The response rate was 94.4% and the MST was 11.1 months, encouraging result. Because of patient selections, it is difficult to make wholly valid conclusions about the most effective regimen based only on Phase II results. However, three-drug combinations containing irinotecan with G-CSF support may have better survival and feasibility than the doublets. The disadvantage is that triplet regimens require G-CSF support, which may make out-patient treatment difficult.

4. Irinotecan containing regimen for LD SCLC

Two meta-analyses showed that the addition of TRT to chemotherapy in patients with LD SCLC improves survival at two and three years by 5.4% [50,51]. In these meta-analyses, non-platinum-based combination chemotherapies were commonly used, with only a few trials using platinum-based chemotherapy. Cisplatin and etoposide plus TRT is now widely regarded as the standard regimen for LD SCLC, and presents acceptable toxicity [52]. Turrisi *et al.* reported results of once-daily versus twice-daily (b.i.d) TRT with four cycles of cisplatin and etoposide. Results showed that the MST was significantly superior in the b.i.d arm (23 versus 19 months) [53].

Irinotecan showed potent radiosensitising effects in human lung tumour xenografts which were related to the cell cycle [54]. Kubota *et al.* reported a pilot study of concurrent etoposide and cisplatin plus accelerated hyperfractionated TRT followed by irinotecan and cisplatin for LD SCLC (JCOG9903) [21]. Treatment consisted of etoposide 100 mg/m^2 on days 1 – 3, cisplatin 80 mg/m^2 on day 1, and concurrent b.i.d TRT of 45 Gy beginning on day 2. The IP regimen started on day 29 and consisted of irinotecan 60 mg/m^2, days 1, 8, 15 and cisplatin 60 mg/m^2 on day 1, with three 28-day cycles. A total of 31 patients were accrued. Although a pilot study, the MST was 20.2 months and 1-, 2- and 3-year survival rates were 76%, 41%, and 38%, respectively. This encouraging regimen proved safe with acceptable toxicities. A randomised Phase III trial comparing EP with IP following EP plus concurrent TRT for LD SCLC is now underway (JCOG0202).

The WJTOG also conducted a similar regimen [55]. Treatment included cisplatin 80 mg/m^2 on day 1 and etoposide 100 mg/m^2 on days 1 – 3 with concurrent TRT (1.5 Gy/b.i.d, a total dose of 45 Gy) followed by 3 cycles irinotecan 60 mg/m^2 on days 1, 8 and 15 and cisplatin 60 mg/m^2. The results of 51 patients were almost identical to JCOG9903; overall response and CR rate was 87.8% and 40.8%, respectively; Grade 4 toxicity included neutropenia (83.7%), anaemia (10.2%), thrombocytopenia (0%), diarrhoea (2%) and infection (2%); the MST was 21.5 months and 2-year survival rate was 45.7%.

A Phase II study of IP induction followed by concurrent b.i.d TRT with EP chemotherapy for LD SCLC was conducted [56] and also showed encouraging results. Treatment consisted of two cycles of cisplatin 40 mg/m^2 and irinotecan 80 mg/m^2 on days 1 and 8 of a 3-week cycle. This was followed by two 3-week cycles of cisplatin 60 mg/m^2 on days 43 and 64, and etoposide 100 mg/m^2 on days 43 – 45 and 64 – 66, with concurrent b.i.d TRT total of 45 Gy beginning on day 43. Thirty-five patients were accrued. The MST was 25 months (but it should be noted that this is a single institution Phase II study).

In these studies, irinotecan was used on an induction or adjuvant setting, and both regimens were encouraging. However, randomised study in which both modalities are compared has not been conducted.

There have been a few trials of concurrent chemoradiotherapy including irinotecan for patients with SCLC as well as NSCLC. Recently, a combined modality treatment of IC and TRT followed by bevacizumab (antiangiogenic anti-VEGF antibody) in the treatment of LD SCLC was conducted in a Phase II trial by the Minnie Pearl Cancer Research Network [57]. Induction therapy consisted of irinotecan 50 mg/m^2 on days 1 and 8, carboplatin AUC = 5 on day1, TRT 1.8 Gy single daily dose to total dose of 61.2 Gy (34 fractions), beginning with the 3rd cycle. Chemotherapy was repeated every three weeks for four cycles. As a maintenance therapy, bevacizumab 10 mg/kg i.v. every 2 weeks was given until disease progression, or a maximun of 10 doses (20 weeks) were administered. The response rate was 81% with 28% CR. This regimen was well tolerated with rare grade 4 toxicity and no treatment-related deaths. One-year progression-free and overall survival were 68% and 71%, respectively. These results suggest that irinotecan can be safely administered with TRT concurrently.

Sohn *et al.* also reported a Phase II study of IP with concurrent TRT in LD SCLC [58]. Chemotherapy of irinotecan 60 mg/m^2 on days 1, 8 and 15 and cisplatin 40 mg/m^2 on days 1 and 8 were repeated every 4 weeks until a maximum of 6 cycles. TRT of 2 Gy/day was commenced on day 1 of the second chemotherapy cycle up to a total of 54 Gy. The results are not concluded at this time.

Langer *et al.* reported a Phase I study of IP and either b.i.d TRT (45 Gy) or once daily RT (70 Gy) to determine if irinotecan can be safely integrated with concurrent TRT and cisplatin in LD SCLC [59]. Acute DLT was defined as grade 4 oesophagitis, pneumonitis, or diarrhoea; grade 4 neutropenic fever; or any attributable grade 5 fatal toxicity (≤ 90 days after RT). Although preliminary, there has been no attributable DLT in the 26 patients that have been enrolled. In combination with cisplatin 60 mg/m^2 every 3 weeks x 4 and either b.i.d TRT or once daily TRT, irinotecan 40 mg/m^2 on days 1 and 8 was safe and feasible. Irinotecan at 50 mg/m^2 on days 1 and 8 every 3 weeks x4 was also feasible in combination with cisplatin and b.i.d TRT. These reports allow us to conclude that irinotecan can be administered with radiotherapy sequentially or concurrently.

5. Expert opinion and conclusion

Irinotecan is effective against SCLC. For the treatment of ED SCLC, IP regimen is at least comparable to EP regimen.

The degree of myelosuppression in the IP regimens was less than that of the EP regimens. However, diarrhoea was more often observed in the IP than the EP regimens, and can lead to severe side effects when the IP regimen is used incautiously. Pharmacogenetic study of irinotecan may prompt one to use the drug in a safer way to avoid severe toxicities. McLeod suggests that at the least, irinotecan 300 – 350 mg/m^2 every 3 weeks should not be given to patients with a known UGT1A1*28 genotype until more definitive guidelines are established [60]. However, the use of UGT1A1*28 genotyping to predict toxicity is controversial and its clinical implications are unclear. Furthermore, whether or not these recommendations are also applicable to patients with SCLC should be elucidated upon because a lower dose of irinotecan is usually administered weekly for the treatment of SCLC, rather than the every 3 or 4 weeks for colorectal cancers. In the coming decade, we must confront the metabolic and pharmacogenomic differences in various populations for the treatment of cancer. For this, international cooperative studies are warranted and indeed of immense importance.

Considering patient safety, irinotecan can indeed be administered relatively safety in patients with SCLC, provided there is careful monitoring of patients, especially regarding diarrhoea and myelosuppresion. Further studies to avoid severe toxicities are needed to advance the safe use of this otherwise promising drug.

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EGFR and ErbB2 mutation status in Japanese lung cancer patients

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Much evidence has accumulated that the epidermal growth factor receptor *(EGFR)* and its family members are strongly implicated in the development and progression of lung cancers. Somatic mutations of the *EGFR* gene were found in about 25–40% of Japanese lung cancer patients. More recently, *erbB2* mutations are found in about 4% of European-derived lung cancer patients. We have investigated *EGFR* and *erbB2* mutation status in 95 surgically treated nonsmall cell lung cancer (NSCLC) cases from Nagoya City University Hospital. Seventy-five adenocarcinoma cases were included. The presence or absence of *EGFR* and *ernB2* mutations of kinase domains were analyzed by reverse transcription polymerase chain reaction (RT-PCR) amplifications and direct sequences. We have also investigated *erbB2* mutation status in 27 surgically treated NSCLC cases followed by treatment with gefitinib from Kinki-chuo Chest Medical Center. *EGFR* mutations (CTG→CGG; L858R) were found from 14 of 95 lung cancer patients. We also detected the deletion 1a-type mutations from 9 patients and deletion 4-type mutations from 6 patients in exon 19. In exon 20, 4 mutations including 2 novel mutations were found. Total *EGFR* mutations were present in 35 patients (36.8%). These mutation statuses were significantly correlated with gender (women 73.3% *vs.* men 20%, $p < 0.0001$), smoking status (never smoker 69.4% *vs.* smoker 16.9%, $p < 0.0001$), pathologic subtypes (adenocarcinoma 45.1% *vs.* nonadenocarcinoma 12.5%, $p = 0.0089$) and differentiation status of the lung cancers (well 51% *vs.* moderately or poorly 18.4%, $p = 0.0021$). On the other hand, *erbB2* mutation was only found from 1 of 95 patients, at exon 20. This patient was female and a never smoker with adenocarcinoma. This 12 nucleotide insertion mutation (2324–2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775–776 ins YVMA). There was no *erbB2* mutation in 27 gefitinib-treated NSCLC patients. In total, we have found only 1 *erbB2* mutation from 122 (0.8%) Japanese NSCLC patients. There was a significantly higher erbB2 positive (2+/3+) ratio in *EGFR* mutant patients (13/25, 52.0%) compared to *EGFR* wild-type patients (10/62, 16.1%; $p = 0.0247$). The NSCLC specimen with *erbB2* mutation showed 1+ immunoreactivity. The *EGFR* mutation status might correlate with the clinicopathologic features related to good response to gefitinib, such as gender, smoking history and pathologic subtypes of lung cancers. However, *erbB2* mutation is rare from Japanese lung cancer and is of limited value for molecular target therapy.

Key words: EGFR; lung cancer; mutations; erbB2; Japanese

Lung cancer is a major cause of death from malignant diseases, due to its high incidence, malignant behavior and lack of major advancements in treatment strategy.[1] Lung cancer was the leading indication for respiratory surgery (42.2%) in 1998 in Japan.[2] More than 15,000 patients underwent surgical operations at Japanese institutions in 1998.[2] The clinical behavior of the lung cancer is largely associated with its stage. The cure of the disease by surgery is only achieved in cases representing an early stage of lung cancer.[3]

There is much accumulated evidence that epidermal growth factor receptor *(EGFR)* and its family members are strongly implicated in the development and progression of numerous human tumors, including lung cancer.[4,5] The erbB family comprises 4 structurally related receptors: ErbB1 (EGFR), ErbB2 (HER2-neu), ErbB3 and ErbB4. On ligand stimulation, the receptor forms either homodimers or heterodimers, which activate their cytoplasmic domain. This tyrosine-auto-phosphorylated region functions as a docking site for messenger proteins, which initiate cascades of cytoplasmic and nuclear mitogenic pathways.[6] Inhibition of this pathway is facilitated by several newly developed compounds that have shown promising results in preclinical and clinical trials.[7] The *EGFR* tyrosine kinase inhibitor, gefitinib, was approved in Japan for the treatment of nonsmall cell lung cancer (NSCLC) since 2002. Trastuzumab is a recombinant DNA-derived monoclonal antibody that selectively binds to p185 HER2, the protein product of *erbB2*. Trastuzumab was approved for breast cancer[8] and clinical trials for NSCLC is underway.[9,10]

Recently, we have found that novel *EGFR* mutations' status at ATP binding pockets in Japanese NSCLC patients were correlated with the clinicopathologic features related to good response to gefitinib.[11] These *EGFR* mutations are predominantly found in Japanese lung cancer patients (about 25%) when compared to USA patients (about 8%[12–14] to 10%[15]). Kasaoka *et al.* have reported that the *EGFR* mutation ratio is 40% of Japanese lung cancer patients.[16] Actually, *EGFR* mutations in lung cancer have been correlated with clinical response to gefitinib therapy *in vivo* and *in vitro*.[11–13] More recently, it has been reported that novel *erbB2* mutations at kinase domain were found in 4% of European-derived NSCLC patients.[17]

To determine the *EGFR* and *erbB2* mutation status in Japanese lung carcinoma for screening purposes, we investigated *EGFR* and *erbB2* mutation status by the RT-PCR amplifications and direct sequences. The findings were compared to the clinicopathologic features of lung cancer.

Material and methods

Study subjects

The study group included 95 lung cancer patients who had undergone surgery (but did not receive gefitinib) at the Department of Surgery II, Nagoya City University Medical School between 1997 and 2002. We have also investigated erbB2 mutation status for 27 NSCLC patients who had undergone surgery followed by treatment with gefitinib at the National Hospital Organization, Kinki-chuo Chest Medical Center. The lung tumors were classified according to the general rule for clinical and pathologic record of lung cancer in Japan.[18] All tumor samples were immediately frozen and stored at −80°C until assayed.

The clinical and pathologic characteristics of the 95 lung cancer patients are as follows: 52 cases at stage I, 9 at stage II and 34 at stage III–IV. The mean age was 64.9 years (range, 42–82). Among the 95 lung cancer patients, 71 (74.7%) were diagnosed as adeno-

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carcinoma, 17 (17.9%) were squamous cell carcinoma and 4 (4.2%) were adenosquamous cell carcinoma. The samples from these patients had never been sequenced for *EGFR* before.

PCR assays for EGFR and erbB2

Total RNA was extracted from lung cancer tissues and adjacent nonmalignant lung tissues using Isogen kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. RNA concentration was determined by spectrophotometer and adjusted to a concentration of 200 ng/ml. About 10 cases were excluded because tumor cells were too few to sufficiently extract tumor RNA. RNA (1 μg) was reverse transcribed by Superscript II enzyme (Gibco BRL, Gaithersburg, MD) with 0.5 μg oligo $(dT)_{12\text{-}16}$ (Amersham Pharmacia Biotech, Piscataway, NJ). The reaction mixture was incubated at 42°C for 50 min and then at 72°C for 15 min. We then used 1 μl of each DNA for PCR analyses. The PCR reactions were performed using LA-Taq kit (Takara Bio, Shiga, Japan) in a 25 μl reaction volume. The primer sequences for *EGFR* gene for kinase domain (exons 18–21) were as follows: the forward primer, 5-CTCTTACACCCAGTGGAGAA-3 and the reverse primer, 5-CATCCACTTGATAGGCACTT-3 (572 bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 40 sec, 60°C for 40 sec, 72°C for 45 sec The primer sequences for *erbB2* gene for kinase domain (exons 19–22) were as follows: the forward primer, 5-CGCTTTTGGCACAGTCTACA-3 and the reverse primer, 5-GGGATCCCATCGTAAGGTTT-3 (594bp). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 40 sec, 60°C for 40 sec, 72°C for 45 sec. The products were purified by Qiagen PCR purification kit (Qiagen, Valencia, CA). Amplified cDNAs were separated on 1% agarose gels, and the bands were visualized by ethidium bromide and photographed under ultraviolet transillumination. These samples were sequenced by ABI prism 3100 analyzer (Applied Biosystems Japan, Tokyo, Japan) and analyzed by BLAST and chromatograms by manual review.

Immunohistochemistry

Tissue blocks were cut into 4 mm sections and mounted on silane-coated slides. The slides were then deparaffinized in xylene, dehydrated in a grade alcohol series and blocked for endogenous peroxidase with 3% H_2O_2 in absolute methanol. After microwave pretreatment in Blockace solution, immunostaining was done at 4°C overnight with a rabbit polyclonal c-erbB2 oncoprotein antibody (A04085, DakoCytomation, Glostrup, Denmark) at a 1:200 dilution. The expression of erbB2 was scored as follows: −, no discernible staining or <10% of cell stained; 1+, >10% of cytoplasmic staining or plasma membrane staining with weak intensity; 2+, >10% of plasma membrane staining with moderate intensity; and 3+, >10% of plasma membrane staining with strong intensity.

Statistical methods

Statistical analyses were done using the Mann-Whitney U-test for unpaired samples and Wilcoxon's signed rank test for paired samples. Linear relationships between variables were determined by means of simple linear regression. Correlation coefficients were determined by rank correlation using Spearman's test and χ^2test. The overall survival of lung cancer patients was examined by the Kaplan-Meier methods, and differences were examined by the log-rank test. All analysis was done using the Stat-View software package (Abacus Concepts, Berkeley, CA) and was considered significant when the p-value was less than 0.05.

Results

EGFR gene mutation status in Japanese lung cancer patients

Using the primer sets for *EGFR* kinase domain, a PCR product of 572 bp was obtained. When we visualized the PCR products with 1% agarose gel, these samples were further studied. In exon 18, there was no G719S mutation found from this study. In exon 19, 9 patients had the del 1a type mutation, 6 patients had the deletion 4 type mutation and 1 patient had the del 1b type mutation. Seven were male and 10 were female. Thirteen were nonsmokers and 4 were smokers. Fifteen patients had adenocarcinoma, 1 had squamous cell carcinoma and 1 had adenosquamous cell carcinoma. Three of the tumors were moderately differentiated, 2 were poorly differentiated and 11 were well differentiated. Five of 15 adenocarcinomas showed bronchioloalveolar carcinoma (BAC) pattern at the edge of tumor. Thus *EGFR* mutation status at exon 19 was significantly correlated with gender ($p = 0.0172$) and tobacco-smoking ($p = 0.0008$) but not with pathologic stages (stage I *vs.* II–IV, $p = 0.9144$), subtypes (adenocarcinoma *vs.* nonadenocarcinoma, $p = 0.2675$) and differentiation of lung cancer (well *vs.* moderately or poorly differentiated, $p = 0.3812$).

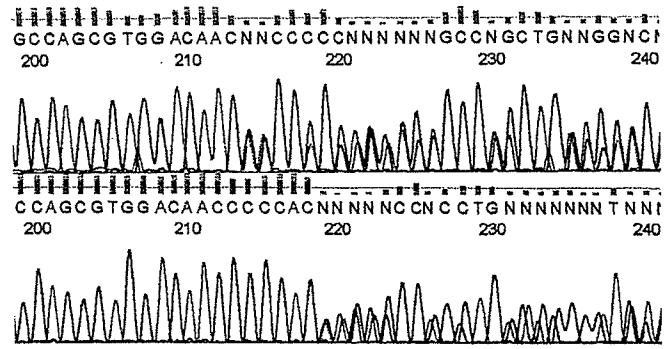

FIGURE 1 – Novel EGFR mutation at exon 20. Top: a male well-differentiated adenocarcinoma patient had the novel 2312–2313 insertion CAA. Bottom: a female, well-differentiated adenocarcinoma patient had the novel 2319–2320 insertion AACCCCCAC.

In exon 20, 3 patients had theheterozygous in-frame insertion mutations. Two were male and 1 was female. All 3 were smokers. A female, well-differentiated adenocarcinoma patient had the novel 2319-2320 insertion AACCCCCAC. A male well-differentiated adenocarcinoma patient had the novel 2312-2313 insertion CAA (Fig. 1). We have found one point mutation, C2369T (T790M). This patient also has the predominant L858R mutation (Fig. 2).

For exon 21, 14 patients had the L858R mutation and 1 patient had the L861Q mutation. Four were male and 11 were female. Twelve were nonsmokers and 3 were smokers. All 15 patients had adenocarcinoma, 1 was moderately differentiated and 14 were well differentiated. Six of 15 adenocarcinomas exhibited the BAC pattern at the edge of the tumor. Thus, exon 21 mutation status was significantly correlated with gender ($p = 0.0005$), smoking status ($p = 0.0007$), pathologic stages ($p = 0.0152$), the pathologic subtypes ($p = 0.0329$) and differentiation of lung cancer ($p = 0.0033$).

The mutations detected in lung cancer specimens from 95 lung cancer patients are summarized in Table I. Taken together, 36 mutations were found from 35 lung cancer samples in our analysis. Total *EGFR* mutations were present in 35 patients (36.8%). These mutation statuses were significantly correlated with gender (women 73.3% *vs.* men 20%, $p < 0.0001$), smoking status (never smoker 69.4% *vs.* smoker 16.9%, $p < 0.0001$), pathologic subtypes (adenocarcinoma 45.1% *vs.* nonadenocarcinoma 12.5%, $p = 0.0089$) and differentiation status of the lung cancers (well 51% *vs.* moderately or poorly 18.4%, $p = 0.0021$).

The overall survival of 95 lung cancer patients from Nagoya City University, with follow-up through December 30, 2003, was studied in reference to the *EGFR* mutation status. The patient with the mutation in the *EGFR* gene ($n = 35$, 4 were dead) had a significantly better prognosis than the patient with wild-type *EGFR* ($n = 60$, 20 were dead; log-rank test $p = 0.0143$, Breslow-Gehan-Wilcoxon test $p = 0.0220$), although the observation period was short (Fig. 3). But a multivariate analysis revealed that pathologic

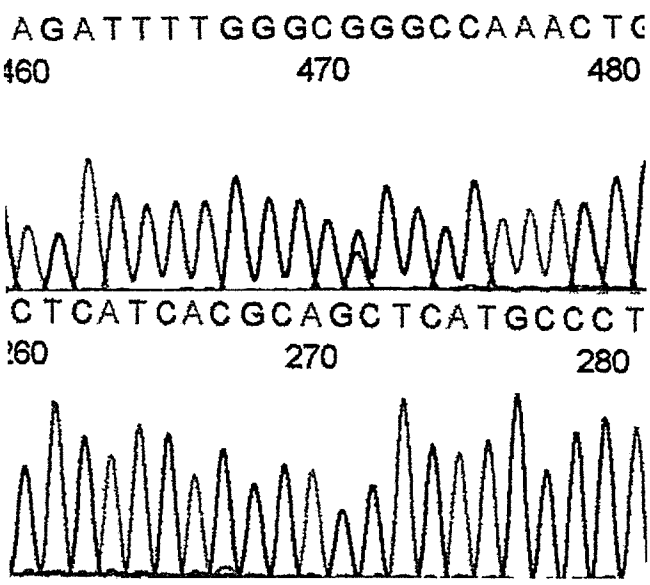

FIGURE 2 – The premoninant L858R (2573 T to G) mutation in exon 21 (top) and T790M (2369 C to T) mutation at exon 20 (bottom) within the EGFR kinase domain.

TABLE I – CLINICOPATHOLOGIC DATA OF 95 LUNG CANCER PATIENTS

Factors	*EGFR* gene status		
	Mutation patients	Wild-type patients	*p*-value
Mean age (years)			
64.9 ± 9.0	35	60	
Stage			
I	25 (72.4%)	27 (45.8%)	0.0274
II–IV	10 (28.6%)	32 (54.2%)	
Lymph node metastasis			
N0	8 (22.9%)	21 (35.0%)	0.3119
N+	27 (77.1%)	39 (65.0%)	
BI			
Never smoker	25 (71.4%)	11 (34.0%)	0.001
Smoker	10 (28.6%)	49 (66.0%)	
Differentiation			
Well	26 (78.8%)	23 (42.6%)	0.0021
Moderately or poorly	7 (21.2%)	31 (57.4%)	
Pathologic subtypes			
Adeno	32 (91.4%)	39 (74.7%)	0.0089
Nonadeno	3 (8.6%)	21 (25.3%)	
Age			
≤ 65	19 (54.3%)	29 (48.3%)	0.7269
> 65	16 (45.7%)	31 (51.7%)	
Gender			
Male	13 (37.1%)	52 (86.7%)	< 0.0001
Female	22 (62.9%)	8 (13.3%)	

N+, lymph node metastasis positive; Adeno, adenocarcinoma.

stage (p = 0.0006) was the only significant factor but not *EGFR* mutation (p = 0.1824).

ErbB2 *gene mutation status in Japanese lung cancer patients*

We identified only one *erbB2* mutation from 95 NSCLC patients. This 12-nucleotide insertion mutation (2324-2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775-776 ins YVMA) (Fig. 4). This patient was a female non-smoker with well-differentiated adenocarcinoma, without *EGFR* mutation. Adjacent normal lung tissue exhibited a wild-type sequence for the *erbB2* gene, suggesting that this mutation was somatic. We have also done sequencing for 27 gefitinib-treated NSCLC patients. Among 27 patients, 9 patients had *EGFR* mutations (data not shown). However, no *erbB2* mutation was found within the kinase domain. Totally, we have found only 1 *erbB2* mutation from 122 (0.8%) Japanese NSCLC patients.

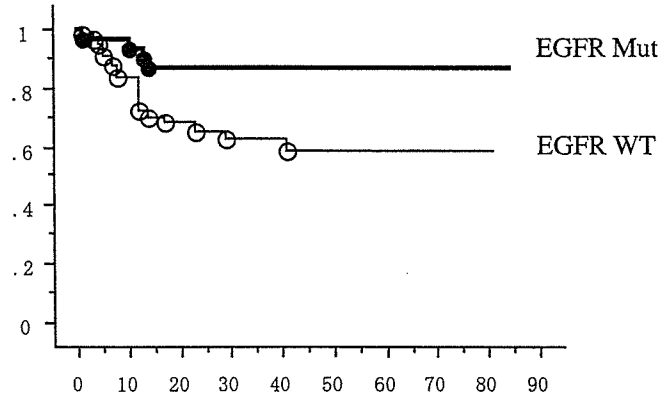

FIGURE 3 – The patient with a mutation in the *EGFR* gene (n = 35, 4 were dead) had a significantly better prognosis than the patient with wild-type *EGFR* (n = 60, 20 were dead) (log-rank test, p = 0.0143; Breslow-Gehan-Wilcoxon test, p = 0.0220), although the observation period was short.

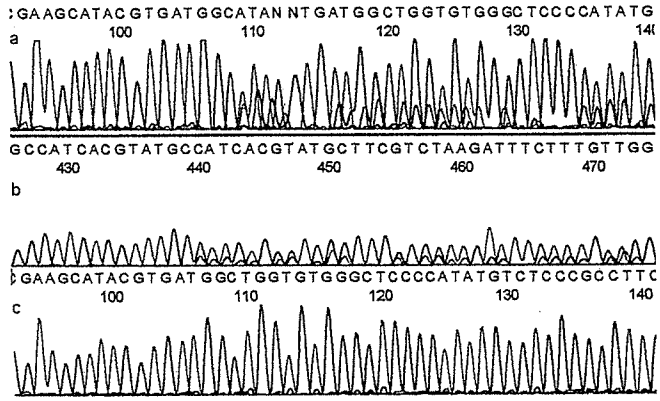

FIGURE 4 – Detection of the insertion mutation in the *erbB2* gene in genomic DNA extracted from lung cancer. *(a)* The 12 nucleotide insertion mutation (2324–2325 ins ATACGTGATGGC) was located in the exon 20 at kinase domain (775–776 ins YVMA). *(b)* Reverse sequence was performed and confirmed. *(c)* Adjacent normal lung tissue showed a wild-type sequence for the *erbB2* gene.

Immunohistochemistry

The immunohistochemical evaluation was done according to the scoring system described in Material and Methods. Immunohistochemistry was done only for 87 patients because the tissue blocks were not available for other patients. The erbB2-positive (2+/3+) ratio was 26.4% (23/87). There was a significantly higher erbB2-positive ratio in *EGFR*-mutant patients (13/25, 52.0%) compared to *EGFR* wild-type patients (10/62, 16.1%) (p = 0.0247). The patient with *erbB2* mutation exhibited 1+ immunoreactivity (Fig. 5).

Discussion

We obtained findings that *EGFR* mutation status was significantly correlated with gender and smoking history of lung cancers. This was in agreement with the recent reports that *EGFR* gene mutations are common in lung cancers from never smokers[13,14] and females with adenocarcinoma.[11,14] However, our analysis also suggested that *erbB2* mutation might be less common in Japanese NSCLC patients.

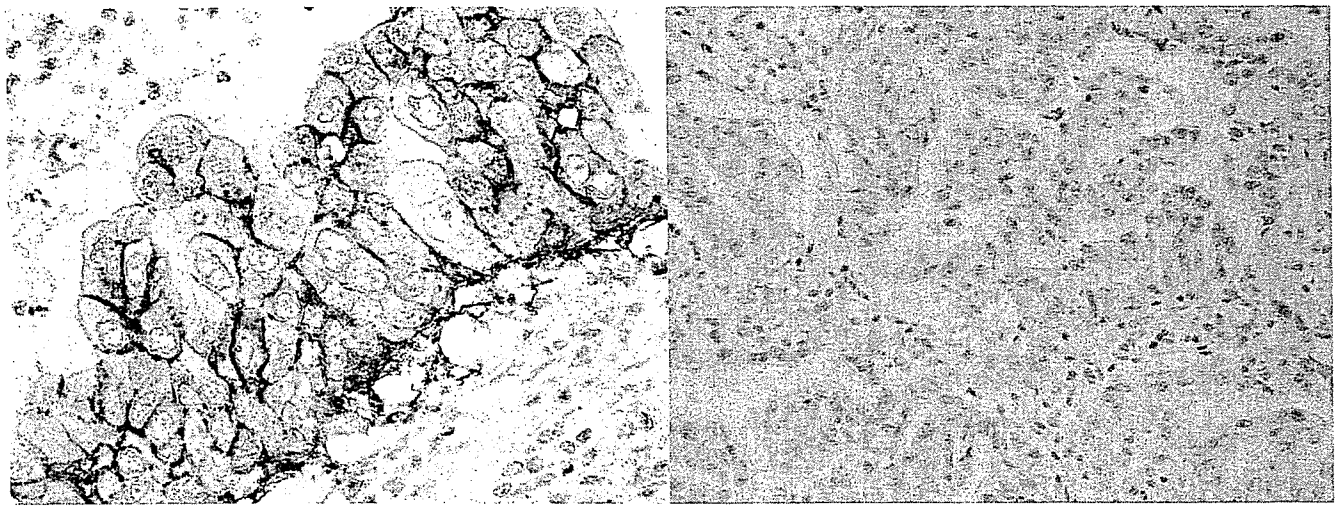

FIGURE 5 – Immunohistochemistry for ErbB2. Left: erbB2-positive (3+) section. Right: the NSCLC specimen with *erbB2* mutation exhibited 1+ immunoreactivity.

Overexpression of EGFR/ErbB2 and ErbB ligands is correlated with advanced diseases and poor patient prognosis.[19] Although *EGFR* is more abundantly expressed in lung carcinoma,[20,21] *erbB2* overexpression is less common; it is found in <35% of patients with nonsmall cell lung cancers, mainly in those with adenocarcinoma.[21] Amplification of *EGFR* and *erbB2* mRNA[22] or overexpression of their proteins[23] has been found to relate to survival in patients with NSCLC, although contradictory results have also been reported.[24,25] The drug trastuzumab, a humanized antibody against the extracellular domain of erbB2, has been approved for treatment of metastatic breast cancer and is most effective in breast cancer with *erbB2* amplification. Preliminary results suggested that the combination of chemotherapy and trastuzumab is well tolerated for NSCLC.[21] However, results from phase II trials of trastuzumab as a treatment for NSCLC have not shown any advantage for most patients[22] and have provided insufficient evidence to proceed to phase III trials.[23] Because the presence of a mutation appears to be a determination of response to therapy, as is the case with gefitinib and *EGFR* mutations, we therefore investigated the *erbB2* and *EGFR* gene mutation status. However, we have found only 1 *erbB2* mutation from 122 Japanese lung cancer patients. More recently, Shigematsu *et al.* reported that *erbB2* mutations were found in 3% (8/269) of Japanese NSCLC.[26] The single *erbB2* mutation we have found was the same as the one repeatedly found by Shigematsu *et al.*[26] Because very few NSCLC patients have gene amplification of *erbB2*, trastuzumab in the treatment of NSCLC might have a limited role.[9] Lung cancers that coexpress both EGFR and erbB2 appear to have more virulent behavior.[27] In addition, EGFR-erbB2 heterodimers are associated with a stronger and more sustained proliferative signal than EGFR homodimers.[22,28] Blockade of a signaling pathway may in theory be overcome by compensatory activation of a separate pathway in the same tumor cell. Because there was a significantly higher erbB2-positive ratio in *EGFR*-mutant patients, blockade of both may ultimately yield superior results.

Because so many *EGFR* mutation phenotypes were discovered, it would be of interest to determine whether resistance to *EGFR* inhibition emerges through secondary mutation as is the case in imatinib-treated chronic myelogenous leukemia.[29] In our analysis, a female never smoker adenocarcinoma patient had the predominant L858R mutation as well as T790M mutation. Actually, this case was untreated with EGFR kinase inhibitors. Threonine 315 to isoleucine substitution in the Abl kinase domain was a critical structural determinant controlling inhibitor sensitivity of STI571.[29] Introduction of bulkier hydrophobic side chains at the Thr-790 position fully preserved the cellular kinase activity of the *EGFR* in the presence of selective kinase inhibitors, indicating potential mechanisms of molecular resistance formation as previously found for BCR-Abl at T315I. Previous *in vitro* study showed that mutation of T790M in the *EGFR* revealed a hotspot for resistance formation against gefitinib,[30] also *in vivo*.[31]

Over the decades, the incidence of lung adenocarcinoma has increased worldwide. Most individuals with lung adenocarcinoma (especially women) are nonsmokers,[32] who are corresponding with the sensitive population to gefitinib. In Taiwan, *EGFR* mutation ratio from adenocarcinoma was also high (55%, 38 of 69), and all of the adenocarcinomas with *EGFR* mutation were well to moderately differentiated.[33] These data were compatible for our results. Because well-differentiated adenocarcinoma patients had a better prognosis,[34] *EGFR* mutant patients showed better prognosis in our univariant analysis. The reason why many mutations were especially found in Asian, female nonsmoker adenocarcinoma remains unknown. Human papilloma virus type 16/18 infections,[35] cooking oil fume,[36] nutritional status, genetic susceptibility, immunologic infection, tuberculosis and asthma[32] have been investigated as causes of lung cancer occurring in nonsmoking women.

The findings of the breakdown of *EGFR* mutations among the 3 exons were interesting. The exon 21 mutations correlated with pathologic stage and subtype, unlike mutations in exon19. Since exon 21 mutations are more closed to the activation loop of *EGFR*, these may be more correlated with gefitinib sensitivity. Especially since 3 patients with exon 20 mutations were smokers, all of the mutations might not be equally correlated with sensitivity for gefitinib.

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Second Primary Cancers in Patients with Stage III Non-Small Cell Lung Cancer Successfully Treated with Chemo-radiotherapy

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Background: Patients successfully treated for non-small cell lung cancer (NSCLC) remain at risk for developing second primary cancer (SPC). The purpose of the current study is to assess the incidence of SPC and the impact of smoking status on the SPC in long-term survivors with stage III NSCLC after chemo-radiotherapy.

Methods: Using the database from the Japan National Hospital Lung Cancer Study Group between 1985 and 1995, information was obtained on 62 patients who were more than 3 years disease-free survivors. Details of clinical information and most smoking history were available from the questionnaire.

Results: Nine of the 62 patients developed SPC 3.9–12.2 years (median, 6.2 years) after the initiation of the treatment. The site of SPC was 2 lung, 1 esophagus, 2 stomach, 1 colon, 1 breast, 1 skin and 1 leukemia. Among these nine, three cancers occurred inside the radiation field. The relative risk of any SPC was 2.8 [95% confidence interval (CI) 1.3–5.3]. The risk changed with the passage of time and it increased significantly (5.2 times at or beyond 7 years) after the treatment. In univariate analysis, the patients who were male, had more cumulative smoking and continued smoking, had an increased risk of SPC [relative risk (RR) 2.7, CI 1.1–5.3; RR 3.0, CI 1.2–6.2; RR 5.2, CI 1.6–11.7, respectively]. In multivariate analysis, factors including smoking status and histological type had no effect on the development of a SPC.

Conclusion: The patients with stage III NSCLC successfully treated with chemo-radiotherapy were at risk for developing SPC and this risk increased with time.

Key words: second primary cancer – non-small cell lung cancer – chemo-radiotherapy

INTRODUCTION

The introduction of combined modality therapy as chest radiotherapy (RT) and chemotherapy for patients with stage III non-small cell lung cancer (NSCLC) has resulted in achieving ~15% long time survivors (123). However, patients successfully treated for NSCLC as well as small cell lung cancer (SCLC) remain at risk for developing second primary cancer (SPC) (4). The risk of SPC in patients with NSCLC has been studied mainly in cohorts of surgically resected patients for stage I NSCLC (567). These reports suggest that the risk of developing SPC and second primary lung cancer (SPLC) is 1–4% and 1–2% per patient per year, respectively, and it appears to increase with the passage of time. Another study including stages I and II patients treated with chest RT confirmed a similar trend that the risk of developing SPC and SPLC is 4.3 and 1.4% per patient per year, respectively (8). Unlike the studies of the patients with SCLC (9–11), these did not provide adequate follow-up information to determine relative risk. Also, there has been no report to date to evaluate the risk of SPC associated with the treatment of RT with chemotherapy as well as smoking status in stage III NSCLC patients.

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PATIENTS AND METHODS

Information was obtained on 1643 patients with stage III NSCLC between 1985 and 1995, using the database from the National Hospital Study Group for Lung Cancer, including

National Hospital Organization Kinki-chuo Chest Medical Center, National Hospital Organization Toneyama Hospital and National Hospital Organization Okinawa Hospital. Among them, 547 patients were treated with chemo-radiotherapy with or without surgery. Of the 547, the 62 patients were more than 3 years disease-free survivors. The patients who relapsed within the 3 years were excluded in this study. Details of clinical information after the treatment and smoking history of the patients were obtained by a questionnaire, which was completed by directly interviewing the patients or the relatives of deceased patients, or by checking the patient's medical records.

Smoking cessation was defined as completely stopping smoking within 6 months after initiation of treatment. Smoking-related cancers include cancer of the lung, larynx and oral cavity, including pharynx, esophagus, pancreas, bladder, kidney, stomach and uterine cervix. A second primary lung cancer was diagnosed according to the criteria provided by Martini and Melamed in 1975 (12). The period of the study was taken as starting from the first day of therapy, and the date of second cancer was taken as the day of histological or cytological documentation of cancer.

For estimation of the expected values of SPC development, the period of risk began 3 years after initiation of treatment and ended with the date of death, date of last follow-up or date of diagnosis of a SPC, whichever occurred first. Age, gender and period-specific rates for cancer incidence within the period 1985–98 obtained from the Research Group for Population-based Cancer Registration in Japan were applied to the appropriate person-years of observation (13). Statistical methods for risk estimation were based on the assumption that observed number of second cancers followed a Poisson distribution (14). To calculate excess risks per 10 000 patients per year in subgroups with significant relative risks, the expected number of cases was subtracted from the number observed. The difference was divided by person-years of observation, and multiplied by 10 000. The risk of a SPC with a specific exposure as smoking was estimated by comparing the patients without the specific exposure, using Poisson regression methods adjusting for gender, histology (squamous cell carcinoma versus non-squamous cell carcinoma) and cumulative smoking amount before the treatment of NSCLC (40 pack-years > versus ⩾40 pack-years) (15).

RESULTS

The 62 questionnaires completed for each patient showed that none of the patients had past history of cancer of any site nor received previous chemotherapy or RT. The patient characteristics are summarized in Table 1. The end of observation to count the person-years was 31 December 1998. The median follow-up from initiation of therapy was 6.2 years (range 3.1–12.2 years). Of the 62 patients, nine developed SPC in 435 person-years of follow-up. Forty-six patients have remained free of cancer since initial treatment. Three other patients relapsed with NSCLC and still remain alive receiving second line chemotherapy. Of the 62 patients, 13 have died: 5 from recurrent NSCLC, 4 from SPC, 4 from other causes. Regarding chemotherapy for initial treatment, 39 patients were treated with cisplatin (CDDP) + mitomycin (MMC) + vindesine (VDS), 16 with CDDP + VDS, 4 with carboplatin, 2 with CDDP + irinotecan, with 1 with CDDP + MMC + inorelbine. In the treatment of RT, 66 Gy were given to 5 patients, 60 Gy to10, 56 Gy to 28, 50 Gy to 15 and 40 Gy to 4. Of the 62 patients, surgery was performed in 24 patients after the chemo-radiotherapy.

Table 1. Patient characteristics (n = 62)

Gender	
Male	50
Female	12
Age (median, range)	61, 34–80
Histology	
Squamous cell carcinoma	30
Adenocarcinoma	21
Large cell carcinoma	10
Adenosquamous carcinoma	1
Stage	
IIIA	32
IIIB	30
Surgery	
Yes	24
No	38
Smoking (median, range)	40 pack-years, 0–120
Stop smoking	
Yes	29
No	16
Unknown	17

For smoking status, information was obtained for all the 62 patients before the treatment, but was available for 45 patients after the treatment. Of the 45 patients treated in the analysis, 16 patients continue to smoke and 19 patients stopped smoking. For assessment, 10 never smokers were also added to the 19 stopped patients, and the 29 patients were categorized to the stop smoking group.

Details of nine patients who developed SPC out of the 62 patients are shown in Table 2. There has been no SPC among the ten never smokers. Two patients (cases 5 and 9) developed a SPLC in different lobes from the original NSCLC. Both tumors arose from the ipsilateral side and both patients continued to smoke after the treatment. One of the two lung cancers developed inside the radiation field. The other malignancies consisted of carcinoma of the esophagus, stomach, colon, skin, breast and acute myelogenous leukemia. Two SPC with skin and breast cancer (cases 6 and 8) also developed inside the radiation field.

Table 3 shows the relative and absolute risks of SPC after initiation of therapy for NSCLC. The risk for development of any SPC increased significantly to 2.8 [95% confidence interval (CI) 1.3–5.3]. In spite of the overall increase in risk, there was no significant increase in relative risk of developing a particular cancer. When smoking-related cancers are combined, there was still no significant increased relative risk in the development of SPC.

Table 2. Characteristics of nine patients with second primary cancers

Patient	Age	Gender	CFI (years)	P His	SPT/His	
1	70	M	3.9	LA	Stomach/AD	
2	69	M	11.5	AD	Colon/AD	
3	61	M	6.3	SQ	Esophagus/SQ	
4	65	M	4.5	SQ	Stomach/AD	
5	62	M	5.6	SQ	Lung/SQ	
6	58	M	4.5	AD	Skin/SQ	inside RT field
7	66	M	8.1	SQ	AML	
8	54	F	10.4	LA	Breast/AD	inside RT field
9	66	M	7.9	AD, SQ	Lung/Undiff	inside RT field

CFI, cancer-free interval; P, Primary; His, Histology; AD, adenocarcinoma; LA, large cell carcinoma; SQ, squamous cell carcinoma; Undiff, undifferentiated carcinoma; AML, Acute myeloid leukemia; RT, radiotherapy.

Table 3. Risk of second primary cancers

Site	Obs	E	O/E	95% CI	Absolute risk*
All cancers	9	3.23	2.8	1.3–5.3	238.9
Esophagus	1	0.12	8.6	0.1–47.7	
Stomach	2	0.81	2.5	0.3–8.9	
Colon	1	0.39	2.5	0.1–14.1	
Lung	2	0.50	4.0	0.4–7.2	
Skin	1	0.03	36.2	0.4–201.3	
Breast	1	0.03	36.7	0.4–204.1	
Leukemia	1	0.03	30.9	0.4–171.5	
Smoking-related	5	1.81	2.8	0.9–6.4	

Obs, observed; E, expected.
*Excess risk per 10 000 persons per year.

Next, the effect of the passage of time was evaluated. The relative risk for 3–4 years after the treatment was 2.2 (95% CI 0.1–23.9) and 1.8 (95% CI 0.1–23.9) for 5–6 years, and 5.2 (95% CI 1.4–13.2) for at or beyond 7 years. The risk changed with the passage of time and it increased significantly (5.2 times at or beyond 7 years) after the treatment. The absolute risk was 600.1 per 10 000 persons per years.

Table 4 shows the results of univariate analysis on the relative risk for a SPC. The risk was significant but modestly increased relative to the general population in male and more cumulative smoking amount (2.7 times; 95% CI 1.1–5.3 and 3 times; 95% CI 1.2–6.2, respectively). Among those who continued to smoke, there was a significantly increased relative risk (5.2 times; 95% CI 1.6–11.7). In contrast, those who stopped smoking showed only a 1.8-fold increase (95% CI 0.3–5.9), which was not significantly different from the general population.

Finally, we assessed multivariate analysis and examined the relationship between continued smoking habits and the risk of a SPC, adjusted for gender, histology type and cumulative smoking amount. The results are shown in Table 5. We could not demonstrate that factors such as continued smoking habits, gender, histology type and cumulative smoking amount had effect on the development of a SPC.

Table 4. Risk of second primary cancers by histology, gender and smoking status

	Obs	O/E	95% CI	Absolute risk*
Histology				
SQ	4	2.7	0.7–6.9	
Non-SQ	5	2.6	0.9–6.7	
Gender				
Male	8	2.7	1.1–5.3	246.7
Female	1	4.3	0.1–23.9	
Surgery				
Yes	4	3.6	0.9–9.2	
No	5	2.3	0.7–5.4	
Smoking				
≤40 pack-years	2	2.2	0.2–8.0	
≥40 pack-years	7	3.0	1.2–6.2	324.2
Intercurrent smoking				
Yes	3	1.8	0.3–5.9	
No	5	5.2	1.6–11.7	430.5

SQ, squamous cell carcinoma; Obs, observed.
*Excess risk per 10 000 persons per year.

Table 5. Relative risk of second primary cancers estimated by multivariate analysis

Risk factor	Relative risk	95% CI
Cumulative smoking (<40 pack-years/≥40 pack-years)	1.4	0.2–8.4
Intercurrent smoking (yes/no)	2.3	0.5–10.8
Histology (SQ/non-SQ)	3.3	0.2–3.3
Gender (male/female)	1.0	0.1–11.2

SQ, squamous cell carcinoma.

DISCUSSION

There has been a large body of work that evaluated the risk of SPC in the patients with NSCLC in the treatment of surgery or RT alone (5678). Although the number of survivors in patients with stage III NSCLC has increased by combined modality therapy as chemotherapy and RT, there has been no report to date to evaluate the risk of SPC in these patients. Additionally, Ng and co-workers (16) reported that the relative risk of SPC was 6.1 with the combined chemotherapy and RT and 4.0 with the RT alone, showing a significant difference ($P = 0.03$) in the surviving patients in Hodgkin's disease. Given that, we focused on the NSCLC patients treated with chemo-radiotherapy.

In our study, 9 patients out of 62 long-term survivors of stage III NSCLC treated with chemo-radiotherapy had a SPC. The relative risk for any SPC (2.8; 95% CI 1.3–5.3) compared with the general population was significantly increased. Instead of many reports examining the risk, these do not provide adequate follow-up information to determine relative risk in the patients with NSCLC. Most studies only show a percent risk per patient per year (5–8). In the current study, the overall rate of developing SPC is estimated at 2.9% per patient per year, which is in agreement with the rates in most surgical series. Ginsberg and Rubinstein (5) reported that SPC occurrence rate was 1.7% per patient per year on 247 patients operated for T1 N0 NSCLC. Other studies showed the rate of 2.8% by Martini et al. (6) and 2.4–3.6% by Thomas and Rubinstein (7). In the current study, we also confirmed the effect of the passage of time on developing SPC. Thomas and Rubinstein (7) reported that the rate of SPC increased from 2.4% for the first 5 years after surgical resection to 3.6% after the fifth year.

We previously studied the relative risk of SPC in the SCLC patient successfully treated with chemotherapy with or without RT (9). Our results showed a similar trend as previous studies (10,11) and demonstrated that the patient had a significantly increased relative risk of 3.6 (95% CI 2.0–5.9) and that the patients who continued to smoke demonstrated a significantly increased risk for a SPC (4.3, 95% CI 1.1–15.9, $P = 0.03$) compared with those who stopped smoking.

Unlike the results of SCLC patients study, the risk of SPC in NSCLC patients was lower, and the impact of continued smoking on developing SPC in the patients was less significant, but the reason for this observation is not completely understood. According to the case–control study from Japan (17), lung cancer risk reduction due to smoking cessation appeared to be greater in SCLC than squamous cell carcinoma or adenocarcinoma, and SCLC seems to be more smoking-related than NSCLC. However, there have been a couple of germline polymorphism as cytochrome P 450 1A1 (CYP1A1) and glutathione *S*-transferase class mu (GSTM1), reported, which is implicated in smoking-related carcinogenesis (18,19). Therefore, SCLC patients are speculated to have a higher potential to develop a SPC, particularly smoking-related cancers.

Among NSCLC patients, there seems to be a special group of roentgenographically occult early stage squamous cell carcinoma of the lung. In this patient group, the rate of occurrence of SPC, particularly SPLC was estimated at 3–4% per patient per year (20,21). The risk for SPLC seemed to be substantially higher than that of 1–2% in the NSCLC patients treated with surgery or RT from the previous study and treated with chemo-radiotherapy from our study. Therefore, the group should be given a special focus and be divided from the general population of NSCLC patients in the research of risk of SPC. Most of the patients can be cured by surgery, photodynamic therapy, brachytherapy and chest RT because of its early clinical stage (22), and are not included in our study. Roentgenographically occult early stage squamous cell carcinoma of the lung is associated with the concept of field cancerization (23), and smoking status seems to be very important to evaluate the risk of SPC, which awaits further examination.

A relatively small sample size and rare events such as SPC in this study resulted in large confidence intervals for the estimates. It is still difficult to conclude the effect of continued smoking on the development of SPC. Cigarette smoking causes not only developing cancers but also cardiovascular and lung damage as well (24,25). It may be speculated that continued smokers died off early when interpreting the results. The cessation of smoking is still warranted among patients with stage III NSCLC treated by chemo-radiotherapy.

In conclusion, stage III NSCLC patients treated with chemo-radiotherapy were at risk of developing SPC and this risk increased with time. A large sample size study in a longer follow-up period may be required in further research to conclude the effect of continued smoking on the development of SPC. SPC in another particular group such as roentgenographically occult early stage squamous cell carcinoma of bronchus also awaits further studies.

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Fractionated Administration of Irinotecan and Cisplatin in Japanese Patients With Extensive-Stage–Disease Small-Cell Lung Cancer

TO THE EDITOR: We read with great interest the recent article by Hanna et al,[1] in which they reported irinotecan and cisplatin (IP) regimen was not superior to the etoposide and cisplatin (EP) regimen for extensive-stage–disease (ED) small-cell lung cancer (SCLC), even though Noda et al[2] clearly showed the superiority of IP regimen over EP regimen. We previously fractionated the schedule of IP to obtain the synergistic effect of the two drugs and to reduce toxicities.[3] The recommended doses of irinotecan and cisplatin on days 1 and 8 were determined to be 50 mg/m^2 and 60 mg/m^2, respectively. However, the phase II study for ED SCLC was stopped early because of poor outcomes in the interim analysis.[4] Despite the small number of patients in our study, the median survival time and 1-year survival rates were similar to those reported in the study by Hanna et al (Table 1). The delivered doses of irinotecan and cisplatin in their study were 1.8 times and 0.7 times as much as those of our study, respectively (Table 1). In comparison to the study by Noda et al, we should have modified fractionated administration by escalating the dose of irinotecan and reducing that of cisplatin to improve the outcomes. However, both irinotecan and cisplatin in the Hanna et al study showed more dose intensity than that reported in the Noda et al study. Hanna et al suggested that IP might therefore be a better regimen for Japanese patients. We considered fractionated administrations of IP to be inferior to the original schedules of IP (cisplatin on day 1 and irinotecan on days 1, 8, and 15) for not only American but also Japanese patients with ED SCLC based on the findings of our study.

Another explanation for the negative results of the Hanna et al study might be due to salvage chemotherapy. More patients on the IP arm received subsequent treatment with etoposide (47.2% *v* 22.6%) whereas more patients on the EP arm received subsequent treatment with topoisomerase I inhibitors including irinotecan or topotecan (33% *v* 24.1%).[1] Noda et al did not describe the use of salvage chemotherapy, which might have affected the survival difference in both arms. Because chemotherapy with fluorouracil, leucovorin, and irinotecan (FOLFIRI), followed by fluorouracil, leucovorin, and oxaliplatin (FOLFOX), had almost the same efficacy as that with FOLFOX followed by FOLFIRI in the treatment of advanced colorectal cancer,[5] IP followed by EP might therefore have had the same efficacy as EP followed by IP in the treatment of ED SCLC. To achieve a prolonged survival, the administration of all three active cytotoxic drugs (cisplatin, irinotecan, and etoposide) during the treatment course may thus be necessary.

Table 1. Irinotecan and Cisplatin for the Treatment of Extensive-Stage–Disease Small-Cell Lung Cancer

Characteristic	Study		
	Noda et al[2]	Hanna et al[1]	Takigawa et al[4]
Age, years			
Median	63	63	61
Range	30-70	37-82	41-74
Performance status 0 or 1, %	92.2	92.3	100
Delivered dose, mg/m^2/wk			
Irinotecan	36.2	39	21.4
Cisplatin	14.3	18	25.7
Median survival, months	12.8	9.3	9.4
1-year survival rate, %	58.4	35	40
Time to progression, months	6.9	4.1	5.6

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

IN REPLY: Takigawa and colleagues consider the fractionated schedule of irinotecan and cisplatin (IP) to be inferior to the original schedule given in the study by Noda et al[1] and point to this as one possible explanation for the lack of survival advantage for the IP regimen in our study[2] published in the May 1, 2006, issue of the *Journal of Clinical Oncology*. A second point raised by these authors is that salvage chemotherapy may have affected the survival outcomes and suggest the best outcomes may be achieved with the combination of all three agents (cisplatin, etoposide, and irinotecan).

Regarding the first point, we acknowledged in our paper that the fractionated regimen of IP may be inferior to the regimen in the study by Noda et al.[1] The authors cite their own study of fractionated IP as evidence of this point.[3] However, the response rate of 80% and median time to progression of 5.6 months in their study (n = 15) was similar to that seen with the Noda IP regimen. In addition, as the authors acknowledge the dose intensity of irinotecan was 1.8 times greater with irinotecan in our study compared with theirs. The Southwest Oncology Group is completing a much larger trial in patients with extensive disease small-cell lung cancer utilizing the two arms of the Noda trial.[1] The results from this trial will provide the answer to this question of dose/schedule of IP. However, given the lack of positive phase III trials testing a number of active agents in various combinations, schedules, and dosages in extensive disease small-cell lung cancer over the last 25 years, it seems unlikely that a change in schedule of IP which provides less dose intensity (as does the original schedule of IP compared with our regimen) will positively affect survival outcomes.

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A phase I and pharmacological study of amrubicin and topotecan in patients of small-cell lung cancer with relapsed or extensive-disease small-cell lung cancer

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Lung cancer;
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Pharmacokinetics

Summary Cisplatin-based chemotherapy is considered to be a standard treatment in patients with relapsed or extensive-disease (ED) small-cell lung cancer (SCLC), the survival benefit remains modest. Relapsed or ED-SCLC patients were enrolled. Topotecan and amrubicin were administered on Days 1–5 and on Days 3–5, respectively. Nine patients received a total of 24 cycles. Since all three patients experienced dose-limiting toxicity (grade 4 neutropenia lasting for more than 4 days, grade 3 febrile neutropenia, and grade 4 thrombocytopenia) at the third dose level (topotecan: 0.75 mg/m^2, amrubicin 40 mg/m^2), the maximum tolerated dose was determined to be this dose level. Objective response was observed in six patients (67%). The maximum concentration (C_{max}) and area under the plasma concentration–time curve (AUC) of amrubicin increased in a dose-dependent manner. Amrubicin did not influence the pharmacokinetics of topotecan. The C_{max} and AUC of amrubicin were correlated with the duration of grade 4 neutropenia. The mean C_{max} of topotecan on day 2 in responders (22.9 ± 3.6) was significantly higher than that in non-responders (10.9 ± 0.4). This phase I study showed the safety and activity of two-drug combination of amrubicin and topotecan in patients with relapsed or ED-SCLC.

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1. Introduction

Recently, therapy with a cisplatin (CDDP)-based two-drug combination has been used as the standard treatment for small-cell lung cancer (SCLC) cases with extensive-disease (ED). In particular, the combination of irinotecan (CPT-11) and CDDP has been reported to be highly effective in previously untreated patients with ED-SCLC [1]. However, since the majority of responders showed early relapse, and salvage chemotherapy for SCLC usually yields disappointing results, the long-time survival rate was extremely low [2–5]. Accordingly, in order to achieve better treatment results for SCLC, new effective combination regimens need to be sought for patients with relapsed or refractory SCLC after standard chemotherapy. Recently, several new agents with novel mechanisms of actions have been developed and been shown to be highly effective for the treatment of SCLC [6].

Amrubicin (AMR), a novel and entirely synthetic anthracycline, inhibits DNA topoisomerase II activity. It has been shown to be active against previously untreated SCLC, with an overall response rate and median survival time (MST) of 78.8% and 11.0 months, respectively [7].

Topotecan (TOP), a unique semi-synthetic water-soluble analog of camptothecin, exhibits inhibitory activity against DNA topoisomerase I, and has been shown to have favourable anti-tumour activity against SCLC, with a response rate of 39% and MST of 9.0 months [8].

DNA topoisomerases I and II are functionally correlated and act in concert. Both enzymes are believed to be essential for the maintenance of cell viability. Therefore, combined use of agents targeted against the DNA topoisomerases I and II may be expected to completely inhibit both DNA and RNA synthesis and exert synergistic cytotoxicity [9–11]. There have been some reports of the effectiveness of such a combination of drugs, namely, irinotecan (CPT-11) and etoposide (VP-16), in patients with SCLC [12].

Based on these results, we conducted a phase I trial of the two-drug combination chemotherapeutic regimen of AMR and TOP in patients with relapsed or ED-SCLC. The primary objective of this trial was to determine the maximum tolerated dose (MTD) of the two-drug regimen. The secondary objectives were to investigate the anti-tumour activity of the regimen and influence of the administration sequence of the two drugs on the pharmacokinetics and clinical toxicity of the combination regimen.

2. Materials and methods

2.1. Eligibility criteria

Patients were recruited based on the following eligibility criteria: pathologically proven SCLC; relapsed disease or ED-SCLC; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1 or 2; age ≤75 years; presence of evaluable lesion; no chemotherapy within 4 weeks prior to study entry; adequate haematological (WBC count ≥3000/μL, neutrophil count ≥1500/μL, haemoglobin level ≥9.5 g/dL, platelet count $\geq 15 \times 10^4$/μL), renal (serum creatinine ≤ 1.5 mg/dL), hepatic (total bilirubin ≤ 1.5 mg/dL, serum transaminases ≤2.5× upper limit of normal range) and pulmonary function ($PaO_2 \geq 60$ Torr) reserves; receipt of written informed consent. Patients with symptomatic brain metastasis or evidence of preexisting interstitial pulmonary disease on the chest radiograph were excluded from the study. Pretreatment evaluations included a complete history, physical examination, laboratory tests, chest radiography, electrocardiography, computed tomography (CT) of the chest and abdomen, magnetic resonance imaging (MRI) of the brain, and a radionuclide bone scan. Staging was conducted according to the tumour, node, metastasis system [13]. The protocol was approved by the institutional review board of the NHO Minami-Okayama Hospital and Okayama University Medical School.

2.2. Treatment scheme

TOP, diluted in 100 mL of physiological saline, was administered by intravenous infusion over 1 h on days 1–5. AMR, diluted in 20 mL of physiological saline, was administered as a bolus intravenous injection over 5 min on days 3–5, after completion of the TOP infusion. Each patient was premedicated with i.v. dexamethasone (8 mg) and granisetron (3 mg). The starting doses of TOP and AMR were 0.75 and 30 mg/m^2, respectively, which were 60–70% of the recommended doses in previous phase II monotherapy studies [8,14–16]. The following five dose escalations of TOP/AMR (mg/m^2) were planned: 0.75/30, 0.75/35, 0.75/40, 1.0/40 and 1.0/45.

The treatment was repeated every 4 weeks at the same dose levels up to four cycles, unless disease progression or unacceptable toxicity was observed, or the patient refused further treatment. Initiation of the next cycle of chemotherapy was delayed until recovery of the WBC count to ≥3000/μL, neutrophil count to ≥1500/μL, platelet count to $\geq 15 \times 10^4$/μL, and resolution of non-haematologic toxicities to ≤grade 1. After completion or discontinuation of this regimen, patients were permitted to receive standard chemotherapy for SCLC.

2.3. Assessment of toxicity and dose escalation

Toxicity was graded according to the National Cancer Institute-Common Toxicity Criteria ver 2.0 [17]. Dose-limiting toxicity (DLT) was defined as development of at least one of the following adverse events: any non-haematologic toxicities ≥grade 3, except for alopecia, nausea, vomiting and general malaise; platelet count $\leq 2 \times 10^4$/μL; grade 4 leukopenia; persistence of grade 4 neutropenia for more than 4 days; grade 3 or more severe neutropenia with fever ≥38 °C or evidence of infection; failure to recover sufficiently from toxicities by Day 29, before beginning the next cycle of treatment.

Initially, three patients were enrolled at each dose level. If fewer than two patients experienced DLT, the next group of patients was treated at the next higher dose level. If all three patients developed the DLT, the dose level was determined to be the MTD. The recommended dose was also defined as one below the MTD. If two patients experienced the DLT, six patients in total were administered the same dose level. If half or more of these six patients developed DLT, the dose was determined to be the MTD. Dose escalation above the starting dose in individual patients was

not allowed. If grade 4 leukopenia, grade 4 neutropenia, or febrile neutropenia was noted, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

2.4. Assessment of antitumour activity

The standard response evaluation criteria in solid tumors was used to evaluate the responses [18]. Complete and partial response (PR) were confirmed by two observations not less than 4 weeks apart.

2.5. Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis were obtained during the second and third days of the first cycle, from an indwelling venous catheter placed in the arm contralateral to that used for the drug infusion. Five milliliters of blood were collected in heparinised tubes before the drug administration, at the end of the TOP infusion, and 0.5, 3, 8 and 23 h after the end of the TOP infusion on both Days 2 and 3 in the first cycle. After centrifugation, the plasma specimens were stored at −80 °C until the assays. The plasma concentrations of AMR, amrubicinol (13-OH-AMR: active form of AMR) and TOP were measured by high-performance liquid chromatography (HPLC). The area under the plasma concentration–time curve (AUC) was calculated using WINNONLIN Standard Edition, Version 1.5. Differences in the pharmacokinetic parameters among three dose levels in the first cycle were evaluated by the Kruskal–Wallis test, and those between Days 2 and 3 in the first cycle were evaluated by Mann–Whitney's *U*-test. The correlations between the pharmacokinetic parameters and the clinical toxicities or responses were assessed with Spearman's rank test. Statistical analyses were performed using the STATVIEW 5.0 program (Brainpower, Calabasas, CA). A *p*-value of less than 0.05 was considered to denote statistical significance.

3. Results

3.1. Patients' characteristics

Nine patients with relapsed or ED-SCLC were enrolled between April and November 2003. There were eight men and one woman, with a median age of 62 years (range, 51–75 years). All patients had a good performance status (PS 0 in five patients and PS 1 in four patients). Five patients (56%) had received prior chemotherapy (CDDP + VP-16 in three, CDDP + CPT-11 in one, and carboplatin + VP-16 in one). Three patients had sensitive disease and two had refractory disease.

A total of 24 chemotherapy cycles were administered. Three patients (33%) received only one cycle of chemotherapy, because of unacceptable toxicity (two patients) or the patient's refusal to undergo further treatment (one patient). At the first dose level (TOP 0.75 mg/m^2, AMR 30 mg/m^2), one patient developed DLT (grade 3: diarrhoea, stomatitis and febrile neutropenia, grade 4: leukopenia, neutropenia lasting for more than 4 days and thrombocytopenia). At the second dose level (TOP 0.75 mg/m^2, AMR 35 mg/m^2), one patient developed DLT (grade 4 neutropenia lasting for more than 4 days). At the third dose level (TOP 0.75 mg/m^2, AMR 40 mg/m^2), all three patients experienced DLT (grade 4 neutropenia lasting for more than 4 days in one, grade 4 neutropenia lasting for more than 4 days and grade 3 febrile neutropenia in one patient each, and grade 4 thrombocytopenia in one). Therefore, the third dose level was deemed to be MTD, and the recommended doses for the phase II study were the second dose levels, that is, 0.75 mg/m^2 for TOP, and 35 mg/m^2 for AMR.

Table 1 Grade 2 or more severe haematological toxicity (all courses)

Toxicity	Grade	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
Leukopenia	2	0	1 (11%)	1 (13%)
	3	6 (86%)	8 (89%)	3 (38%)
	4	1 (14%)	0	4 (50%)
Neutropenia	2	1 (14%)	0	2 (25%)
	3	2 (29%)	3 (33%)	0
	4	4 (57%)	6 (67%)	6 (75%)
Thrombocytopenia	2	1 (14%)	4 (44%)	0
	3	1 (14%)	0	5 (63%)
	4	1 (14%)	0	0
Anaemia	2	1 (14%)	5 (56%)	3 (38%)
	3	1 (14%)	2 (22%)	2 (25%)
	4	2 (29%)	0	1 (13%)

3.2. Haematological toxicity

The main toxicity of this drug combination was myelosuppression. Analysis of the toxicity during all courses of chemotherapy is shown in Table 1. Grade 3 or 4 leukopenia was observed during all the seven courses (100%) at the first dose level, eight courses (89%) at the second dose level, and seven courses (88%) at the third dose level. Similarly, grade 3 or 4 neutropenia was also frequently observed, necessitating G-CSF administration in eight patients. Grade 3 or 4 thrombocytopenia was observed less frequently at the first and second dose level, however it was observed during five courses (63%) at the third dose level, with two patients requiring platelet transfusion. Although grade 3 or 4 anaemia was observed less frequently, three patients required red blood cell transfusion.

3.3. Non-haematological toxicity

The non-haematological toxicities observed are summarised in Table 2. Febrile neutropenia occurred during one course (14%) at the first dose level, two courses (22%) at the second dose level, and four courses (50%) at the third dose level, however, it was reversible in all cases with only appropriate supportive care. Other toxicities, including diarrhoea, were mild, and did not require any intensive management.

There seemed to be different severity in toxicity profiles in patients with or without prior chemotherapy; grade 4 neutropenia and leucopenia were observed in 5 (38%) of 13 courses versus none of 11 courses in previously treated and untreated patients, respectively. Additionally, febrile neutropenia occurred in only patients with prior chemotherapy (7 [54%] of 13 courses versus none of 11 courses, respectively). However, in our study, pretreated patients tended to be incidentally accrued at higher dose level, which might be rather contributed to the difference in severity of toxicity profiles than prior chemotherapy itself was.

3.4. Antitumour activity

All patients were assessable for response. Although none of the cases showed complete response, six patients (67%),

Table 2 Grade 2 or more severe non-haematologic toxicity (all courses)

Toxicity	Grade[a]	Dose level		
		1	2	3
No. of treated patients		3	3	3
No. of courses evaluated		7	9	8
No. of courses in which toxicity was encountered (%)				
Febrile neutropenia	3	1 (14%)	2 (22%)	4 (50%)
Nausea/vomiting	2	0	1 (11%)	0
	3	0	0	0
Hepatotoxicity	2	1 (14%)	0	0
	3	0	0	0
Infection	2	0	0	0
	3	0	1 (11%)	0
Diarrhoea	2	0	1 (11%)	0
	3	1 (14%)	0	0

[a]No grade 4 or more severe toxicities were observed.

Table 3 Pharmacokinetic parameters of the drugs at dose levels 1–3

		Level 1 (AMR 30 mg/m^2) [number of points: 3]	Level 2 (AMR 35 mg/m^2) [number of points: 3]	Level 3 (AMR 40 mg/m^2) [number of points: 3]	p
AMR	C_{max} (ng/mL)	319.4 ± 109.5	401.6 ± 76.1	447.5 ± 33.5	0.49
	AUC (ng h/mL)	1195.6 ± 445.5	1615.1 ± 194.6	1849.8 ± 90.2	0.58
13-OH-AMR	C_{max} (ng/mL)	23.2 ± 13.3	28.9 ± 2.5	28.3 ± 2.5	0.73
	AUC (ng h/mL)	196.2 ± 169.7	191.2 ± 95.3	299.4 ± 88.2	0.67
TOP (day 2)	C_{max} (ng/mL)	20.3 ± 2.9	21.6 ± 7.9	18.8 ± 7.5	0.73
	AUC (ng h/mL)	64.2 ± 5.1	54.3 ± 15.7	45.1 ± 5.9	0.25
TOP (day 3)	C_{max} (ng/mL)	22.1 ± 1.7	15.0 ± 1.1	16.8 ± 1.7	0.09
	AUC (ng h/mL)	71.4 ± 6.7	53.2 ± 6.2	56.5 ± 1.9	0.19

Each data represents the mean values and standard errors. *Abbreviations*: AMR, amrubicin; TOP, topotecan; C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

Table 4 Pharmacokinetic parameters of topotecan on days 2 and 3

	Day 2 (topotecan alone) [number of points: 9]	Day 3 (topotecan combined with amrubicin) [number of points: 9]	p
Parameters			
T_{max} (h)	0.5	0.5	
C_{max} (ng/mL)	20.2±3.3	18.0±1.3	0.83
AUC (ng h/mL)	54.5±5.8	60.4±3.9	0.23

Each data represents the mean values and standard errors. Abbreviations: C_{max}, maximum concentration; AUC, area under the plasma concentration–time curve.

including one receiving only the first dose level, showed PR. It is worthy of note that 4 out of the 5 (80%) relapsed patients showed PR, although only 2 out of 4 (50%) chemo-naïve patients showed PR. The median time to progression was 4.0 (95% CI: 0.8–6.8) months.

3.5. Pharmacokinetic and pharmacodynamic analysis

Pharmacokinetic parameters were determined in samples obtained on the second and third days of the first cycle in all nine patients. The maximum concentration (C_{max}) and AUC of AMR increased in a dose-dependent manner, although statistical significance was not reached (Table 3). The C_{max} and AUC of TOP were almost comparable among the first three dose levels, suggesting that the AMR dose did not influence the pharmacokinetics of TOP (Table 3). The C_{max} and AUC of

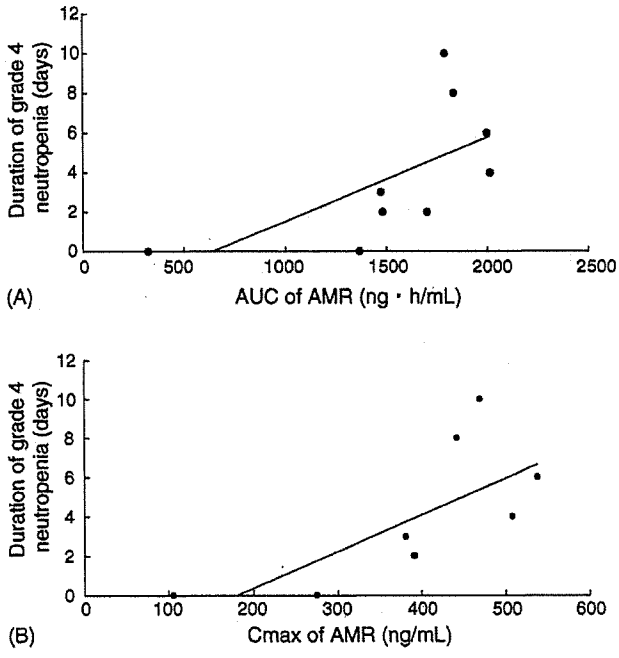

Fig. 1 (A) The correlation between the area under the plasma concentration–time curve (AUC) of AMR (amrubicin) on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0288$), and (B) the correlation between the maximum concentration (C_{max}) of AMR on day 2 and the duration of grade 4 neutropenia in the first cycle (Spearman rank test, $p=0.0225$).

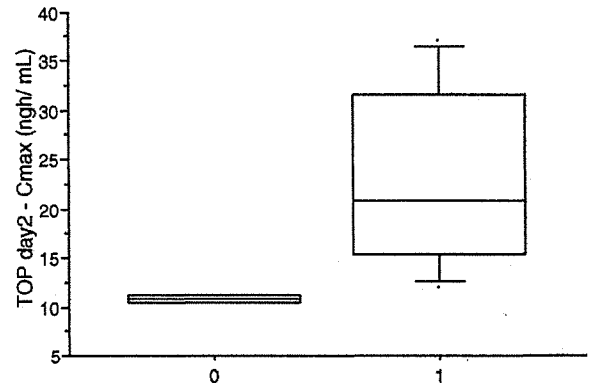

Fig. 2 Correlation between the maximum concentration (C_{max}) of topotecan on day 2 and objective tumour response in the first cycle. "0" denotes stable disease and progressive disease and "1" denotes partial response. The mean C_{max} of seven responders and two non-responders were 22.9±3.6 and 10.9±0.4, respectively (Mann–Whitney's U-test, $p=0.0404$).

13-OH-AMR were not significantly different even with dose escalation of AMR. 13-OH-AMR was not detectable in any of the samples collected from the first patient and two of the samples collected from the second patient at the first dose level, in three samples collected from the two patients at the second dose level, and in one sample collected from the patients at the third dose level, although AMR was detectable in all of these samples. However, the serum concentrations of 13-OH-AMR were higher than 20 ng/mL (minimum detectable value) in all the other patients. We also evaluated differences in the pharmacokinetic parameters of TOP between Day 2 (TOP alone) and Day 3 (TOP plus AMR), in order to investigate the effect of concurrent administration of AMR on the pharmacokinetics of TOP. As listed in Table 4, there were no significant differences. In the correlation of toxicity profiles with the pharmacokinetic parameters, the AUC and C_{max} of AMR were correlated with the duration of grade 4 neutropenia ($p=0.0288$ and 0.0225, respectively; Fig. 1A and B). In addition, the mean C_{max} of TOP on Day 2 in 7 responders (22.9±3.6) was significantly higher than that in 2 non-responders (10.9±0.4, $p=0.0404$; Fig. 2).

4. Discussion

Although the combined use of DNA topoisomerase I and II inhibitors is theoretically attractive, preclinical studies have demonstrated mixed results [19,20–23]. There have been