Table 2 | **Summary of results from Phase II IDEAL trials**

Trial	Patient population	Gefitinib dose (mg /day)*	Number of patients	Objective response (%)	Disease control rate (%)	Symptom improvement rate (%)	Potential prognostic factors	Safety	CTC grade 3 or 4 drug-related AEs (%)	Withdrawals due to drug-related AEs (%)
IDEAL 1	PS 0–2; stage IIIA–IV disease; one or two previous CT regimens	250 500	104 106	18.4 19.0	54.4 51.4	40.3 37.0	Female gender; adenocarcinoma tumour histology; Japanese patients	Drug-related AEs generally mild (NCI-CTC grade 1 or 2) and were more common with 500 mg/day; most frequent AEs were mild skin and GI toxicities	8.7 30.2	1.9 9.4
IDEAL 2	PS 0–2; stage IIIB-IV disease; over two previous CT regimens	250 500	102 114	11.8 8.8	42.2 36.0	43.1 35.1	Female gender; adenocarcinoma tumour histology	Drug-related AEs generally mild (NCI-CTC grade 1 or 2) and were more common with 500 mg/day; most frequent AEs were mild skin and GI toxicities	6.9 17.5	1.0 4.0

*Patients were randomized to receive either 250 or 500 mg/day in each trial. AE, adverse event; CT, chemotherapy; GI, gastrointestinal; NCI-CTC, National Cancer Institute Common Toxicity Criteria (version 2.0); PS, performance status. Table compiled from data in REFS 39–41.

The reason for these regional differences in the occurrence of ILD is unknown, although it might be related to an increased susceptibility to ILD within the Japanese population that is independent of treatment with gefitinib. A higher rate of ILD in Japan, compared with other countries, has been reported in patients who were treated with the antirheumatic drug leflunomide[50]. Furthermore, a recent review has identified national differences in the terms used to describe the pulmonary side effects of drugs[51]. A retrospective analysis of 1,976 patients who have received gefitinib in Japan indicates that risk factors for ILD might include smoking status, male gender and pre-existing idiopathic pulmonary fibrosis[49]. Given that ILD is a known complication of lung cancer and has also been associated with chemotherapy and radiotherapy treatment[52], the small risk of developing ILD-type events during treatment with EGFR-TK inhibitors should not prevent patients with NSCLC from receiving these drugs.

Approval of gefitinib. Based on the results of the Phase II IDEAL trials, gefitinib was approved in Japan on July 5, 2002 for the treatment of inoperable or recurrent NSCLC. Subsequently, gefitinib has gained approval for the treatment of previously treated NSCLC in over 30 countries, including the United States.

Understanding responses

Clinical characteristics. Results from the IDEAL trials showed that in an unselected population of patients with pretreated NSCLC, treatment with gefitinib 250 mg/day resulted in clinical benefits (disease stabilization or tumour regression) in about 40–50% of patients, and an objective response in 12–18% of patients[39,40]. However, retrospective analyses of these trial data indicate that certain patient subgroups have a higher probability of achieving an objective tumour response than others. For example, in both IDEAL studies, objective tumour responses were more likely to be seen in female than male patients and in patients with adenocarcinoma NSCLC tumours than tumours of other histological types[39,40]. Furthermore, in the IDEAL 1 study, the response rates in Japanese patients were higher than those observed in non-Japanese patients[39]. Other studies have indicated further demographic factors to add to the list of potential predictive markers of gefitinib response, including patients with tumours of the bronchioloalveolar carcinoma histological subtype and patients with a history of non-smoking[53].

A similar analysis of data from the BR21 study of erlotinib showed that female gender, adenocarcinoma histology and a history of non-smoking could also be predictive of a patient's response to erlotinib[54]. Although tumour EGFR levels and the appearance of rash had initially been postulated as prognostic markers of response, it has become clear during the clinical development of gefitinib that neither of these are effective or reliable predictors of drug response[55,56]. Much research is underway to determine mechanisms of patient responsiveness to EGFR-TK inhibitors. The results of these studies could help in the identification of patients who are likely to benefit most from this class of drugs.

EGFR-TK mutations. Our understanding of why some patient subgroups are more likely to respond to gefitinib than others is limited. However, the recent exciting discovery that some patients with a marked response to gefitinib have somatic EGFR-TK mutations and the finding that the frequency of these mutations is highest in those patient subgroups previously associated with the greatest response to gefitinib[22] (FIG. 2) could provide a partial explanation. The new data indicate that somatic mutations in exons 18–21 in the ATP-binding region of the TK domain of the *EGFR* gene might predict those patients who are likely to have an objective response to gefitinib[21,22]. Whilst investigating whether mutation of receptor TKs has a causal role in the development of NSCLC, Paez *et al.* searched for somatic genetic alterations in NSCLC primary tumour biopsies from 119 unselected patients[22]. Although genes encoding 47 different TK receptors were analysed for mutations, mutations were only observed in the *EGFR* gene. Eighteen different mutations were

found in exons 18–21, which cluster around the TK domain of *EGFR*. These mutations were more frequent in women than in men (20% versus 9%), in adenocarcinomas than in other histologies (21% versus 2%) and in patients from Japan than in patients from the United States (26% versus 2%).

These findings spurred investigation of whether EGFR-TK mutations might be a determinant of gefitinib sensitivity. Paez *et al.* searched for EGFR mutations in tumour samples from five patients who responded to gefitinib (four had achieved a partial response and one had experienced rapid symptom improvement). They found that biopsy samples from all of these tumours had EGFR-TK mutations, whereas none were evident in the tumour samples from four patients who had progressed during gefitinib treatment[22]. Simultaneously with the publication of these results, Lynch *et al.* published the results of their investigation into EGFR-TK mutations in primary tumours from a small number of patients with NSCLC. They identified somatic mutations in the TK domain of EGFR in eight of the nine patients studied who had achieved an objective response with gefitinib and in none of the seven patients studied who had progressed on gefitinib[21]. Following release of these landmark data, analysis of the mutation status of tumours from other patients who have been treated with EGFR-TK inhibitors is being carried out.

So far, three classes of EGFR mutations have been identified — missense mutations, deletions and in-frame insertions (FIG. 2)[21]. Functional analysis in fibroblasts that expressed two mutant forms of EGFR — the L858R missense mutation and L747-P753insS deletion — have provided insight into how these mutations affect the function of gefitinib[21]. These studies showed that activation of mutant EGFR is characteristically more intense and prolonged than that of the activated wild-type receptor, and also that much lower concentrations of gefitinib are needed to completely inhibit this mutant receptor, compared with the wild-type receptor[21]. These studies indicate that these mutations stabilize the interaction between the EGFR-TK domain and ATP or its competitive inhibitor (for example, gefitinib). *In vitro* studies have shown that tumour cell lines that express these mutant forms of EGFR are more susceptible to apoptosis following gefitinib exposure, compared with wild-type cells[57].

Data from Sordella *et al.* also indicate that apoptotic pathways in NSCLC tumours that express mutant forms of EGFR differ from those in wild-type cells[58]. Cells with mutant EGFR preferentially activate the AKT and signal transducer and activator of transcription (STAT) anti-apoptotic signalling pathway, and EGFR inhibition with gefitinib results in rapid cell death. This could underlie the marked responses to gefitinib in patients with mutant EGFR[58]. However, the functional impact of all the EGFR-TK mutations discovered so far, and their clinical significance, is not yet known[57].

These studies raise the possibility of predicting, on the basis of somatic EGFR mutations, which patients are most likely to achieve an objective response with gefitinib and other EGFR-TK inhibitors. However, other mechanisms might be involved in determining sensitivity to gefitinib and other EGFR-TK inhibitors. At least one patient with gefitinib-responsive NSCLC did not have any of these mutations[21], and in one study exploring treatment with erlotinib, one non-responder had EGFR-TK mutations whereas five patients with stable disease did not[57]. Furthermore, the clinical benefits of treatment with gefitinib and other EGFR-TK inhibitors are not restricted to objective response. It is also very important to consider the large proportion of patients who achieve disease stability or symptom improvement that do not seem to be explained by somatic EGFR-TK mutations. Identifying markers of tumour response to EGFR-TK inhibitors is a complex process, and much more research is required to clarify the full clinical implications of the EGFR-TK mutations and to understand how treatment outcome can be predicted.

While these findings raise the possibility of a diagnostic test for EGFR mutation status being developed, there are several practical implications for this. In particular, will one test ever identify all the possible mutations? Although the original publication by Lynch *et al.* described a total of 7 distinct mutations[21], this number continues to grow, with over 40 distinct mutations reported 1 month later at the American Society for Clinical Oncology annual meeting 2004. The effects of the different types of mutations on downstream signalling pathways also differs, making it difficult, at this time, to specify which particular mutations should be screened for. As these EGFR mutations are somatic, rather than germline, any test to determine mutation status will also require direct tumour biopsy material, rather than being performed on more easily accessed tissue such as blood, skin or buccal mucosa. At present, mutation analysis is a complex and time-consuming

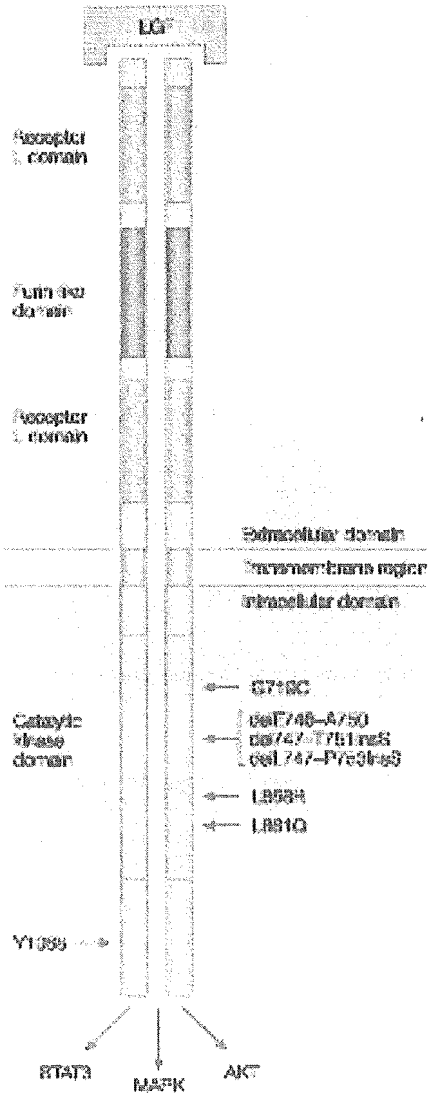

Figure 2 | **The epidermal growth factor receptor.** The epidermal growth factor receptor (EGFR) contains two extracellular L domains, along with a furin-like extracellular domain. These are connected, through the transmembrane region, to an intracellular domain that contains the catalytic kinase domain, along with a tyrosine phosphorylation site (Y1068). This region, when phosphorylated, leads to the activation of signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK) and AKT signalling pathways. The locations of the activating EGFR tyrosine-kinase mutations identified by Lynch *et al.* in tumours from patients with NSCLC who had responded to gefitinib (listed on right side of molecule) are all located within the catalytic kinase domain of the receptor[21]. It is suggested that non-small-cell lung cancer tumour cells that express mutant forms of EGFR preferentially activate the AKT and STAT-mediated anti-apoptotic signalling pathways, so EGFR inhibition with gefitinib results in rapid cell death[58]. Figure modified with permission from REF. 21 © (2004) Massachusetts Medical Society.

procedure, requiring specialist expertise and equipment, and is only available at a limited number of medical research institutions. Furthermore, unless the biopsy sample contains a significant proportion of cancer cells, it is very difficult to establish that a particular tumour does not have cells that express mutant forms of EGFR.

Other factors. Other techniques and biomarkers are being investigated to identify patients with NSCLC who are most likely to respond to certain EGFR inhibitors. These include immunohistochemical assays to evaluate expression levels of EGFR-related proteins, fluorescence *in situ* hybridization (FISH) analysis to identify amplified genes, and gene-expression and proteomic analyses to identify other markers of response to gefitinib. Preliminary evaluation of response, based on *EGFR* mutations and amplification of *EGFR* (as determined by FISH), in a small number of patients (about 20) showed that all responders carried either amplifications or mutations in *EGFR*, or both[59]. Analysis of RNA samples isolated from tumour specimens of 17 patients, of whom 2 had a partial response and 3 had stable disease, revealed that expression levels of several genes, including *STAT5A*, *STAT5B* and gene encoding γ-catenin, is correlated with clinical response[60]. So, EGFR mutations are not the only story in gefitinib sensitivity — other mechanisms are also potentially involved.

Assessing gefitinib response

Results of the IDEAL trials showed that over 40% of symptomatic patients with refractory NSCLC experienced symptom improvement within 8–10 days of starting gefitinib therapy, and that this correlated with response and increased survival (REFS 39,40,61; and R.S.H *et al.*, unpublished observations). Approximately 90% of tumour responses in the trials were seen within the first 2 months. Given the absence of a simple diagnostic test for determining EGFR mutation status, and the fact that EGFR mutations do not seem to account for the full benefit of gefitinib, the most practical way to determine if a patient will benefit from gefitinib currently is to initiate up to 8 weeks of trial therapy.

Combination therapies

In parallel with the IDEAL studies, gefitinib was investigated as a first-line treatment (treatment when a patient has not received any previous therapy for advanced disease) in combination with chemotherapy in two Phase III trials, called Iressa NSCLC Trial Assessing Combination Treatment (INTACT) 1 and INTACT 2. Patients in these trials had either locally advanced stage III disease that was not curable with surgery or radiotherapy, or stage IV disease. Patients in INTACT 1 received gefitinib in combination with gemcitabine and cisplatin[62], whereas patients in INTACT 2 received a combination of gefitinib, paclitaxel and carboplatin[63]. Although preclinical studies[26] had shown synergy among these drugs, and two earlier Phase I studies[64,65] had indicated that first-line combination therapy with gefitinib and platinum-based chemotherapy was feasible, the INTACT trials did not report an increase in survival times among patients who received gefitinib in addition to platinum-based chemotherapy. Similarly, in clinical trials called Tarceva Responses in Conjunction with Paclitaxel and Carboplatin (TRIBUTE), which tested the addition of erlotinib to carboplatin and paclitaxel therapy, and the Tarceva Lung Cancer Investigation Trial (TALENT), which tested erlotinib in combination with cisplatin and gemcitabine, no increases in patient survival time were observed[66,67].

The reasons for these disappointing results are unknown, although the possibility that EGFR-TK inhibitors and chemotherapy have antagonistic effects has been proposed[68]. Antagonism between cytostatic and cytotoxic agents has been demonstrated between tamoxifen and chemotherapy in patients with breast cancer receiving adjuvant therapy[69]. Gefitinib has both antiproliferative and pro-apoptotic effects. Its antiproliferative effects are the result of p27-mediated G1 cell-cycle arrest of EGFR-dependent tumour cells that, in a similar way to tamoxifen, could render tumour cells less sensitive to cytotoxic agents. Conversely, the pro-apoptotic effects of gefitinib could increase the antitumour effects of chemotherapy. The challenge is to dissociate the antiproliferative effects from the apoptotic effects of gefitinib when it is used in combination with chemotherapy. Early preclinical studies in human tumour xenograft models involving the combination of gefitinib and chemotherapy indicated that intermittent gefitinib administration was significantly superior to continuous dosing[70]. The antiproliferative effects of gefitinib could require continuous kinase inhibition to maintain cell-cycle arrest, whereas sensitization to apoptosis might require temporary inhibition of the survival (anti-apoptotic) pathways.

In the INTACT 2 trial, a subset of patients with adenocarcinoma histology who had received chemotherapy for over 90 days seemed to have a slightly better survival outcome if they also received gefitinib. This indicates the possible efficacy of gefitinib monotherapy in maintenance therapy[63] — as a cytostatic agent that maintains tumour regression after chemotherapy. So, instead of concomitant administration, scheduling gefitinib after chemotherapy might benefit patients with NSCLC. This sequential approach is now being investigated in a US Cooperative Group Phase III study, in which patients with inoperable stage III NSCLC receive gefitinib or placebo following treatment with chemoradiation and consolidation docetaxel.

Several key classes of agents that target specific cellular mechanisms are in different phases of clinical development in combination with EGFR inhibitors. As these agents have the potential to target different signalling pathways involved in cancer pathogenesis, they have the potential to be used in combination. For example, antitumour activity of erlotinib in combination with the angiogenesis inhibitor bevacizumab has been reported in a Phase I/II study of patients with recurrent NSCLC who had received one or more chemotherapy regimen[71]. An example of an agent that selectively targets two key pathways in tumour growth (the EGFR and VEGFR pathways) is ZD6474, which is now in Phase II development[72].

Ongoing development in NSCLC

How can the use of gefitinib in patients with NSCLC be optimized, both as a monotherapy and in sequence with chemotherapy? Although in most countries where gefitinib is approved the licence is for use only in pretreated patients with advanced NSCLC, the drug is now being investigated in patients with all stages of lung cancer. In one Phase II trial conducted in Japan, first-line gefitinib 250 mg/day therapy resulted in an overall tumour response rate of 30%, but 4 of the 40 patients in this trial developed ILD[73]. Another Phase II study evaluated gefitinib 250 mg/day as first-line treatment in patients with NSCLC and poor performance status. Treatment was well tolerated, resulted in a disease control rate (the proportion of patients with partial or complete tumour regression or stable disease) of 48.3%, and a tumour response rate of 5.2%[74]. These data support the further investigation of gefitinib as a first-line therapy. Large trials are underway to define its full potential in patients with NSCLC, which might include use as adjuvant, first-, second- and third-line treatment and as maintenance therapy (TABLE 3).

Table 3 | **Gefitinib trials underway in patients with non-small-cell lung cancer**

Name	Phase	Design	Gefitinib therapy*	Number of patients	Patient status	Drug compared with	Primary end point	Sponsor
BR19	III	Double blind	Adjuvant	1,160	Tumour surgically removed, with stage IB, II and IIIA (N2) NSCLC	Placebo	Overall survival	NCIC, EORTC
EORTC 08021-ILCP	III	Double blind	Adjuvant	736	Stage IIIB/IV NSCLC, PS 0–2, first-line CT	Placebo	Survival, progression-free survival, toxicity	EORTC, ILCP
INVITE	II	Open label	First line	192	Age ≥70 years, PS ≤2, stage IIIB/IV NSCLC	Vinorelbine	Progression-free survival	AstraZeneca
INSTEP	II	Double blind	First line plus BSC	200	PS 2 or 3, stage IIIB/IV NSCLC	Placebo plus BSC	Progression-free survival	AstraZeneca
INTEREST	III	Open label	Second/third line	1,440	Locally advanced or metastatic NSCLC	Docetaxel	Overall survival	AstraZeneca
ISEL	III	Double blind	Second/third line plus BSC	1,692	Stage IIIB/IV NSCLC, PS 0–2	Placebo plus BSC	Overall survival	AstraZeneca
V15-32	III	Open label	Second/third line	484	Stage IIB/IV NSCLC, PS 0–2	Docetaxel	Survival	AstraZeneca
SWOG 0023	III	Double blind	Maintenance	840	Patients with stage III NSCLC who have received CT/RT with consolidation docetaxel	Placebo	Overall survival, progression-free survival	SWOG

*Patients receive 250 mg/day. BSC, best supportive care; CT, chemotherapy; EORTC, European Organisation for Research and Treatment of Cancer; ILCP, Italian Lung Cancer Project; NCIC, National Cancer Institute of Canada; NSCLC, non-small-cell lung cancer; PS, performance status; RT, radiotherapy; SWOG, Southwest Oncology Group.

Using gefitinib to treat other cancers

Studies are also underway to evaluate the ability of gefitinib to treat patients with other solid tumours, such as head and neck cancer, breast cancer and CRC. Gefitinib 500 mg/day has shown encouraging single-agent activity and favourable tolerability as first- or second-line therapy in a Phase II study of 52 patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SCCHN)[75]. Of the 47 patients evaluable for response, 10.6% had an observed response and a substantial number had disease control (53%). Median time to progression was 3.4 months and overall survival was 8.1 months. Gefitinib was well tolerated and the study findings support further investigation of gefitinib in patients with SCCHN. Two key international trials are now recruiting patients and will assess the potential of gefitinib as a first- and second-/third-line therapy in patients with head and neck cancer. In a Phase II study, first-line treatment with gefitinib will be combined with chemoradiotherapy, and in the Phase III trial, gefitinib monotherapy (250 and 500 mg/day) is being compared with methotrexate as a second- or third-line treatment. Data from a Phase I study in patients with metastatic and/or locally recurrent SCCHN indicate that the cyclooxygenase-2 inhibitor celecoxib increases the antitumour activity of gefitinib, with a response rate of 33.3%[76].

Although results from trials investigating the clinical benefit of gefitinib monotherapy in patients with breast cancer have shown few objective responses[77,78], alternative approaches, such as combining gefitinib with endocrine treatment, seem more promising. Many oestrogen-receptor-positive breast tumours initially respond to antihormone therapy. These responses, however, are often incomplete and prolonged treatment results in resistance, induction of EGFR expression and the emergence of highly proliferative cells. Recent preclinical data in oestrogen-receptor-positive breast cancer cells indicate that combining gefitinib with tamoxifen or fulvestrant, either as cotreatment[79,80] or pretreatment[81], induces an additive antitumour effect and prevents the emergence of EGFR-positive antihormone resistance. Gefitinib is believed to overcome antihormone resistance by eliminating crosstalk between the oestrogen receptor and ERBB2 (also known as HER2) signalling pathways[81]. Recent pharmacokinetic data from patients with breast cancer who were treated with gefitinib show that concentrations of gefitinib in tumours (2.3–25.8 μg/g) were much higher than in plasma (0.10–0.42 μg/g)[82]. This tumour/plasma ratio (54-fold) was much higher than that observed in animal models, and confirms that gefitinib is extensively distributed to breast tumour tissue. The trial is designed to identify molecular alterations in human breast cancer tissue after short-term exposure to gefitinib. Clinical trials are now being designed to further investigate the effects of gefitinib on breast tumours.

Gefitinib 500 mg/day therapy has also shown activity in patients with metastatic CRC, when administered in combination with FOLFOX-4, a combination of three chemotherapy drugs — oxaliplatin, leucovorin and fluorouracil. In a Phase II trial, patients with advanced CRC who had received previous therapy or no previous therapy received FOLFOX-4 for 14 days, and thereafter gefitinib was added to the treatment regimen[83]. Although the trial is ongoing, results are available from 50 patients. Patients who had not been treated with chemotherapy had a response rate of 78%, and patients who did not respond to previous chemotherapy had a response rate of 36%. These data are encouraging compared with those usually observed with FOLFOX-4 therapy alone in patients with metastatic CRC.

Results have also been reported in patients with gastric cancer. In a Phase II trial of 75 patients with advanced metastatic gastric cancer, gefitinib therapy resulted in disease control in 13 patients (13.9%), of whom 1 had a partial response after receiving 250 mg/day and 12 had stable disease after receiving either 250 mg/day or 500 mg/day of gefitinib. Again, the drug was generally well tolerated at both doses, although the lower dose was associated with fewer drug-related adverse events[84].

Future directions

Over 190,000 patients have been treated with gefitinib worldwide. Since their onset, studies of gefitinib have generated a large body of data that have contributed to the ongoing clinical advancement of this drug and provides useful knowledge that could assist the development of other EGFR-TK inhibitors. Initially, clinical trials with gefitinib focused primarily on patients with advanced NSCLC, but ongoing trials are providing encouraging evidence for its potential in treating earlier-stage disease and several other tumour types. Investigations are also underway to find out how gefitinib can be combined with chemotherapy and other novel agents. The discovery of EGFR mutations and the potential identification of other markers that predict patient response could help to optimize the use of gefitinib in the future. Nonetheless, understanding the basis of stable disease and symptom improvement remains an important challenge.

Roy S. Herbst is at the Department of Thoracic/Head and Neck Medical Oncology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 432, Houston, Texas 77030, USA.

Masahiro Fukuoka is at the Department of Medical Oncology, Kinki University School of Medicine, 377-2, Ohno-higashi, Osakasayama City, Osaka 589-8511, Japan.

José Baselga is at the Medical Oncology Service, Vall d'Hebron University Hospital, Universitat Autonoma de Barcelona, P Vall d'Hebron 119-129, 08035 Barcelona, Spain.

Correspondence to R.S.H.
e-mail: rherbst@mdanderson.org

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Competing interests statement

The authors declare competing financial interests: see web version for details.

Online links

DATABASES

The following terms in this article are linked online to:

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breast cancer | colorectal cancer | head and neck cancer | non-small cell lung cancer | ovarian cancer

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Epidermal growth factor receptor information and resources: http://www.egfr-info.com/
Information on gefitinib: http://www.iressa.com/
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Phase I–II study of amrubicin and cisplatin in previously untreated patients with extensive-stage small-cell lung cancer

Y. Ohe[1]*, S. Negoro[2], K. Matsui[3], K. Nakagawa[4], T. Sugiura[5], Y. Takada[6], Y. Nishiwaki[7], S. Yokota[8], M. Kawahara[9], N. Saijo[7], M. Fukuoka[4] & Y. Ariyoshi[10]
On behalf of the Amrubicin SCLC Study Group

[1]Department of Internal Medicine, National Cancer Center Hospital, Tokyo; [2]Department of Clinical Oncology, Osaka City General Hospital, Osaka; [3]Department of Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Osaka; [4]Department of Medical Oncology, Kinki University School of Medicine, Osakasayama, Osaka; [5]Department of Internal Medicine, Aichi Cancer Center Hospital, Nagoya, Aichi, [6]Department of Radiology, Hyogo Medical Center for Adults, Akashi, Hyogo; [7]Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwa, Chiba; [8]Department of Internal Medicine, National Hospital Organization Toneyama National Hospital, Toyonaka, Osaka; [9]Department of Internal Medicine, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Osaka; [10]Aichi Prefectural Hospital, Okazaki, Aichi, Japan

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Background: Amrubicin, a totally synthetic 9-amino-anthracycline, demonstrated excellent single-agent activity for extensive-stage small-cell lung cancer (ED-SCLC). The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy with amrubicin and cisplatin, and to assess the efficacy and safety at their recommended doses (RD).

Patients and methods: Eligibility criteria were patients having histologically or cytologically proven measurable ED-SCLC, no previous systemic therapy, an Eastern Cooperative Oncology Group performance status of 0–2 and adequate organ function. Amrubicin was administered on days 1–3 and cisplatin on day 1, every 3 weeks.

Results: Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2/day and cisplatin 60 mg/m^2) and three patients at level 2 (amrubicin 45 mg/m^2/day and cisplatin 60 mg/m^2). Consequently, the MTD and RD were determined to be at level 2 and level 1, respectively. The response rate at the RD was 87.8% (36/41). The median survival time (MST) was 13.6 months and the 1-year survival rate was 56.1%. Grade 3/4 neutropenia and leukopenia occurred in 95.1% and 65.9% of patients, respectively.

Conclusions: The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC.

Key words: anthracycline, cisplatin, phase I–II, small-cell lung cancer

Introduction

Small-cell lung cancer (SCLC) is one of the most chemosensitive solid tumors, and the outcome of SCLC patients is slowly but surely improving. Combination chemotherapy consisting of cisplatin plus etoposide and concurrent twice-daily thoracic radiotherapy has yielded a 26% 5-year survival rate in limited-stage (LD) patients [1]. Despite the high response rate to combination chemotherapy, however, local and distant failure is very common, especially in extensive-stage (ED) patients. Moreover, resistance to chemotherapeutic agents develops easily after failure of initial treatment. Thus, long-term survivors are still very rare among patients with ED-SCLC. To improve the outcome of SCLC patients, several strategies, such as high-dose chemotherapy, dose-intensive chemotherapy, alternating chemotherapy and introduction of new drugs, have been investigated [2–6]. However, only the introduction of new agents has improved the outcome of SCLC patients. Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin alternating cyclophosphamide, doxorubicin and vincristine had been mainly used for SCLC in North America. Recently, a Japanese trial [Japan Clinical Oncology Group (JCOG) 9511] demonstrated the superiority of the combination of irinotecan and cisplatin for ED-SCLC patients over the combination of etoposide and cisplatin [6]. The development of more active chemotherapy, and especially the introduction of effective new drugs, is therefore essential to improve the survival of SCLC patients.

Amrubicin (SM-5887) is a totally synthetic anthracycline and a potent topoisomerase II inhibitor [7–14]. It has antitumor activity, and is more potent than doxorubicin against various mouse experimental tumors and human tumor

*Correspondence to: Dr Y. Ohe, Department of Internal Medicine, National Cancer Center Hospital 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-7006; E-mail: yohe@ncc.go.jp

xenografts. Amrubicin and its 13-hydroxy metabolite, amrubicinol, inhibit purified human DNA topoisomerase II [11]. Amrubicinol is 10–100 times more cytotoxic than amrubicin [9]. The potent therapeutic activity of amrubicin is caused by the selective distribution of its highly active metabolite, amrubicinol, in tumors [9]. In an experimental animal model, amrubicin did not exhibit any chronic cardiotoxicity potential, and no deleterious effects on doxorubicin-induced cardiotoxicity in dogs was observed [14]. In a phase II study of amrubicin using a schedule of 45 mg/m^2 on days 1–3 every 3 weeks, in 33 previously untreated ED-SCLC patients, an overall response rate of 76% and a complete response (CR) rate of 9% were reported [15]. Moreover, median survival time (MST) was 11.7 months in the single-agent phase II study of amrubicin. Amrubicin is one of the most active new agents for SCLC. Thus, we conducted a phase I/II study of amrubicin plus cisplatin for untreated ED-SCLC, because cisplatin is considered as one of the most important drugs in the treatment of SCLC. The aims of this trial were to determine the maximum-tolerated doses (MTD) of combination therapy of amrubicin with cisplatin, to assess the efficacy and safety for ED-SCLC at their recommended doses (RD), and to examine the pharmacokinetics of the drug combination.

Patients and methods

Patient selection

Patients with histologically and/or cytologically documented SCLC were eligible for this study. Each patient was required to meet the following criteria: extensive-stage disease [16]; no prior therapy for primary lesion; measurable lesion; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; expected survival time >2 months; age 20–74 years; adequate hematological function [white blood cell (WBC) count 4000–12 000/mm^3, neutrophils ≥2000/mm^3, platelets ≥100 000/mm^3, hemoglobin ≥10 g/dl]; adequate hepatic function [total bilirubin within 1.5× the upper limit of normal; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5× the upper limit of normal]; adequate renal function (creatinine within the upper limit of normal); partial pressure of arterial oxygen 60 torr; no abnormality requiring treatment on electrocardiogram; left ventricle ejection fraction >60%; written informed consent. Patients with symptomatic brain metastasis, pleural effusion that required drainage, non-steroidal anti-inflammatory drug or glucocorticoid use for >50 days, pericarditis carcinomatous, active infection, varicella, superior vena cava syndrome, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), gastric and/or duodenal ulcer, severe heart disease, severe renal disease, active concomitant malignancy, symptomatic pneumonitis and/or pulmonary fibrosis and pregnant/nursing women were excluded. This study was approved by the Institutional Review Board at each hospital.

Patient evaluation

Pretreatment evaluation consisted of complete blood cell counts, differential, routine chemistry measurements, progastrin-releasing peptide (ProGRP), neuron-specific enolase, electrocardiogram, echocardiography, chest radiograph, chest and abdominal computed tomography (CT) scan, whole-brain magnetic resonance imaging (MRI) or CT scan, and isotope bone scan. Complete blood cell counts, differential and routine chemistry measurements were performed at least once a week during the chemotherapy.

Treatment schedule

At level 1, chemotherapy consisted of cisplatin 60 mg/m^2 on day 1 and amrubicin 40 mg/m^2 on days 1–3. Amrubicin was administered as an intravenous injection over 5 min and cisplatin was administered as a drip infusion over 60–120 min with adequate hydration. At level 2 the dose of amrubicin was increased to 45 mg/m^2 on days 1–3. Level 3 was planned with cisplatin 80 mg/m^2 on day 1 and amrubicin 45 mg/m^2 on days 1–3. The chemotherapy was repeated every 3 weeks for four to six courses. Intrapatient dose escalation was not allowed. Administration of granulocyte colony-stimulating factor (G-CSF) was permitted prophylactically for patients expected to experience grade 3 neutropenia during the first course. Prophylactic administration of G-CSF was only permitted at second or later courses.

The administrations of both cisplatin and amrubicin were postponed if patients met the following criteria: WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; AST and ALT >5× the upper limit of normal; total bilirubin >1.5× the upper limit of normal; creatinine >1.3× the upper limit of normal; ECOG PS 3 or 4; active infection; grade 2 or worse non-hematological toxicity, except for alopecia, anorexia, nausea, vomiting or fatigue.

The administrations of both cisplatin and amrubicin were withdrawn if patients met the following criteria: tumor regression <15% after first course or <30% after second course; WBC <3000/mm^3; neutrophils <1500/mm^3; platelets <100 000/mm^3; no recovery from grade 3 or 4 non-hematological toxicity at 6 weeks after the start of previous chemotherapy; abnormality of electrocardiogram requiring treatment for more than 6 weeks; left ventricle ejection fraction <48%; treatment delay of >4 weeks.

The dose of amrubicin was decreased 5 mg/m^2/day if patients met the following criteria: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 neutropenia with fever; platelets <20 000/mm^3 during the previous course. The dose of cisplatin was decreased to 75% if creatinine increased to >1.5× the upper limit of normal during the previous course.

The dose-limiting toxicity (DLT) was defined as follows: grade 4 leukopenia or neutropenia for ≥4 days; grade 3 febrile neutropenia; platelets <20 000/mm^3; grade 3 or worse non-hematological toxicity except for nausea, vomiting, anorexia, fatigue, hyponatremia and infection. Initially, three patients were treated at each dose level. If DLT was not observed in any of the three patients, dose escalation was carried out. If DLT was observed in one of three patients, an additional three patients were entered at the same dose level. If DLT was observed in three or more of six patients, or two or three of the initial three patients, we considered that dose to be the MTD. If DLT was observed in one or two of six patients, dose escalation was also carried out. Dose escalation was determined based only on the data from the first course of chemotherapy.

Response and toxicity evaluation

Response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) and tumor markers were excluded from the criteria [17]. CR was defined as the complete disappearance of all clinically detectable tumors for at least 4 weeks and no new lesions. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameters of target lesion, taking as reference the baseline sum longest diameter, the required non-progression in non-target lesions and no new lesions for at least 4 weeks. Stable disease (SD) included: regression of target lesions insufficient to meet the criteria for PR, a <20% increase in the sum of the longest diameter of target lesion, taking as reference the smallest sum longest diameters recorded since the treatment started, the required non-progression in non-target lesions and no new lesions for at least 6 weeks. Progressive disease (PD) indicated a >20% increase in the sum of the longest diameters of target lesion, taking as reference the smallest sum longest diameter recorded since the treatment started

and/or unequivocal progression of existing non-target lesions and/or appearance of new lesions. The evaluation of objective tumor response for all patients was performed by an external review committee.

Toxicity grading criteria of the National Cancer Institute Common Toxicity Criteria (version 2.0) was used for evaluation of toxicity.

Statistical analysis

This study was designed to reject response rates of 70% (P0) at a significance level of 0.05 (one-tailed) with a statistical power of 80% to assess the activity of the regimen as a 85% response rate (P1) at the recommended dose. The upper limit of rejection was 29 responses (CR + PR) among 37 evaluable patients. Overall survival was defined as the interval between the first administration of the drugs in this study and death or the last follow-up visit. Median overall survival was estimated using the Kaplan–Meier method [18].

Table 1. Characteristics of treated patients

	Phase I	Phase II	Total
Number of patients	7	37	44
Gender			
Male	5	31	36
Female	2	6	8
Age (years)			
Median	65	64	64.5
Range	54–73	50–74	50–74
ECOG PS			
0	0	5	5
1	7	32	39
2	0	0	0
Stage			
IIIB	0	2	2
IV	7	35	42
Prior therapy			
Yes	0	1	1
No	7	36	43
Serum ALP			
Normal	7	29	36
Elevated	0	7	7
Serum LDH			
Normal	3	14	17
Elevated	4	23	27
Na			
Normal	6	35	41
Decreased	1	2	3
Number of metastases			
0	0	2	2
1	4	27	31
2	3	6	9
3	0	1	1
4 or more	0	1	1

In one patient, serum ALP level could not be measured.
ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Pharmacokinetic analysis

Pharmocokinetic analysis was performed in patients entering the phase I section of this study. One milliliter of the blood was taken from the patients before administration of amrubicin, and at 0 min, 15 min, 1, 2, 3, 4, 8 and 24 h after administration on days 1 and 3 in the first course of chemotherapy. Concentrations of amrubicin and its active metabolite, amrubicinol, in plasma and red blood cells were measured as reported elsewhere [9].

Results

Patient characteristics

Between April 2001 and December 2002, 45 patients with ED-SCLC were enrolled and 44 were treated in this study (Table 1). One patient did not receive the protocol treatment because atrial fibrillation was observed just before administration on day 1 of the first course. All treated patients were assessed for response, survival and toxicity. The median age of the treated patients was 64.5 years (range 50–74). There were 36 males and eight females. Five patients had an ECOG PS 0 and 39 patients had PS 1. Only one patient received surgery for brain metastasis as a prior therapy.

MTD and DLT in the phase I study

Four patients were enrolled at dose level 1 (amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1) and three patients at level 2 (amrubicin 45 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1). Toxicities in the phase I study are listed in Table 2. No DLT were observed during the first course of level 1. At level 2, grade 4 neutropenia for ≥4 days and febrile neutropenia occurred in one patient, and febrile neutropenia and grade 3 constipation occurred in another patient. Consequently, the MTD and RD were determined to be level 2 and level 1, respectively.

Pharmacokinetics of amrubicin and its active metabolite, amrubicinol

Pharmacokinetic parameters of amrubicin in plasma were almost identical on days 1 and 3 at the two dose levels (Table 3). No clear dose relationship in the area under the concentration–time curve (AUC) of amrubicin in the plasma was observed. The AUC of amrubicinol in red blood cells tended to increase on day 3 at both doses (Table 4). No clear dose relationship in the AUC of amrubicinol in red blood cells was observed. Combination with cisplatin did not alter the pharmacokinetics of amrubicin and amrubicinol (data not shown).

Treatment received in patients treated at the RD

Forty-one patients were treated at the RD: amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1. Of 41 patients, 32 (78%) patients received more than three

Table 2. Toxicities during the first course in the phase I study

	Level 1 ($n=4$)					Level 2 ($n=3$)				
Amrubicin	40 mg/m^2 days 1–3					45 mg/m^2 days 1–3				
Cisplatin	60 mg/m^2 day 1					60 mg/m^2 day 1				
	Grade (NCI CTC)					Grade (NCI CTC)				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	0	1	1	2	0	0	0	1	1	1
Neutropenia	0	0	0	2	2	0	0	0	0	3
Febrile neutropenia	4	–	–	0	0	1	–	–	2	0
Hemoglobin	1	1	2	0	0	2	1	0	0	0
Thrombocytopenia	1	2	0	1	0	0	2	0	1	0
Stomatitis	3	0	1	0	0	3	0	0	0	0
Nausea	1	1	2	0	–	1	1	0	1	–
Constipation	3	0	1	0	0	1	0	1	1	0
Hyponatremia	2	1	0	0	1	1	2	0	0	0
Hypocalcemia	3	0	1	0	0	3	0	0	0	0

Dose limiting toxicity at level 2: febrile neutropenia, two patients; grade 4 neutropenia ≥4 days, one patient; grade 3 constipation, one patient.
NCI CTC, National Cancer Institute Common Toxicity Criteria.

Table 3. Pharmacokinetics of amrubicin in plasma

Dose	n	Day	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	V_d (l)	CL (l/h)	AUC_{0-24h} (ng h/ml)
40 mg/m^2	4	1	0.11 ± 0.04	2.29 ± 0.31	46.6 ± 11.0	13.6 ± 1.8	2995 ± 434
	4	3	0.08 ± 0.01	2.89 ± 0.34	50.0 ± 10.6	11.6 ± 1.9	3511 ± 514
45 mg/m^2	3	1	0.13 ± 0.05	2.39 ± 0.34	56.3 ± 10.6	14.9 ± 1.8	3052 ± 402
	3	3	0.09 ± 0.03	2.27 ± 0.18	51.9 ± 3.7	14.2 ± 2.3	3217 ± 479

$T_{1/2\alpha}$, half-life at distribution phase; $T_{1/2\beta}$, half-life at elimination phase; V_d, volume of distribution; CL, clearance; AUC, area under the concentration–time curve.

courses of chemotherapy, and 10 (31%) of these 32 patients needed dose reduction of amrubicin at the fourth course (Table 5). Of 41 patients, 22 (54%) patients completed four courses of chemotherapy without dose modification. The main cause of dose reduction was myelosuppression, especially leukopenia and neutropenia.

Objective tumor response and overall survival

The objective tumor responses are given in Table 6. Four CRs and 32 PRs occurred, for an objective response rate of 87.8% [95% confidence interval (CI) 73.8% to 95.9%] in 41 patients treated at the RD. The objective response rate for all 44 patients was 88.6% (95% CI 75.4% to 96.2%). The overall survival times of the 41 patients treated at the RD are shown in Figure 1. The MST of the 41 patients was 13.6 months (95% CI 11.1–16.6), with a median follow-up time for eight censored patients of 16.4 months (95% CI 14.2–18.8). The 1- and 2-year survival rates were 56.1% and 17.6%, respectively. The MST of all 44 patients was 13.8 months (95% CI 11.1–16.6). The 1- and 2-year survival rates of all 44 patients were 56.8% and 21.4%, respectively.

Table 4. Pharmacokinetics of amrubicinol in red blood cells

Dose	n	Day	$T_{1/2}$ (h)	AUC_{0-24h} (ng·h/ml)
40 mg/m^2	4	1	21.0 ± 3.1	1412 ± 314
	4	3	20.7 ± 4.8	2159 ± 622
45 mg/m^2	3	1	19.6 ± 6.1	1098 ± 277
	3	3	18.1 ± 5.7	2027 ± 332

$T_{1/2}$, elimination half-life; AUC, area under the concentration–time curve.

Table 5. Treatment received in patients treated at the recommended dose

Cycle	n	Amrubicin (mg/m^2)			Cisplatin (mg/m^2)	
		40	35	30	60	45
1	41	41			41	
2	36	30	6		36	
3	33	26	5	2	33	
4	32	22	8	2	32	
5	18	9	5	4	18	
6	13	6	3	4	12	1

Table 6. Response rates

	n	CR	PR	SD	PD	NE	Response rate (%) (95% CI)
All	44	4	35	3	0	2	88.6 (75.4–96.2)
Treated at RD	41	4	32	3	0	2	87.8 (73.8–95.9)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; 95% CI, 95% confidence interval; RD, recommended dose.

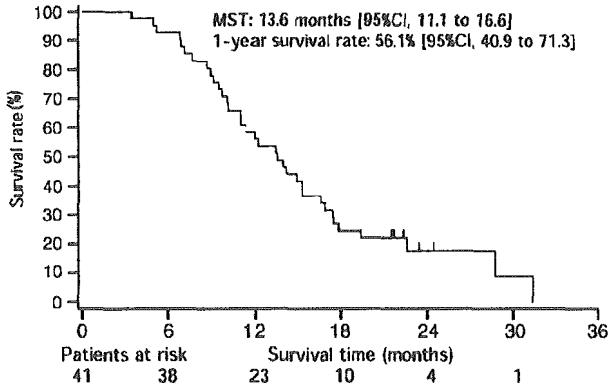

Figure 1. Overall survival of patients with extensive-stage small-cell lung cancer who were treated with amrubicin and cisplatin at the recommended dose. MST, median survival time; 95% CI, 95% confidence interval.

Toxicity in patients treated at the RD

The worst grades of hematological and non-hematological toxicities experienced by each patient are listed in Table 7. Hematological toxicity, especially leukopenia and neutropenia, was common and relatively severe. Grade 3 or worse leukopenia and neutropenia occurred in 65.9% and 95.1% of patients, respectively. Febrile neutropenia was observed in two patients at level 2. Grade 3 or worse anemia and thrombocytopenia occurred in 53.7% and 24.4% of patients, respectively. Four patients received platelet transfusions. Common non-hematological toxicities were gastrointestinal toxicity, such as anorexia, nausea, vomiting, constipation, diarrhea and stomatitis. Gastric ulcers developed in three patients. Hepatic and renal toxicity were not common in this study. Grade 3 or worse hyponatremia and hypokalemia occurred in 22% and 9.8% of patients, respectively. One patient developed myocardial infarction; however, cardiac toxicity was not common. No treatment-related deaths were observed.

Discussion

Doxorubicin and epirubicin are classified as active agents for SCLC, for which single-agent activity is a >20% response rate [19]. Doxorubicin has been used as a constituent of combination therapy for SCLC in the CAV (cyclophospamide, doxorubicin and vincristine) and CAP (cyclophosphamide, doxorubicin and cisplatin) regimens. Epirubicin has shown

Table 7. Toxicity in patients treated at the recommended dose ($n = 41$)

	Grade (NCI CTC)					Grade 3/4 (%)
	0	1	2	3	4	
Leukopenia	1	0	13	20	7	65.9
Neutropenia	0	1	1	7	32	95.1
Febrile neutropenia	41	–	–	0	0	0.0
Hemoglobin	1	8	10	17	5	53.7
Thrombocytopenia	9	14	8	10	0	24.4
Stomatitis	22	13	5	1	0	2.4
Anorexia	1	14	13	13	0	31.7
Nausea	3	15	14	9	0	22.0
Vomiting	20	8	11	2	0	4.9
Constipation	24	1	13	3	0	7.3
Diarrhea	26	12	1	2	0	4.9
Gastric ulcer	38	0	1	2	0	4.9
Bilirubin	24	12	4	1	0	2.4
Hyponatremia	18	14	–	7	2	22.0
Hypokalemia	31	6	–	4	0	9.8
Hyperkalemia	33	3	4	1	0	2.4
Hypocalcemia	31	5	4	0	1	2.4

NCI CTC, National Cancer Institute Common Toxicity Criteria.

50% and 48% response rates in two clinical studies in 41 and 80 previously untreated patients, respectively, with ED-SCLC [20, 21]. However, currently, combination modalities containing doxorubicin or epirubicin are not being used in the therapy of SCLC, in preference to combination therapy with cisplatin and etoposide. Since amrubicin has shown excellent single-agent activity [15], it can be expected to be superior to other anthracyclines in the treatment of SCLC. Additionally, the present results of combination therapy with cisplatin support the view that amrubicin may be a promising agent that overcomes the therapeutic plateau of SCLC.

Amrubicin is one of the most promising new agents for the treatment of SCLC. In a previous phase II study of amrubicin 45 mg/m^2 on days 1–3 every 3 weeks as a monotherapy for chemonaive ED-SCLC, a 76% overall response rate and 11.7 month MST were observed [15]. The overall response rate and MST were comparable to those achieved with standard combination chemotherapy, such as etoposide plus cisplatin [5, 6]. Moreover, only a few patients treated in the phase II study received salvage chemotherapy consisting of cisplatin and etoposide [15]. The major toxicity of amrubicin as a monotherapy was hematological toxicity: grade 4 leukopenia and neutropenia were seen in 12.1% and 39.4% of patients, respectively, and thrombocytopenia and anemia of grade 3 or worse in 21.2%. Hepatic, renal and cardiac toxicities with amrubicin were not common. Cisplatin is a key drug for the treatment of SCLC and its hematological toxicity, such as leukopenia and neutropenia, is not severe. Thus, we conducted a phase I–II study of amrubicin and cisplatin treatment for chemonaive ED-SCLC to determine the MTD of this combination therapy, to

assess the efficacy and safety of the drugs delivered at their RD in chemonaive ED-SCLC, and to examine pharmacokinetics.

The topoisomerase I inhibitor, irinotecan, is also very effective for SCLC [6]. Combinations of topoisomerase I and topoisomerase II inhibitors, such as irinotecan plus etoposide, have been reported as active combination chemotherapy for SCLC [22]. Thus, combination of irinotecan and amrubicin is another candidate for new combination chemotherapy for SCLC. A phase I study of irinotecan and amrubicin for chemonaive non-SCLC was performed in National Cancer Center Hospital (unpublished data). However, the MTD was less than irinotecan 60 mg/m^2 on days 1 and 8 and amrubicin 35 mg/m^2 on days 2–4, due to relatively severe myelotoxicity. We considered that amrubicin <35 mg/m^2 on days 2–4 with irinotecan 60 mg/m^2 on days 1 and 8 was insufficient to treat SCLC.

In this study, we determined the RD to be amrubicin 40 mg/m^2 on days 1–3 and cisplatin 60 mg/m^2 on day 1 every 3 weeks, and 41 patients were treated at the RD. Main toxicities of this combination chemotherapy were myelosuppression, especially leukopenia and neutropenia, and gastrointestinal toxicities including anorexia, nausea, vomiting, constipation, diarrhea, stomatitis and gastric ulcer. Of 41 patients, 32 (78%) patients received four or more courses of chemotherapy, and 22 (54%) patients completed four courses of chemotherapy without dose modification. One patient developed myocardial infarction; however, other cardiac toxicity, including decrease in left ventricle ejection fraction, was not observed in up to six courses of chemotherapy. The total dose of amrubicin was 720 mg/m^2. Grade 3 or 4 hyponatremia occurred in nine (22%) patients; however, most of the patients were asymptomatic. No unexpected toxicities and no treatment-related deaths were observed in this study. Toxicities observed in this study were manageable.

Four CRs and 32 PRs occurred, for an objective response rate of 87.8% (95% CI 73.8% to 95.9%) in 41 patients treated at the RD. In most patients, ProGRP levels changed in parallel with tumor responses. The MST of the 41 patients was 13.6 months, and the 1-year survival rate was 56.1%. These results were better than recently reported results for irinotecan and cisplatin in chemonaive ED-SCLC: an objective response rate of 84% and MST of 12.8 months [6]. The combination of amrubicin and cisplatin has demonstrated an impressive response rate and MST in patients with previously untreated ED-SCLC. A possible reason for the better results is overselection of patients, because we used unusual exclusion criteria such as non-steroidal anti-inflammatory drug or adrenal cortical steroid use for >50 days, and gastric and/or duodenal ulcer. However, in a phase II study, this kind of bias is not uncommon.

Combination chemotherapy with etoposide plus cisplatin or etoposide plus cisplatin, alternating with cyclophosphamide, doxorubicin and vincristine, had been considered as standard chemotherapy for SCLC in North America and Japan. A Japanese phase III trial (JCOG 9511) demonstrated that treatment with four cycles of irinotecan plus cisplatin every 4 weeks yielded a highly significant improvement in survival in ED-SCLC patients over standard etoposide plus cisplatin, with less myelosuppression [6]. Based on the results of the JCOG 9511 trial, irinotecan plus cisplatin is considered to be the reference chemotherapy arm for ED-SCLC in future trials in Japan [23]. The JCOG are preparing a phase III clinical trial of amrubicin and cisplatin for previously untreated ED-SCLC to compare combination therapy of irinotecan with cisplatin.

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