al. 2004). In addition, the two previously reported prognostic models fitted our results for Japanese patients with CUP quite well. Though the investigators published a regression tree analysis in 1,000 consecutive patients in a US population (Hess et al. 1999), that kind of analysis requires a much larger data set and was not feasible in this study. Even using a simple prognostic assessment, however, the verification of prognostic models using an independent data set may be useful for establishing therapeutic strategies for patients with CUP.

Concerning the indications for chemotherapy, previous studies have suggested that patients in the poor-risk group should not be offered chemotherapy routinely, since the median survival time of poor-risk patients is less than 4 months (van der Gaast et al. 1995; Culine et al. 2002). However, in our study, the median survival times of the poor-risk patients according to the prognostic models were 10.1 and 6.7 months, respectively. Whether, poor-risk patients should be offered palliative therapy or chemotherapy may be difficult to determine without conducting a randomized trial. Since survival times have been prolonged by advances in new anticancer drugs, the utility of platinum-based combination chemotherapy or single-agent chemotherapy for poor-risk CUP patients should be carefully considered in a prospective trial.

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COMMENTARY

The next step to approaching central nervous system metastasis in HER-2-positive metastatic breast cancer patients

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Trastuzumab is a recombinant humanized monoclonal antibody which inhibits tumor cell growth by targeting the HER-2/neu receptor. The efficacy of trastuzumab has been demonstrated by randomized controlled trials not only of metastatic breast cancer[1] but also in adjuvant treatment of primary breast cancer[2,3] This agent has dramatically changed the treatment strategy of HER-2-positive breast cancer.

Increased central nervous system (CNS) metastasis related to the use of trastuzumab has been of concern. Considering that breast cancer itself is a tumor which is often accompanied by CNS metastasis[4] the incidence of this may be still high.

In this issue of the *Asia–Pacific Journal of Clinical Oncology*, Dawson *et al.* reported the results of a retrospective study of 28 patients with HER-2-positive breast cancer treated with trastuzumab. In their study, the incidence of CNS metastasis was 39%, median survival time from diagnosis of CNS metastasis was 12.1 months. The uniqueness of their study is a long follow-up period and the quality of pathological information: 93% of HER-2-positive cases were confirmed by immunohistochemical staining (IHC) or Fluorolescence *in situ* hybridization (FISH). The limitation of their study was the small sample size and lack of control group.

We also investigated 70 patients with HER-2-positive breast cancer consecutively treated with trastuzumab from 1999 to 2002 in National Cancer Center Hospital, Japan.[5] All tumors were confirmed to be HER-2 positive by either IHC or FISH. The median follow-up time was 36 months. In our cohort, the incidence of CNS metastasis was 40% (28 patients out of 70) and median overall survival was 11 months, both observation being very similar to Dawson's study. This may be due to consistency in patient background such as the high proportion of patients to be confirmed as HER-2 positive and similar median follow up period.

The findings from previous and current studies are summarized in Table 1. There are some studies addressing the high incidence of CNS metastasis after treatment with trastuzumab, although others do not. The incidence varies from 10 to 48%.[6–10] This variation may be due to the nature or biases of retrospective studies, such as patient selection method or the method of defining of HER-2 positivity. The cohorts with high proportion of pathologically proven HER-2-positive cases seem to have higher incidence of CNS metastasis than those with low proportion of HER-2-positive cases. Only in one study was the incidence of brain metastasis very high, 48%. This is perhaps due to selection bias in that study, because patients were selected from an imaging database.

A short follow-up can bias survival data. Survival from diagnosis of CNS metastasis until death varies form 5 months to 25 months, as shown in the Table 1. However, if we confine to studies with adequate follow up period, the survival comes around one year, from 11 to 13 months.

Although patient background should be taken into account when we see the result of the studies, in overall, we may conclude that for breast cancer patients with CNS metastasis with properly examined HER-2 status and adequate follow-up period, the incidence and median survival of CNS metastasis is around 25–40% and about 12 months. The high incidence is more likely because of better systemic control by trastuzumab rather than the biology of HER2-posituve tumor.[11] With new active agents developed against systemic disease, e.g. bevacizumab, an antivascular endothelial growth factor monoclonal antibody, the increase in CNS

Table 1 Findings from previous and current studies of patients with HER-2-positive breast cancer

Author	N	BM%	HER2conf%	TTC	mf/u	OS	Source
Miller[8]	155	15	80	–	–	5 month	*Ann Oncol* 2003
Bendell[5]	122	34	–	6 month	22 month	13 month	*Cancer* 2003
Clayton[6]	93	25	100	10 month	11 month	6 month	*Br J Cancer* 2004
Lai[7]	79	48	47	–	–	25 month	*Cancer* 2004
Shmueli[9]	41	31	100	10 month	–	–	*Eur J Cancer* 2004
Dawson	28	39	93	12.1 month	31 month	12 month	This issue
Matsumoto	70	40	100	11.5 month	38 month	11 month	This issue

N, number of patients; BM%, percentage of patients with brain metastasis; Her2conf%, percentage of patients whose HER2 status was pathologically confirmed; TTC, time from start of trastuzumab to diagnosis of CNS metastasis; mf/u, median follow up period; OS, overall survival after CNS metastasis diagnosis.

metastasis has become of a further threat, because most available agents cannot cross the blood brain barrier (BBB).

Unfortunately we currently have limited treatment options for CNS metastasis, those are the local treatments such as irradiation, surgery or intrathecal chemotherapy. Dawson *et al.* mentioned two possible treatment approaches against CNS metastasis. The first one is to use more aggressive local therapy, such as prophylactic cranial irraditaion or repeating stereotactic radio-surgery. Little is known about the safety and efficacy of such aggressive treatment. Considering the limitation of radiotherapy such as irreversible late toxicity or difficulties in re-irradiation, we need to investigate the way to select the best suitable patient population for aggressive local therapy. A second approach involves using new systemic agent which can pass through BBB. Temozolamide (TMZ) is a novel oral alkylating agent which can enter the BBB. The addition of TMZ to radiotherapy for newly diagnosed glioblastoma resulted in a clinically meaningful and statistically significant survival benefit, proven in recently reported phase III study.[12] Assessing this kind of new agents may make a breakthrough.

Furthermore, the current standard care does not recommend routine CNS screening, because there is no evidence to support the benefit of early diagnosis and treatment of CNS metastasis. Rather, some studies have recommended that prognoses of patients with symptomatic or asymptomatic CNS metastasis seem to be the same, but depending mainly on the control of extracranial metastasis.[9] This suggests that improved local treatment for CNS metastasis may improve the outcome of selected patients with better systemic control. Therefore, efforts for developing new surveillance strategy should be combined with new treatment development strategy. Rigorous challenges against CNS metastasis in both treatment and surveillance are now strongly warranted.

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Progress in the field of molecular biology and application of biotechnology to medical oncology

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Abstract

Recent progress in the field of molecular biology has been expected to contribute to progress in the field of clinical medicine. Personalized medicine could be achieved by pharmacogenomics. Prospective clinical studies using biomarkers are considered to be important. Investigators should plan the study design and carefully perform such studies.

Key words: Pharmacogenomics, DNA chip, biomarker, prediction

Introduction

Remarkable progress has been made in the field of molecular biology in the 20th century (Table 1). The entire human genome has been sequenced by the Human Genome Project. The 21st century is, therefore, called the "Post Genome" era and further advances in the clinical application of biotechnology are expected. Applied biotechnology is also useful for both diagnostic and therapeutic oncology. Here, we shall discuss the application of biotechnology to the field of medical oncology.

Table 1 Progress in the field of molecular biology during the 20th century

year	event
1890	Mendelism
1926	Genes on chromosome (Mogan)
1944	DNA as gene component (Eibree)
1953	Double helix of DNA (Watson & Crick)
1956	Replication enzyme of DNA (Komburg)
1973	Recombination technology (Cohen)
1985	PCR (Mullis)
1990	Start the Human Genome Project
1998	Deciphering the human genome proceed to multicellular organism
2001	Decoding of the human genome by Celera Genomics Co.

Tissue Banking

Genome biology is expected to be applied to drug development. Drug development, such as that of cytotoxic anticancer drugs and molecular target drugs in the field of oncology, is one of the most upcoming fields. The first and most important step of drug screening is target identification and the search for seeds. The next step is screening of the compounds, followed by preclinical and clinical studies. It is considered that genomic information effectively contributes to the target identification and its validation. To obtain data about the human genome, analysis of human materials is essential. This approach is called the "Reverse Translational Research". In the clinical setting, it is also called "Molecular Correlative Study". These approaches are adopted by government-supported projects both in Japan and abroad. Pharmaceutical companies also aggressively conduct a search for seeds. Mega-pharmas, in particular, have already established the banking system for human materials. Japan has also started a banking system, but it seems to be still immature and Japan still falls behind other countries. The process of collecting clinical samples is called "Tissue Banking" or simply "Banking".

Pharmacogenomics

The approach mentioned above is also applied in the clinical setting. One of the well-recognized approaches is "Personalized Medicine,"

that allows therapy to be customized to individuals by analyzing the individual's genome. Analysis of the genome is called "pharmacogenomics" when it is related to treatment with drugs. "Pharmacogenomics" is a word combining "genomics" and "pharmacology". Broadly, pharamcogenomics includes the analysis of gene products, such as RNA and proteins. The pharmacogenomic approach is considered to contribute to health and welfare. The US and other governments are encouraging this strategy. For example, the US government provides guidance to the industry on the process of Investigational New Drug (IND), New Drug Application (NDA), and Biologic Licence Application (BLA). In our country, the Ministry of Health, Welfare, and Labour has requested for genomic information obtained by the genomic testing in clinical studies for pharmaceutical companies.

Application of pharamcogenomics is expected in three major stages: discovery, preclinical, and clinical stages (Table 2). Three examples are provided as follows; i) research on gene-related diseases; ii) relationship between gene polymorphism and response to drug treatment; iii) genomic tests for the prediction of drug responses. Examples 2 and 3 are considered to be closely associated with cancer treatment and will directly contribute to the exclusion of patients with severe toxicities or to the selection of responders and non-responders to a particular treatment. The markers obtained by pharmacogenomics are called as "biomarkers".

Biomarkers for molecule-targeting drugs

We would like to consider biomarkers for target-based drugs. 1) Overexpression of the target molecule: this is often detected by immunohitochemical analysis. Amplification of target molecules is detected by FISH, CISH or PCR. Somatic mutations in tumor tissues are detected by direct sequencing or other PCR based assays. For the purification of tumor tissues, the microdissection technique is useful. There are biomarkers for conventional cytotoxic drugs. ERCC1 is an enzyme involved in DNA repair and its transcript levels have been reported to be related to the responses to platinum-containing regimens (e.g., cisplatin plus gemicitabine) in non-small cell lung cancer patients.[1] Thus, biomarkers could be determinants for predicting the sensitivity and responses of tumors to cytotoxic drugs.

As mentioned above, the EGFR somatic mutation in lung cancer is a hot topic. Strong correlation has been observed between EGFR somatic mutations and clinical responses to an EGFR-specific tyrosine kinase inhibitor, gefitinib. Thus, the EGFR mutation is a definite biomarker, and other somatic mutations of oncogenes in tumors have been also reported. These mutations could be used as new biomarkers to clarify subpopulations of patients that would respond to molecule-targeting drugs. Currently, trials for new molecule-targeting therapeutics are now underway for solid tumors. Treatment with angiogenesis inhibitors and antibodies are expected to improve the outcome of patients. New biomarkers need to be continually sought for this type of therapeutics.

Now, these molecular correlative studies are called as "Critical Path Research" in the field of drug development (Fig. 1).

Considering the background of aggressiveness of biomarker research, the average response to drugs is much lower than that of other diseases (Fig. 2).

The average response rate to anticancer drugs is 20-30%, which is inadequate. In order to improve the response rate to anticancer drugs, selection of subpopulations of patients that

Table 2 Three broad applications of pharmacogenomics

Discovery
Target identification
Mechanisms of Action
Target differentiation
Biomarker identification
Preclinical Toxicology
Toxicogenomics
In vivo mechanism of action
Biomarker identification
Clinical
In vivo mechanism of action
Biomarker development and validation

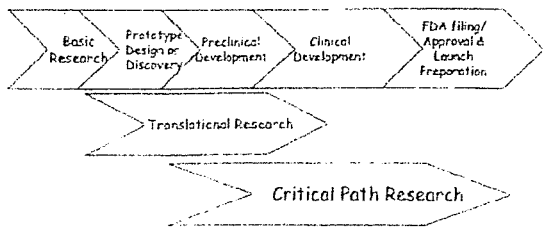

Fig. 1 Critical path. Significant benefit of bringing innovative products faster to the public.

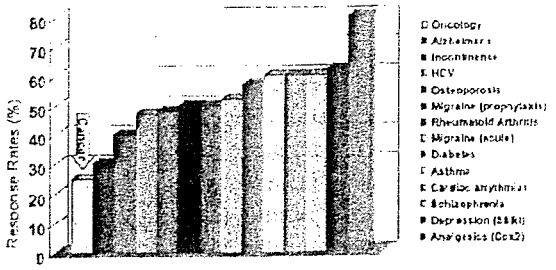

Fig. 2 The need for better predictive markers
(Paul Warning, Genentech, modified)

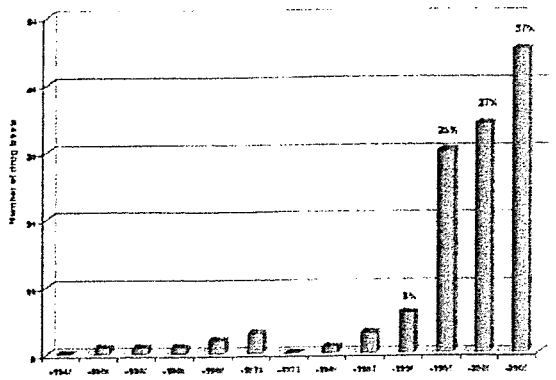

Fig. 3 Labels of approved drugs with pharmacogenomic information
(Frueh FW, CDER/FDA, modified)

would potentially show response is one strategy. At the same time, the labeling of drugs with pharmacogenomic data has been increasing recently (Fig. 3)

Government-related regulatory institutions in the US (Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologic Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH)) developed a "Guideline for Industry," by which pharmaceutical companies are required to submit pharmacogenomic data. How should investigators assess/evaluate the data ? Essentially, we should recognize three categories of pharmacogenomic information while selecting the treatment strategy : 1) test required, 2) test recommended, 3) information only.

Trastuzumab (Herceptin®) for breast cancer is a good example of the first ; testing for anti-Her2 by FISH analysis (Herceptest®) is required for the administration of Trastuzumab. Although EGFR somatic mutation, EGFR immunohistochemistry, and FISH for EGFR are considered to be good biomarkers for predicting the response to EGFR-targeting drugs, they belong to the "Test only" category. It is not within the scope of this review to discuss why these differences exist. Anyway, applied pharmacogenomics is very important in the selection of appropriate subpopulations, and an increase in the number of "Test required" biomarkers is warranted.

Another point for discussion is that the pharmacogenomic approach has so far focused on the prediction or evaluation of adverse events Single- nucleotide polymorphisms of metabolizing enzymes, such as p450 or UDP-glucuronoyltransferases (UGT)[2] are closely related to the toxicity profile of drugs. Therefore, tests for these genes are also included in the label of the drugs. The available evidence actually contributes to identify subpopulations of patients likely to show severe side effects. On the other hand, there is not much evidence, in terms of biomarkers, to distinguish accurately between responders and non-responders. It is important to consider the latter approach when considering personalized medicine.

Drug-diagnostic co-development

As mentioned before, the importance of pharmacogenomics has been discussed worldwide. Last year, the FDA proposed the new concept "drug-diagnostic co-development", although it is still in the draft stage and needs open discussion. What is the "co-development" ? "Co-development" means : 1) Critical Path Research for biomarkers that would distinguish responders from non-responders in clinical studies ; 2) research for avoiding severe toxicities ; 3) clinical studies for POC (proof of concept) by monitoring pharmacodynamic markers. The endpoints of these approaches are to set the appropriate doses for each subpopulation or responders. Investigators should consider the study designs flexibly in these approaches. For example, randomized phase II studies and randomized discontinuation studies may be given more consideration. In addition, for the selection of biomarkers in Critical Path Research, more strict validation will be necessary, because the tests using the biomarkers will directly affect the treatment of each patient.

Problems in pharamcogenomics and future perspectives

Biomarker researches can be divided into two categories, "hypothesis-driven" and "hypothesis-free" ; the former is to prove the power of preex-