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Seminars in Oncology

Geographic Variation in the Second-Line Treatment of Non–Small Cell Lung Cancer

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Although there is broad agreement on management options for treating different stages of non–small cell lung cancer (ie, surgery for stage I and II disease, combined treatment modalities for stage III disease, and platinum-based chemotherapy as initial treatment for appropriate patients with stage IV disease), there is considerable geographic variation in practice patterns. These variations reflect a number of factors, including health care economics, the influence of national and regional regulatory bodies, the nature of physician and patient interaction, and probable biological differences between different populations in terms of drug metabolism and inherent susceptibility to both drug activity and toxicity. The approaches taken by three different geographic regions, the United States, European Union, and Japan, are evaluated. Clinically, the most striking differences in activity and toxicity between different regions have been seen with the epidermal growth factor receptor inhibitors gefitinib and erlotinib. Japanese patients experience significantly greater response and a greater degree of interstitial lung disease than patients in the European Union and North America (ie, US and Canada). Similar differences in efficacy and toxicity have also been noted with cytotoxic chemotherapy agents in the first-line setting. These geographic and ethnic differences in toxicity and efficacy will need to be considered in the design and comparison of future clinical trials.
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Lung cancer is the most lethal malignancy in the developed world, and was expected to account for over one million deaths worldwide in 2005.[1] Non–small cell lung cancer (NSCLC) accounts for approximately 85% of these cases.[2] The vast majority of cases are secondary to tobacco use. Other etiologies include asbestos and radon exposure as well as a genetic contribution.

Although standards of care have been established for different stages of the disease, there is considerable geographic variation in practice patterns. Three major geographic factors influence the choice of second- and third-line therapy. First is the influence of the regulatory agencies that govern the approval of antineoplastic agents. Second is the influence of the specific national healthcare system, including factors governing reimbursement to patients and physicians for treatment. Finally, and most significantly, is the emerging recognition that there are biological differences between different populations in terms of drug metabolism and inherent efficacy. This article will briefly review the approaches taken to second-line therapy in three different areas of the world: the United States, European Union (EU), and Japan.

Overview of Second-Line Therapy

Docetaxel

The first agent to show unequivocal activity in the second-line treatment of NSCLC was docetaxel. A National Cancer Institute of Canada trial compared docetaxel at 75 mg/m^2 or 100 mg/m^2 versus best supportive care. This trial found superior quality and length of life for patients treated with 75 mg/m^2 docetaxel.[3] An industry-sponsored study in the United States compared docetaxel at either 75 or 100 mg/m^2 versus a physician choice of either vinorelbine or ifosfamide. Again, quality of life and survival were superior for docetaxel 75 mg/m^2.[4] The concordant results of these two trials support

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the conclusion that docetaxel 75 mg/m^2 every 3 weeks has a clear role in this setting. Docetaxel has been approved for treatment of previously treated NSCLC in the United States, EU, and Japan.

Pemetrexed

Pemetrexed, a new antifolate agent that has shown activity in mesothelioma, has been tested in the second-line treatment of NSCLC. A phase III trial randomizing patients to either pemetrexed (500 mg/m^2 every 3 weeks with vitamin B_{12} and folate supplementation) or docetaxel (75 mg/m^2 every 3 weeks) showed a similar level of activity but superior tolerability.[5] There was considerably less myelotoxicity and alopecia in the pemetrexed arm, and significantly fewer patients required hospitalization after treatment than with docetaxel. Activity, in terms of response rate, median survival time, and 1-year survival rate, was superimposable for pemetrexed and docetaxel. Pemetrexed has been approved in the United States and EU for the second-line treatment of advanced NSCLC.

Gefitinib

Gefitinib was the first drug to receive approval for third-line therapy of NSCLC anywhere in the world (Japan). This approval was controversial as its basis was response rate rather than a more unequivocal outcome of patient benefit, such as survival rate.[6] The drug had previously failed to show benefit (in terms of response or survival) as a first-line treatment when combined with standard chemotherapy.[7,8]

Two large phase II trials of gefitinib monotherapy, the Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 and IDEAL 2 studies, evaluated the agent in pretreated NSCLC. Both studies determined response and survival. The IDEAL 1 trial, conducted primarily in Japan and Europe, also evaluated the safety profile and symptom improvement, while the IDEAL 2 trial, conducted in North America, evaluated symptom improvement as an additional primary endpoint.[9,10] The response rates for dosages of 250 mg/day and 500 mg/day were 18.4% and 19% in IDEAL 1, and 12% and 9% in IDEAL 2, respectively. Many patients, even those with a poor performance status (ie, performance status 2–3) experienced symptom improvement (most notably in pulmonary symptoms of dyspnea and chest pain) within 2 weeks of starting gefitinib treatment. This improvement in quality-of-life scales, though questionable as there was no randomization against either best supportive care or another agent, was the major impetus for granting conditional approval to market the agent in the United States. Approval was granted under the provision that appropriate randomized trials be conducted. Gefitinib has not received approval in the EU, although it has been approved in Switzerland.

Subset analysis shows that female sex, adenocarcinoma (and, in particular, bronchioloalveolar histology), and nonsmoking status are predictors of response.[10,11] Female sex was a particularly strong predictor in both IDEAL trials. In the primarily North American IDEAL 2 study, 50% of women experienced symptomatic response versus 31% of men ($P = .006$). Radiographic regression was also seen in 19% of women versus only 3% of men ($P = .001$). Two groups in Boston, MA have recently reported that mutations in the aATP-binding pocket of the epidermal growth factor receptor (EGFR) tyrosine kinase (TK) domain predict for clinical benefit from gefitinib.[12,13] While others have confirmed the presence of mutations, the role of mutations versus other alterations in *EGFR* (copy number, expression as measured by fluorescence in situ hybridization) have also been proposed as predictors of response to EGFR TK inhibitors (TKIs). It remains unclear as to whether any of these molecular variables predict independently for outcome.[14]

The role of gefitinib has recently been questioned because of the results of the Iressa Survival Evaluation in Lung Cancer (ISEL) trial.[15] This trial, undertaken in countries in which gefitinib had not received approval (ie, countries other than the United States and Japan) randomized patients between gefitinib and placebo. The ISEL trial was conducted in cooperation with 210 institutes in 28 countries (not including Japan). An advantage was shown in terms of response rate.[15] However, a trend toward improved survival did not achieve statistical significance. The subset analysis in Asian and non-Asian patients showed that female sex and adenocarcinoma histology were more common characteristics in Asian patients (Table 1). The US Food and Drug Administration has recently restricted use of gefitinib to patients who are currently being treated with the agent and who demonstrate benefit, and those enrolled in clinical trials.

Erlotinib

Erlotinib is an agent very similar to gefitinib in terms of structure and activity. It too has been evaluated as a second-line drug in the treatment of NSCLC, showing 'promising results' in terms of response and survival in phase II trials.[16]

However, unlike gefitinib, a phase III trial was unequivocally positive. The National Cancer Institute of Canada led a study (JBR-21) comparing erlotinib with best supportive care in third-line therapy. This large study (more than 700 patients) provided definitive evidence of benefit in terms of survival for this agent.[17] Improvements in response (9% v >1%), median survival (6.7 v 4.7 months; $P < .001$), 1-year survival (31% v 21%), and symptomatology (cough, dyspnea, pain) were observed.[17] Erlotinib has been approved in the United States and EU for the second- and third-line therapy of advanced NSCLC.

Geographic Variations in Treatment

Variations in the efficacy and safety of second-line NSCLC therapies have been observed across geographic regions, and have had an impact on the choice of treatment options within the three key pharmaceutical markets of the United States, the EU, and Japan.

United States

As described above, three agents have been approved by the US Food and Drug Administration for use in the second-line

Table 1 A Comparison of Gefitinib Monotherapy Data Across Geographic Regions

Characteristics	Japanese[40]	Non-Japanese[40]	American[10]	Asian[15]	Non-Asian[15]
No. of patients by gefitinib dose					
250 mg/m²	51	53	102	235	894
500 mg/m²	51	55	114	0	0
Demographics					
Median age (yrs)	60	61	61	61	62
Age range (yrs)	28–77	38–85	30–84	NA	NA
Female (%)	37	22	43	40	31
PS 0–1 (%)	91	83	80	72	64
Stage IV (%)	80	81	89	NA	NA
Adenocarcinoma (%)	76	56	66	64	44
No. of prior chemotherapy regimens (%)					
1	53	59	1	54	48
2	47	41	41	46	52
3 or more	0	0	58	0	0
Treatment efficacy					
Response rate (%)	28	10	10	12	7
Median survival (mos)	12	9.9	6–7	9.5	5.2
1-year survival (%)	50	NA	24–27	44	21
Grade 3–4 toxicity (%)					
Diarrhea	4	3	3	NA	NA
Skin rash	3	5	2	NA	NA
ALT elevation	7	1	1	NA	NA
Interstitial lung disease	2	0	0	2	0.001

Abbreviations: ALT, alanine aminotransferase; NA, not applicable; PS, performance status.

setting: docetaxel, pemetrexed, and erlotinib. Erlotinib also has approval in the third-line setting. Gefitinib, which had been granted an accelerated approval-based on the phase II data from the IDEAL studies,[18] has been re-labeled in light of data from the ISEL trial.[19] At present it may only be prescribed in a non-investigational setting for patients who are already receiving the agent and who have demonstrated benefit.

Agent Selection. Controversy exists over which of the three approved agents should be used in the second-line setting. Several factors enter into consideration in the United States. First, docetaxel has also received approval as a first-line agent and is frequently used in this setting with carboplatin or cisplatin. Therefore, a patient who has already received this agent and has progressed would not be a suitable candidate to receive the drug again in the second-line setting. Second, there are no trials comparing the value (in terms of patient benefit) of any of the second-line agents in this setting. As a result, clinical judgement and economic issues are relevant. Third, there appears to be an emerging trend for physicians to use erlotinib in patients who have demonstrated the greatest degree of benefit, ie, non-smokers, women, those patients with adenocarcinoma histology, and those with Asian ancestry. It is possible that selection of patients in the future will also be driven by objective biological markers, ie, the presence of *EGFR* gene mutations or increased *EGFR* copy number. Pemetrexed is therefore used in the remaining population. For most practitioners the superimposable results in terms of survival for pemetrexed and docetaxel, coupled with its superior toxicity profile, make pemetrexed the preferred agent when both drugs are considered for second-line therapy.

Economics. Economic issues are of considerable importance given the expense of the agents. Most insurance programs in the United States will cover the cost of administration of intravenous agents but vary considerably regarding the coverage for oral agents. The cost of gefitinib (USD $2,000 to $3,000/month) is considerable. An assistance program sponsored by the manufacturer is available.

European Union

It is difficult to separate any side effects or outcome differences between the EU countries and North America. Several of the trials described above, including JBR-21 and the randomized trial of pemetrexed versus docetaxel, were conducted with significant accrual from European countries. Approvals within Europe are granted by the European Medicines Agency; a separate Committee for Proprietary Medicinal Products provides clinical expertise for the review process. Pemetrexed, erlotinib, and docetaxel are the agents currently approved in the EU for use as second-line therapy.

Japan

Japan was the first country to approve gefitinib for use in the treatment of lung cancer. Drug approvals in Japan are granted by the Ministry of Health, Labor, and Welfare. The Japanese have a significant preference for oral medications, a factor that is likely to have contributed to the rapid approval of gefitinib.[20]

Approximately 50% of the patients enrolled into the IDEAL 1 trial were Japanese.[9] The remainder were from Europe, Australia, and South Africa, and were predominantly white. Significant differences emerged regarding both efficacy and toxicity; there was no comparison of survival. The response rate was clearly higher for the Japanese (27.5% v 10.4%; $P = .0023$). There were no pharmacokinetic differences to explain this response difference. However, in a multivariate analysis, ethnicity did not emerge as an independent factor for response. Baseline factors such as performance status, sex, and histology appear to explain the ethnic differences.

In the ISEL study, the response rate and median survival time were 12% and 9.5 months in Asian patients and 7% and 5.2 months in non-Asian patients, respectively (Table 1).[15] Mutations of the *EGFR* gene, recently identified in patients with gefitinib-responsive lung cancer,[12,13] correlated well with clinical response to gefitinib and patient survival in retrospective case series studies.[21,22] The relatively high frequency of the mutations in East Asian patients (27% to 34%), compared with 14% or less in American patients, may explain the geographical difference in the efficacy of gefitinib.[12,23] The frequencies of grade 3–4 common toxicities of gefitinib, including diarrhea, skin rash, and alanine transferase elevation, were the same among the study populations (Table 1).

Treatment-Associated Interstitial Lung Disease. Because of the limited number of patients evaluated in clinical trials, it is sometimes difficult to identify and analyze uncommon toxicity before marketing a drug. Interstitial lung disease (ILD) associated with administration of gefitinib came to light in October 2002, 4 months after approval of this agent in Japan.[24] In the IDEAL studies, two Japanese patients developed grade 3–4 ILD (2%), while no patients outside Japan experienced ILD. In the ISEL study, the incidence of grade 3–4 ILD was 2% in Asian patients and .001% in non-Asian patients. In a retrospective evaluation of 112 Japanese patients, the incidence of ILD was 5.4%. The primary risk factor was a prior history of pulmonary fibrosis.[24] Between July 2002 and December 2004, there were 86,800 patients with NSCLC who were estimated to have received gefitinib in Japan. According to the Ministry of Health, Labor, and Welfare 1,473 patients were suspected of having ILD associated with the use of gefitinib and 588 patients died of ILD.[25] A prospective survey of gefitinib toxicity in 3,354 NSCLC patients treated at 698 hospitals in Japan between June and December 2003 showed that the incidence of ILD was 5.8% and the mortality rate was 2.5%.[26] Risk factors for the development of ILD identified in the Japanese population were preceding pulmonary fibrosis, smoking history, poor performance status, and male sex.[24,26,27] ILD tends to appear rapidly after initiation of therapy.[28]

In an analysis by the US Food and Drug Administration comparing the incidence of ILD associated with gefitinib treatment in North America and Japan, there was an incidence of approximately 2% from a Japanese postmarketing experience and 0.3% in approximately 23,000 patients in the United States expanded-access program.[18]

It is interesting to note that ILD has been associated with weekly docetaxel therapy in Japanese patients. In a phase II study, docetaxel as a single agent was administered at a dose of 35 mg/m^2 on days 1, 8, and 15 every 4 weeks in 48 patients with advanced or recurrent NSCLC. Of these, 33 patients had had no prior chemotherapy and 15 had received one prior chemotherapy treatment. Patients who had previously undergone thoracic radiotherapy, who had preceding ILD or pulmonary fibrosis, or who had severe pulmonary emphysema were excluded from the study. Of the 48 patients in the study, five (10.4%) developed grade 3–4 ILD.[29] The incidence of ILD associated with weekly administration of docetaxel in other countries varies with reports: grade 3–4 pulmonary toxicity was noted in seven of 35 (20%) patients in a Spanish study,[30] one of 63 (1.6%) in a French study,[31] none of 110 patients in an Italian study, and none of 30 patients in an American study.[32,33] It is unclear from these data whether the development of ILD represents a toxicity to which Japanese patients are predisposed, or is a diagnosis that is made more frequently in Japan for other reasons.

Differences in Efficacy and Toxicity. The differences between Western populations and the Japanese (and other non-Western ethnicities) in both the efficacy and toxicity of an anticancer agent are an emerging issue. Two recent trials comparing carboplatin plus paclitaxel with other combinations for first-line therapy of NSCLC were conducted in the United States (by the Southwest Oncology Group) and Japan (Japan Cooperative Oncology Group, Four Arm Comparative Study).[34] The carboplatin plus paclitaxel arm was similar in both studies (differing only by a slightly lower dose of paclitaxel in the Japanese study), and criteria for entry, dose modifications, toxicity, and response assessment were identical. Considerable differences in toxicity and activity were noted between the two studies. The rate of febrile neutropenia was five-fold greater (16% v 3%; $P < .0001$) in the Japanese trial, while the rate of neuropathy was substantially lower (5% v 16%; $P = .001$). The response rates were similar, while the 1-year survival rate was better in the Japanese trial (51% v 37%; $P = .009$).

Distribution of Genetic Polymorphisms for Thymidylate Synthase

Another area of growing interest in this field is the observation that the activity of antifolate agents may be related to germline differences in the expression of the target enzyme, thymidylate synthase (TS). Pemetrexed, though a multitargeted antifolate, appears to have its primary activity at TS. TS expression is controlled in part by the *TS* enhancer region (*TSER*) within the 5′ untranslated region of the *TS* gene. Recent work has shown that the *TSER* is polymorphic with significant ethnic variation and relates to the activity of the agents. Tandem repeats of 28 base pairs have been identified,

Table 2 Geographic Differences in the Incidence of TSER*3 Polymorphism[35]

Population	Individuals Homozygous for *TSER*3* (%)
White	28
African-American	24
Southwest Asian	40
Chinese	67

and expression of the gene is increased with additional repeats. A triple tandem repeat (*TSER*3*) demonstrates 2.6-fold greater expression than the double repeat (*TSER*2*). There is considerable variation in this polymorphism both within and between ethnic groups (Table 2).[35]

Increased expression of this enzyme can alter both the activity and pharmacology of folate antagonist agents. For example, the activity of 5-fluorouracil activity in colon cancer is influenced by the *TSER* polymorphism.[36] Patients homozygous for *TSER*3* show increased intratumoral levels of TS protein. Higher levels of TS are associated with poorer response rates and survival. In lung cancer, there is evidence from Japanese studies that elevated TS levels correlate with increased proliferation and decreased sensitivity to antifolate agents (specifically 5-fluorouracil).[37,38] Preliminary data indicate that *TS* gene polymorphisms are prognostic for patients treated with platinum-based chemotherapy.[39] Studies are currently in preparation to determine whether *TS* gene polymorphisms are a predictive or prognostic factor (or both) for treatment with pemetrexed in NSCLC.

Conclusion

Second- and third-line treatments have now emerged as a standard of care throughout the world. Regulatory agencies in the United States and EU have approved docetaxel, pemetrexed, and erlotinib for second-line use. Japan was the first country to approve an EGFR TKI (gefitinib) for second-line use. There appears to be a substantially greater response to both gefitinib and erlotinib in Japan, but also a significant risk of life-threatening pneumonitis. Moreover, this variation in efficacy and side-effect profile appears to be present in other Asian populations. These ethnic differences may be surrogates for differences in genetic aspects of drug metabolism or potential differences in tumor susceptibility. The findings of a recent 'common arm' study performed in the United States and Japan in first-line therapy, as well as the studies of the two EGF TKIs, clearly demonstrate that the benefits and risks of anticancer agents may differ between populations. It is clear that the benefits and risks of anticancer agents differ between populations.

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Phase 1 Clinical Study of Pegylated Liposomal Doxorubicin (JNS002) in Japanese Patients with Solid Tumors

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Background: Pegylated liposomal doxorubicin (PLD, JNS002) is a formulation of doxorubicin encapsulated polyethylene-glycol coated liposomes with prolonged circulation time and unique toxicity profile. This phase 1 study was aimed at investigating the maximum tolerated dose (MTD), recommended dose, toxicity, pharmacokinetics, and antitumor activity in Japanese patients with solid tumors.

Methods: Patients with solid tumors not amenable to standard forms of treatment were eligible. PLD was administered as an intravenous infusion every 4 weeks. Dose escalation of PLD was planned from 30 to 60 mg/m^2 in 10 mg/m^2 increments. The pharmacokinetics of total doxorubicin (encapsulated plus non-encapsulated) in plasma were examined for the first cycle of treatment.

Results: Fifteen patients, aged 49–69 (median; 56) years with advanced solid tumors were enrolled. The major non-hematological toxicities were hand–foot syndrome (HFS), rash and stomatitis. Myelosuppression, especially leukopenia and neutropenia were major hematological toxicities. Although HFS was not severe, a delay of doses for subsequent cycles was required with multiple dosing. The peak plasma concentration and the area under the concentration time curve of PLD increased proportionally to the dose. Objective response was observed in one patient and the normalization of tumor marker values in another. These two patients had been diagnosed with ovarian cancer.

Conclusion: The recommended dose for phase 2 clinical studies of PLD in Japanese patients was 50 mg/m^2 every 4 weeks. The encouraging results prompted us to plan a subsequent clinical study of PLD against ovarian cancer.

Key words: Phase 1 study – drug delivery system – Pegylated liposomal doxorubicin – JNS002

INTRODUCTION

Pegylated liposomal Doxorubicin (PLD) is a formulation of doxorubicin hydrochloride encapsulated in long circulating STEALTH® liposomes and formulated for intravenous administration. PLD was designed to enhance the efficacy and reduce the toxicities of doxorubicin such as myelosuppression, alopecia and cardiotoxicity by altering the plasma pharmacokinetics and tissue distribution of the drug.

This pegylated-liposome system can evade non-specific capture by the reticuloendothelial system because the outer shell of the liposome is covered with a hydrophilic PEG. This character is the basis of the so-called 'stealth effect' (1). The diameter of the liposome is small (100 nm) but is still large enough to avoid renal secretion. Meanwhile, in the solid tumor tissues, it was found that solid tumors generally possess the pathophysiological characteristics: hypervasculature, secretion of vascular permeability factors stimulating extravasation of macromolecules within the cancer and absence of effective lymphatic drainage from tumors that impedes the efficient clearance of macromolecules accumulated in solid tumor tissues. These characteristics of solid tumors are the basis of the enhanced permeability and retention effect, the EPR effect (2,3). Taking these data together, conventional low-molecular-weight anticancer agents disappear before reaching the tumor tissues and exerting their cell-killing effect. However, macromolecules and nanoparticles including liposomal carrier should have time to reach, exit from tumor capillaries and stay for a long time in tumor tissue, by means of the EPR effect (2–5). Following intravenous injection of PLD into tumor-bearing mice,

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doxorubicin levels measured in tumors are substantially higher than those seen in animals receiving comparable doses of non-encapsulated drug (6). It appears that PLD accumulates preferentially in tumor tissues with increased microvascular permeability, such as in the case of most tumors with active neoangiogenesis (7,8). At these tumor sites, the accumulating liposomes gradually break down releasing doxorubicin to the surrounding tumor cells (9,10). Antitumor efficacy of PLD has been evaluated in a variety of murine tumor models and human xenograft tumor models. In addition, it was also known to be effective against spontaneously arising malignancies in dogs (11).

Based on the previous clinical data, PLD is an active agent available for the treatment of AIDS-related Kaposi's sarcoma (12,13) and has shown significant activity against some solid tumors, including ovarian and breast cancer, in phase 1 and 2 studies (14–16). Phase 1 study in the USA and Israel of PLD in patients with solid tumor pointed at a major change in the toxicity profile of doxorubicin, characterized by dominant and dose-limiting mucocutaneous toxicities in the form of palmar–plantar erythrodysesthesia (PPE, known also as hand–foot syndrome, HFS) (17) and stomatitis, mild myelosuppression, minimal alopecia and no apparent cardiac toxicity (14). With the aim of establishing an effective treatment against malignant solid tumors using this promising new formulation of doxorubicin hydrochloride, we initiated a clinical study of PLD in Japan. The objectives of this phase 1 study were (i) to determine the maximum tolerated dose (MTD) and recommended dose of PLD, (ii) to identify the toxicity profile, (iii) to assess its pharmacokinetic (PK) profile, and (iv) to observe any antitumor activities.

PATIENTS AND METHODS

Patients

Patients with malignant solid tumors were eligible if they met the following criteria: (i) histologic or cytologic confirmation of malignant solid tumor; (ii) tumors resistant to standard therapies or for which there was no effective treatment; (iii) $\geq$20 years and $\leq$74 years of age; (iv) Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; (v) life expectancy of at least 3 months; (vi) no chemotherapy, hormonal therapy, radiation therapy, or surgery within 4 weeks prior to the registration (in case of nitrosoureas or mitomycin for previous treatment: 6 weeks); (vii) adequate bone marrow activity (white blood cell count $\geq$4000/μl and $\leq$12 000/μl, absolute neutrophil count $\geq$2000/μl, platelet count $\geq$100 000/μl, and hemoglobin level $\geq$9.0 g/dl), adequate hepatic function (serum total bilirubin [Tbil] level $\leq$1.5 times the normal upper limit, transaminase $\leq$2.5 times the normal upper limit), adequate renal function (serum creatinine [Cr] level $\leq$1.5 times the normal upper limit), and adequate cardiac function (left ventricular ejection fraction [LVEF] $\geq$55%) by echocardiography; (viii) no severe complications such as uncontrollable infections, heart disease, diabetes and psychogenic disorders; (ix) written informed consent given. Patients with any one of the following conditions were excluded from the study: pregnancy or lactation; symptomatic brain metastasis; doxorubicin dose given prior to study $\geq$300 mg/m^2; a history of hypersensitivity reactions to doxorubicin or ingredients of PLD; hepatic B or C virus or human immunodeficiency virus infection; prior extensive radiation therapy (>30% of bone marrow reserves), and others.

The protocol was approved by the institutional review board of the National Cancer Center and the study was performed in keeping the good clinical practice (GCP) regulations. The study was closed for accrual in March 2004.

Drug Administration

PLD was supplied by Janssen Pharmaceutical K. K. (Tokyo, Japan) as a dispersion including 50 mg of doxorubicin hydrochloride in STEALTH® liposome per vial (2 mg/ml). An amount prescribed less than 90 mg was diluted in 250 ml of 5% glucose solution and that of 90 mg or more was diluted in 500 ml of 5% glucose solution prior to administration. Diluted PLD was infused intravenously at a rate of 1.0 mg/min from the start to the end of infusion to minimize the risk of infusion reactions.

Patients were administered PLD on day 1 of each 28-day cycle and they received two or more cycles in principle. All patients were admitted for the first cycle of treatment to be monitored carefully, giving consideration to unexpected adverse events. Subsequent cycles were performed in the outpatient setting. Although no standard premedication was given, infusion reaction, nausea and vomiting were treated as needed.

STUDY DESIGN

Based on the results of previously reported clinical study (14), the starting dose of PLD was 30 mg/m^2 (Level 1) and dose escalation in 10 mg/m^2 increments was planned up to 60 mg/m^2 (Level 4). At each dose level, three patients were scheduled for entry. Three additional patients were scheduled for treatment at the same dose level if any of the predefined dose limiting toxicities (DLTs) was observed in one of the initial three patients. The MTD was defined as the dose level at which any of the DLTs was observed in two or more of three to six patients. Intrapatient dose escalation was not allowed. The treatment was repeated every 4 weeks, unless patients developed progressive disease or DLTs. In this study the DLTs were defined as follows: (i) grade 3 or more nonhematological toxicity except for nausea/vomiting, anorexia and general malaise; (ii) grade 3 or more febrile neutropenia; (iii) grade 4 hematological toxicity except grade 4 neutropenia not lasting for 5 days, according to the Japanese version of NCI-Common Toxicity Criteria prepared by the Japan Clinical Oncology Group (JCOG). As multiple dosing is required for PLD to show the optimal antitumor effect, the

recommended dose was determined after an overall review of the results obtained for the following: status of manifestation of DLT in cycle 1; status of manifestation and disappearance of toxicity in cycle 2 and subsequent cycles; frequency and nature of treatment delay/discontinuation; pharmacokinetics; and antitumor effect. Tumor responses were evaluated according to RECIST (response evaluation criteria in solid tumors) criteria.

PHARMACOKINETICS

Pharmacokinetic (PK) evaluation was performed in all patients during the initial cycle of treatment, and in patients who could be administered repeatedly during the second cycle of treatment. Venous blood samples (5 ml, anticoagulant: EDTA) were taken before dosing, at the end of infusion and 1, 4, 8, 24, 34, 48, 96, 168 and 240 h after completion of infusion, and then before dosing, at the end of infusion in the second cycle. Blood samples were immediately placed in ice water and centrifuged at 4°C, 1000×g for 10 min, and plasma was aliquoted and stored at −20°C or below in polyethylene tubes until analysis.

The concentrations of total (encapsulated plus non-encapsulated) doxorubicin and its major metabolite doxorubicinol in plasma were measured by validated reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection (excitation wavelength: 480 nm and emission wavelength: 560 nm) which is a modification of the measurement method previously reported (18).

The PK parameters (C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half-life; AUC, area under the concentration–time curve; V_c, volume of distribution; CL, total clearance) were calculated by non-compartmental analysis using WinNonlin™ (Pharsight) software.

In addition, an assessment was made of the correlation between C_{max} and AUC with the dose (mg/m^2) of liposomal doxorubicin administered. Moreover, the presence or absence of accumulation was verified by comparing the individual plasma concentrations of doxorubicin and doxorubicinol determined before dosing and at the end of infusion in the second cycle with the corresponding measured values in the initial cycle.

RESULTS

Patients' Characteristics

From April 2003 to January 2004, 15 patients were entered in this study. Their characteristics are listed in Table 1. There were five men and 10 women with good performance status and the median age was 56 (range, 49–69) years. The predominant types of tumor were ovarian cancer and non-small cell lung cancer. Seven patients had received surgical resection for primary tumors, all 15 patients had received prior chemotherapy and 11 had more than three prior regimens. Two patients had received anthracycline; one at a cumulative dose of 273 mg/m^2 and the other at 100 mg/m^2. A total of 67 cycles of PLD was administered, and the median number of cycles administered per patient was three (range, 1–15). All patients were included in the toxicity evaluation.

Table 1. Patients' characteristics

	Number of patients
Total number of patients	15
Male/female	5/10
Age (years)	
Median	56
Range	49–69
ECOG* performance status	
0	5
1	10
Primary cancer	
Ovary	6
Non-small cell lung	6
Breast	1
Esophagus	1
Thymic cancer	1
Prior treatment	
Surgery	7
Chemotherapy	15
Radiation	3
Number of prior chemotherapy regimens	
1	3
2	4
≥3	8

*Eastern Cooperative Oncology Group.

Toxicity

The major toxicities in the first cycle and all cycles are listed in Table 2. The principal non-hematological toxicities were skin toxicities consisting of HFS and skin rash, and stomatitis.

HFS and rash as major skin toxicities occurred in 12 (80.0%) and 10 (66.7%) patients, respectively. These toxicities were generally mild (≤grade 2, Table 2) with clinical symptoms including erythema, swelling, itching, pain and desquamation. The median time to onset of HFS and grade 2 HFS were 39 days (cycle 2) and 96 days (cycle 3.5) after treatment initiation, and the median duration of grade 2 HFS was 7 days. The median time to onset of rash and grade 2 rash were 29 days (cycle 1.5) and 64.5 days (cycle 2.5) after treatment initiation and the median duration of grade 2 rash was 5 days. These skin toxicities increased in

Table 2. Major toxicities

Dose (mg/m^2)	No. of patients	CTC grade																					
		HFS			Rash			Stomatis			Nausea			Anorexia			Leu			Neu			Anemia		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1st cycle																									
30	6	–	–	–	1	1	–	–	2	–	3	–	–	2	1	–	3	2	–	2	1	–	3	2	–
40	3	1	1	–	2	–	–	1	–	–	1	–	–	1	–	–	–	2	–	–	1	1	1	–	–
50	6	–	–	–	–	1	–	2	2	–	2	–	–	2	–	–	2	3	–	–	2	3	3	–	–
All cycles																									
30	6	1	2	–	2	1	–	1	2	–	4	–	–	4	1	–	2	3	–	1	2	–	3	2	–
40	3	1	2	–	1	1	–	–	1	–	2	–	–	1	–	–	–	2	–	–	1	1	1	–	–
50	6	4	2	–	1	4	–	2	2	–	2	–	–	2	–	–	2	3	1	–	2	3	1	3	–

HFS, hand–foot syndrome; Leu, leukemia; Neu, neutropenia; anemia, hemoglobin decrease.

frequency and severity at high dose or with multiple doses of PLD. In level 1 and 3 cohorts, treatment delays owing to skin toxicities were observed in six of 29 cycles and 10 of 32 cycles, respectively. However, these skin toxicities were manageable by delay of the next infusion and commonly used dermatologic medications including vitamin B_2, B_6 tablets, antihistamine and steroid tablets/ointment.

Stomatitis was observed in eight patients (53.3%) and was generally mild (≤grade 2, Table 2). The median times to onset of stomatitis and grade 2 stomatitis were 15 days (cycle 1) and 17 days (cycle 1) after treatment initiation, and the duration of grade 2 stomatitis was 7 days. This toxicity tended to occur after cycle 1, but resolved relatively promptly.

The principal hematological toxicities were leukopenia and neutropenia, and there was only one patient with grade 3 leukopenia in level 3 and there were 4 patients with grade 3 neutropenia in level 2 and 3 (1 and 3 patients, respectively, Table 2). The nadir time to leukopenia and neutropenia was approximately 3 weeks after treatment initiation. Although leukopenia and neutropenia increased in severity at high dose (50 mg/m^2) compared with at low dose (30 mg/m^2), they were manageable with just delay of subsequent treatment. No patient developed neutropenic fever, thrombocytopenia or grade 4 hematological toxicities in any dose levels. No patient required administration of granulocyte colony-stimulating factor or blood transfusion.

The left ventricular ejection fraction (LVEF) was determined at baseline and serially by heart ultrasonography. There was one patient each with grade 1 LVEF decrease after the administration of PLD cumulative dose of 40 and 100 mg/m^2, respectively. Seven patients developed cardiotoxicity that was reported as an adverse event (Table 3). All of them were grade 1. One patient, who had received 100 mg/m^2 anthracycline as previous treatment, experienced supraventricular arrhythmia, AV block and sinus arrhythmia after the administration of a PLD cumulative dose of 150 mg/m^2 (total doxorubicin dose of 250 mg/m^2). No patient required treatment for cardiotoxicity.

Table 3. Cardiotoxicities

	30 mg/m^2 ($n = 6$)	40 mg/m^2 ($n = 3$)	50 mg/m^2 ($n = 6$)
Cardiac disorder	2	2	3
Supraventricular arrhythmia	0	0	3
AV block	0	0	1
Myocardial	0	1	0
Palpitation	1	1	0
Pericardial effusion	1	1	0
Sinus arrhythmia	0	0	1
Ventricular	0	1	1

Grade 1 or 2 infusion reactions developed in 4 patients and they appeared within 10 min after initiation of infusion. All symptoms caused by infusion reaction disappeared within 60 min without any medication, interruption of infusion or infusion rate adjustment.

Three DLTs were recognized in one patient administered 30 mg/m^2 of PLD with grade 3 diarrhea, grade 3 infection not accompanied by neutropenia, and grade 3 hypoxia. Diarrhea and infection were recovered and improved at the end of the observation period, respectively, while hypoxia lasted. There was no DLT at the level of 40 or 50 mg/m^2. There were no treatment-related deaths in this study.

Antitumor Activity

All of 15 patients were evaluable for antitumor response. One and eight out of 15 evaluable patients had achieved

partial response and stable disease, respectively. The patient who achieved partial response (PR) was a 53-year-old female diagnosed as ovarian cancer with three lesions in peritoneum and one instance of pelvic lymph node metastasis. The duration of response was 441 days. In the case of the other patient with ovarian cancer who was evaluated as not evaluable (NE), the elevated tumor marker CA125 (241 U/ml) prior to the study entry was normalized (11 U/ml) after the second cycle of PLD.

PHARMACOKINETICS

Pharmacokinetic evaluation was performed using plasma samples obtained from all 15 patients during the initial cycle of treatment, and for 11 patients during the second cycle of treatment. Pharmacokinetic parameters are summarized in Table 4 and the mean plasma doxorubicin concentration–time profiles are illustrated in Fig. 1. Plasma doxorubicin concentrations after administration of PLD showed a monophasic decline, consistent with a one-compartment model. Total doxorubicin exhibited a long $t_{1/2}$ (range of mean values: 86.3–95.3 h), slow clearance (range of mean values: 11.0–13.1 ml/h/m^2), and small volume of distribution (range of mean values: 1.47–1.57 l/m^2) that was similar to the plasma volume.

The plasma C_{max} and AUC values increased proportionally with the dose of PLD ($P < 0.0001$ respectively, Fig. 2), suggesting linear pharmacokinetics in this dose range. Moreover, PLD did not significantly accumulate in plasma when administered at intervals of 4 weeks or longer. Plasma concentrations of doxorubicinol, the major metabolite of doxorubicin, were lower than the lower limit of quantitation in most samples (data not shown).

DISCUSSION

We report a phase 1 study of pegylated liposomal doxorubicin (PLD) given every 4 weeks in Japanese patients with solid tumors. The major non-hematological toxicities were HFS, rash and stomatitis. Myelosuppression especially, leukopenia and neutropenia were the most common hematological toxicities. HFS is rarely seen with standard doses of conventional doxorubicin and other liposomal anthracycline agents (19–21). In our study, grade 3 or higher skin toxicities were not observed but it was indicated that they increased in frequency and severity by multiple administration of PLD. These skin toxicities were manageable by delay of next infusion and commonly used dermatologic medications. Lyass et al. reported that severity of HFS was correlated with $t_{1/2}$ of PLD ($P = 0.0083$), and prevention of recurrence of HFS was best achieved by delay of the next infusion (22). The effect of dose interval on skin toxicity may be related to the turnover time of keratinocytes and epidermal transit time that are in the order of 3–4 weeks (23). Thus, prevention of recurrence of skin toxicity seems to be best achieved by delay of the next infusion because of allowing adequate time for recovering of keratinocytes.

The severity of stomatitis and the nadir leukocyte count were reported as correlated with dose level and C_{max} of PLD. In our study, these toxicities observed in Japanese patients tended to increase in severity along with dose escalation. As the results of our PK analysis revealed that C_{max} increased linearly with dose, it can be suggested that the toxicity profile observed in Japanese patients is similar to that reported by Lyass et al. (22). Prevention of increase in severity of these toxicities seems to be best achieved by dose reduction.

Cardiotoxicities observed in Japanese patients were all grade 1 in our study. The most serious toxicity of conventional doxorubicin therapy is cumulative-dose-related cardiotoxicity (24). Although no retrospective and prospective studies have identified a maximum 'cardiac safe' cumulative dose of PLD which may induce chronic heart failure, the result of a recent direct comparison study conducted in patients with metastatic breast cancer between PLD and conventional doxorubicin therapy showed that the risk of cardiotoxicity with PLD was significantly lower than that with conventional doxorubicin (21). Our study result and previous clinical studies suggest that PLD can be used in place of conventional doxorubicin to reduce the risk of cardiotoxicity without reducing the efficacy of therapy.

All infusion reactions appeared within 10 min after initiation of PLD infusion at rate of 1 mg/min and all of them were generally mild. However some cases that required discontinuation of treatment were reported (21). So it is very important to monitor the patients' condition carefully during the initial 10–15 min after start of PLD infusion. Infusion reaction was correlated with the initial PLD infusion rate—a lower infusion rate reduces the risk of infusion reaction (25).

Only one patient treated at level 1 developed DLTs and no patients developed DLT in level 2 and 3. However, the

Table 4. PK parameters

Dose (mg/m^2)	No. of patients	C_{max} (μg/ml)		AUC (μg h/ml)		$t_{1/2}$ (h)		CL (ml/h/m^2)		Vd (l/m^2)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
30	6	19.312	(2.502)	2512.7	(783.5)	89.50	(24.05)	13.14	(4.84)	1.569	(0.187)
40	3	25.605	(2.866)	3228.0	(789.6)	86.30	(14.72)	12.99	(3.70)	1.568	(0.174)
50	6	34.057	(3.293)	4663.3	(1061.8)	95.33	(25.32)	11.10	(2.05)	1.471	(0.130)

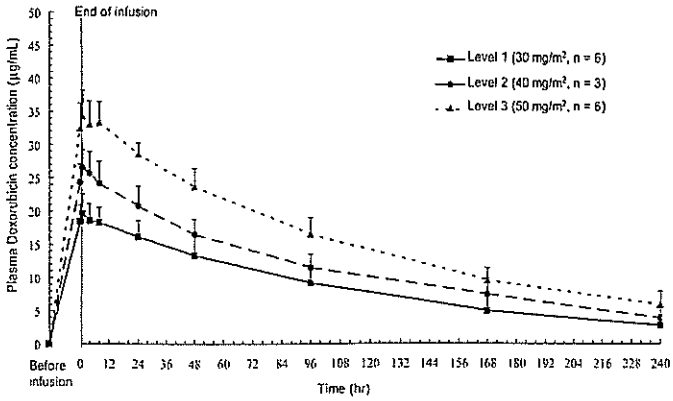

Figure 1. Mean plasma concentration–time curve for doxorubicin infused as PLD.

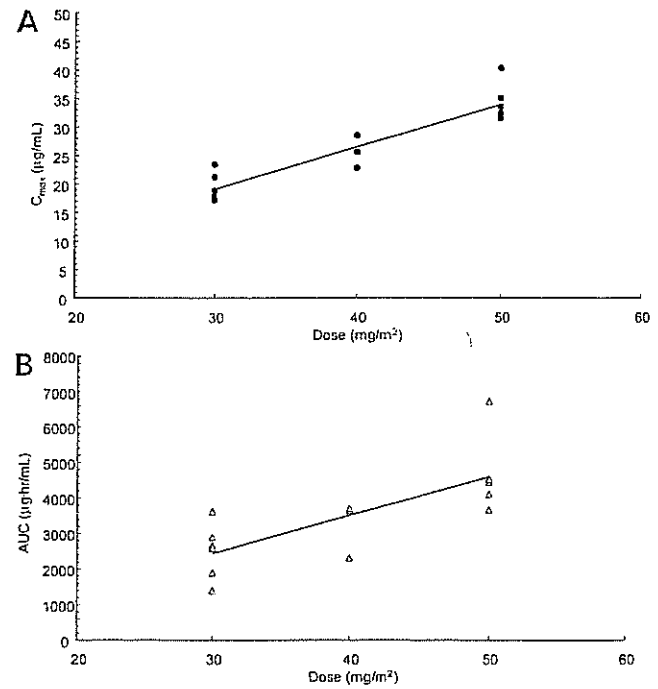

Figure 2. Relationships between (A) dose and C_{max} ($r^2 = 0.861$) and (B) dose and AUC ($r^2 = 0.575$) for doxorubicin infused as PLD.

independent data monitoring committee did not recommended further dose escalation beyond level 3. We accepted this recommendation for the following reasons. First, the repeated dosing toxicity of PLD in level 3, which was the approved dosage established in Europe and the USA, was acceptable. Second, among six patients treated at level 3, delay of therapy was required in three patients because of leukopenia in the present phase 1 study. Of these three, two patients also developed HFS leading to delay of therapy. In the level 3 cohort, HFS causing delay of therapy was observed in 10 of 32 cycles in total. Based on these findings, further dose escalation over level 3 seemed to be difficult as PLD requires multiple dosing to show antitumor activity. Third, from the results of PK analysis, PLD did not significantly accumulate in plasma when administered at intervals of 4 weeks or longer by level 3. Fourth, antitumor effect was already obtained in patients with ovarian cancer in the present study. From the above-mentioned facts, we concluded that level 3 (50 mg/m^2) was the recommended dose for subsequent phase 2 study. HFS showed an aggravating trend with repeated JNS002 treatment in our study, but did not lead to a severe toxicity. However, repeated JNS002 treatment in the previous phase 1 study in USA and Israel resulted in a severe dose-limiting toxicity. Therefore, further studies should be carefully conducted in a greater number of patients paying attention to the severity of HFS.

Regarding pharmacokinetics of PLD, the profile clarified in our study is largely consistent with previous findings in overseas studies indicating that PLD has an extremely long circulation time with a slow clearance and a small volume of distribution (22,26,27). Lyass et al. provided the results of correlation analysis that dose and C_{max} are strongly correlated with stomatitis and nadir leukocyte count, whereas plasma $t_{1/2}$ is significantly correlated with HFS which is one of the important cause for prolongation of dosing interval leading to delay of treatment for consequent cycle (22). The half-life values in the present study (86–95 h) are comparable to those reported previously (80–84 h, Hamilton et al. (26); 62–86 h, Lyass et al. (22); 75–91 h, Hubert et al. (27)).

PLD is already approved for the treatment of AIDS-KS and ovarian cancer in Europe and the USA, and breast cancer in Europe. Also in our study of six patients with ovarian cancer, one had achieved partial response and one had achieved normalization of the tumor marker CA125. This result is very encouraging in planning for further clinical studies in Japanese patients with ovarian cancer.

In conclusion, we confirmed the tolerance of the recommended dose (50 mg/m^2) in Europe and the USA, which was intravenous infusion of PLD every 4 weeks in Japanese patients, and one partial response and one normalization of CA125 were observed in patients with ovarian cancer. We concluded that the recommended dose in phase 2 clinical study was 50 mg/m^2 every 4 weeks. At present, a phase 2 clinical study in Japanese patients with ovarian cancer is ongoing.

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

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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

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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

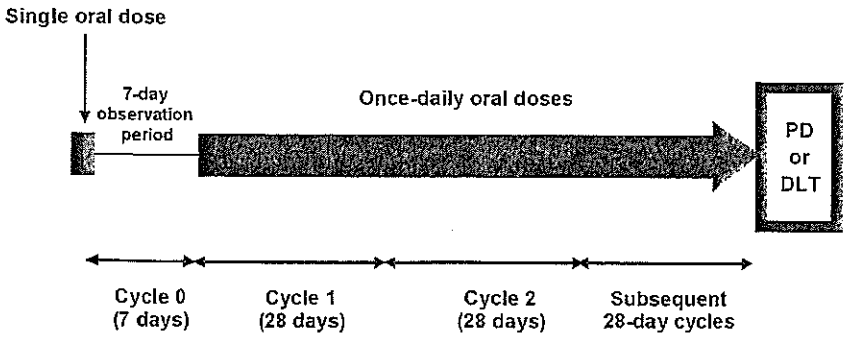

FIGURE 1. Study design. PD, progressive disease; DLT, dose-limiting toxicity.

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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.

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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)		
Adverse Event*	G1/2	G3	G1/2	G3	G1/2	G3	G1/2	G3	Total (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.

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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.

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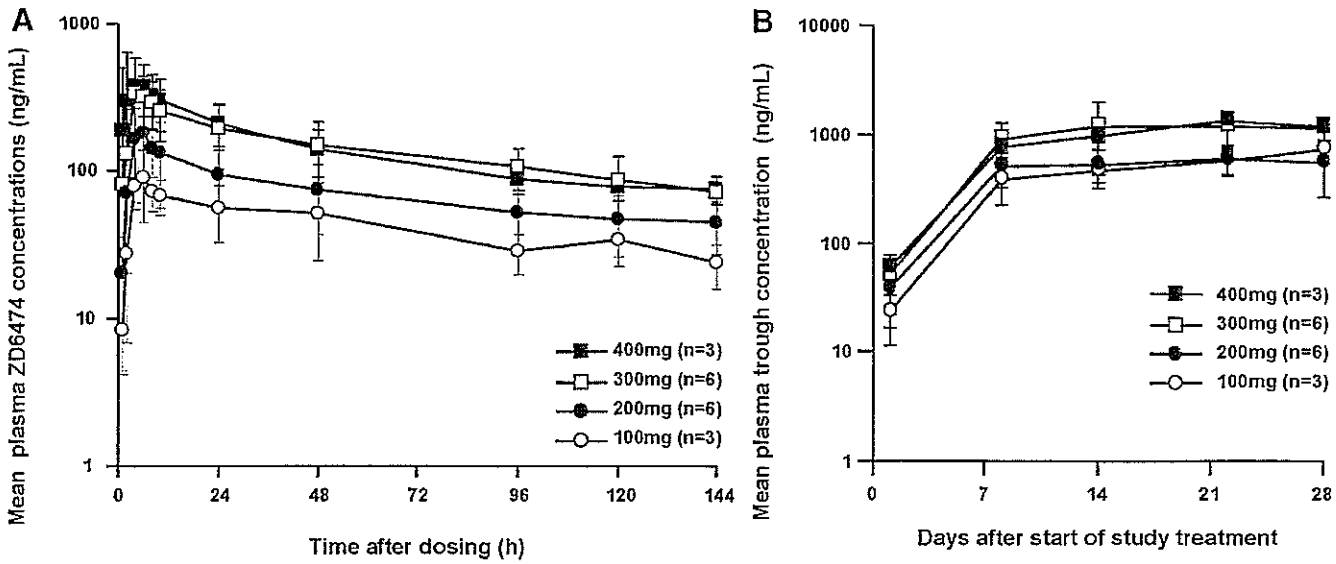

FIGURE 2. (*A*) Mean (±SD) plasma concentration of ZD6474 after a single oral dose. (*B*) Mean (±SD) plasma trough concentration of ZD6474 during continuous oral dosing for 28 days (cycle 1).

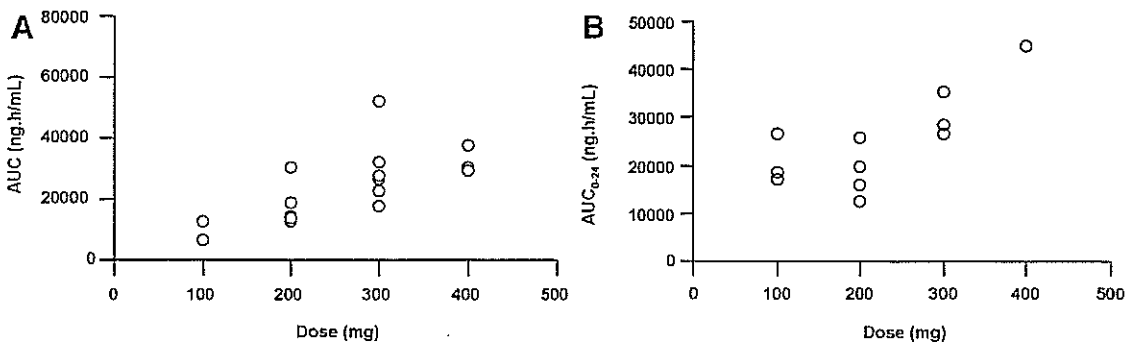

FIGURE 3. (*A*) Relationship between AUC and dose after a single oral dose of ZD6474. (*B*) Relationship between AUC_{0-24} and dose after 28-day multiple doses of ZD6474. AUC, area under the curve from zero to infinity; AUC, area under the curve from 0 to 24 hours.

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

					Partial Response	
Patient No.	Age (yr)	Sex	Initial ZD6474 Dose (mg)	Dose Reduction[a]	Time to Onset (days)	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).

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