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Phase I Study of Irinotecan and Amrubicin in Patients with Advanced Non-Small-Cell Lung Cancer

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Abstract. *Background: Combination chemotherapy of irinotecan and amrubicin for advanced non-small cell lung cancer (NSCLC) has not been fully evaluated. To determine the maximum-tolerated dose (MTD), a phase I study in patients with advanced NSCLC was conducted. Materials and Methods: Patients with stage IIIB/IV NSCLC were enrolled in this study. Both patients with and without prior chemotherapy were also eligible. The drugs were administered on days 1 and 8, every 3 weeks. The starting doses of irinotecan and amrubicin were 60 and 35 mg/m^2, respectively. Results: Nineteen patients received a total of 53 courses. Grade 4 neutropenia was observed in 23% of courses. Anaemia and thrombocytopenia were generally mild. Grade 3 febrile neutropenia occurred in 5 courses. Other grade 3 or greater non-haematological toxicities were observed in only 4 out of 52 courses (grade 3 infection and hyponatremia). The maximum-tolerated doses (MTDs) of irinotecan and amrubicin were 100 and 45 mg/m^2, respectively. Objective response was obtained in 2 patients (10.5%), who had received prior chemotherapy. Conclusion: This combination was well tolerated, but produced only a modest anti-tumour effect for advanced NSCLC. Further investigation into the role of this regimen as a salvage chemotherapy may be warranted in relapsed patients.*

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Lung cancer is the leading cause of cancer deaths in many countries (1). Although cisplatin-based chemotherapy has been conducted extensively in patients with advanced non-small cell lung cancer (NSCLC) over the past two decades, the survival benefit remains modest and further improvement of treatment outcome is needed (2). Recently, several new agents with novel mechanisms have been developed and have shown to be highly effective for NSCLC (3). Irinotecan, a unique semi-synthetic derivative of camptothecin, is a topoisomerase I inhibitor shown to have favourable anti-tumour activity for advanced NSCLC as a single agent, with a response rate of 21-32% (4, 5). Amrubicin, a totally synthetic anthracycline, is a topoisomerase II inhibitor and also effective for NSCLC as a single agent, with a response rate of 25% (6), although other anthracyclines are now considered to have little benefit in the treatment of advanced NSCLC. Combined use of topoisomerase I and II inhibitors has been demonstrated to be complementary in preclinical studies (7, 8). The toxicity profiles of these two drugs are different (4-6). Although several large randomized phase III studies have been conducted to compare survival, response and toxicity from a platinum-based doublet containing a single new agents to a non-platinum-based doublet consisting of two new agents, it has not yet been determined which is more effective (9). Thus, there is still room for investigation of non-platinum regimens.

Table I. *Planned dose level.*

Dose level	Irinotecan (mg/m^2)	Amrubicin (mg/m^2)
1	60	35
2	80	35
3	80	40
4	100	40
5	100	45
6	100	50

Table II. *Patient characteristics.*

Number of patients		19
Age	median (range)	67 (48-74)
Gender	male	12
	female	7
Performance status	0 / 1	11 / 8
Histology	adenocarcinoma	16
	squamous cell carcinoma	3
Stage	IIIB	2
	IV	7
	postoperative recurrence	10
No. of prior chemotherapy regimens		
	0 / 1 / 2 / 3	12/ 6 / 0 / 1

Based on such a background, a phase I study of combination chemotherapy in patients with advanced NSCLC was designed. The primary objective was to determine the maximum-tolerated dose (MTD) for each drug, with a secondary objective of assessing anti-tumour activity.

Patients and Methods

Eligibility criteria. Patients were required to fulfil the following eligibility criteria: pathologically proven, advanced and inoperable NSCLC; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1, or 2; age ≤75 years; presence of evaluable lesions; adequate reserves of haematological function (white blood cell (WBC) count >4000 /μL, neutrophil count >2000 /μL, haemoglobin level >9.5 g/dL, platelet count >10x10^4 /μL), renal function (serum creatinine <1.5 mg/dL), hepatic function (total bilirubin <1.5 mg/dL, serum transaminases <2.5x upper limit of normal range) and pulmonary function (PaO_2 ≥60 Torr); and acquisition of written informed consent. Both patients with and without prior chemotherapy were included. Patients with symptomatic brain metastasis were excluded from the study. Baseline pretreatment evaluations included a complete history, physical examination, laboratory tests, chest radiograph, electrocardiogram, computed tomography (CT) scans of the chest and abdomen, magnetic resonance imaging (MRI) of the brain, and a radionuclide bone scan. Staging was assessed according to the tumour, node and metastasis system (10). The protocol was approved by the institutional review board of each participating institute.

Treatment schedules. Amrubicin, diluted in 20 mL physiological saline, was intravenously administered over 5 minutes on days 1 and 8. Soon after completion of amrubicin infusion, irinotecan, diluted in 250 mL physiological saline, was intravenously administered over 1 hour on the same days. Each patient was premedicated with intravenous administration of dexamethasone (8 mg) and granisetron (3 mg) 30 minutes before bolus infusion of amrubicin. Treatment was repeated every 3 weeks, with 5 dose levels planned. The starting doses of irinotecan and amrubicin were 60 and 35 mg/m^2, respectively, which were two-thirds of the recommended doses as single agents (Table I). Administration of irinotecan and amrubicin on day 8 was delayed until day 15 if haematological toxicity of grade 3 or greater, non-haematological toxicity of grade 2 or greater, or diarrhoea was observed on the day of administration. If these toxicities did not improve by day 15, this administration was cancelled in that course. Patients were treated with at least 2 courses of chemotherapy unless there was disease progression, unacceptable toxicity, or withdrawal of informed consent. Initiation of the next course of chemotherapy was delayed until recovery of WBC count to 3000 /μL or greater, neutrophil count to 1500 /μL or greater, platelet count to 10x10^4 /μL or greater, and resolution of non-haematological toxicities to grade 1 or less.

Assessment of toxicity and dose escalation. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, Version 2.0). Dose-limiting toxicity (DLT) was defined as development of at least one of the following adverse events: any non-haematological toxicity of grade 3 or greater, except alopecia, nausea, or vomiting; platelet count <2x10^4 /μL; grade 4 leukopenia; grade 4 neutropenia lasting for 4 days or longer; cancellation of irinotecan and amrubicin administration on day 8; or inability to begin the next course of treatment by day 56 due to failure to recover from toxicity.

For each dose level, 3 or 6 patients were scheduled to enter. If fewer than 2 out of 3 or 3 out of 6 patients experienced DLT, the next group of patients was treated at the next higher dose level. MTD was defined as the dose level that produced any DLT in 3 or more patients out of a maximum of 6 patients, and further dose escalation was not permitted. All treatment courses were analysed to determine DLT and MTD, although the decision to elevate dose level was based on toxicity in the first course. Dose escalation above starting doses in an individual patient was not allowed. The recommended dose was defined as the dose level below MTD. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia were observed, use of granulocyte colony-stimulating factor was permitted. The dose could be reduced in subsequent courses if patients experienced DLT in the previous course.

Table III. *Haematological toxicity of grade 2 or greater (all courses).*

		Dose level				
Toxicity	Grade	1	2	3	4	5
No. of treated patients		3	3	3	6	4
No. of courses evaluated		4	5	8	31	5
No. (%) of courses encountered						
Leukopenia	2	2 (50%)	2 (40%)	3 (38%)	9 (29%)	0
	3	2 (50%)	0	0	5 (16%)	4 (80%)
	4	0	0	0	0	1 (20%)
Neutropenia	2	0	3 (60%)	1 (13%)	9 (29%)	0
	3	4 (100%)	0	3 (38%)	8 (26%)	1 (20%)
	4	0	1 (20%)	0	7 (23%)	4 (80%)
Thrombocytopenia	2	0	0	0	1 (3%)	0
	3	0	0	0	1 (3%)	0
	4	0	0	0	0	0
Anaemia	2	1 (25%)	4 (80%)	0	3 (10%)	2 (40%)
	3	0	0	0	0	0
	4	0	0	0	0	0

Assessment of anti-tumour activity. Standard Response Evaluation Criteria in Solid Tumours (11) was used to evaluate responses. The best overall response was defined as the best response recorded from the start of treatment until disease progression or recurrence.

Results

Patient characteristics and treatment delivery. Nineteen patients with advanced NSCLC were enrolled between May, 2003 and January, 2004 in 4 institutes. The patient characteristics are listed in Table II. A total of 53 courses were evaluated, with a median number of 2 courses (range: 1 to 8). Eight patients (42%) received only 1 course of chemotherapy, because of disease progression in 6 patients, patient refusal and unacceptable toxicity (1 patient each). The median total delivered dose of amrubicin was 140 mg/m^2, ranging from 70 to 640 mg/m^2. Administration of irinotecan and amrubicin on day 8 was delayed for 1 week in 1 course at dose level 4 because of diarrhoea. All patients and courses were assessable for safety.

Haematological toxicity. Grade 4 neutropenia occurred in 12 (23%) out of 53 courses (Table III). In 2 courses, grade 4 neutropenia continued for 4 and 6 days, and grade 4 leukopenia continued for 4 days in 1 course despite G-CSF support, but they were not accompanied by any febrile episodes. Anaemia and thrombocytopenia were relatively mild, and no transfusions were required.

Non-haematological toxicity. Non-haematological toxicity was generally mild and no patient experienced grade 4 or greater toxicitiy (Table IV). Febrile neutropenia occurred in 5 (9%) out of 53 courses. Grade 3 infection occurred in 3 out of 53 courses; 2 in the first course, on day 4 at dose level 2 and on day 25 at dose level 4. These toxicities were reversible with appropriate supportive care. Grade 3 hyponatremia was observed in 1 out of 53 courses, but was reversible and mild. No other severe toxicities, such as diarrhoea or cardiotoxicity, occurred.

Maximum-tolerated dose. In the first course, DLT was observed in 1 of 3 patients at dose level 2 (grade 3

Table IV. *Non-haematological toxicity of grade 2 or greater (all courses).*

		Dose level				
Toxicity	Grade	1	2	3	4	5
No. of treated patients		3	3	3	6	4
No. of courses evaluated		4	5	8	31	5
No. (%) of courses encountered						
Febrile neutropenia	3	1 (25%)	0	0	2 (6%)	2 (40%)
Nausea/vomiting	2	0	1 (20%)	4 (50%)	0	0
	3	0	0	0	0	0
Hepatotoxicity	2	1 (25%)	0	0	0	0
	3	0	0	0	0	0
Infection	2	0	0	0	0	0
	3	0	2 (40%)	0	1 (3%)	0
Diarrhoea	2	0	2 (40%)	0	0	1 (20%)
	3	0	0	0	0	0
Hyponatremia	2	0	0	0	0	0
	3	0	1 (20%)	0	0	0

No patient developed grade 4 or greater toxicity.

infection), in 2 of 6 at dose level 4 (grade 3 febrile neutropenia and grade 3 infection) and in 3 of 4 at dose level 5 (persistence of grade 4 neutropenia and grade 4 leukopenia, persistence of grade 4 neutropenia, and grade 3 febrile neutropenia). There were no treatment-related deaths. Accordingly, the MTDs of irinotecan and amrubicin were determined to be 100 and 45 mg/m^2, respectively (dose level 5). The recommended doses of irinotecan and amrubicin were therefore concluded to be 100 and 40 mg/m^2, respectively (dose level 4).

Anti-tumour activity. All patients were assessable for response. Objective tumour response was obtained in 2 (11%) of 19 patients. Both patients were treated at dose level 4 and had received prior chemotherapy (single agent vinorelbine therapy and combination chemotherapy of carboplatin and gemcitabine, respectively).

Discussion

The present study demonstrated that the two-drug combination of irinotecan and amrubicin using a fractionated administration schedule was well tolerated in patients with advanced NSCLC. MTDs of irinotecan and amrubicin were 100 and 45 mg/m^2, respectively. Dose-intensities of irinotecan and amrubicin at the MTD were 67 and 30 mg/m^2/week, respectively. The MTD for irinotecan was higher than in the two-drug combination with cisplatin (12) and the MTD for amrubicin was slightly lower than when used as a single agent (6).

As expected, myelosuppression was the major toxicity in this study, however, it was reversible and not life-threatening. One of the major toxicities associated with anthracyclines is cardiotoxicity (13). However, in a preclinical study using dogs, Noda *et al.* reported that amrubicin had neither cardiotoxicity nor deteriorating effects on pre-existing cardiomyopathy (14). Also, previous clinical trials involving 74 patients with small cell lung cancer demonstrated that amrubicin had no cardiotoxicity, consistent with our results (15, 16). Similarly, irinotecan is not reported to have any major potential cardiotoxicity (17). Thus, this regimen may be used safely even in patients with pre-existing cardiac dysfunction. Additionally, it is of note that diarrhoea was mild in our study, since one of the DLTs of single agent irinotecan was diarrhoea (17). The difference in incidence of diarrhoea might be attributable to the difference in treatment schedule or in supportive care. Further confirmation is warranted.

Previous studies have shown that development of cellular resistance to topoisomerase II inhibitors confers an increased sensitivity to topoisomerase I inhibitors (7). The reverse effect, in which resistance to a topoisomerase I inhibitor enhances the sensitivity to topoisomerase II inhibitors, has also been reported (8). This enhanced sensitivity may be related to a compensatory role by each topoisomerase

enzyme to a deficiency in the other. Despite positive experimental findings, combined use of topoisomerase inhibitors has produced controversial *in vitro* results. When administered simultaneously, some reports have indicated synergistic or additive cytotoxic effects in various tumour cell lines (18), while others have demonstrated antagonistic effects (19). However, Bertrand *et al.* have shown that sequential administration of camptothecin and etoposide resulted in additive cytotoxicity in colon cancer cell lines (20). Therefore, further characterisation of the maximum attainable effect from a topoisomerase I and II inhibitors combination is required.

In Japan, two phase II studies investigating a combination of irinotecan and topoisomerase II inhibitor, etoposide, have been conducted (21, 22). Masuda *et al.* evaluated this combination chemotherapy in patients with relapsed small cell lung cancer (21). Irinotecan was administered at a dose of 70 mg/m^2 on days 1, 8 and 15, and etoposide was given at a dose of 80 mg/m^2 on days 1 to 3. This combination produced a response rate of 71%, far exceeding the response rates of 40-50% previously reported for the combination of cisplatin and etoposide (23). However, Oshita *et al.* investigated the same combination using a different schedule in chemo-naïve patients with metastatic NSCLC, in which both irinotecan and etoposide were administered on days 1 to 3 (22). This concurrent administration yielded a disappointing response rate of 21%, less than the 32% response rate of irinotecan alone (4). Considering the data, it may be preferable to avoid simultaneous administration of topoisomerase I and II inhibitors. This scheduled-dependency, as well as insufficient doses of the two drugs (15 out of 19 were treated with doses lower than MTD) probably contributed to the lower efficacy in this study. More preclinical and clinical investigations are needed to clarify the optimum sequence and administration schedule for both drugs.

In this study, objective response was obtained in 2 patients who had prior chemotherapy, whereas no chemo-naïve patients achieved objective response. This may be attributed to differences in the expression levels of target molecules of chemotherapeutic agents, in addition to the small sample size. Naruse *et al.* reported that K562/TPA, a human leukemic phorbol ester-resistant subline, was 400-fold more sensitive to the EGFR tyrosine kinase inhibitor gefitinib than the K562 parental cell, and that EGFR protein expression was detected in K562/TPA but not in K562 parent cells. They speculated that the high sensitivity of the multiple drug-resistant cell line K562/TPA is due to acquired EGFR expression from exposure to cytotoxic agents (24). Similarly, determining protein levels of topoisomerases before and after chemotherapy may be useful for characterising differences in response to this regimen between relapsed and chemo-naïve patients.

In conclusion, the combination of irinotecan and amrubicin was well tolerated, but produced only a modest anti-tumour effect for advanced NSCLC. However, further investigation into the role of this regimen as salvage chemotherapy may be warranted in relapsed patients, because relapsed patients responded to the regimen and there have been no reports evaluating topoisomerase I and II inhibitors combination trials in relapsed patient with NSCLC.

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