Table 4 Hematologic toxicity (worst of any course)

Level	No. of patients	Leukopenia			Neutropenia			Anemia			Thrombocytopenia		
		Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
1	9	5	0	0	2	4	0	5	3	0	2	0	0
2	7	2	3	0	1	3	1	2	3	0	2	1	2
3	2	1	1	0	1	1	0	2	0	0	0	0	0

Table 5 Non-hematologic toxicity (worst of any course)

Level	No. of patients	Nausea/vomiting			Diarrhea			Infection			Pulmonary			Liver			Renal		
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade ≥3	Grade 2	Grade ≥3	Grade 1	Grade ≥2	Grade 2	Grade ≥3
1	9	5	0	1	0	3	1	0	1	1	0	0	0	0	1	0	0	0
2	7	0	0	0	3	2	0	0	0	0	0	0	0	0	1	0	0	0
3	2	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0

Table 6 Therapeutic response

Level	No. of patients	Previously treated patients (refractory)	Response			
			CR	PR	NC	PD
1	9	6 (3)	1	6	2	0
2	7	3 (1)	1	6	0	0
3	2	0	0	2	0	0

Overall response rate (ORR) = 16/18 (89%); RR for previously untreated patients = 9/9 (100%); RR for previously treated patients = 7/9 (78%). CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

in the second course due to toxicities experienced during the first course. The reasons for dose reduction were thrombocytopenia in two patients, neutropenia in one patient and both thrombocytopenia and neutropenia in one patient. However, no patients experienced further toxicities after dose reduction. Median percentage of irinotecan dose intensity (mg/m^2/week), expressed as the actual delivered dose as a percentage of the projected dose, was 84% (range: 48–100%). Of the 162 projected irinotecan infusions, 18 dose omissions occurred during the study period due to leukopenia in five cases, thrombocytopenia in four cases, diarrhea in eight cases and patient refusal in one case. Therefore, the percentage of actual irinotecan infusions, based on actually delivered infusions as a percentage of projected infusions, was 89% (144/162).

3.3. Toxicity

Hematologic and non-hematologic toxicities are listed in Tables 4 and 5. Grade 3 or 4 neutropenia, anemia, and thrombocytopenia occurred in 50%, 33%, and 17% of patients, respectively. However, neither grade 4 leukopenia nor anemia occurred at all three dose levels. Non-hematologic toxicities were generally mild, and grade 3 diarrhea and grade 3 nausea/vomiting occurred in only one patient each. Other non-hematologic toxicities were also mild, and no grade 3 or 4 toxicities except for gastrointestinal toxicities occurred at all three dose levels.

3.4. Response and survival

Chemotherapeutic responses are listed in Table 6. Of the 18 patients, two showed CRs and 14 PRs, giving a response rate of 89% (16/18). For the nine chemo-naïve patients, the response rate was 100% (9/9). In contrast, of the nine previously-treated patients, seven responded to treatment, giving a response rate of 78% (7/9). Of the four patients with refractory relapses, two responded. The median survival time (MST) and 1-year survival rate for all 18 patients in the study was 13.3 months and 62%, respectively (Fig. 1).

4. Discussion

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. However, four comparative studies, including two phase III [13,14] and two randomized phase II [15,16] trials, have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies.

To our knowledge, this is the first study to evaluate the CI regimen in elderly patients with SCLC. The response rate of the CI regimen was 89%, with an MST of 13.3 months. These were very promising results, especially as this study included only elderly SCLC patients and half of the study group had already received some form of chemotherapy, although this study included both ED and LD patients as the same population. Observed instances of toxicity tended to be mild and no TRDs occurred. Although a near full-dose combination chemotherapy was administered to the elderly SCLC patients in our study, only half of the patients experienced grade 3/4 neutropenia. Furthermore, the irinotecan dose intensity of 84% was relatively high. It is possible that the acceptable toxicities and dose intensity were largely attributable to the prophylactic use of G-CSF and the high-dose loperamide therapy against irinotecan-induced diarrhea. On the other hand, other phase I studies, which also included patients over the age of 70, demonstrated that carboplatin AUC 5 and irinotecan 50 mg/m^2 can be safely administered without G-CSF prophylaxis [17–19]. However, these studies were not specifically designed to the elderly population and the median age of these studies were clearly younger than that of our trial.

Several retrospective analyses [20–22] and a prospective study [23] have shown that standard-dose chemotherapy without G-CSF support can lead to an increased risk of early death and sepsis in older populations. Moreover, American

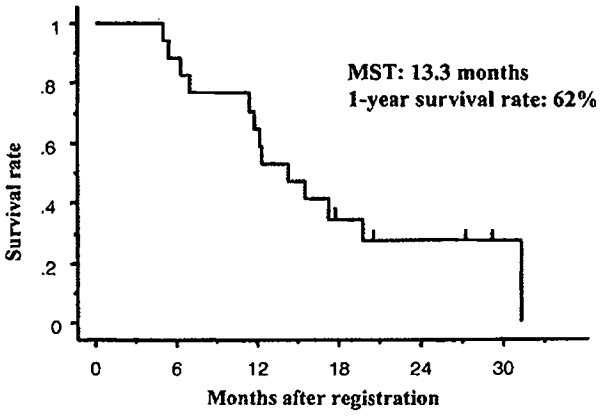

Fig. 1 Overall survival curve.

Society of Clinical Oncology (ASCO) guidelines recommend the use of prophylactic G-CSF in patients at higher risk of chemotherapy-induced infections, including patients with a poor PS or comorbid illness [24]. Therefore, we suggest that the prophylactic use of G-CSF in this study was justified as the CI regimen used was near to the full-dose regimen even though only elderly patients with SCLC were studied.

As our study consisted of a heterogeneous patient population, including patients that had been previously treated, or over 75 years of age, three dose levels were used according to individual patient characteristics. Furthermore, stage was also different among the patients. Therefore, the limitation of this study was that it was neither considered phase I nor II study and was not designed based on the proper statistical methodology. However, at the time of study proposal, no prospective trial using carboplatin plus irinotecan regimen for elderly patients with SCLC was reported. Furthermore, we did not know whether this combination was feasible and effective for elderly SCLC patients. Therefore, dose levels were selected by patient characteristics and this study was designed as a prospective study to evaluate feasibility and efficacy for the elderly SCLC patients. For this reason, it may be difficult to mention on the efficacy of this treatment because of wide patient selection and uncommon study design. In terms of future trials using the CI regimen, level 1 or 2 appeared to be the appropriate dose level for previously untreated elderly patients with adequate organ function because majority of the patients were registered in level 1 and 2. However, phase I/II study using the CI regimen, which is based on the proper statistical method, is warranted for evaluating toxicity and efficacy in the chemo-naïve elderly SCLC patients with specific stage.

Recently, we reported a phase III trial that compared the CE regimen to a split doses of PE (SPE) regimen in elderly or poor-risk patients with ED-SCLC (JCOG 9702) [25]. Although the CE regimen led to pronounced but manageable thrombocytppenia, other toxicities, palliation scores, response rate, and overall survival rate were very similar between the two treatments. However, the CE regimen did not require hydration and could be given in an outpatient setting. Based on the results of this phase III study, many JCOG members prefer the CE regimen over the SPE regimen and consider it to be more suitable for use as a control treatment in future phase III trials.

Compared with the MST obtained for the JCOG 9702 trial (10.6 months for CE versus 9.8 months for SPE), the MST of 13.3 months for the CI regimen in the current study is promising, although the current study included both ED and LD patients as the same population and also included both treated and untreated patients. Furthermore, although 90–95% of the patients in the JCOG 9702 trial experienced grade 3 or 4 neutropenia [25], the toxicity of the current study was 50% and seemed to be generally mild. However, JCOG has also shown that IP is more effective than PE for treating non-elderly patients with ED-SCLC in a phase III trial [6]. Taking these findings together, we are now considering a comparative trial of CE versus CI in elderly patients with ED-SCLC.

In conclusion, the CI regimen was an effective and nontoxic regimen in elderly patients with SCLC, and should be evaluated in future phase III trials.

Acknowledgements

Supported in part by Grants-in-Aid for Cancer Research and for the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor, and Welfare (Tokyo).

References

[1] Morita T. A statistical study of lung cancer in the annual of pathological autopsy cases in Japan, from 1958 to 1997, with reference to time trends of lung cancer in the world. Jpn J Cancer Res 2002;93:15–23.

[2] Yoshimi I, Ohshima A, Ajiki W, Tsukuma H, Sobue T. A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka cancer registry, Japan and in the surveillance, epidemiology and end results program, USA. Jpn J Clin Oncol 2003;33:98–104.

[3] Kaneko S, Ishikawa KB, Yoshimi I, Marugame T, Hamashima C, Kamo K, et al. Projection of lung cancer mortality in Japan. Cancer Sci 2003;94:919–23.

[4] Fukuoka M, Furuse K, Saijo N, Nishiwaki Y, Ikegami H, Tamura T, et al. Randomized trial of cyclophosphamide, doxorubicin, and vincristine versus cisplatin and etoposide versus alternation of these regimens in small cell lung cancer. J Natl Cancer Inst 1991;83:855–61.

[5] Roth BJ, Johnson DH, Einhorn LH, Schacter LP, Cherng NC, Cohen HJ, et al. Randomized study of cyclophosphamide, doxorubicin, and vincristine versus etoposide and cisplatin versus alternation of these two regimens in extensive small cell lung cancer: a phase III trial of the Southeastern Cancer Study Group. J Clin Oncol 1992;10:282–91.

[6] Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small cell lung cancer. New Engl J Med 2002;346:85–91.

[7] Okamoto H, Watanabe K, Nishiwaki Y, Mori K, Kurita Y, Hayashi I, et al. Phase II study of area under the plasma-concentration-versus-time curve-based carboplatin plus standard-dose intravenous etoposide in elderly patients with small cell lung cancer. J Clin Oncol 1999;17:3540–5.

[8] Okamoto H, Nagatomo A, Kunitoh H, Kunikane H, Watanabe K. A phase I clinical and pharmacologic study of a carboplatin and irinotecan regimen combined with recombinant human granulocyte-colony stimulating factor in the treatment of patients with advanced non-small cell lung carcinoma. Cancer 1998;82:2166–72.

[9] Calvert AH, Newell DR, Gumbrell LA, O'Reilly S, Burnell M, Boxall FE, et al. Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 1989;7:1748–56.

[10] Abigerges D, Armand JP, Chabot GG, Costa LD, Fadel E, Cote C, et al. Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. J Natl Cancer Inst 1994;86:446–9.

[11] World Health Organization. WHO handbook for reporting results of cancer treatment. Geneva, Switzerland: Word Health Organization; 1979 (WHO Offset Publication No. 48).

[12] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.

[13] Medical Research Council Lung Cancer Working Party. Comparison of oral etoposide and standard intravenous multidrug chemotherapy for small cell lung cancer: a stopped multicentre randomized trial. Lancet 1996;348:563–6.

[14] Souhami RL, Spiro SG, Rudd RM, Ruiz de Elvira MC, James LE, Gower NH, et al. Five-day oral etoposide treatment for advanced small cell lung cancer: randomized comparison with intravenous chemotherapy. J Natl Cancer Inst 1997;89:577–80.
[15] Pfeiffer P, Rytter C, Madesen EL, Moeholt K, Hansen O, Bentzen S, et al. Five-day oral etoposide treatment for advanced small cell lung cancer: randomized comparison with intravenous chemotherapy. J Natl Cancer Inst 1997;89:1892–3.
[16] Ardizzoni A, Favaretto A, Boni L, Baldini E, Castiglioni F, Antonelli P, et al. Platinum-etoposide chemotherapy in elderly patients with small cell lung cancer: results of a randomized multicentre phase II study assessing attenuated-dose or full-dose with lenograstim prophylaxis—a Forza Operativa Nazionale Italiana Carcinoma Polmonare and Gruppo Studio Tumori Polmonari Veneto (FONICAP-GSTPV) study. J Clin Oncol 2005;23:569–75.
[17] Fukuda M, Oka M, Soda H, Terashi K, Kawabata S, Nakatomi K, et al. Phase I study of irinotecan combined with carboplatin in previously untreated solid cancers. Clin Cancer Res 1999;5:3963–9.
[18] Sato M, Ando M, Minami H, Ando Y, Ando M, Yamamoto M, et al. Phase I/II and pharmacologic study of irinotecan and carboplatin for patients with lung cancer. Cancer Chemother Pharmacol 2001;48:481–7.
[19] Schmittel A, Schulze K, Hutter G, Krebs P, Thiel E, Keilholz U, et al. Phase I dose escalation study of carboplatin to a fixed dose of irinotecan as first-line treatment of small cell lung cancer. Onkologie 2004;27:280–4.
[20] Findlay MP, Griffin AM, Raghavan D, McDonald KE, Coates AS, Duval PJ, et al. Retrospective review of chemotherapy for small cell lung cancer in the elderly: dose the end justify the means? Eur J Cancer 1991;27:1597–601.
[21] Clamon GH, Audeh MW, Pinnick S. Small cell lung carcinoma in the elderly. J Am Geriat Soc 1982;30:299–302.
[22] Radford JA, Ryder WD, Dodwell D, Anderson H, Thatcher N. Predicting septic complications of chemotherapy: an analysis of 382 patients treated for small cell lung cancer without dose reduction after major sepsis. Eur J Cancer 1992;29A: 81–6.
[23] Timmer-Bonte JN, de Boo TM, Smit HJ, Biesma B, Wilschut FA, Cheragwandi SA, et al. Prevention of chemotherapy-induced febrile neutropenia by prophylactic antibiotics plus or minus granulocyte colony-stimulating factor in small cell lung cancer: a Dutch randomized phase III study. J Clin Oncol 2005;23:7974–84.
[24] Ozer H, Armitage JO, Bennett CL, Crawford J, Demetri GD, Pizzo PA, et al. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. J Clin Oncol 2000;18: 3558–85.
[25] Okamoto H, Watanabe K, Kunikane H, Yokoyama A, Kudoh S, Ishizuka N, et al. Randomized phase III trial of carboplatin plus etoposide vs. split doses of cisplatin plus etoposide in elderly or poor-risk patients with extensive disease small cell lung cancer (ED-SCLC): JCOG 9702. J Clin Oncol 2005;23(16S, Part II of II):1094S (late breaking abstract).

A Literature Review of Molecular Markers Predictive of Clinical Response to Cytotoxic Chemotherapy in Patients with Lung Cancer

Ikuo Sekine, MD, John D. Minna, MD,† Kazuto Nishio, MD,‡ Tomohide Tamura, MD,* and Nagahiro Saijo, MD**

Background: To find candidate genes for a predictive chemosensitivity test in patients with lung cancer by using a literature review.
Methods: Using MEDLINE searches, "in vitro chemosensitivity associated genes" and articles on association of the gene alteration with clinical chemosensitivity in lung cancer patients were selected. We calculated odds ratios (ORs) and their 95% confidence intervals (95% CIs) of response rates for patients who had tumors with or without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.
Results: Of the 80 in vitro chemosensitivity-associated genes identified, 13 genes were evaluated for association with clinical chemosensitivity in 27 studies. The median (range) number of patients in each study was 50 (range, 28-108). The response rates of lung cancer with high and low P-glycoprotein expression were 0% and 73% to 85%, respectively ($p < 0.001$). Glutathione S-transferase pi expression (OR 0.22, 95% CI 0.06-0.79), excision repair cross-complementing 1 alterations (combined OR 0.53, 95% CI 0.28-1.01; $p = 0.055$), and tumor suppressor p53 mutation (combined OR 0.25, 95% CI 0.12-0.52) were associated with clinical chemosensitivity.
Conclusion: In total, 80 in vitro chemosensitivity-associated genes were identified in the literature, and high and low P-glycoprotein, glutathione S-transferase pi expression, excision repair cross-complementing 1 alterations, and tumor suppressor p53 mutation were candidates for future clinical trials of chemosensitivity tests in lung cancer patients.

Key Words: chemotherapy, drug response, molecular markers, prediction, lung cancer

(*J Thorac Oncol.* 2006;1: 31–37)

Lung cancer is the leading cause of death in many countries despite extensive basic research and clinical trials. Approximately 80% of patients with lung cancer have developed distant metastases either by the time of initial diagnosis or during recurrence after surgery for local disease. Systemic chemotherapy against lung cancer, however, has limitations in efficacy such that patients with distant metastases rarely live long.[1]

Tumor response to chemotherapy varies among patients, and objective tumor response rates to standard chemotherapy regimens are approximately 20 to 40% in patients with non—small-cell lung cancer and 60 to 90% in patients with small-cell lung cancer. Thus, it would be extremely useful to know in advance whether patients have tumors that respond to chemotherapy agents and whether the tumors would be resistant to such therapy. For this purpose, cell culture-based chemosensitivity tests have been investigated for more than 20 years, but they are not widely accepted because of technical problems such as the large amount of material required, a low success rate for the primary culture, length of time required, and poor correlation with the clinical response.[2–5]

To overcome these obstacles, DNA-, RNA-, and protein-based chemosensitivity tests have been created, but gene alterations that are predictive of the clinical drug response are not established. Recently, as many as 400 genes whose expression was associated with drug response were identified by cDNA microarray studies, but their functions do not seem to be related to drug sensitivity or resistance.[6–10] In addition, the genes identified by microarray studies were highly unstable and depended on the selection of patients used for gene identification.[11,12] The purpose of this study was to provide an overview of gene alterations in lung cancer that are associated with chemotherapy drug response to identify candidate genes for predictive chemosensitivity tests in patients with lung cancer.

MATERIALS AND METHODS

Because one set of genes associated with chemosensitivity is those directly involved in drug resistance mechanisms, we conducted a MEDLINE search for articles on tumor drug resistance published in the years 2001–2003. This search yielded 112 studies, including several review articles. By searching manually through these articles, we identified 134 genes or gene families that may be involved in drug resistance based on their function. We conducted the second MEDLINE searches for papers of in vitro studies on the 134 genes or gene families by using their names as a keyword.

*Divisions of Thoracic Oncology and Internal Medicine, National Cancer Center Hospital, Tokyo, Japan; †Hamon Center for Therapeutic Oncology Research, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX; ‡Pharmacology Division, National Cancer Center Research Institute, Tokyo, Japan.

ISSN: 1556-0864/06/0101-0031

From the 134 genes, we selected genes that met the following definition of "in vitro chemosensitivity associated genes": 1) alteration of the gene was identified in a human drug-induced resistant, solid tumor cell line; 2) transfection of the gene induced drug resistance; or 3) down-regulation of the gene or its encode protein increased drug sensitivity. In this latter category, we included studies in which the gene expression or function was suppressed by antisense RNA, hammerhead ribozyme, or an antibody against the gene product. We excluded studies in which drugs were used to inhibit function because the specificity of the drug against the target may not have been complete. We performed a third MEDLINE search for articles on the association between the gene alteration and chemosensitivity of lung cancer cell lines by using the name of the gene as a keyword. Articles in which the association was evaluated in 20 or more cell lines were included in this study. Finally, we searched MEDLINE for studies on the association between the gene alteration and clinical drug response in patients with lung cancer by using the name of the gene as a keyword. Articles in which the association was evaluated in 25 or more patients with advanced lung cancer were included in this study. Studies in which gene expression was evaluated with microarray were excluded because result analysis and interpretation of this technique have not been established, as indicated by the fact that the list of genes identified by microarray studies was highly variable without overlap between these gene sets.[11,12] Clinical studies on concurrent chemoradiotherapy were excluded. We constructed 2 × 2 tables from the response data and calculated odds ratios (ORs), their variances, and 95% confidence intervals (95% CIs) for the patients who had tumors with gene alteration relative to those who had tumors without gene alteration. Combined ORs and 95% CIs were estimated using the DerSimonian-Laird method.[13] When a response rate was 0, association with gene alteration was evaluated using the χ^2 test because 95% CIs for ORs cannot be calculated. The name of each gene was standardized according to Human Gene Nomenclature Database of National Center for Biotechnology Information.

RESULTS

Of the 134 genes or gene families found, a gene alteration in drug-induced resistant cells, an increased or decreased resistance in transfected cells, and an altered sensitivity in gene down-regulated cells were reported for 45, 57, and 32 genes, respectively. In total, 80 genes met the definition of "in vitro chemosensitivity associated gene" (Table 1).

Gene alteration was associated with in vitro chemosensitivity in 15 (50%) of 30 studies on 15 (56%) of 27 gene alterations (Table 2). Clinical drug response was evaluated in 27 studies on 13 gene alterations. The methods used to identify gene alteration included immunohistochemical protein expression analysis (n = 18), polymerase chain reaction (PCR)-based mRNA expression analysis (n = 3), and PCR-based mutation analysis (n = 6). All but one clinical study was retrospective, and the median (range) number of patients in each study was 50 (28-108). Gene alteration was associated with clinical response in 8 of the 27 (30%) studies (Table 2).

TABLE 1. In Vitro Chemosensitivity-Associated Genes

Transporters: ABCA2, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, ABCG2, MVP, ATP7A, ATP7B, SLC29A1, SLC28A1, SLC19A1
Drug targets: TUBB, TUBB4, TUBA, TYMS, TOP1, TOP2A, TOP2B, DHFR,
Target-associated proteins : MAP4, MAP7, STMN1, KIF5B, HSPA5, PSMD14, FPGS
Intracellular detoxifiers: GSTP1, GPX, GCLC, GGT2, MT, RRM2, AKR1B1
DNA damage recognition and repair proteins: HMGB1, HMGB2, ERCC1, XPA, XPD, MSH2, MLH1, PMS2, APEX1, MGMT, BRCA1, GLO1
Cell cycle regulators: RB1, GML, CDKN1A, CCND1, CDKN2A, CDKN1B
Mitogenic signal regulators: ERBB2, EGFR, KRAS2, HRAS, RAF1
Survival signal regulators: AKT1, AKT2
Integrin: ITGB1
Transcription factors: JUN, FOS, MYC, NFKB1
Apoptosis regulators: TP53, MDM2, TP73, BCL2, BCL2L1, MCL1, BAX, BIRC4, BIRC5, TNFRSF6, CASP3, CASP8, HSPB1

We evaluated the association between transporter P-glycoprotein/multidrug resistance 1 (ABCB1) expression and clinical chemosensitivity in four studies. The response rate of lung cancer with high ABCB1 expression was consistently 0%, whereas that for lung cancer with low ABCB1 expression was 73 to 85% (Table 3). Among drug targets, only topoisomerase II-beta (TOP2B) expression was associated with clinical drug response in patients with small-cell lung cancer (OR 0.29, 95% CI 0.09-0.95). The intracellular detoxifier glutathione s-transferase pi (GSTP1) was associated with both in vitro and clinical drug response (OR 0.22, 95% CI 0.06-0.79) (Table 4). DNA repair gene excision repair cross-complementing 1 (ERCC1) alterations were associated with drug response among patients with non—small-cell lung cancer with marginal statistical significance; the combined OR (95% CI) for ERCC1 alteration was 0.53 (0.28-1.01; p = 0.055) (Table 5). Tumor suppressor p53 (TP53) mutation was the only alteration associated with drug response among patients with non—small-cell lung cancer among genes involved in cell cycle and apoptosis. A combined OR (95% CI) for TP53 among patients with non—small-cell lung cancer was 0.25 (0.12-0.52) (Table 6). B-cell CLL/lymphoma 2 (BCL2) and its family protein expression was not associated with clinical drug response (Table 7).

DISCUSSION

We identified 80 in vitro chemosensitivity-associated genes in our literature search. Of these, 13 were evaluated clinically in 27 studies; ABCB1, TOP2B, GSTP1, and ERCC1 expression and TP53 mutation were associated with changes to drug responses among patients with lung cancer.

Classical drug resistance is believed to be the result of molecular changes inhibiting the drug-target interaction. ABCB1, an ATP-binding cassette protein, acts as an energy-dependent transmembrane efflux pump and decreases the intracellular accumulation of anticancer drugs, including anthracyclines, vinca alkaloids, taxanes, and epipodophyllotox-

TABLE 2. Chemosensitivity-Associated Genes and Association with Chemosensitivity

		Association with chemosensitivity					
		In vitro studies (*n*)			Clinical studies (*n*)		
Category	No of Genes	Total	Yes	%	Total	Yes	%
Transporter	15	9	5	55	4	4	100
Drug target	8	2	1	50	5	1	20
Target-associated protein	7	0	0		0	0	
Intracellular detoxifier	7	3	3	100	1	1	100
DNA repair	10	1	1	100	6	0	0
Damage recognition protein	2	0	0		0	0	
Cell cycle	6	4	2	50	2	0	0
Mitogenic signal	5	3	1	33	1	0	0
Survival signal	2	0	0		0	0	
Transcription factor	4	3	0	0	0	0	
Cell adhesion-mediated drug-resistance protein	1	0	0		0	0	
Apoptosis	13	5	2	40	8	2	25
Total	80	30	15	50	27	8	30

TABLE 3. ABCB1 (P-Glycoprotein) and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio
Yeh et al.[30]	Non-small cell	Paclitaxel	IHC	Low	35	80	0
				High	15	0	$p < 0.001$*
Kawasaki et al.[31]	Small cell	CAV or EP	IHC	Low	26	85	0
				High	4	0	$p < 0.001$*
Hsia et al.[32]	Small cell	EP	IHC	Low	37	73	0
				High	13	0	$p < 0.001$*
Savaraj et al.[33]	Small cell	CAV, CEV, or EP	RT-PCR	Low	24	75	0
				High	7	0	$p < 0.001$*
Combined odds ratio for ABCB expression in patients with SCLC: 0							

IHC, Immunohistochemical analysis; RR, response rate; RT-PCR, reverse transcriptase-polymerase chain reaction.
*Calculated using the χ^2 test because the confidence interval cannot be calculated.

ins. Overexpression of this protein gives tumor cells a multidrug resistance phenotype in vitro, which is thought to be associated with clinical chemoresistance.[14] Our review showed that the response rate of tumors with ABCB1 overexpression was 0 in all studies of lung cancer, whereas that for lung cancer tumors with low ABCB1 expression was 73 to 85% (Table 3).

There is a close relationship between drug sensitivity and quantitative and qualitative alterations of the drug's target, including tamoxifen sensitivity and estrogen receptor expression and trastuzumab response and Her-2/neu overexpression in breast cancer,[15] imatinib resistance and BCR-ABL gene amplification and mutations in Philadelphia chromosome-positive leukemias,[16] and imatinib response and KIT gene mutations in gastrointestinal stromal tumors.[17] In all of these cases, the target molecule is a receptor or a mutated tyrosine kinase located at the entry of growth-stimulating signal transduction pathways. Recently, gefitinib, a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), has been developed, and two large phase II trials showed a response rate of 18% and 12% in patients with non—small-cell lung cancer who were previously treated with conventional chemotherapy.[18,19] Responses to the drug have been unpredictable, but mutations of the EGFR gene were identified in patients with gefitinib-responsive lung cancer.[20,21] Furthermore, all mutations in these tumors were restricted to the activation loop of the kinase domain of EGFR, which are in distinct contrast to mutations in extracelluar and regulatory domains of EGFR in glioblastoma, which are unresponsive to gefitinib.[22] Thus, molecular developments of structure and function of the targets hold the promise of targeted cancer therapy. The target molecules of many anticancer cytotoxic agents have not been clearly defined; therefore, the relationship between the target molecule status and sensitivity to the agent has not been established. TOP2B expression was associated with drug response in patients with small-cell lung cancer, with a response rate of 71% for high TOP2B expression tumors versus 90% for low TOP2B expression tumors (OR 0.29, 95% CI 0.09-0.95).[23] This result, however, is in contrast with the idea that a higher

TABLE 4. Drug Targets, Intracellular Detoxifier, and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio (95% CI)
Beta-tubulin class III							
Rosell et al.[34]	Non-small cell	Paclitaxel, Vinorelbine	Real-time PCR	Low	13	46	0.39
				High	24	25	(0.09-1.62)
Topoisomerase II-alpha							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	65	85	0.65
				High	23	80	(0.20-2.17)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	30	47	0.67
				High	8	38	(0.14-3.40)
Topoisomerase II-beta							
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	48	90	0.29
				High	35	71	(0.09-0.95)
Dingemans et al.[35]	Non-small cell	Platinum-based	IHC	Low	18	50	0.86
				High	13	46	(0.21-3.58)
Glutathione s-transferase pi							
Nakanishi et al.[36]	Non-small cell	Cisplatin-based	IHC	Low	17	47	0.22
				High	37	16	(0.06-0.79)

CI, confidence interval; IHC, immunohistochemical analysis; PCR, polymerase chain reaction; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.

TABLE 5. DNA Repair Genes and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Alteration	Patients (*n*)	RR (%)	Odd ratio (95% CI)
Excision repair cross-complementing 1 expression							
Lord et al. [37]	Non-small cell	Cisplatin, gemcitabine	Real-time PCR	Low	23	52	0.38
				High	24	36	(0.11-1.26)
Excision repair cross-complementing 1 (ERCC1) polymorphism at codon 118							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	C/C	54	54	0.61
			Hybridization	C/T or T/T	53	42	(0.28-1.31)
Combined odds ratio (95% C.I.) for ERCC1 alteration in patients with NSCLC0.53 (0.28-1.01, $p = 0.055$)							
Xeroderma pigmentosum group D polymorphism							
At codon 231							
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	G/G	100	48	1.08
			Hybridization	G/A or A/A	8	50	(0.26-4.57)
At codon 312							
Camps et al.[39]	Non-small cell	Cisplatin, gemcitabine	PCR Sequencing	G/G	18	17	3.33
				G/A or A/A	15	40	(0.66-16.7)
At codon 751							
Camps et al.[39]	Non-small cell	Cisplatin, gemcitabine	PCR Sequencing	A/A	22	23	2.04
				A/C or C/C	16	38	(0.49-8.45)
Ryu et al.[38]	Non-small cell	Cisplatin-based	PCR	A/A	96	49	0.74
			Hybridization	A/C	12	42	(0.22-2.51)
Combined odds ratio (95% CI) for XPD polymorphism in patients with NSCLC: 1.38 (0.68-2.78).							

CI, confidence interval; PCR, polymerase chain reaction; RR, response rate; NSCLC, non—small-cell lung cancer; XPD, xeroderma pigmentosum group D.

TABLE 6. Cell Cycle Regulators, Mitogenic Signals, Tumor Protein p53, and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Alteration	Patients (*n*)	RR (%)	Odds ratio (95% CI)
Retinoblastoma 1 expression							
Gregorc et al.[40]	Non-small cell	Cisplatin-based	IHC	Low	61	51	0.45
				High	41	32	(0.20-1.03)
Cyclin-dependent kinase inhibitor 1A, p21 expression							
Dingemans et al.[23]	Small cell	CEV, EP	IHC	Low	63	90	0.57
				High	22	71	(0.17-1.92)
Kirsten rat sarcoma 2 viral oncogene homolog mutation							
Rodenhuis et al.[41, a]	Aenocarcinoma	Ifosfamide, carboplatin	PCR-MSH	Normal	46	26	0.65
				Mutated	16	19	(0.16-2.70)
Tumor protein p53 (P53) mutation							
Nakanishi et al.[36]	Non-small cell	Cisplatin-based	IHC	Normal	11	45	0.19
				Mutated	29	15	(0.04-0.94)
Gregorc et al.[40]	Non-small cell	Cisplatin-based	IHC	Normal	56	57	0.26
				Mutated	46	26	(0.11-0.62)
Combined odds ratio (95% CI) for P53 mutation in patients with NSCLC: 0.25 (0.12-0.52)							
Kawasaki et al.[31]	Small cell	CAV or EP	IHC	Normal	10	70	1.3
				Mutated	20	75	(0.24-6.96)
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Normal	47	85	0.81
				Mutated	45	82	(0.27-2.45)
Combined odds ratio (95% C.I.) for P53 mutation in patients with SCLC: 0.93 (0.37-2.35).							

CI, confidence interval; IHC, immunohistochemical analysis; PCR-MSH, polymerase chain reaction-mutation specific hybridization; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.
[a]Prospective study.

TABLE 7. B-Cell CLL/Lymphoma 2 (BCL2) Family Expression and Clinical Response to Chemotherapy

Author	Histology	Drugs	Method	Expression	Patients (*n*)	RR (%)	Odds ratio (95% CI)
BCL2							
Krug et al.[42]	Non-small cell	Docetaxel, vinorelbine	IHC	Low	26	46	1.75
				High	5	60	(0.25-12.3)
Dingemans et al.[23]	Small cell	CEV or EP	IHC	Low	20	79	1.36
				High	71	85	(0.38-4.86)
Takayama et al.[43]	Small cell	CAV or EP	IHC	Low	17	76	0.50
				High	21	62	(0.12-2.08)
Combined odds ratio (95% CI) for BCL2 expression in patients with SCLC: 0.87 (0.33-2.32)							
BAX (BCL2-associated X protein)							
Krug et al.[42]	Non-small cell	Docetaxel, vinorelbine	IHC	Low	9	56	0.72
				High	19	47	(0.15-3.54)

CI, confidence interval; IHC, immunohistochemical analysis; RR, response rate; CEV, cyclophosphamide, etoposide, and vincristine; EP, etoposide and cisplatin.

expression of topoisomerase II enzymes correlates with greater chemosensitivity in patients with breast cancer.[24]

In addition to genes involved in classical drug resistance, genes that act downstream of the initial damage induced by a drug-target complex are thought to play an important role in chemosensitivity.[25] ERCC1 is a key enzyme in nucleotide excision repair, one of the key pathways by which cells repair platinum-induced DNA damage. High levels of ERCC1 mRNA have been associated with platinum resistance in the treatment of ovarian and gastric cancer.[26,27] The codon 118 in exon 4 of ERCC1 gene is polymorphic with the nucleotide alteration AAC to AAT. Although this base change results in coding for the same amino acid, it may affect gene expression based on the usage frequency of synonymous codons.[28] The associations between drug response and both ERCC1 gene expression and polymorphism at codon 118 in patients with non—small-cell lung cancer have been reported in the literature. A combined OR (95%

CI) for these ERCC1 alterations was 0.53 (0.28-1.01, p = 0.055), although each study failed to show statistical significant association. Thus, ERCC1 may be a candidate for evaluation of the predictability of drug response in future clinical trials.

TP53, which is mutated or deleted in more than half of lung cancer cells, has a remarkable number of biological activities, including cell-cycle checkpoints, DNA repair, apoptosis, senescence, and maintenance of genomic integrity. Because most anticancer cytotoxic agents induce apoptosis through either DNA damage or microtubule disruption, mutated TP53 may decrease chemosensitivity by inhibiting apoptosis or, in contrast, may increase chemosensitivity by impairing DNA repair after drug-induced DNA damage.[29] This review showed that mutated TP53 was associated with poor drug response in patients with non—small-cell lung cancer (Table 6).

No other genes located downstream (including xeroderma pigmentosum group D, retinoblastoma 1, cyclin-dependent kinase inhibitor 1A, Kirsten rat sarcoma 2 viral oncogene homolog, B-cell CLL/lymphoma 2, and B-cell CLL/lymphoma 2-associated X protein) were associated with clinical drug response (Tables 5-7). The association was evaluated for only 8 of 43 *in vitro* chemosensitivity-associated downstream genes; therefore, key genes may be among the remaining 35 genes. Most clinical studies included a limited number of patients with various background characteristics such as tumor stage and chemotherapy regimen administered, which resulted in low statistical power to identify the association. Finally, because all but one study was retrospective, the quality of tumor samples may vary, and it is therefore unclear whether the gene alteration was detected in all samples. Thus, in future prospective clinical studies, the method of tumor sample collection and preservation, as well as immunohistochemistry and polymerase chain reaction-based methods, should be standardized, and the sample size of patients should be determined with statistical consideration.

The recently developed microarray technique enables investigators analyze mRNA expression of more than 20,000 genes at once, and as many as 100 to 400 genes were selected statistically as chemosensitivity-related genes.[6–8,10] Among them, however, only a limited number of genes were functionally related to chemosensitivity, and only ABCB1 and BAX corresponded with the 80 chemosensitivity-associated genes identified in this literature review, which were picked because of their known function and contribution to in vitro chemosensitivity. Thus, it will be interesting to evaluate the role of expression profile of these genes using microarray analysis.

The association between the expression and alterations of genes and clinical drug responses should be studied further in prospective trials. ABCB1, GSTP1, ERCC1, and TP53, and other genes identified by exploratory microarray analyses should be evaluated in those trials. Simple methods to identify gene alterations, such as immunohistochemistry and polymerase chain reaction-based techniques, will be feasible in future clinical trials because of their simplicity, cost, and time. The median number of patients in retrospective studies analyzed in this review was 50 (range, 28-108). In future prospective trials, sample size consideration for statistical power will also be important.

In conclusion, we identified 80 in vitro chemosensitivity-associated genes in a review of the literature; ABCB1, GSTP1, and ERCC1 expression and TP53 mutation were associated with drug responses among patients with lung cancer.

ACKNOWLEDGMENTS

This study was supported in part by Lung Cancer SPORE Grant P50CA70907, ATP010019, and Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan. We thank Yuko Yabe and Mika Nagai for their collection and arrangement of numerous papers.

REFERENCES

1. Sekine I, Saijo N. Novel combination chemotherapy in the treatment of non-small cell lung cancer. *Expert Opin Pharmacother* 2000;1:1131–1161.
2. Gazdar AF, Steinberg SM, Russell EK, et al. Correlation of in vitro drug-sensitivity testing results with response to chemotherapy and survival in extensive-stage small cell lung cancer: a prospective clinical trial. *J Natl Cancer Inst* 1990;82:117–124.
3. Cortazar P, Johnson BE. Review of the efficacy of individualized chemotherapy selected by in vitro drug sensitivity testing for patients with cancer. *J Clin Oncol.* 1999;17:1625–1631.
4. Cortazar P, Gazdar AF, Woods E, et al. Survival of patients with limited-stage small cell lung cancer treated with individualized chemotherapy selected by in vitro drug sensitivity testing. *Clin Cancer Res* 1997;3:741–747.
5. Shaw GL, Gazdar AF, Phelps R, et al. Individualized chemotherapy for patients with non-small cell lung cancer determined by prospective identification of neuroendocrine markers and in vitro drug sensitivity testing. *Cancer Res* 1993;53:5181–5187.
6. Mariadason JM, Arango D, Shi Q, et al. Gene expression profiling-based prediction of response of colon carcinoma cells to 5-fluorouracil and camptothecin. *Cancer Res* 2003;63:8791–8812.
7. Kikuchi T, Daigo Y, Katagiri T, et al. Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. *Oncogene* 2003;22:2192–2205.
8. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003;362:362–369.
9. Dan S, Tsunoda T, Kitahara O, et al. An integrated database of chemosensitivity to 55 anticancer drugs and gene expression profiles of 39 human cancer cell lines. *Cancer Res* 2002;62:1139–1147.
10. Girard L, Sekine I, Shah J, et al. Correlation between in vitro drug sensitivity and microarray-based gene expression signatures in lung and breast cancer. In Proceedings of the 95th Annual Meeting of American Association for Cancer Research, Orlando, FL, March 27–31, 2004.Pp. 1098.
11. Ein-Dor L, Kela I, Getz G, et al. Outcome signature genes in breast cancer: is there a unique set? *Bioinformatics* 2006;21:171–178.
12. Michiels S, Koscielny S, Hill C. Prediction of cancer outcome with microarrays: a multiple random validation strategy. *Lancet* 2006;365: 488–492.
13. Armitage P, Berry G, Matthews JNS. Statistical Methods in Medical Research, 4th ed. Oxford: Blackwell Science Ltd, 2002.
14. Sekine I, Saijo N. Polymorphisms of metabolizing enzymes and transporter proteins involved in the clearance of anticancer agents. *Ann Oncol* 2001;12:1515–1525.
15. Ellis M, Hayes DF, Lippman ME. Treatment of metastatic breast cancer. In Harris J, Lippman ME, Morrow M, Osborne CK (Eds.), Diseases of

the Breast, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2003.Pp. 1101-1159.
16. Gambacorti-Passerini CB, Gunby RH, Piazza R, et al. Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. *Lancet Oncol* 2003;4:75–85.
17. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol.* 2003;21:4342–4349.
18. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–2246.
19. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–2158.
20. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
21. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304: 1497–1500.
22. Minna JD, Gazdar AF, Sprang SR, et al. Cancer: a bull's eye for targeted lung cancer therapy. *Science* 2004;304:1458–1461.
23. Dingemans AM, Witlox MA, Stallaert RA, et al. Expression of DNA topoisomerase IIalpha and topoisomerase IIbeta genes predicts survival and response to chemotherapy in patients with small cell lung cancer. *Clin Cancer Res* 1999;5:2048–2058.
24. Di Leo A, Isola J. Topoisomerase II alpha as a marker predicting the efficacy of anthracyclines in breast cancer: are we at the end of the beginning? *Clin Breast Cancer* 2003;4:179–186.
25. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* 2002;108:153–164.
26. Dabholkar M, Vionnet J, Bostick-Bruton F, et al. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest* 1994;94:703–708.
27. Metzger R, Leichman CG, Danenberg KD, et al. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998;16:309–316.
28. Yu JJ, Mu C, Lee KB, et al. A nucleotide polymorphism in ERCC1 in human ovarian cancer cell lines and tumor tissues. *Mutat Res* 1997;382: 13–20.
29. Brown JM, Wouters BG. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. *Cancer Res* 1999;59:1391–1399.
30. Yeh JJ, Hsu WH, Wang JJ, et al. Predicting chemotherapy response to paclitaxel-based therapy in advanced non-small-cell lung cancer with P-glycoprotein expression. *Respiration* 2003;70:32–35.
31. Kawasaki M, Nakanishi Y, Kuwano K, et al. Immunohistochemically detected p53 and P-glycoprotein predict the response to chemotherapy in lung cancer. *Eur J Cancer* 1998;34:1352–1357.
32. Hsia TC, Lin CC, Wang JJ, et al. Relationship between chemotherapy response of small cell lung cancer and P-glycoprotein or multidrug resistance-related protein expression. *Lung* 2002;180:173–179.
33. Savaraj N, Wu CJ, Xu R, et al. Multidrug-resistant gene expression in small-cell lung cancer. *Am J Clin Oncol* 1997;20:398–403.
34. Rosell R, Scagliotti G, Danenberg KD, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. *Oncogene* 2003;22:3548–3553.
35. Dingemans AC, van Ark-Otte J, Span S, et al. Topoisomerase IIalpha and other drug resistance markers in advanced non-small cell lung cancer. *Lung Cancer* 2001;32:117–128.
36. Nakanishi Y, Kawasaki M, Bai F, et al. Expression of p53 and glutathione S-transferase-pi relates to clinical drug resistance in non-small cell lung cancer. *Oncology* 1999;57:318–323.
37. Lord RV, Brabender J, Gandara D, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. *Clin Cancer Res* 2002;8:2286–2291.
38. Ryu JS, Hong YC, Han HS, et al. Association between polymorphisms of ERCC1 and XPD and survival in non-small-cell lung cancer patients treated with cisplatin combination chemotherapy. *Lung Cancer* 2004; 44:311–316.
39. Camps C, Sarries C, Roig B, et al. Assessment of nucleotide excision repair XPD polymorphisms in the peripheral blood of gemcitabine/cisplatin-treated advanced non-small-cell lung cancer patients. *Clin Lung Cancer* 2003;4:237–241.
40. Gregorc V, Ludovini V, Pistola L, et al. Relevance of p53, bcl-2 and Rb expression on resistance to cisplatin-based chemotherapy in advanced non-small cell lung cancer. *Lung Cancer* 2003;39:41–48.
41. Rodenhuis S, Boerrigter L, Top B, et al. Mutational activation of the K-ras oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: a prospective study. *J Clin Oncol* 1997;15:285–291.
42. Krug LM, Miller VA, Filippa DA, et al. Bcl-2 and bax expression in advanced non-small cell lung cancer: lack of correlation with chemotherapy response or survival in patients treated with docetaxel plus vinorelbine. *Lung Cancer* 2003;39:139–143.
43. Takayama K, Ogata K, Nakanishi Y, et al. Bcl-2 expression as a predictor of chemosensitivities and survival in small cell lung cancer. *Cancer J Sci Am* 1996;2:212.

A Phase I Dose-Escalation Study of ZD6474 in Japanese Patients with Solid, Malignant Tumors

Tomohide Tamura, MD, Hironobu Minami, MD,† Yasuhide Yamada, MD, PhD,* Noboru Yamamoto, MD, PhD,* Tatsu Shimoyama, MD,* Haruyasu Murakami, MD,* Atsushi Horiike, MD,* Yasuhito Fujisaka, MD,* Tetsu Shinkai, MD, PhD,† Makoto Tahara, MD, PhD,† Kenji Kawada, MD,† Hiromichi Ebi, MD,† Yasutsuna Sasaki, MD, PhD,† Haiyi Jiang, MD,‡ and Nagahiro Saijo, MD, PhD**

Introduction: ZD6474 (vandetanib) is an orally available inhibitor of vascular endothelial growth factor receptor, epidermal growth factor receptor, and RET receptor tyrosine kinase activity. This study assessed the safety and tolerability of escalating doses of ZD6474 in Japanese patients with solid, malignant tumors.

Methods: Adult patients with solid tumors refractory to standard therapy received a once-daily oral dose of ZD6474 (100–400 mg) in 28-day cycles, until disease progression or unacceptable toxicity was observed.

Results: Eighteen patients were treated at doses of 100 mg (n = 3), 200 mg (n = 6), 300 mg (n = 6), and 400 mg (n = 3). Dose-limiting toxicities at the completion of cycle 2 were hypertension (n = 3), diarrhea (n = 1), headache (n = 1), toxic skin eruption (n = 1), and alanine aminotransferase increase (n = 1). A dose of 400 mg/day was considered to exceed the maximum tolerated dose (MTD). Toxicities were manageable with dose interruption and/or reduction. Objective tumor response was observed in four of nine patients with non-small cell lung cancer (NSCLC) at doses of either 200 or 300 mg. Terminal half-life was about 90–115 hours. Plasma trough concentrations achieved steady-state conditions after approximately 1 month of daily dosing.

Conclusions: It was concluded that a dose of 400 mg/day was considered to exceed the MTD, and doses for phase II study were thought to be not more than 300 mg/day. The objective response observed in some NSCLC patients is encouraging for further studies in this tumor type.

Key Words: Phase I study, ZD6474, Vandetanib, Non-small cell lung cancer

(*J Thorac Oncol.* 2006;1: 1002–1009)

*National Cancer Center Hospital, Tokyo, Japan; †National Cancer Center Hospital East, Chiba, Japan; and ‡AstraZeneca KK, Osaka, Japan.

Address for correspondence: Tomohide Tamura, MD, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: ttamura@ncc.go.jp

ISSN: 1556-0864/06/0109-1002

Vascular endothelial growth factor (VEGF) is a potent stimulator of angiogenesis and plays an essential role in the formation and maintenance of the vasculature by activating protease expression, endothelial cell proliferation and migration, and capillary vessel formation.[1–4] Enhanced secretion of VEGF from tumor tissue induces vascular permeability and results in the development of a network of highly permeable, immature vessels that are characteristic of pathological angiogenesis.[5] Although VEGF binds to VEGFR-1 (Flt-1) and VEGFR-2 (KDR or Flk-1) on vascular endothelial cells, activation of VEGFR-2 alone is sufficient to stimulate VEGF-mediated angiogenesis.[6] Pathological angiogenesis is necessary for the progression of solid, malignant tumors,[7] and inhibition of VEGF-dependent signaling has been identified as a key antiangiogenic strategy.[8,9] The clinical value of inhibiting VEGF signaling in colon cancer,[10] non-small cell lung cancer (NSCLC),[11] and breast cancer[12] has been confirmed with bevacizumab, an anti-VEGF antibody.

Epidermal growth factor receptor (EGFR)-dependent signaling is an important pathway contributing to the growth and metastasis of tumor cells, and aberrant EGFR tyrosine kinase activity has been reported in a number of human tumors.[13,14] One consequence of upregulated EGFR tyrosine kinase activity is increased expression of proangiogenic factors, including VEGF,[15,16] which may lead to possible paracrine and autocrine stimulation of angiogenesis.

ZD6474 (vandetanib; ZACTIMA) is a novel inhibitor of VEGFR, EGFR, and RET tyrosine kinase activity.[17–20] As such, ZD6474 has the potential to inhibit two key pathways in tumor growth: VEGF-dependent tumor angiogenesis, and EGFR- and RET-dependent tumor cell proliferation and survival. Indeed, preclinical evaluation of ZD6474 has demonstrated potent inhibition of VEGF-dependent signaling and angiogenesis in vivo, as well as dose-dependent inhibition of tumor growth, including profound regression in established PC-3 prostate tumors. More recently, the results of a phase I study of ZD6474 conducted in the United States and Australia showed that once-daily continuous oral dosing was generally well tolerated in patients with advanced tumors.[21]

We report the results of a phase I, open-label, nonrandomized, multicenter clinical study of ZD6474 in Japanese patients with advanced solid tumors. The primary objective

of the study was to assess the safety and tolerability of escalating oral doses of ZD6474, with the aim of establishing the maximum tolerated dose (MTD) and the recommended doses for further phase II study assessment. Additional objectives included evaluation of antitumor activity and assessment of single- and multiple-dose pharmacokinetics.

PATIENTS AND METHODS

Patients

Adult patients between 20 and 74 years of age with solid, malignant tumors refractory to standard therapies, or for which no appropriate therapy exists, were eligible for inclusion. Patients were required to have a life expectancy ≥3 months and a World Health Organization performance status of 0 or 1. The main exclusion criteria were significant cardiac, hematopoietic, hepatic or renal dysfunction; severe complications (including active double cancers); any gastrointestinal disease that would affect drug bioavailability; poorly controlled hypertension; CNS tumors and metastases; systemic anticancer therapy or radiotherapy within the previous 4 weeks; unresolved adverse effects from prior anticancer therapy or radiotherapy; and incomplete recovery from prior surgery. All patients provided written informed consent. The trial was approved by the ethics committee of institutional review board and was conducted in accordance with the Declaration of Helsinki and guidelines for good clinical practice.

Study Design

This was an open-label, nonrandomized, multicenter dose-escalation study. Patients received a single oral dose of ZD6474 (100, 200, 300, or 400 mg), which was followed by a 7-day observation period (cycle 0; Figure 1). On day 8, patients started a once-daily ZD6474 dosing regimen at the same dose as they had received in cycle 0 for a total of 28 days (cycle 1). Further 28-day treatment cycles were repeated at the same dose. A dose-limiting toxicity (DLT) was defined as any toxicity of at least grade 3 according to common toxicity criteria (CTC version 2.0) that was related to ZD6474 treatment, or grade 2 diarrhea daily for >7 days or grade 3 diarrhea despite maximum antidiarrheal support; ≥grade 2 skin toxicity for >7 days that affected the patient's subjective well-being and required cessation of treatment, despite supportive care; and QT or corrected QT (QTc) prolongation ≥490 msec, or a rise of ≥60 msec from baseline QT or QTc to ≥460 msec. QTc values were obtained using Bazett's[22] method of correction.

The initial dose of ZD6474 was set at 100 mg/day, based on the minimum toxic effect dose in rats as well as safety data from U.S./Australian phase I study. Dose escalation was performed when a minimum of three patients per dose level had completed cycle 1 (28 days) without experiencing a DLT. The MTD was defined as the dose of drug at which 33.3% of patients experienced a DLT during cycle 1 that was not controlled with symptomatic therapy. Once the MTD was established, three or more additional patients were enrolled at the two highest dose levels below the MTD. This was to further characterize the safety, tolerability, and biological activity of ZD6474.

Assessment of Safety and Tolerability

The primary objective was to assess the safety and tolerability of escalating oral doses of ZD6474. After full physical examination at enrollment, adverse events (AEs) were recorded at each scheduled study visit.

Electrocardiograms (ECGs) were recorded at the screening visit, on days 1 (baseline) and 2 of cycle 0, and three times per week up to day 21 of cycle 1. If no prolongation of QT or QTc occurred, ECGs were performed weekly up to day 14 of cycle 2, every 2 weeks until the end of cycle 3 and monthly during subsequent cycles; and 29 days after the last dose. Vital signs (blood pressure, pulse rate, and body temperature) were measured before and 2, 4, 6, 8, and 10 hours after the drug administration on day 1, and then every 24 hours until day 7 of cycle 0; every 24 hours until day 15 of cycle 1; weekly thereafter until the end of cycle 2; once every 2 weeks during subsequent cycles; and at withdrawal.

Blood chemistry and hematological assessments were performed at the screening visit; predose of cycle 0; predose and on days 8, 15, 22, and 29 of cycles 1 and 2; every 2 weeks (days 15 and 29) during subsequent cycles; at withdrawal; and on days 15 and 29 after the last dose. Electrolytes were measured weekly for patients who experienced diarrhea or vomiting. Urinalysis was performed at the screening visit; on day 2 of cycle 0; on days 15 and 29 of cycle 1; on day 29 during subsequent cycles; at withdrawal; and on days 15 and 29 after the last dose.

Pharmacokinetic Assessment

The pharmacokinetic profile of ZD6474 was assessed after both single and multiple dosing. During cycle 0, blood

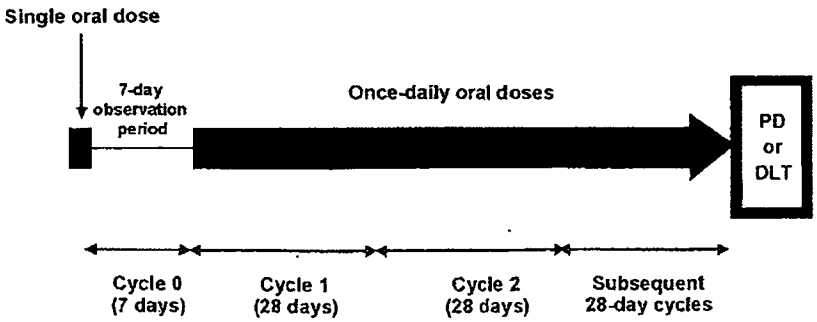

FIGURE 1. Study design. PD, progressive disease; DLT, dose-limiting toxicity.

samples were collected before and 1, 2, 4, 6, 8, 10, 24, 48, 96, 120, and 144 hours after administration. During cycle 1, blood samples were collected before administration on days 1, 8, 14, 22, and 28 and 2, 4, 6, 8, 10, and 24 hours after administration on day 28. Samples were also collected before administration on days 15 and 29 of cycles 2 and 3, before administration on day 29 of subsequent cycles, and at withdrawal. Plasma concentrations of ZD6474 were determined using high-performance liquid chromatography with mass spectrometry (LC-MS/MS). C_{max} and t_{max} were determined by visual inspection of the plasma concentration time data for ZD6474 for each patient on each sampling occasion. Where there were adequate data, ZD6474 plasma elimination half-life ($t_{½}$) was determined by log-linear regression of those points considered to constitute the terminal phase. The area under the plasma concentration time curve (AUC_{0-t}) was calculated using the linear trapezoidal rule. The accumulation ratio based on AUC_{0-24} was calculated by ratio of AUC_{0-24} after 28-day multiple doses to AUC_{0-24} after a single dose.

Assessment of Tumor Response

Tumor response was evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines[23] at the end of each treatment cycle. Baseline tumor assessments were performed before the start of single dosing.

Statistical Analyses

All analyses were descriptive, with no formal statistical analysis performed on the data from this study. AEs were coded according to both the Medical Dictionary for Regulatory Activities (MedDRA) coding system and the CTC grading system.

RESULTS

Patient Characteristics

All 18 patients (11 male, 7 female) enrolled in the study received ZD6474 treatment and were evaluable for safety, efficacy, and pharmacokinetics. Initially, three patients each were enrolled in the 100-, 200-, 300-, and 400 mg groups. Subsequently, three additional patients were enrolled in the 200- and 300-mg groups. Overall, 3, 6, 6, and 3 patients received ZD6474 100, 200, 300, and 400 mg, respectively.

The overall patient population profile is summarized in Table 1. Median duration of ZD6474 treatment was 56.5 (22–556) days. The median duration (range) of each dose group was 43.0 (30–45), 191.5 (29–556), 76.5 (25–124), and 37.0 (22–42) days in the 100-, 200-, 300-, and 400-mg groups, respectively. The reasons for discontinuation were radiological or clinical disease progression (n = 12), AEs (n = 5), or disease-related postrenal failure (n = 1).

Safety and Tolerability

All patients experienced at least one AE. Drug-related AEs by CTC grade with an incidence of at least 20% of the overall population are summarized in Table 2. The most common drug-related AEs were rash (n = 13), prolongation of QTc interval (n = 12), diarrhea (n = 11), and proteinuria (n = 11). There were various types of rash such as acne, dermatitis acneform, macular rash, maculopapular rash, pustular rash, erythema, folliculitis, photosensitivity rash, follicular rash, and skin eruption. Although there were no skin disorders of grade 3 or 4 severity, one patient in the 300-mg group developed grade 2 toxic skin eruption, which persisted for 7 days despite medical treatments and local supportive care. Because of this, the event was defined as DLT, and the study treatment was discontinued.

TABLE 1. Patient Characteristics

	ZD6474 Dose				
	100 mg (n = 3)	200 mg (n = 6)	300 mg (n = 6)	400 mg (n = 3)	Total (n = 18)
Male/female	1/2	5/1	3/3	2/1	11/7
Median age, yr (range)	50 (44–67)	52.5 (41–72)	55.5 (31–68)	53 (40–62)	52 (31–72)
Performance status (0/1)	1/2	2/4	2/4	1/2	6/12
Primary tumor diagnosis (n)					
NSCLC	1	3	3	2	9
Colorectal	1	1	1	1	4
Breast	0	1	0	0	1
Stomach	0	0	1	0	1
Other*	1	1	1	0	3
Number of prior cancer treatments†	3	6	6	3	18
Chemotherapy	3	6	5	3	17
Radiotherapy	1	1	3	1	6
Median duration of ZD6474 treatment, days (range)	43 (30–45)	191.5 (29–556)	76.5 (25–124)	37.0 (22–42)	56.5 (22–556)

*Various other tumor types.
†Includes surgery, chemotherapy, immunotherapy, hormonal therapy, and radiotherapy.
NSCLC, non-small cell lung cancer.

TABLE 2. Common Drug-Related Adverse Events by CTC Grade

	ZD6474 Dose								
	100 mg (n = 3)		200 mg (n = 6)		300 mg (n = 6)		400 mg (n = 3)		Total
Adverse Event*	G1/2	G3	G1/2	G3	G1/2	G3	G1/2	G3	(n = 18)
Rash (NOS)	1	0	6	0	4	0	2	0	13
Electrocardiogram QT corrected interval prolonged	2	0	4	0	4	0	2	0	12
Diarrhea (NOS)	1	0	4	0	3	1	2	0	11
Proteinuria	1	0	4	0	4	0	2	0	11
Fatigue	1	0	1	1	2	0	3	0	8
Hypertension† (NOS)	0	0	1	2	1	1	1	1	7
Blood lactate dehydrogenase increased	0	0	4	0	1	0	2	0	7
ALT increased	0	0	3	0	1	0	1	1	6
Anorexia	1	0	2	0	2	0	1	0	6
AST increased	0	0	3	0	1	0	2	0	6
β-N-acetyl-D-glucosaminidase increased	0	0	4	0	1	0	1	0	6
Hematuria	1	0	2	0	0	0	2	0	5
Headache	0	0	1	0	1	1	2	0	5
Lymphopenia	0	1	2	0	1	0	1	0	5
Blood alkalinephosphatase	0	0	3	0	1	0	1	0	5
Nausea	0	0	1	0	2	0	1	0	4

*Medical dictionary for regulatory activities (MedDRA) preferred term.
†Includes one patient with an adverse event reported as blood pressure increased.
CTC, common toxicity criteria; NOS, not otherwise specified; ALT, alanine aminotransferase; AST, asparate aminotransferase.
No grade 4 drug-related adverse events were reported.

All episodes of QT or QTc prolongation in this study were asymptomatic and considered by the investigator to be drug related. QTc prolongation necessitated dose interruption in 7 of 12 patients, 6 of whom were able to resume ZD6474 treatment at a reduced dose. The remaining patient was discontinued from the study after experiencing QTc prolongation, despite resuming treatment at a reduced dose.

No grade 4 drug related AE was observed. Seven patients experienced grade 3 drug-related AEs. The most common grade 3 drug-related AE was hypertension. One patient who had grade 3 hypertension in the 300-mg group was urgently hospitalized for hypertension and headache (both of grade 3) at 6 weeks after the start of multiple dosing. The symptoms were relieved 3 weeks after dose interruption, and the treatment with ZD6474 was resumed at a reduced dose of 150 mg/day. Eight patients had dose interruption, and five patients discontinued study treatment because of AEs. Drug-related AEs that led to treatment discontinuation were increased alanine aminotransferase, fatigue, hypoacusis, prolonged QTc interval, and toxic skin eruption (all n = 1).

Mean arterial blood pressure increased in most patients after multiple dosing with ZD6474. Hypertension or increased blood pressure was reported as an AE in eight patients (n = 4, grade 1 or 2; n = 4, grade 3). In five of these eight patients, the AE required treatment with standard antihypertensive medication (primarily Ca^{2+}-channel blockers or ACE inhibitors). There were no clinically relevant hematological toxicities. Elevations of ALT, asparate aminotransferase, and alkalinephosphatase reported as AE were in 6, 6, and 5 patients, respectively. Urinalysis revealed raised β-N-acetyl-D-glucosaminidase (n = 6) and proteinuria (n = 11), but all of these events were classified as CTC grade 1. Elevations of serum creatinine level were observed in three patients.

In total, five patients experienced drug-related DLTs up to the completion of cycle 2 (Table 3). Because 33.3% of patients in the 400-mg cohort developed a DLT during cycle 1, 400 mg was considered to exceed the MTD.

Pharmacokinetic Evaluation

Pharmacokinetic parameters following a single oral dose and multiple oral doses of ZD6474 (100–400 mg) are shown in Tables 4. Plasma concentration of ZD6474 decreased biphasically (Figure 2A). The terminal half-life seemed to be independent of the dose and was estimated to be approximately 100 hours; this may be underestimated because up to 40% of the AUC was extrapolated. Mean plasma trough concentrations of ZD6474 during continuous oral dosing indicate that steady state is achieved after about 1 month of treatment (Figure 2B). Based on the $AUC_{0-24\ h}$ on days 1 and 28, exposure to ZD6474 increased approximately sixfold after multiple dosing compared with a single dose. The relationship between AUC and dose after a single dose and 28-day multiple dosing was shown in Figure 3A and B, respectively. Exposure to ZD6474 as assessed by AUC after a single oral dose seemed to show an increase with dose. There was an approximately threefold interindividual variability in AUC at the same dose level.

TABLE 3. Drug-Related Dose-Limiting Toxicity (DLT) at the Completion of Cycle 2

ZD6474 (mg)	Patients Enrolled	Patients Developing DLT	DLT*	
			Cycle 1	Cycle 2
100	3	0/3	None	None
200	3	0/3	None	None
	3	1/3	Hypertension	None
	(additional cohort)			
300	3	1/3	None	Hypertension, diarrhea, headache†
	3	1/3	None	Toxic skin eruption
	(additional cohort)			
400	3	2/3	Hypertension	Alanine aminotransferase increased

*All DLTs were CTC grade 3 except for grade 2 toxic skin eruption.
†Observed in the same patient.
CTC, common toxicity criteria; ALT, alanine aminotransferase.

Tumor Response

Tumor responses were evaluated in 18 patients. No complete response was observed, but four patients achieved a confirmed partial response (three patients in the 200-mg group and one patient in the 300-mg group), all of whom had NSCLC with adenocarcinoma. Prior cancer treatments in these four patients included chemotherapy (n = 4), surgery (n = 2), and radiotherapy (n = 2). Each of the responders experienced dose interruptions/reduction because of AEs, but their responses were maintained at a reduced dose of 100 or 200 mg/day; the individual time to onset of response was 36, 64, 70, and 103 days, with a respective duration of response of 90, 230, 246, and 438 days (Table 5). Three of the four responders subsequently discontinued treatment because of AEs. Representative CT scans from two responders are shown in Figure 4.

DISCUSSION

In this phase I dose-escalation study, once-daily oral dosing with ZD6474 was generally well tolerated at doses up to and including 300 mg in Japanese patients with solid, malignant tumors. Pharmacokinetic analyses confirmed that once-daily oral dosing was appropriate for ZD6474, which had an estimated half-life of approximately 5 days. Notably, partial tumor response was observed in four out of nine patients with refractory NSCLC.

The most common drug-related AEs were rash, QTc prolongation, diarrhea, and proteinuria. QTc prolongation was reported at all doses studied, with no clear evidence of dose dependency. All patients with QTc prolongation were asymptomatic, and most did not require withdrawal of ZD6474 treatment. QTc prolongation was reversible and can be managed through dose interruption or dose reduction.

TABLE 4. Pharmacokinetic Parameters of ZD6474 After a Single Dose (Cycle 0) and After Multiple Dosing for 28 Days (Cycle 1)

	ZD6474 Dose			
Parameters After a Single Dose	100 mg (n = 3)	200 mg (n = 6)	300 mg (n = 6)	400 mg (n = 3)
Mean C_{max}, ng/mL (SD)	103 (42)	186 (92)	392 (198)	447 (240)
Median t_{max}, hr (range)	6 (4–6)	4 (4–6)	5 (4–8)	6 (2–6)
Mean $AUC_{0-24\ h}$, μg·hr/ml (SD)	1.5 (0.5)	2.8 (1.5)	5.6 (2.5)	6.7 (3.0)
Mean AUC, μg·hr /ml (SD)	10.1 (3.5)	16.8 (6.9)	29.4 (11.8)	32.1 (4.7)
Mean $t_{½}$, hr (SD)	115 (46)	101 (14)	90 (14)	114 (45)
Parameters After Multiple Dosing	**100 mg (n = 3)**	**200 mg (n = 4)**	**300 mg (n = 3)**	**400 mg (n = 1)**
Mean C_{max}, ng/mL (SD)	1200 (583)	922 (259)	1580 (302)	2050
Median t_{max}, hr (range)	4 (4–6)	6 (4–10)	6 (6–6)	4
Mean AUC_{0-24h}, μg·hr/ ml (SD)	20.5 (5.0)	18.3 (5.7)	29.9 (4.6)	44.6
Accumulation index* (SD)	14.2 (1.8)	6.2 (1.9)	5.3 (1.2)	6.5

*Day 28 $AUC_{0-24\ h}$/day 1 $AUC_{0-24\ h}$.
AUC, area under the curve to infinity; $AUC_{0-24\ h}$, area under the curve to 24 hr; C_{max}, maximum concentration; SD, standard deviation; t_{max}, time to maximum concentration; $t_{½}$, terminal half-life.

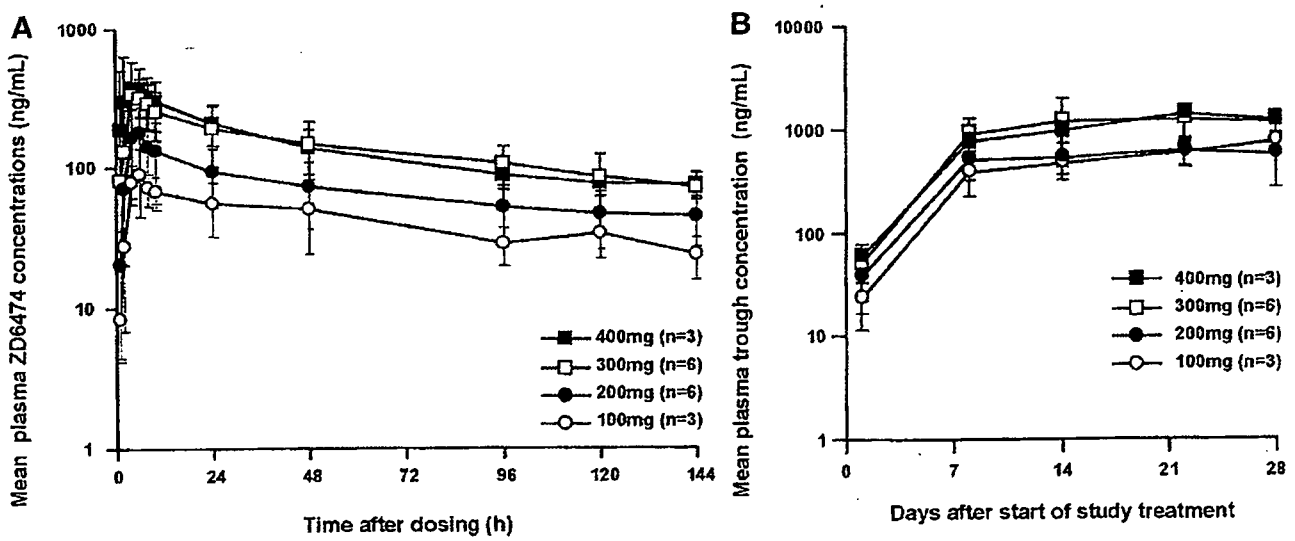

FIGURE 2. (*A*) Mean (±SD) plasma concentration of ZD6474 after a single oral dose. (*B*) Mean (±SD) plasma trough concentration of ZD6474 during continuous oral dosing for 28 days (cycle 1).

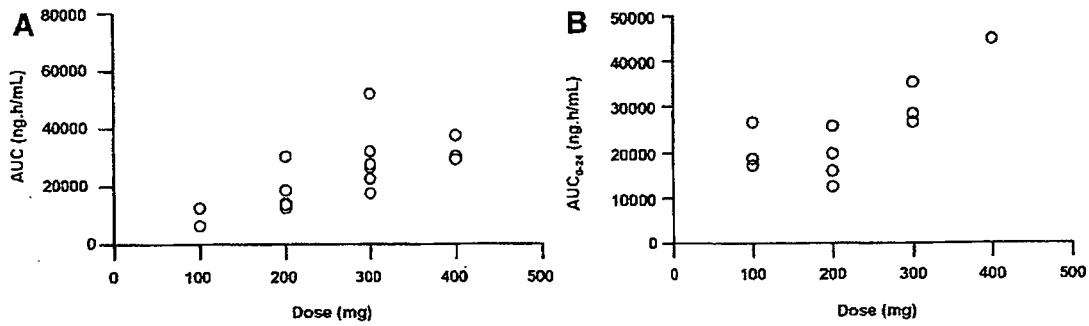

FIGURE 3. (*A*) Relationship between AUC and dose after a single oral dose of ZD6474. (*B*) Relationship between AUC_{0-24} and dose after 28-day multiple doses of ZD6474. AUC, area under the curve from zero to infinity; AUC, area under the curve from 0 to 24 hours.

There were some T-wave and U-wave changes in ECG, but there was no consequent arrhythmia finding in ECG. However, ECG monitoring should continue in future clinical trials.

Hypertension was also reported as a drug-related AE in seven patients, but no patients withdrew from the study as a result of hypertension, and all cases were controllable with dose adjustment or appropriate drug therapy. Rash and hypertension were also reported as relatively common AEs in a larger phase I study of ZD6474, which was conducted in the United States and Australia.[21] These events could be indicative of target inhibition by ZD6474. Also, because synthesis

TABLE 5. Summary of Partial Responders

Patient No.	Age (yr)	Sex	Initial ZD6474 Dose (mg)	Dose Reduction[a]	Partial Response: Time to Onset (days)	Partial Response: Duration (days)[b]
301	72	M	200	200→100 mg (day 28)	64	+230
304	54	M	200	200→100 mg (day 42)	103	438
305	41	M	200	200→100 mg (day 276)	70	+246
406	50	F	300	300→200 mg (day 79)	36	+90

[a]Dose reduction was attributable to AEs: QT/QTc prolongation (#301); hypertension (#304); rash (#305); toxic skin eruption (#406).
[b]Dose discontinuation was attributable to: hypoacusis (#301); disease progression (#304); fatigue (#305); toxic skin eruption (#406).

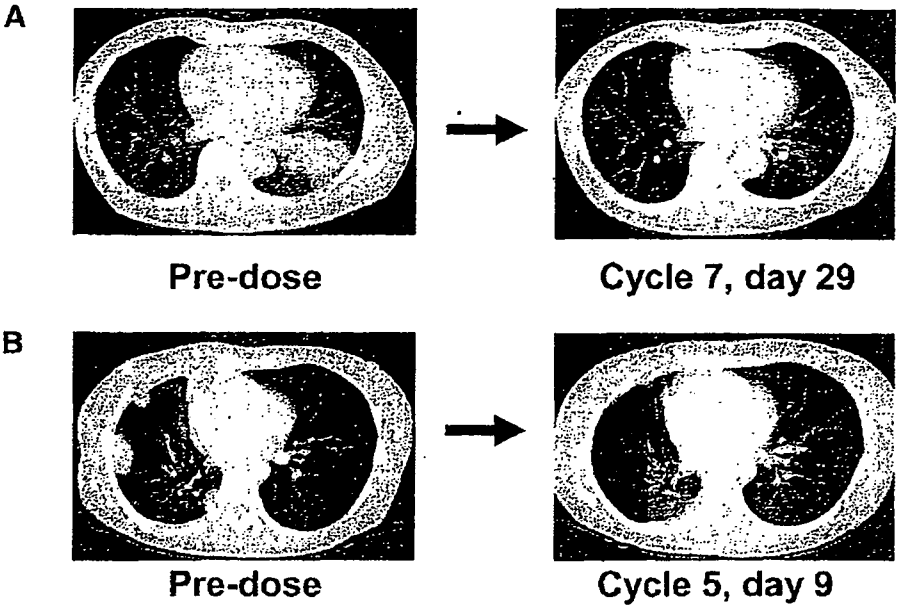

FIGURE 4. Representative CT scans before and after ZD6474 treatment in two NSCLC (adenocarcinoma) patients with partial responses. Baseline scans were performed within 4 weeks before the first dose. Male, 72 years (#301), initial ZD6474 dose = 200 mg. Female, 50 years (#406), initial ZD6474 dose = 300 mg.

of the vasodilator nitric oxide is downstream of VEGF-induced angiogenesis signaling,[24] inhibition of VEGFR-dependent signaling by ZD6474 may decrease nitric oxide production and lead to hypertension. Hypertension and elevated ALT levels were reported as DLTs in the 400-mg dose group during the period up to completion of cycle 2. As a result, this dose was considered to exceed the MTD.

Rash may be a consequence of EGFR inhibition, with the consideration that dose-dependent development of rash was reported in studies of other EGFR inhibitors, erlotinib[25] and gefitinib.[26,27] Because different types of rash, including erythema and photosensitivity, were observed in this study, it seems that the rash induced by ZD6474 may be more varied and systematic than was reported with those EGFR inhibitors.

Pharmacokinetic assessment in this study has confirmed that ZD6474 offers a convenient once-daily oral dosing schedule that is sufficient to achieve steady-state exposure. In this respect, the pharmacokinetic characteristics of ZD6474 in this Japanese study did not differ from those obtained in the U.S./Australian study.[21]

Although this study was primarily designed to assess safety and tolerability, secondary assessment of efficacy revealed that four out of nine patients with NSCLC exhibited a partial response to ZD6474 treatment at initial daily doses of 200 mg (n = 3) and 300 mg (n = 1). It is worth noting that partial tumor response was maintained in these patients (range 90–438 days) despite subsequent reductions in daily dose. This finding has prompted evaluation of ZD6474 in patients with NSCLC in phase II studies.[28–30] Although EGFR mutational status was not determined for any patients in the current study, a recent preclinical study showed that the antiproliferative effects of ZD6474 were augmented in an NSCLC cell line harboring EGFR containing a small in-frame deletion mutation.[31] Characteristics predicting response to gefitinib such as female gender, adenocarcinoma, nonsmoking status, Asian ethnicity, and EGFR mutations should be investigated in future studies.

Multiple signaling pathways contribute to tumor-related angiogenesis and tumor growth and metastasis. As such, novel therapies that target a single molecule or biochemical pathway may have less clinical efficacy than agents with more than one mode of action. Because ZD6474 is a selective inhibitor of VEGFR-2 and EGFR tyrosine kinase activity, this agent may be particularly beneficial in tumor types that display aberrant activity of both signaling pathways. However, the relative contribution of VEGFR-2 and EGFR tyrosine kinase inhibition to the clinical activity of ZD6474 in specific tumor types, as well as to the toxicity profile of ZD6474, remains to be determined.

In conclusion, these data indicate that ZD6474 at oral doses up to 300 mg/day was tolerated in Japanese patients with advanced tumors. A dose of 400 mg/day was considered to exceed the MTD, and doses of ≤300 mg/day were considered appropriate for evaluation in a further phase II study.[29]

Targeting multiple pathways in cancer may be necessary to provide sustained clinical benefit to patients, and ZD6474 has the potential to inhibit two key pathways in tumor growth by targeting VEGFR-dependent tumor angiogenesis and EGFR-dependent tumor cell proliferation and survival. Phase III development of ZD6474 in NSCLC has been initiated, and the clinical development program continues to investigate efficacy in other tumor types.

ACKNOWLEDGMENTS

This study, including editorial assistance provided by Dr. John Matthew, was supported financially by AstraZeneca. ZACTIMA is a trademark of the AstraZeneca group of companies.

REFERENCES

1. Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. *Semin Oncol* 2002; 29:10–14.
2. Keck PJ, Hauser SD, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 1989;246:1309–1312.
3. Lamoreaux WJ, Fitzgerald ME, Reiner A, et al. Vascular endothelial growth factor increases release of gelatinase A and decreases release of tissue inhibitor of metalloproteinases by microvascular endothelial cells in vitro. *Microvasc Res* 1998;55:29–42.
4. Pepper MS, Montesano R, Mandriota SJ, et al. Angiogenesis: a paradigm for balanced extracellular proteolysis during cell migration and morphogenesis. *Enzyme Protein* 1996;49:138–162.
5. Benjamin LE, Golijanin D, Itin A, et al. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J Clin Invest* 1999;103:159–165.
6. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocr Rev* 1997;18:4–25.
7. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–257.
8. Ellis LM, Liu W, Fan F, et al. Role of angiogenesis inhibitors in cancer treatment. *Oncology (Huntingt)* 2001;15:39–46.
9. Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. *Oncologist* 2004;(9 Suppl 1):2–10.
10. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–2342.
11. Sandler AB, Gray R, Brahmer J, et al. Randomized phase II/III trial of paclitaxel (P) plus carboplatin (C) with or without bevacizumab (NSC # 704865) in patients with advanced non-squamous non-small cell lung cancer (NSCLC): An Eastern Cooperative Oncology Group (ECOG) Trial - E4599. *Proc Am Soc Clin Oncol* 2005; abst LBA4.
12. Miller KD, Wang M, Gralow J, et al. A randomized phase III trial of paclitaxel versus paclitaxel plus bevacizumab as first-line therapy for locally recurrent or metastatic breast cancer: results from the Eastern Cooperative Oncology Group (ECOG) study E2100. *Presented at the American Society of Clinical Oncology Annual Meeting, May 13–17*, 2005, Orlando, Florida.
13. Ciardiello F, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001; 7:2958–2970.
14. Harari PM. Epidermal growth factor receptor inhibition strategies in oncology. *Endocr Relat Cancer* 2004;11:689–708.
15. Gille J, Swerlick RA, Caughman SW. Transforming growth factor-alpha-induced transcriptional activation of the vascular permeability factor (VPF/VEGF) gene requires AP-2-dependent DNA binding and transactivation. *EMBO J* 1997;16:750–759.
16. Goldman CK, Kim J, Wong WL, et al. Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: a model of glioblastoma multiforme pathophysiology. *Mol Biol Cell* 1993;4:121–133.
17. Ciardiello F, Caputo R, Damiano V, et al. Antitumor effects of ZD6474, a small molecule vascular endothelial growth factor receptor tyrosine kinase inhibitor, with additional activity against epidermal growth factor receptor tyrosine kinase. *Clin Cancer Res* 2003;9:1546–1556.
18. Hennequin LF, Stokes ES, Thomas AP, et al. Novel 4-anilinoquinazolines with C-7 basic side chains: design and structure activity relationship of a series of potent, orally active, VEGF receptor tyrosine kinase inhibitors. *J Med Chem* 2002;45:1300–1312.
19. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res* 2002;62:4645–4655.
20. Carlomagno F, Vitagliano D, Guida T, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res* 2002;62:7284–7290.
21. Holden SN, Eckhardt SG, Basser R, et al. Clinical evaluation of ZD6474, an orally active inhibitor of VEGF and EGF receptor signaling, in patients with solid, malignant tumors. *Ann Oncol* 2005;16:1391–1397.
22. Bazett HC. An analysis of the time-relations of electrocardiograms. *Heart* 1920;7:353–370.
23. Therasse P. Measuring the clinical response. What does it mean? *Eur J Cancer* 2002;38:1817–1823.
24. Ziche M, Morbidelli L, Choudhuri R, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 1997; 99:2625–2634.
25. Perez-Soler R. Can rash associated with HER1/EGFR inhibition be used as a marker of treatment outcome? *Oncology (Huntingt)* 2003;17:23–28.
26. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2003;21:2237–2246.
27. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* 2003;290:2149–2158.
28. Heymach JV, Johnson BE, Prager D, et al. A phase II trial of ZD6474 plus docetaxel in patients with previously treated NSCLC: follow-up results *Proc Am Soc Clin Oncol* 2006; abst 7016.
29. Nakagawa K, Kiura K, Shinkai T, et al. A randomized double-blind phase IIa dose-finding study of ZD6474 in Japanese patients with NSCLC. *Proc Am Soc Clin Oncol* 2006; abst 7067.
30. Natale RB, Bodkin D, Govindan R, et al. ZD6474 versus gefitinib in patients with advanced NSCLC: Final results from a two-part, double-blind, randomized Phase II trial. *Proc Am Soc Clin Oncol* 2006; abst 7000.
31. Arao T, Fukumoto H, Takeda M, et al. Small in-frame deletion in the epidermal growth factor receptor as a target for ZD6474. *Cancer Res* 2004;64:9101–9104.

Lippincott Williams & Wilkins
a Wolters Kluwer business
Reprinted in Japan

ANTICANCER RESEARCH 26: 2479-2486 (2006)

A Phase I Study of Irinotecan in Combination with Amrubicin for Advanced Lung Cancer Patients

HIROYASU KANEDA, TAKAYASU KURATA, KENJI TAMURA, HISAO UEJIMA, KAZUHIKO NAKAGAWA and MASAHIRO FUKUOKA

Department of Medical Oncology, Kinki University School of Medicine, Osaka 589-8511, Japan

Abstract. *Background: A combination phase I study was conducted in a cohort of lung cancer patients to determine the maximum tolerated dose (MTD) and toxicities of irinotecan (CPT-11), a topoisomerase I inhibitor, in combination with amrubicin (AMR), a topoisomerase II inhibitor, and to observe their antitumor activities. Patients and Methods: Patients with lung cancer received AMR (35 – 40 mg/m^2 given intravenously over 5 min) for 3 consecutive days, and CPT-11 (50 – 60 mg/m^2 given intravenously over 90 min) after the completion of AMR infusion on days 1 and 8, every 3 weeks. Results: In total, eleven patients were enrolled in this study. The most frequent toxicities were bone marrow suppression, particularly leucopenia and neutropenia, followed by infection, diarrhea and pneumonitis. As a consequence of these toxicities, the MTD and the recommended dose could not be determined. There were two partial responses, which included one patient with small cell lung cancer (SCLC) who had previously received chemotherapy and the other with previously untreated non-small cell lung cancer (NSCLC). Conclusion: These data suggest that the combination of CPT-11 and AMR is not tolerated, as it mediates an unexpectedly strong myelosuppressive effect, and is inactive against both NSCLC and SCLC.*

Lung cancer is the leading cause of cancer deaths worldwide. In spite of the development of new anticancer agents, such as paclitaxel, docetaxel, irinotecan (CPT-11) and gemcitabine, the prognosis of lung cancer is still poor. New agents and new combination chemotherapy regimens are warranted in order to improve the outcome for lung cancer patients. The DNA topoisomerases are essential nuclear enzymes that catalyze the breakage and rejoining of DNA. There are two classes of DNA topoisomerases, type I (topo I) and type II (topo II), which alter the topology of single- and double-stranded DNA, respectively, and are concerned with genetic reactions including DNA replication, transcription and DNA repair (1). To date, several DNA topoisomerase inhibitors, including CPT-11, the anthracyclines and etoposide, have played an important role in lung cancer chemotherapy (2, 3). Moreover, some investigators have reported that the combination of topo I and topo II inhibitors resulted in a synergistic effect in preclinical studies (4). This synergistic effect may be related to their complementary functions. However, other investigators have reported, conversely, that inhibition of both topo I and topo II led to an antagonistic effect (5, 6). Thus, the inhibition of both topoisomerases seems to be a very attractive strategy in the context of lung cancer chemotherapy, although it is not clear whether the combination results in a synergistic, additive or antagonistic effect. Amrubicin (AMR) is a novel, totally synthetic, 9-aminoanthracycline derivative that inhibits topo II. It has more potent antitumor activity and less heart, liver and renal toxicities than doxorubicin, according to *in vivo* studies. Amrubicinol, the C-13 alcohol metabolite of AMR, which also inhibits topo II, has 10 to 100 times more antitumor activity than the parent compound. Based on preclinical study data, intravenous (*i.v.*) administration on 3 consecutive days every 3 weeks was recommended for use in a phase I/II study involving previously untreated advanced non-small cell lung cancer (NSCLC) patients. The dose-limiting toxicities (DLTs) were leucopenia, thrombocytopenia and gastrointestinal disturbance and the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies were 50 mg/m^2/day and

Abbreviations: NSCLC, non-small cell lung cancer; ED-SCLC, extensive-disease small cell lung cancer; PS, performance status; topo I, topoisomerase I; topo II, topoisomerase II; CPT-11, irinotecan; AMR, amrubicin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; RD, recommended dose; MST, median survival time; JCOG, Japan Clinical Oncology Group; FACS, Four Arm Cooperative Study; AUC, area under the concentration-time curve; C_{max}, concentration$_{max}$.

Correspondence to: Masahiro Fukuoka, Department of Medical Oncology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. Tel: +81-72-366-0221, Fax: +81-72-360-5000, e-mail: mfukuoka@med.kindai.ac.jp

Key Words: Irinotecan, amrubicin, lung cancer.

0250-7005/2006 $2.00+.40