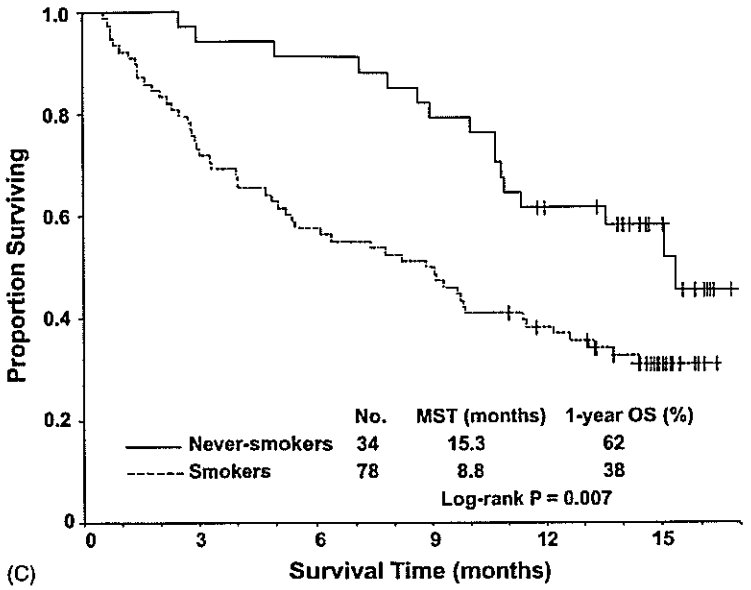

Fig. 2 (*Continued*).

as high as 5.4%. The risk of ILD appears to be around 2–5% if gefitinib is given to patients without careful risk assessment. We think that the incidence can be reduced by patient selection after a thorough risk assessment and that the proper use of gefitinib may enable great benefit, far exceeding its potential risks.

Our analysis of the risk factors for the development of ILD revealed pre-existing PF as a strong risk factor. Of the 112 patients in this study, 12 had PF at the start of gefitinib administration. Four (33%) of these patients subsequently developed ILD, 3 (25%) died as a result, and no response was seen in any of these 12 patients. A panel of experts convened by AstraZeneca Japan retrospectively analyzed 104 patients with NSCLC who developed ILD during gefitinib therapy in Japan and reported that 30 (29%) of them were diagnosed as pre-existing PF by chest X-rays or computed tomography scans taken before gefitinib administration [8]. The panel also noted that the patients with PF had a significantly higher mortality rate after the onset of ILD: it was 77% (23/30) among the patients with PF and 34% (25/74) among the patients without PF ($P < 0.001$) [8]. We conclude that gefitinib treatment may be harmful to patients with PF and recommend that gefitinib not be used if PF is apparent on the chest X-rays.

In our study, all patients were Japanese and a 33% response rate was observed. In the IDEAL 1 trial, 102 Japanese and 106 non-Japanese patients received gefitinib, and the response rate was 27.5% in the Japanese and 10.4% in the non-Japanese [5]. Whether this difference was attributable to ethnicity or an imbalance in other characteristics is unknown, but a high response rate in Japanese patients has been consistently observed in clinical practice.

Both the IDEAL 1 and 2 trials suggested "female gender" and "adenocarcinoma" as predictive factors for tumor response to gefitinib [5,6], and a retrospective analysis of gefitinib monotherapy for advanced NSCLC showed that "adenocarcinoma" (especially with bronchioloalveolar features) and "no history of smoking" were significantly correlated with response to gefitinib [9]. We observed the same tendency with a response rate of 53% in women, 38% in patients with adenocarcinoma, and 63% in never-smokers. "No history of smoking" was a significant predictive factor for response in multivariate analysis, and it was also a significant predictor of longer TTF and longer survival. Since both female gender and adenocarcinoma were significantly associated with no history of smoking, which of these characteristics are true predictive factors remains uncertain. It was also suggested that heavier smokers and male smokers specifically had a lower response rate among the patients with smoking history. Since heavier smokers tended to have a higher risk of ILD, we should carefully assess their risk-benefit ratio of gefitinib therapy before selecting therapeutic strategies.

There are some biological explanations for these clinical characteristics associated with response to gefitinib [10]. Although gefitinib inhibits the intracellular tyrosine kinase domain of EGFR, no correlation between expression of EGFR and response

has been demonstrated [11]. When EGFR and human epidermal growth factor receptor 2 (HER2) are coexpressed, HER2 is the preferred dimerization partner of EGFR, and EGFR-HER2 heterodimers have more signaling potency than EGFR homodimers [12]. Preclinical studies have indicated that tumor cell lines overexpressing HER2 or coexpressing EGFR and HER2 are sensitive to gefitinib [13–16]. Since EGFR/HER2-coexpression is more common in adenocarcinoma of the lung than in squamous cell carcinoma [13,17], the high response rate in adenocarcinoma may be attributable to it. In women, estrogens and estrogen receptors are involved in the development of NSCLC [18], and estrogens binding to its receptors upregulates EGFR and EGFR ligands [19]. The presence of estrogens and its receptors may impact EGFR signaling and the response of NSCLC to gefitinib in women. NSCLC in never-smokers may also have a different biology. Since several studies have indicated fewer mutations of the p53 and K-ras genes in never-smokers than in smokers [20,21], the relation between such tobacco-related mutations and gefitinib response should be investigated. Subgroups of patients who obtain a clinical benefit from gefitinib administration are needed to be identified more precisely, and molecular markers predictive of tumor response should be sought by using DNA microarrays and a proteomics-based approach.

Our analysis suggests that patients who suffer from skin toxicity, diarrhea, or liver toxicity have a greater clinical benefit from gefitinib treatment. A correlation between skin toxicity and survival has also been shown in a study of gefitinib for head and neck cancer [22] and in studies of erlotinib, another EGFR tyrosine kinase inhibitor [23]. Because these findings may be attributable to the responders having taken gefitinib for longer periods and the toxicities in these patients being evaluated more carefully, further studies are needed to confirm them. If the early onset of toxicities has predictive value for survival, it can be used for clinical decision making regarding continuation of gefitinib treatment.

5. Conclusion

When gefitinib is used to treat advanced NSCLC, it confers a higher risk of ILD on patients with PF and a greater clinical benefit on never-smokers, women, patients with adenocarcinoma, and patients with no history of thoracic radiotherapy. Gefitinib therapy is an important treatment option for patients with advanced NSCLC, but the proper use of it based on individual risk-benefit assessments is crucial.

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ANTI-TUMOUR TREATMENT

Treatment of small cell lung cancer in the elderly based on a critical literature review of clinical trials

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KEYWORDS
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Summary At diagnosis, 25–40% of patients with small cell lung cancer (SCLC) are 70 years of age or older, and many of them have been undertreated because of fear of excessive toxicity associated with chemotherapy. Papers retrieved by a Medline search using the key words "elderly or older" and "small cell lung cancer" and by a manual search were classified into the three types: (1) case-series studies, (2) subgroup analyses of phase II and phase III trials by age, and (3) prospective clinical trials in the elderly. Treatment regimens, delivery, toxicity, antitumor activity, and patient survival were reviewed in elderly patients with good and poor general condition. The standard chemotherapy regimens for the general population could be applied to elderly patients in good general condition (performance status of 0–1, normal organ function, and no comorbidity), but etoposide and carboplatin regimen with dose modification was frequently used for unselected elderly patients. A combination of full-dose thoracic radiotherapy and chemotherapy was the treatment of choice for limited SCLC in the elderly. Full cycles of chemotherapy were tolerable by 80% of the elderly patients with good general condition, but two cycles may be optimal for unselected elderly patients. Although the evidence levels based on clinical trials available today are low, these results are helpful for clinical practice and future clinical trials for elderly patients with SCLC.

Introduction

Lung cancer is currently the most common cancer in the world, and it is the leading cause of cancer death in many countries.[1,2] Small cell lung cancer (SCLC) accounts for 15–25% of all lung tumors. For treatment purposes, it is considered separately from other histological types, which are known as non-small cell lung cancer, because by the initial diagnosis SCLC has already metastastized to distant organs in 60–70% of patients, and it is highly sensitive to chemotherapy and radiotherapy. The prognosis of the disease is extremely poor. The 5-year survival rate of patients with limited disease (LD), which is a disease confined to one hemithorax that can be encompassed in a tolerable radiation field, is less than 15–25%, and most patients with extensive disease (ED), which has spread

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beyond the range of LD, die within two years after diagnosis.[3]

At diagnosis, 25–40% of patients with SCLC are 70 years old or older, and the number of patients is expected to increase, because the geriatric population is growing.[3–5] There has been a general tendency among physicians to consider aged people to always have poor tolerance for chemotherapy, and as a result many elderly cancer patients have been undertreated because of fear of excessive toxicity.[5] Thus, it is one of the immediate tasks for medical oncologists to establish treatment of SCLC in the elderly based on evidence obtained in clinical trials.

The decreases in lean body mass, hepatic blood flow, and renal function that accompany aging affect drug distribution, metabolism, and excretion. The clearance of anticancer agents commonly used for the treatment of SCLC, including cisplatin, doxorubicin, etoposide, and ifosfamide, has been shown to be decreased in the elderly.[6] Myelotoxicity is also sometimes severer in this population than in younger populations, because the absolute amount of hematopoietic marrow decreases with age. The incidence of doxorubicin-induced cardiotoxicity is also increased in the elderly, although the mechanism is unknown.[6] These age-related changes in pharmacokinetics and phamacodynamics, however, have not been fully evaluated in the treatment for SCLC in the elderly.

Studies on the treatment of SCLC in the elderly can be classified into the following three types: (1) case-series studies, (2) subgroup analyses of phase II and phase III trials by age, and (3) prospective clinical trials in the elderly. The first type of studies retrospectively analyzes all the elderly cases of SCLC diagnosed at an institution in a given period. They may provide information on the general aspects of elderly patients with SCLC, including performance, comorbidity, and percentages of patients treated with chemotherapy or supportive care alone. The results for outcome of treatment, however, are thought to be highly biased, because the patient populations in these studies are heterogeneous in terms of various prognostic factors. In the second type of studies, treatment outcome is retrospectively compared between an elderly group and a younger group. The patients in these studies are highly selected, because only those who meet strict eligibility criteria are included in clinical trials. Thus, the results of the analyses are understandable, but they are only applicable to the limited population of elderly patients. The most reliable and clinically useful results are obtained in the third type of studies, because the subjects can be freely defined and biases are controlled. Thus far, however, only a limited number of prospective studies on elderly patients with SCLC have been available.

The interpatient variability in activities of daily living, performance status, and comorbidity in elderly patients is so large that it is difficult to establish a standard treatment applicable to all patients. In this review, treatments for patients with good and poor general condition were summarized separately. We believe these summaries are helpful for clinical practice and future clinical trials for elderly patients with SCLC.

Methods

We retrieved papers published during the period from 1981 to 2000 by means of a Medline search using the key words "elderly or older" and "small cell lung cancer" in the Medical Subject Headings and a manual search. The papers were then classified into the three types: (1) case-series studies, (2) subgroup analyses of phase II and phase III trials by age, and (3) prospective clinical trials in the elderly. Among the retrospective studies in the first two categories, only those in which "elderly" was defined as 70 years or older were selected for the analysis. Prospective trials of infirm as well as elderly patients, however, were included in the analysis, because both populations were frequently included in the same trial. Patient characteristics, treatment regimens, treatment delivery, toxicity, antitumor activity, and patient survival were reviewed. The general clinical characteristics of the elderly SCLC patients are summarized on the basis of the results of the first type of studies. In principle, our summary of treatment for elderly patients with good performance status and no comorbidity is based on the results of the second type of studies, and our summary for unselected elderly patients is based on the third type of studies. Evidence levels are provided according to the previously described scale (Table 1).[7]

General clinical characteristics of elderly patients with SCLC

Elderly patients 70 years of age or older accounted for 26–38% (average, 31%) of all of the patients (Table 2). The percentage of limited disease ranged from 36% to 50% in both age groups. The general condition of the elderly patients was worse than in the younger patients; patients with PS 0 or 1 accounted for only 52–69% of the elderly patients, and comorbidity was noted in 63–78%. Optimal treatment, defined as four or more treatment

Table 1 Levels of evidence

I	Evidence obtained from meta-analysis of multiple, well-designed, controlled studies. Randomized trials with low false-positive and low false-negative errors (high power)
II	Evidence obtained from at least one well-designed experimental study. Randomized trials with high false-positive and/or low false-negative errors (low power)
III	Evidence obtained from well-designed, quasi-experimental studies such as non-randomized, controlled single-arm, pre-post, cohort, time, or matched case-control series
IV	Evidence from well-designed, non-experimental studies such as comparative and correlational descriptive and case studies
V	Evidence from case reports and clinical examples

Table 2 Case-series studies on small cell lung cancer in the elderly

Authors (year)	Age	Number of patients (%)	Limited disease (%)	PS 0–1 (%)	Comorbidity (%)	Optimal treatment (%)[a]	TRD (%)	MST (month)
Nou (1996)[8]	<70	235 (68)	50	NA	NA	NA	7	11
	⩽70	110 (32)	48	NA	NA	NA	8	7
Dajczman et al. (1996)[9]	<70	231 (74)	40	80	56	44	5	9
	⩽70	81 (26)	43	52	75	23	5	6
Tebbutt et al. (1997)[10]	<70	102 (67)	46	60	NA	83	NA	No difference
	⩽70	51 (33)	49	55	63	47	4	No difference
Jara et al. (1999)[11]	<70	59 (62)	42	71	58	59	NA	8
	⩽70	36 (38)	36	69	78	39	NA	5

MST, median survival time; NA, not available; PS, performance status; TRD, treatment-related death.
[a] Optimal treatment was defined as four or more treatment cycles, relative total dose of 85% or higher, or no definition described.

cycles, relative total does of 85% or higher, or no definition available, was delivered to 23–47% of the elderly patients compared with 44–83% of the younger patients. The incidence of treatment-related death and patient survival, however, did not differ between the two age groups.

Chemotherapy for elderly patients in good general condition

Among elderly lung cancer patients, 10–30% are in good general condition without comorbidity,[9–13] and the standard chemotherapy for the general population, including cyclophosphamide, doxorubicin and vincristine (CAV), cisplatin and etoposide (PE), and CAV alternating with PE regimens, can be given to this population (Evidence level, IV). Subgroup analyses of phase II and phase III trials of SCLC by age showed that myelosuppression and doxorubicin-induced cardiotoxicity were severer in the elderly patients than in the younger patients, and that their incidence of treatment-related death tended to be higher. About 80% of elderly patients, however, received optimal treatment, and their survival was comparable to that of younger patients (Table 3).[14–16] Thus, the standard chemotherapy should be tried in these patients, although a reduction in treatment cycles and chemotherapy dose, or prolongation of treatment intervals may be needed more often than in younger patients.

Chemotherapy for unselected elderly patients

The standard chemotherapy for younger patients is not indicated for 70–90% of elderly patients because of poor performance status or the presence of complications. Oral etoposide and teniposide has been tried in these patients, but randomized trials showed that it was more toxic and had no survival benefit over the standard chemotherapy (Table 4).[17,18] A randomized trial of two-drug

Table 3 Subgroup analyses of phase III trials of small cell lung cancer by age

Authors (year)	Treatment	Age	Number of patients	Limited disease (%)	PS 0–1 (%)	Optimal treatment (%)[a]	Grade 3–4 toxicity (%)	TRD (%)	MST (month)
Paccagnella et al. (1996)[14]	CAV–PE (±TRT)	<70	254	58	ND	RDI 78	NA	3	12
		⩽70	32	56	ND	RDI 67	NA	9	12
Siu et al. (1996)[15]	CAV–PE (±TRT)	<70	520	100	88	92	Neutropenia[b] (60) Thrombocytopenia (10) Cardiac (0.2)	2	15
		⩽70	88	100	84	82	Neutropenia[b](64) Thrombocytopenia[15] Cardiac (3)	5	13
Yuen et al. (2000)[16]	PE+TRT	<70	331	100	96	90	Neutropenia[b](58) Thrombocytopenia (21) Infection (6)	1	22
		⩽70	50	100	90	78	Neutropenia[b](82) Thrombocytopenia (36) Infection (10)	10	14

CAV, cyclophosphamide, doxorubicin and vincristine; MST, median survival time; NA, not available; ND, no difference; PE, cisplatin and etoposide; PS, performance status; RDI, relative dose intensity; TRD, treatment-related death; TRT, thoracic radiotherapy.

[a] Optimal treatment was defined as four or more treatment cycles.

[b] Grade 4 only.

Table 4 Phase III studies comparing standard and low intensive chemotherapy in elderly or poor risk patients with small cell lung cancer

Authors (year)	Chemotherapy regimen	Number of patients	Age ⩾ 70 (%)	PS ⩾ 2 (%)	RR (%)	Grade 3–4 toxicty (%)	TRD (%)	MST (month)
Girling (1996)[17]	Oral E (50 mg) bid days 1–10	171	Median 67	100	61	Neutropenia[a](14), Infection (4)	14	4.3[b]
	Standard EV or CAV	168	Median 68	100	73	Neutropenia[a] (12), Infection (7)	10	6.1[b]
Souhami et al. (1997)[18]	Oral E (100 mg) bid days 1–5	75	52	48	33	Neutropenia (3), Infection (5)	2	4.8[b]
	Standard CAV/PE	80	44	56	46	Neutropenia (3), Infection (6)	1	5.9[b]
MRC (1996)[19]	EV	156	25	54	55	Leukopenia[a] (4)[b], Stomatitis[c] (34)[b]	1	4.6
	EVMC	154	27	52	54	Leukopenia[a] (16)[b], Stomatitis[c] (54)[b]	7	4.7
James et al. (1996)[20]	Half dose CAV/PE, q11 days	78	Median 63	63	59	Leukopenia (23)[b], Infection (5)	0	6.4
	Standard CAV/PE, q3w	89	Median 63	67	45	Leukopenia (7)[b], Infection (5)	1	5.8
Earl et al. (1991)[21]	Planned CEV	155	Median 65	31	NA	NA	NA	8.2
	Required CEV	145	Median 66	35	NA	NA	NA	6.8

CAV, cyclophosphamide, doxorubicin and vincristine; CEV, cyclophosphamide, etoposide and vincristine; E, etoposide; EV, etoposide and vincristine; EVMC, etoposide, vincristine, methotrexate and cyclophosphamide; MST, median survival time; NA, not available; PE, cisplatin and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death.

[a] Including grade 2–4 toxicity.

[b] Statistically significant.

[c] Including grade 1–4 toxicity.

versus four-drug combinations showed severer toxicity in the four-drug arm with no improvement in survival.[19] A regimen of cisplatin and etoposide (PE) alternating with cyclophosphamide, doxorubicin, and vincristine (CAV) every 10–11 days at half the standard dose failed to reduce toxicity or improve survival compared with the standard PE alternating CAV regimen in a randomized trial.[20] Another randomized trial of cyclophosphamide, etoposide, and vincristine (CEV) given as needed to palliate symptoms, versus CEV given at fixed 3- to 4-week treatment intervals showed that patients randomized to receive chemotherapy as needed had a median interval between cycles of 5 weeks and received only 50% as much total chemotherapy as the patients randomized to the fixed schedule. Although the median survival times were equivalent between both arms, better symptomatic control was achieved with the fixed interval treatment.[21] Thus, these less intensive treatments than the standard treatment are not less toxic or useful for palliation.

The combination of carboplatin and etoposide has been one of the most frequently evaluated regimens in elderly patients with SCLC, and has yielded a response rate of 70–90% and a median survival of 8–10 months for ED and 12–15 months for LD with acceptable toxicity in phase II trials (Table 5).[22,23,25] Modification of the carboplatin dose based on creatinine clearance levels can be especially useful in elderly patients, because many of them have impaired renal function. As a result, this two-drug combination periodically repeated every 3- to 4-weeks has become standard treatment in this patient population (Evidence level, II).

Treatment of elderly patients with limited disease who are in good general condition

A retrospective review of 1208 patients (including 398 SCLC patients, 107 patients more than 70 years of age, 114 patients with PS 2 or higher, and 352 patients with body weight loss greater than 5%) in six EORTC clinical trials (including three for NSCLC, one for SCLC, and two for esophageal cancer) showed that age did not influence the frequency or severity of acute and delayed toxicity of thoracic radiotherapy.[27] Retrospective subset analysis of patients with limited SCLC who were treated with concurrent chemoradiotherapy in phase III trials showed that 80% of the patients 70 years of age or older completed the planned treatment, although hematological toxicity was severer in the elderly group than the younger group (Table 3).[15,16] Only patients with good general condition were included in these trials; 90% had PS 0–1 and 82% had less than 5% body weight loss in the one study,[16] and 84% had PS 0–1 in the other.[15] Thus, the standard chemoradiotherapy can be given to elderly patients in good general condition with PS 0–1, normal organ function and no comorbidity (Evidence level, IV).

Treatment for unselected elderly patients with limited disease

There are three phase II trials of concurrent chemoradiotherapy in this patient population. Although the chemotherapy cycles in these trials were reduced compared with the standard 4–6 cycles, the 5-year survival rates reached to 13–25% with manageable toxicity (Table 6).[28–30] Thus, a combination of full-dose thoracic radiotherapy and two cycles of chemotherapy may be the optimal treatment in unselected elderly patients with limited disease (Evidence level, III).

Discussion

It has been thought to be difficult to establish standard treatments for elderly patients with SCLC, because they form a heterogeneous population in terms of general condition and treatment outcome varies from report to report. However, by classifying studies on the treatment of this population into three types and characterizing subjects included in the studies, relatively consistent results were obtained. To select the optimal treatment for elderly patients, two groups needed to be considered separately: elderly patients in good general condition and all others. The former can be treated with the same strategy as younger patients with minor modifications, if any.

Among elderly patients, 30–50% have PS 2 or higher, and 60–80% have complications in major organs including the kidney, heart, and lung.[6,9–11] They have been treated with oral etoposide or combination chemotherapy at decreased doses or longer intervals. These less intensive treatments than the standard treatment, however, were not less toxic or useful for palliation in the elderly with decreased activity. By contrast, two-drug combination chemotherapy, including a combination of etoposide and carboplatin, produced response rates (RRs) and median survival times (MSTs) comparable to those of younger patients with

Table 5 Phase II trials for elderly or poor risk patients with small cell lung cancer

Authors (year)	Chemotherapy regimen (mg/m^2)	Number of patients	Age $\geqslant 70$ (%)	PS $\geqslant 2$ (%)	RR (%)	Grade 3–4 toxicty (%)	TRD (%)	MST (month)
Evans et al. (1995)[22]	Oral E (100 mg) days 1–7 Carbo (150) day 1	47	Median 69	30	71	Neutropenia (84) Thrombocytopenia (21) Stomatitis (2)	18	LD 14 ED 11
Matsui et al. (1998)[23]	Oral E (40) days 1–14 Carbo[a] day 1	38	100	34	81	Neutropenia (53) Thrombocytopenia (53) Infection (8)	5	LD 15 ED 9
Westeel et al. (1998)[24]	P (30) A (40) V (1) day 1 E (100) days 1, 3, 5	41	100	66	88	Infection (6) Emesis (9)	0	ED 11
Okamoto et al. (1999)[25]	E (100) days 1–3 Carbo[a]day 1	36	100	25	75	Neutropenia (86) Thrombocytopenia (50) Infection (5)	3	LD 12 ED 10
Samantas et al. (1999)[26]	Oral E (100 mg) days 1–12 Carbo (80) weekly	60	Median 66	59	32	Neutropenia (6) Thrombocytopenia (2) Infection (3)	3	5.5

Carbo, carboplatin; E, etoposide; ED, extensive disease; LD, limited disease; MST, median survival time; PAVE, cisplatin, doxorubicin, vincristine and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death.

[a] Dose adjusted for creatinine clearance.

Table 6 Phase II trials of chemoradiotherapy for elderly or poor risk patients with limited small cell lung cancer

Authors (year)	Chemotherapy radiotherapy (Gy/fraction)	Number of patients	Age ⩾ 70 (%)	PS ⩾ 2 (%)	RR (%)	Grade 3–4 toxicty (%)	TRD (%)	MST (month)	5-YS (%)
Westeel et al. (1998)[28]	PAVE × 3, PE × 1 20/5, 30/10, 40/15	25	Median 72	28	92	Thrombocytopenia[a](9) Infection (18) Esophagitis[a](9)	3	16	24
Murray et al. (1998)[29]	CAV × 1, PE × 1 20/5, 30/10	55	67	45	89	Infection(4)	5	13	18
Jeremic et al. (1998)[30]	Carbo + oral E × 2 45/30 (twice daily)	72	100	17	75	Leukopenia (8) Thrombocytopenia (12) Infection (3) Esophagitis (3)	NA	15	13

CAV, cyclophosphamide, doxorubicin and vincristine; Carbo, carboplatin; E, etoposide; MST, median survival time; NA, not available; PAVE, cisplatin, doxorubicin, vincristine and etoposide; PE, cisplatin and etoposide; PS, performance status; RR, response rate; TRD, treatment-related death; 5-YS, five-year survival rate.

[a] Grade 4 only.

acceptable toxicity in elderly patients. Carboplatin is especially useful for the elderly, because it requires only minimum hydration, its non-hematological toxicity is mild, and the dose can be adjusted according to patient's creatinine clearance. Japanese Clinical Oncology Group (JCOG) evaluated toxicity and efficacy of this method in a phase II study (JCOG9409), and showed that grade 4 neutropenia and thrombocytopenia were noted in 44% and 12% of patients, respectively, and that CR and PR were obtained in 6% and 69%, respectively.[25] We started a large phase III trial in 1997, comparing etoposide (80 mg/m^2 days 1–3) and carboplatin (AUC = 5) with etoposide (the same dose) and cisplatin (25 mg/m^2 days 1–3) in elderly patients with SCLC (JCOG 9702). Up to the present, more than 200 patients were registered in this study.

A recent phase III trial showed that a combination of cisplatin and irinotecan was superior to a combination of cisplatin and etoposide in patients with extensive SCLC, but only patients 70 years of age or younger were included in this study.[31] In addition, there is no clinical trial of irinotecan in elderly patients with SCLC. Another anticancer agent promising in the treatment of SCLC is amrubicin, which yielded a response rate of 79% and median survival time of 11 months in patients with extensive SCLC.[32] Further studies are necessary to evaluate these new agents in the treatment of elderly patients with SCLC.

The chemoradiotherapy used in younger patients may be too intensive for most elderly patients with limited SCLC. One approach that avoids excessive toxicity is to reduce the dose of the chemotherapy or radiotherapy. A recent meta-analysis of chemotherapy alone versus chemotherapy plus radiotherapy in patients with limited SCLC demonstrated survival benefit of radiotherapy added to chemotherapy in patients less than 70 years of age, but the benefit disappeared in the older patients.[33] This finding indicates that the standard treatment in this setting might be chemotherapy alone. The currently available phase II studies of treatment of limited SCLC in the elderly, however, showed that two cycles of chemotherapy plus full-dose radiotherapy produced long-term survivors with acceptable toxicity.[28–30] Thus, which modality should be modified remains controversial, but reduced cycles of chemotherapy combined with full-dose radiotherapy appears to be the treatment of choice at present.

The criteria for the classification of elderly patients into two groups in this review were based on PS, function of major organs, and comorbidity. However, they may be inadequate to evaluate this heterogeneous elderly population. In future clinical trials, it will be important to evaluate the influence of cancer treatment on the functional status of the elderly. A comprehensive geriatric assessment designed to improve the health care of elderly people consists mainly of instruments for evaluating activities of daily living, physical function, cognitive function, and emotional status.[34, 35] It has been used as a diagnostic tool to screen for problems and to determine the needs of the geriatric population for in-home assistance, home-health service, or hospital care, but it may be also useful for our purpose.

In conclusion, although the evidence levels based on clinical trials currently available are low, it is possible to select the optimal treatment for elderly patients with SCLC by dividing them into patients in good and poor general condition.

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SYNERGISTIC INTERACTION BETWEEN THE EGFR TYROSINE KINASE INHIBITOR GEFITINIB ("IRESSA") AND THE DNA TOPOISOMERASE I INHIBITOR CPT-11 (IRINOTECAN) IN HUMAN COLORECTAL CANCER CELLS

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Epidermal growth factor receptor [EGFR (HER1, erbB1)] is a receptor with associated tyrosine kinase activity, and is expressed in colorectal cancers and many other solid tumors. We examined the effect of the selective EGFR tyrosine kinase inhibitor (EGFR-TKI) gefitinib ("Iressa") in combination with the DNA topoisomerase I inhibitor CPT-11 (irinotecan) on human colorectal cancer cells. EGFR mRNA and protein expression were detected by RT-PCR and immunoblotting in all 7 colorectal cancer cell lines studied. Gefitinib inhibited the cell growth of the cancer cell lines *in vitro* with an IC_{50} range of 1.2–160 μM by 3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Lovo cells exhibited the highest level of protein and autophosphorylation of EGFR and were the most sensitive to gefitinib. The combination of gefitinib and CPT-11 induced supra-additive inhibitory effects in COLO320DM, WiDR and Lovo cells, assessed by an *in vitro* MTT assay. Administration of gefitinib and CPT-11 had a supra-additive inhibitory effect on WiDR cells and tumor shrinkage was observed in Lovo cell xenografts established in nude mice, whereas no additive effect of combination therapy was observed in COLO320DM cells. To elucidate the mechanisms of synergistic effects, the effect of CPT-11-exposure on phosphorylation of EGFR was examined by immunoprecipitation. CPT-11 increased phosphorylation of EGFR in Lovo and WiDR cells in time- and dose-dependent manners. This EGFR activation was completely inhibited by 5 μM gefitinib and gefitinib-induced apoptosis was enhanced by combination with CPT-11, measured by PARP activation although no PARP activation was induced by 5 μM CPT-11 alone. These results suggested that these modification of EGFR by CPT-11, in Lovo cells, is a possible mechanism for the synergistic effect of CPT-11 and gefitinib. These findings imply that the EGFR-TKI gefitinib and CPT-11 will be effective against colorectal tumor cells that express high levels of EGFR, and support clinical evaluation of gefitinib in combination with CPT-11, in the treatment of colorectal cancers.

Key words: *combination; gefitinib; "Iressa"; colorectal cancer; irinotecan*

Colorectal cancer is a major public health concern. Although chemotherapy appears to be of very limited value in advanced colorectal cancer, there have been many efforts to apply combination chemotherapy in patients with primary disease.[1–3]

The combination of fluorouracil and leucovorin used to be recognized as standard therapy for colorectal cancer, but the topoisomerase I inhibitor, irinotecan (CPT-11), has recently been demonstrated to be active against colorectal cancer that was resistant to prior therapy.[4,5] Moreover, the CPT-11/5-FU/LV combination has been approved as standard chemotherapy by the US FDA for metastatic colorectal cancer.[6] However, patients treated with CPT-11 plus bolus 5-FU/leucovorin have been found to have a 3-fold higher rate of treatment-induced or treatment-exacerbated death than patients treated with other arms of the respective studies.[7] We have therefore been seeking a new combination regimen containing CPT-11 and target-based drugs.

The development of target-based drugs, including receptor tyrosine kinase inhibitors (TKI), is one of the promising strategies for cancer chemotherapy.[8,9] Colorectal cancers express receptors of the type 1 tyrosine kinase family, including epidermal growth factor receptor (EGFR) and c-erbB-2,[10–12] and the EGFR has emerged as a central molecular target for modulation in cancer therapeutics. The correlation between high expression of EGFR and clinically aggressive malignant disease has made EGFR a promising target of therapy for many epithelial tumors, which represent approximately 2/3 of all human cancers. In solid cancers, including colorectal cancers, high EGFR expression correlates with poor prognosis.[11] Gefitinib ("Iressa") is an orally active, selective EGFR-TKI that blocks signal transduction pathways involved in the proliferation and survival of cancer cells and in other host-dependent processes promoting cancer growth.[13,14] In EGFR tyrosine kinase assays, gefitinib has an IC_{50} of 0.033 μM. Inhibition of c-erbB-2 and KDR occurs at doses 100-fold higher than for EGFR inhibition.[15] We have previously demonstrated that gefitinib exerts high growth-inhibitory activity against EGFR-positive tumors in a xenograft model,[16] and gefitinib is therefore expected to be a potent therapeutic agent against EGFR-positive colorectal cancers. In recent years, it has been shown that the combined treatment of established human colorectal cancer xenograft with anti-EGFR drug (cetuximab or gefitinib) and with topoisomerase I inhibitor, topotecan, increase the antitumor activity of these drugs.[17,18] The aim of the present study was to investigate the combination effect of gefitinib and CPT-11 and to elucidate the biochemical mechanism of synergistic interaction in colorectal cancers.

MATERIAL AND METHODS

Drugs and chemicals

Gefitinib (N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl)propoxy] quinazolin-4-amine) was provided by AstraZeneca (Cheshire, UK). Gefitinib was dissolved in dimethyl sulfoxide (DMSO) for the *in vitro* study and suspended in 5% glucose, pH 6, for the *in vivo* study. CPT-11 was obtained from Yakult Honsha (Tokyo, Japan). CPT-11 was dissolved in 45 mg/ml solvitol (pH 3-4) for both the *in vivo* and *in vitro* studies.

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Animals

Female BALB/c nude mice, 6-weeks-old, were purchased from Japan Charles River Co., Ltd. (Atsugi, Japan). All mice were maintained in our laboratory under specific-pathogen-free conditions.

Cells and culture

Human colorectal cancer cell lines WiDR, LS-174T, COLO320DM, COLO320HSR, Lovo, SW480 and HCT116 were obtained from ATCC (Lockville, MD). Lovo cells, SW480 and HCT116 cells were maintained in HAM's F12 medium (GIBCO BRL, Grand Island, NY), Leibovitz's L-15 medium and McCoy's 5A medium (GIBCO BRL), respectively, all supplemented with 10% heat-inactivated fetal bovine serum (FBS). Other cell lines were maintained in RPMI1640 (Nikken Bio Med. Lab., Kyoto, Japan) supplemented with 10% FBS.

Growth-inhibition assay

We used the tetrazolium dye [3,(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, MTT] assay to evaluate the cytotoxicity of various drug concentrations. A 200 ml volume of an exponentially growing cell suspension (5×10^3–1.5×10^4 cells/ml) was seeded into a 96-well microtiter plate and 20 μl of each drug at various concentrations was added. After incubation for 72 hr at 37°C, 20 μl of MTT solution (5 mg/ml in phosphate buffered saline, PBS) was added to each well and the plates were incubated for a further 4 hr at 37°C. After centrifuging the plates at 200*g* for 5 min, the medium was aspirated from each well, and 180 μl of DMSO was added to each well to dissolve the formazan. Optical density was measured at 562 and 630 nm with a Delta Soft ELISA analysis program interfaced with a Bio-Tek Microplate Reader (EL-340, Bio-Metallics, Princeton, NJ). Each experiment was performed in 6 replicate wells for each drug concentration and carried out independently 3 or 4 times. The IC_{50} value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves. Percent survival was calculated as follows: (mean absorbance of 6 replicate wells containing drugs − mean absorbance of 6 replicate background wells)/(mean absorbance of 6 replicate drug-free wells − mean absorbance of 6 replicate background wells) × 100.

RT-PCR

Specific primers designed for EGFR CDS were used for detection of EGFR mRNA as described elsewhere.[16] First-strand cDNA was synthesized from the cells' RNA with an RNA PCR Kit (TaKaRa Biomedicals, Ohtsu, Japan). After reverse transcription of 1 μg of total RNA with Oligo(dT)-M4 adaptor primer, the whole mixture was used for PCR with 2 oligonucleotide primers (5′-AATGTGAGCAGAGGCAGGGA-3′,5′GGCTTGGTTTG-GAGCTTCTC-3′). PCR was performed with initial denaturation at 94°C for 2 min, 25 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec and extension at 72°C for 105 sec).

Immunoprecipitation and immunoblotting

The cultured cells were washed twice with ice-cold PBS, lysed in EBC buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 100 mM NaF, 200 mM Na orthovanadate and 10 mg/ml each of leupeptin, aprotinin and phenylmethylsulfonyl fluoride). The lysate was cleared by centrifugation at 20,000*g* for 5 min, and the protein concentration of the supernatant was measured by BCA protein assay (Pierce, Rockford, IL). For Immunoblotting, 20 μg samples of protein were electrophoretically separated on a 7.5% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membrane was probed with rabbit polyclonal antibody against EGFR (1005; Santa Cruz Biotech, Santa Cruz, CA), HER2/neu (c-18; Santa Cruz), phospho-EGFR specific for Tyr 845, Tyr 1045, and Tyr 1068 (numbers 2231, 2235 and 2234; Cell Signaling, Beverly, MA) and cleaved PARP (number 9544; Cell Signaling) as the first antibody, followed by horseradish peroxidase-conjugated secondary antibody. The bands were visualized by electrochemiluminescence (ECL, Amersham, Piscataway, NJ). For immunoprecipitation, 5×10^6 cells were washed, lysed in EBC buffer, and centrifuged. The resultant supernatants (1,500 μg) were incubated with the anti-EGFR antibody (1005) at 4°C overnight. The immunocomplex were absorbed onto protein A/G-Sepharose beads, washed 5 times with lysate buffer, denatured and subjected to electrophoresis on a 7.5% polyacrylamide gel followed by immunostaining probed with antiphosphotyrosine antibody (PY-20, BD Bioscience Clontech, Tokyo, Japan).

Combined effect of gefitinib and CPT-11 in vitro

The combined effect of gefitinib and CPT-11 on colorectal cancer cell growth was evaluated by the combination index (CI) analysis method.[6] For any given drug combination, CI represents the degree of synergy, additivity or antagonism. CI was expressed in terms of fraction-affected (F_a) values, which represents the percentage of cells killed or inhibited by the drug. Using the mutually exclusive ($\alpha=0$) or mutually nonexclusive ($\alpha=1$) isobologram equation, the F_a/CI plots for each cell line was constructed by computer analysis of the data generated from the median effect analysis. CI values were interpreted as follows: <1.0 = synergism; 1.0 = additive and >1.0 = antagonism.

Using the median-effect method, developed by Chou and Talalay, the dose-response curve was plotted for each drug and for multiple doses of a fixed-ratio combination by using the equation:

$$f_a/f_u = (D/D_m)^m,$$

where, D is the dose-administered, Dm is the dose required for 50% inhibition of growth, f_a is the fraction affected by dose D, f_u is the unaffected fraction and m is a coefffect curve. The dose-response curve was plotted by logarithmic conversion of the equation to determine the m and D_m values, and the dose D_x required for x percent effect $(f_a)_x$ was then calculated as

$$D_x = D_m[f_a]_x/(f_u)x]^{1/m}.$$

Thus, CI can be defined by the isobologram equation

$$CI = (D)_1/(D_x)^1 + (D)_2/(D_x)2 + \alpha(D)_1(D)_2/(D_x)_1(D_x)_2,$$

where $(D_x)_1$ is the dose of Drug-1 required to produce x percent effect alone, and $(D)_1$ is the dose of Drug 1 required to produce the same x percent effect in combination with Drug 2; similarly, $(Dx)_2$ is the dose of Drug 2 required to produce x percent effect alone and $(D)_2$ is the dose of Drug 2 required to produce the same x percent effect in combination with Drug 1. Theoretically, CI is the ratio of the combined dose to the sum of the single-drug doses at an isoeffective level. Consequently, CI values <1 indicate synergism, values >1 indicate antagonism and a value of 1 indicates additive effects. The CI values obtained from both the classical nonconservative ($\alpha=0$) and conservative ($\alpha=1$) isobologram equations are presented in this report.

Growth-inhibition assay in vivo

Experiments were performed in accordance with the United Kingdom Coordinating Committee on Cancer Research Guidelines for the welfare of animals in experimental neoplasia (second edition).

In vivo experiments were scheduled to evaluate the combined therapeutic effect on preexisting tumors of oral or intraperitoneal administration of gefitinib and intravenous injection of CPT-11. The dose of each drug was set based on the results of a preliminary experiment involving administration of each drug alone. Ten days before administration, 1×10^7 WiDR and COLO320DM or 2×10^6 Lovo cells were injected subcutaneously into the back of mice. Five or 6 mice per group were injected with tumor cells. Tumor bearing mice were either given gefitinib, 40 mg/kg/day *p.o.* on days 1–10, or CPT-11, 40 mg/kg/day *i.v.* on days 1, 5 and 9, or

both, or placebo (5%(w/v) glucose solution). Alternatively, gefitinib, 30 or 60 mg/kg, *i.p.* days 1–14, and *i.v.* CPT-11, 16.7 or 33.3 mg/kg, *i.v.* on days 1, 5 and 9, were administered to the mice. Tumor diameters were measured with calipers on days 1, 4, 7, 10, 14, 18 and 22 to evaluate the effects of treatment, and tumor volume was determined by using the following equation: tumor volume = $ab^2/2$ (mm^3) (where *a* is the largest diameter of the tumor and *b* is the shortest diameter). Day "x" denotes the day on which the effect of the drugs was estimated, and day "0" denotes the first day of treatment. All mice were sacrificed on day 22 after measuring their tumors.

Statistical analysis

Differences between the test groups were analyzed by 1-factor ANOVA followed by Fisher's protected least significant difference (PLSD). A value of $p<0.05$ was considered statistically significant.

RESULTS

EGFR and HER2 expression and EGFR autophsophorylation in colorectal cancer cells

We examined EGFR mRNA expression by RT-PCR analysis using 2 specific primers. Approximately 570 bp-long PCR products were amplified in all cell lines that exhibited expression of EGFR mRNA (Fig. 1*a*). Comparison of the protein expression levels of EGFR in colorectal cancer cells by immunoblotting (Fig. 1*b*) revealed high expression in Lovo and WiDR cells. EGFR protein was also detected in LS-174T, COLO320DM, COLO320HSR, HCT116 and SW480 cells, although the expression levels in COLO320DM and COLO320HSR are subtle. The highest expression level of phosphrylated EGFR measured by phospho-specific EGFR antibody (Tyr845, Tyr1045 and Tyr1068) was observed in Lovo cells (Fig. 1*b*). Because the function of EGFR is closely related to that of other HER families including HER2/neu, we also examined the protein level of HER2/neu. High expression of HER2/neu were observed in LS-174T, HCT-116 and SW480 (Fig. 1*b*).

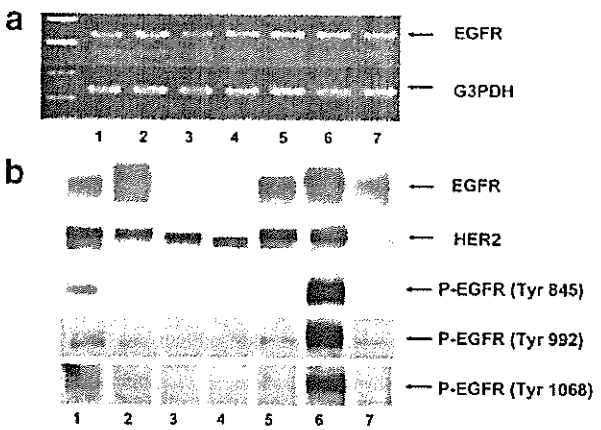


FIGURE 1 – EGFR expression in colorectal cancer cells. (*a*) Expression of EGFR mRNA in each cell line was detected by RT-PCR using specific primers designed for EGFR CDS. Expression of G3PDH mRNA was detected. Twenty-five cycles of PCR amplification were performed for each PCR product. Lanes 1–7 represent LS-174T, WiDR, COLO320DM, COLO320HSR, HCT116, Lovo and SW480 cells, respectively. (*b*) A 20 μg sample of total cell lysates was separated by 7.5% SDS-PAGE, transferred to PVDF membrane, and incubated with a specific anti-human EGFR, HER2/neu and phospho-EGFR (Tyr845, Tyr992 and Tyr1068).

Cellular sensitivity of colorectal cancer cells to gefitinib and CPT-11

The growth inhibitory effect of gefitinib and CPT-11 on colorectal cancer cells was examined by MTT assay. The IC_{50} values of gefitinib for the cell lines ranged from 1.2 μM (Lovo cells) to 160 μM (HCT116 cells) (Table I). No significant relationship was observed between EGFR expression levels and IC_{50} values among these cell lines. However, Lovo cells, which exhibited the highest EGFR expression and its phosphorylation, were the most sensitive to gefitinib. On the other hand, the IC_{50} values of CPT-11 for the cell lines ranged from 5.2 μM (Lovo) to 35 μM (SW480). The range of sensitivity to gefitinib was wider than to CPT-11.

In vitro *combined effect of gefitinib and CPT-11 on colorectal cancer cell lines*

Based on the results of the evaluation of *in vitro* growth-inhibition, 4 cell lines (WiDR, COLO320DM, Lovo, and SW480 cells) were selected for the *in vitro* combination study. Cells were treated with gefitinib or CPT-11 alone or in concomitant combination at fixed molar ratio for 72 hr. The ratios of gefitinib and CPT-11 were set based on the IC_{50} values of each cell line. Growth rate values are averages of data from at least 3 independent experiments. The effects of combinations of gefitinib and CPT-11 on cell growth are shown in Figure 2. CI values of <1, >1 and 1 indicate a supra-additive effect (synergism), antagonistic effect and additive effect, respectively. A low CI index was observed in WiDR, COLO320DM and Lovo cells over a wide range of inhibition levels. Synergistic effects were also observed in the relatively high F_a values in SW480 cells. These results suggest that gefitinib and CPT-11 had a synergistic effect on most of the colorectal cancer cell lines *in vitro*.

In vivo *combination effects of gefitinib and CPT-11*

In order to determine whether the combination of these 2 drugs is also synergistic against colorectal cancer *in vivo*, the growth-inhibitory effect of the combination was evaluated against the colorectal cancer cells in tumor xenografts. The growth inhibitory effect of gefitinib, 30 mg/kg, *i.p.* days 1–10, and CPT-11, 40 mg/kg, *i.v.* days 1, 5 and 9, on WiDR cells was evaluated (Fig. 3*a,b*). Administration of gefitinib or CPT-11 alone suppressed the tumor volume of WiDR cells with a T/C value of 73.9% and 69.2%, respectively, at day 22, (Fig. 3*c*), whereas gefitinib+CPT-11 suppressed WiDR tumors with T/C value of 51.8% at day 22, but this was not statistically significant (Fig. 3*d*, $p=0.164$ by 1-factor ANOVA). A 10% body weight loss was observed until day 15 in mice given the combination, but body weight recovered by day 22 (Fig. 3*e*). No growth inhibitory effect of single or combined therapy of CPT-11 and gefitinib in COLO320DM cells were observed (data not shown). In mice transplanted with Lovo cells, with a high EGFR expression level, marked tumor growth inhibition was achieved with gefitinib+CPT-11 (Fig. 3*f*). The T/C of the combination schedule at day 11 was 22.8% and significantly lower than in the control ($p<0.0012$ by Fisher's PSLD, Fig. 3*g*). A 10% maximum body weight loss until day 15 was also observed in mice treated with the combination (Fig. 3*j*).

Alternatively, the combined effect of oral administration of gefitinib and intravenous administration of CPT-11 was evaluated in mice transplanted with Lovo cells. Gefitinib, 30 or 60 mg/kg *p.o.* days 1–14, and CPT-11, 16.7 or 33.3 mg/kg *i.v.* days 1, 5 and 9, were administered (schedule 2, Fig. 4*a*), and greater growth inhibition was observed in mice treated with this combination, compared to the controls (Fig. 4*b*) A more marked growth-inhibitory effect was observed at a higher dose of CPT-11 (16.7 *vs.* 33.3 mg/kg), but there was no difference between 30 mg/kg and 60 mg/kg of gefitinib in the combination. The combination of gefitinib (30 and 60 mg/kg) and CPT-11 (33.3 mg/kg/*i.v.*) resulted in tumor reduction during treatment that was significant at day 15 (Fig. 4*c*). The T/C values imme-

TABLE 1 – *IN VITRO* GROWTH-INHIBITORY ACTIVITY OF GEFITINIB AND CPT-11 IN HUMAN COLORECTAL CANCER CELLS (MTT ASSAY)[1]

Cell line	gefitinib		CPT-11	
	IC_{50} (μM)	Concentration range (μM)	IC_{50} (μM)	Concentration range (μM)
WiDR	10 ± 1.1	0.83–53	33 ± 7.5	1.6–160
LS-174T	100.4 ± 10.1	N.D.	13	N.D.
COLO320DM	11 ± 3.8	0.63–100	11 ± 0.6	1.6–160
COLO320HSR	22	N.D.	5.5	N.D.
HCT116	177.0 ± 12.2	N.D.	11	N.D.
SW480	23 ± 0.6	1.6–10	35 ± 5.5	1.6–50
Lovo	1.2 ± 0.59	0.31–25	5.2 ± 0.82	0.16–10

[1]The IC_{50} value (μM) of each drug was measured by MTT assay, as described in the Materials and Methods. Each value is a mean ± SD of 3 or 4 independent experiments–N.D., not determined.

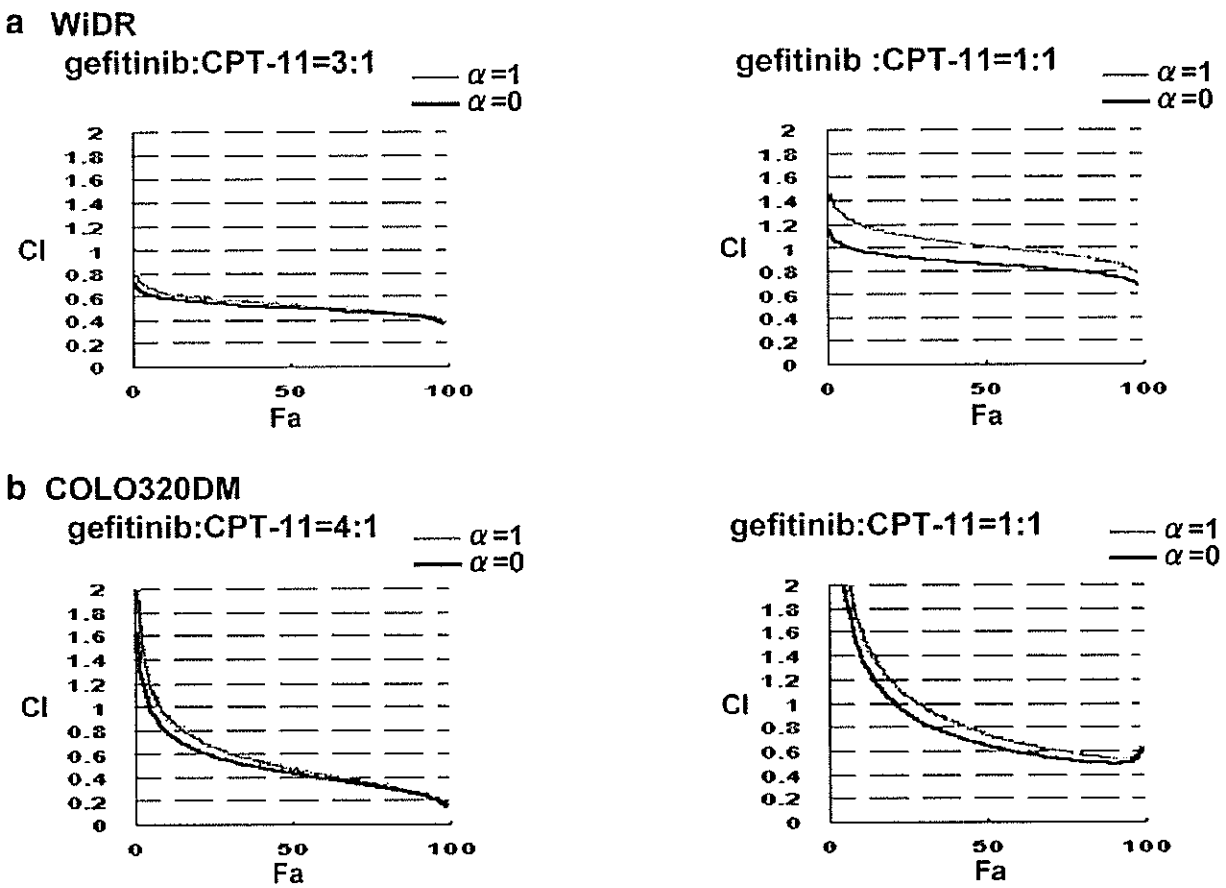


FIGURE 2 – Combination index (CI) plots of interactions between gefitinib and CPT-11. Cells were treated with gefitinib and CPT-11 alone and in combination at fixed molar ratios (molar ratios of gefitinib to CPT-11 of 3:1 and 1:1 [(*a*) WiDR], 4:1 and 1:1 [(*b*) COLO320DM], 1:2 and 1:5 [(*c*) Lovo], 1:1 [(d) SW480]. Using the mutually exclusive (CI) or mutually nonexclusive (CI′) isobologram equation, the affected fraction (F_a)-CI plot for each cell was constructed by computer analysis of the data generated from the median effect analysis. CI values <1 occurred over a wide range of inhibition levels, indicating synergy.

diately after the completion of treatment (at day 15) and at day 22 are summarized in Fig.4*d*. More severe body weight loss was observed, ~20% at day 15, in mice treated with 60 mg/kg of gefitinib alone or with CPT-11, suggesting that CPT-11 does not enhance the body weight loss induced by gefitinib. Body weight recovered by day 22 (Fig. 4*e*). No deaths of were observed during the treatment or observation period.

Induction of EGFR phosphorylation and enhanced gefitinib-induced PARP activation by CPT-11

To elucidate the synergistic effects of CPT-11 and gefitinib, we examined the effect of exposure of CPT-11 on EGFR phosphorylation in Lovo and WiDr cells. Phosphorylated EGFR was detected with anti-phosphotyrosine antibody (PY-20)

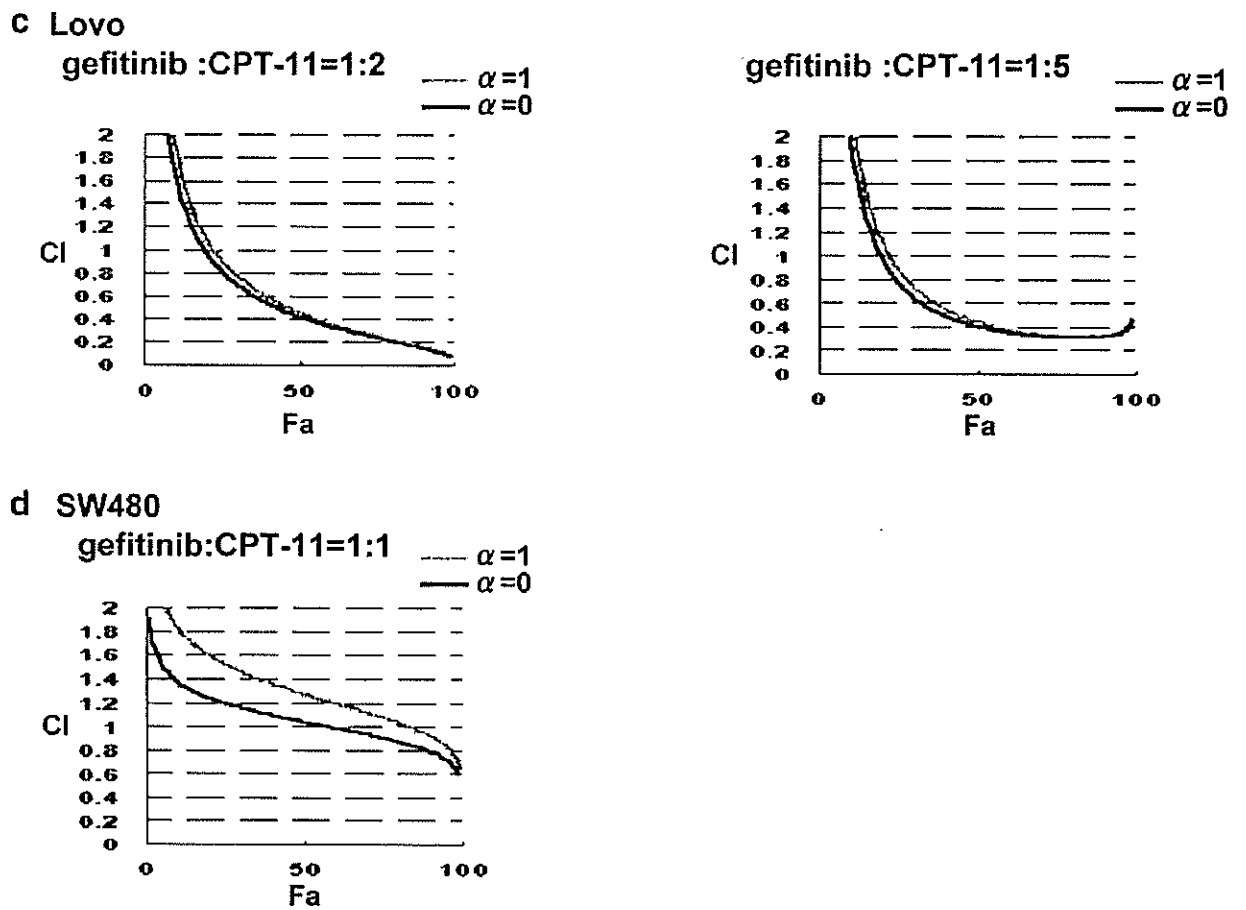


FIGURE 2 – CONTINUED.

against immunoprecipitated EGFR and increased phosphorylation of EGFR was observed after exposure to CPT-11 in Lovo cells in dose- and time- dependent manner (3–24 hr) (Fig. 5*a*). The dose-dependent activation of EGFR by CPT-11 was also obtained in WiDR cells (Fig. 5*b*). CPT-11-induced phosphorylation of EGFR was observed without ligand-stimulation. The EGFR activation was completely inhibited by 24 hd exposure of 5 μM gefitinib. gefitinib-induced apoptosis measured by PARP activation was enhanced by combination with CPT-11, although no PARP activation was induced by CPT-11 alone (Fig. 5*c*). These results suggest that the modification of EGFR by CPT-11 increases the cellular sensitivity to gefitinib, resulting the synergistic effect of CPT-11 and gefitinib. We also observed the effect of gefitinib on the expression and the activity of topoisomerase I by immunoblotting and decatenatnion assay. No modification of topoisomerase I by gefitinib was observed (data not shown).

DISCUSSION

Evidence has suggested that the new EGFR-targeting drug gefitinib is active against gastrointestinal malignancies as well as non-small cell lung cancer. Combination of gefitinib with cytotoxic drugs has been evaluated in the U.S. and Europe,[19,20] but combination with CPT-11 has not been evaluated. CPT-11 is a potent DNA-targeting drug in patients with colorectal cancer that is refractory to treatment with fluorouracil and leucovorin,[4,5] although a higher rate of treatment-induced toxicity was suspected in a retrospective analysis.[7] In preclinical study, Ciadiello *et al.*[17,18] reported that supra-additive combination effect of EGFR-targeting drug (cetuximab or gefitinib) and topoisomerase I inhibitor, topotecan was observed in human colorectal cancer GEO xenograft. We have therefore studied the synergistic potential for a new combination regimen containing CPT-11 and gefitinib. The synergistic potential of CPT-11 combined with gefitinib demonstrated in our study suggests that the gefitinib/CPT-11 combination is a promising regimen for colorectal cancer patients. Schedule 2, administration of oral gefitinib and intravenous CPT-11 designed in a xenograft model, was based on possible clinical administration of the drugs, and thus a treatment schedule consisting of intermittent *i.v.* CPT-11 and continuous gefitinib *p.o.* may be applicable to colorectal cancer in humans.

In xenograft models, body weight loss was observed when administered in combination as well as when each drug was administered alone. However, body weight loss rapidly recovered immediately after the completion of administration, and no deaths were observed. Diarrhea is the dose-limiting toxicity of CPT-11 in humans,[7] and it is also observed in patients treated with gefitinib.[21,22] However, no diarrhea or related phenomena were observed in the mouse model treated with combinations of these

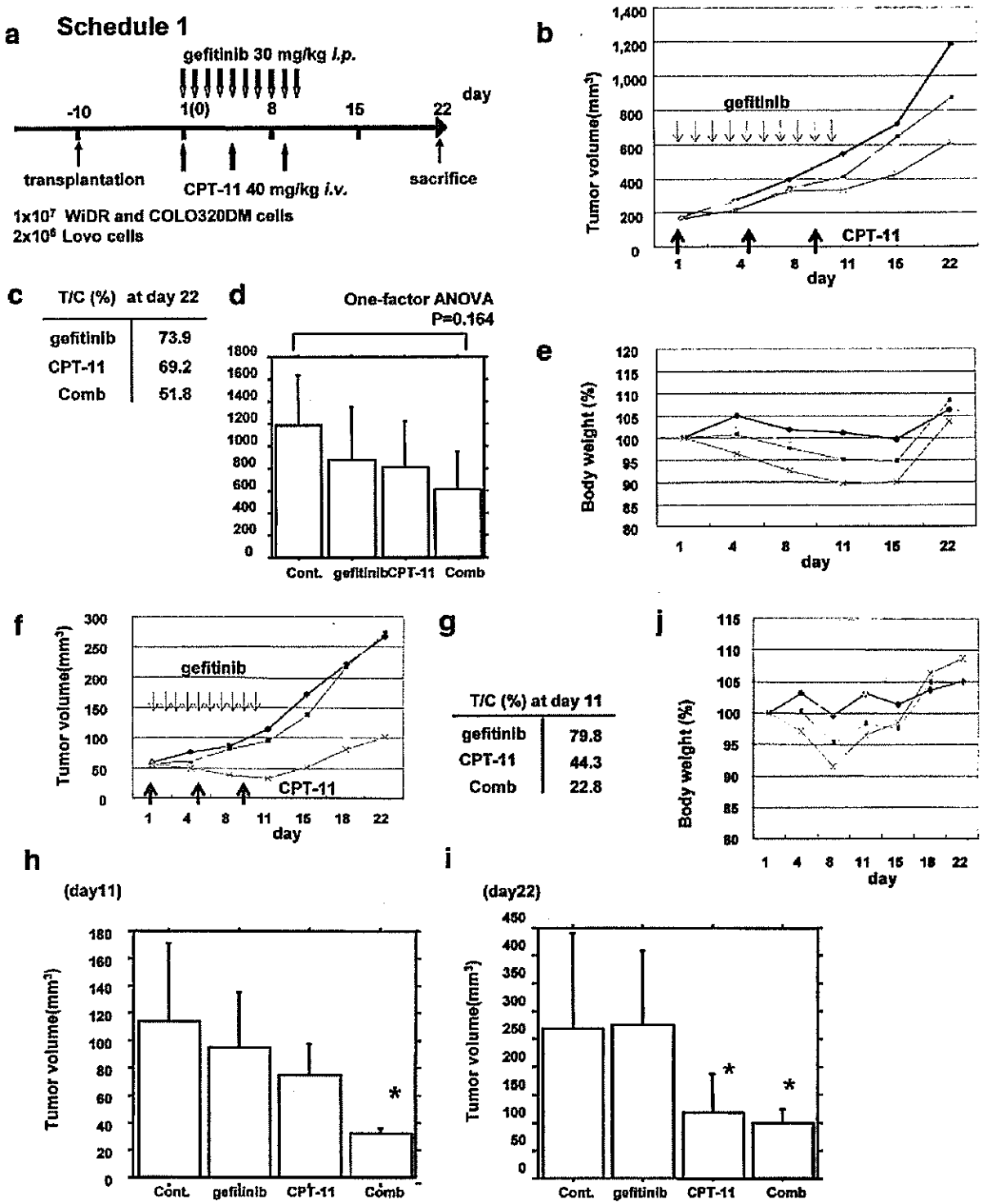
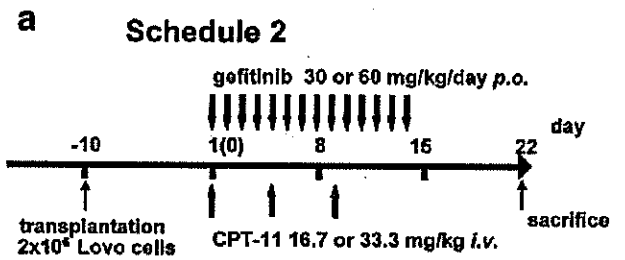
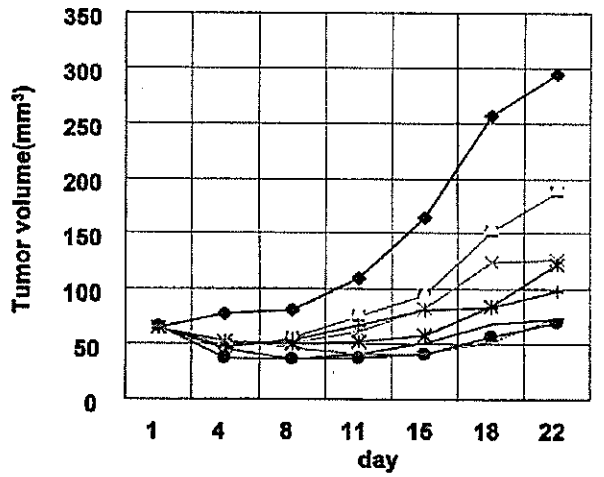
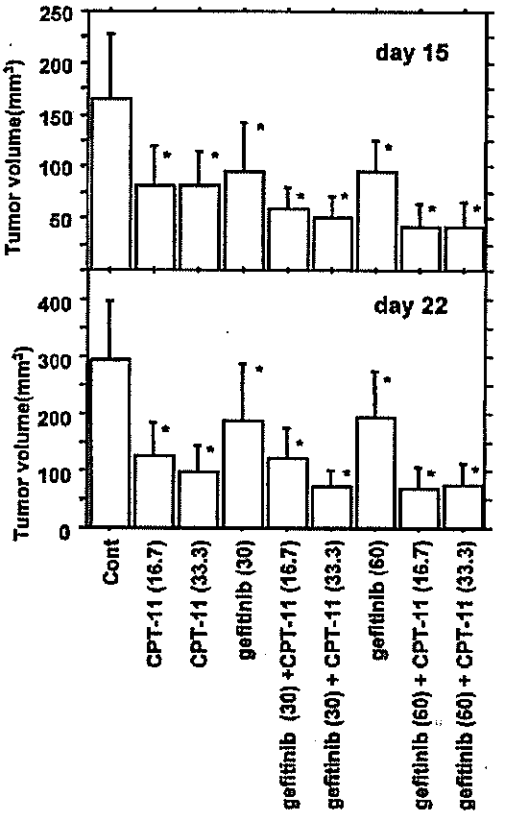


FIGURE 3 – *In vivo* combined effect of gefitinib and CPT-11 on WiDR and Lovo tumor xenografts. (*a*) Treatment schedule. (*b*) (WiDR) and F (Lovo), Tumor growth curves. Female nude mice bearing WiDR or Lovo xenografts were randomly allocated to treatment with 5% (w/v) glucose solution (diamond), gefitinib (square), CPT-11 (triangle), or the combination (×). Tumor volume was calculated as described in Material and Methods. Each data point represents the mean tumor volume of 5 mice. E (WiDR) and J (Lovo) Percent change in body weight in the gefitinib (hatched square) and combination (×) group. C (WiDR) and G (Lovo) Ratio of tumor volume in the control (C) to tumor volume in the treatment group (T) at day 22 and day 15. D (WiDR), H and I (Lovo) Histogram of mean tumor volume at day 11 and day 22 bars, *S.D.* Statistical analysis was performed by 1-factor ANOVA, followed by Fisher's PLSD between 2 groups, as described in the Material and Methods section. *Significant difference ($p<0.05$; Fisher's PLSD) compared to the control.







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	T/C (%) at day 15
gefitinib 30	57.6
gefitinib 60	58.4
CPT-11 16.7	49.6
gefitinib 30+CPT-11 16.7	35.4
gefitinib 60+CPT-11 16.6	25.1
CPT-11 33.3	49.3
gefitinib 30+CPT-11 33.3	30.9
gefitinib 60+CPT-11 33.3	25.3

	T/C (%) at day 22
gefitinib 30	63.7
gefitinib 60	65.7
CPT-11 16.7	43.0
gefitinib 30+CPT-11 16.7	41.6
gefitinib 60+CPT-11 16.6	23.6
CPT-11 33.3	33.2
gefitinib 30+CPT-11 33.3	24.9
gefitinib 60+CPT-11 33.3	25.3

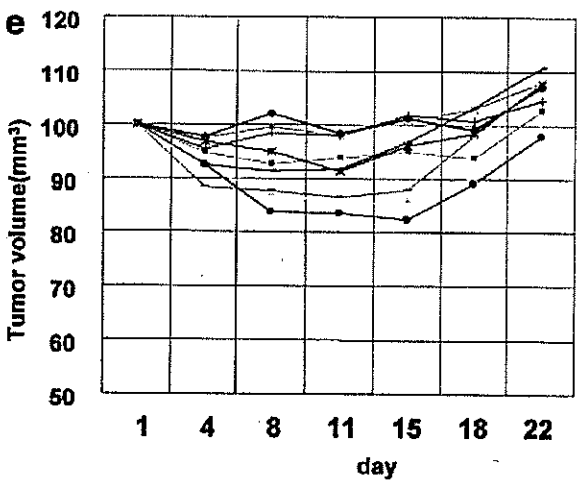

FIGURE 4 – The dose-dependent effect of combination therapy on Lovo cells *in vivo*. (*a*) Treatment schedule. (*b*) Significant growth-inhibition was observed in mice treated with the combination. Mice were allocated to 9 groups (6 mice/group) [closed diamond, 5%(W/V) glucose solution; ×, CPT-11 16.7 mg/kg; + CPT-11 33.3 mg/kg; square, gefitinib 30 mg/kg; star, gefitinib 30 mg/kg + CPT-11 16.7 mg/kg; blue line, gefitinib 30 mg/kg + CPT-11 33.3 mg/kg; open triangle, gefitinib 60 mg/kg; circle, gefitinib 60 mg/kg + CPT-11 16.7 mg/kg; light blue line, filled square, gefitinib 60 mg/kg + CPT-11 33.3 mg/kg]. (*c*) Mean tumor volumes and results of the statistical analysis at days 15 and 22, bars, *S.D.* *Significant difference ($p<0.05$) compared to the control. (*d*) T/C(%) at day 15 and 22. (*e*) Treatment-related body weight loss occurred in mice treated with gefitinib 60 mg/kg (triangle, circle, and light blue line).