# MET Tyrosine Kinase Inhibitor Crizotinib (PF-02341066) Shows Differential Antitumor Effects in Non-small Cell Lung Cancer According to *MET* Alterations

*Junko Tanizaki, MD, Isamu Okamoto, MD, PhD, Kunio Okamoto, MD, PhD, Ken Takezawa, MD, PhD, Kiyoko Kuwata, PhD, Haruka Yamaguchi, PhD, and Kazuhiko Nakagawa, MD, PhD*

**Introduction:** Tyrosine kinase inhibitors (TKIs) targeted to MET are undergoing clinical trials in patients with solid tumors, but the precise mechanism of the antitumor activity of these drugs remains unclear. We examined the antitumor action of the MET-TKI crizotinib (PF-02341066) in lung cancer cells that are positive or negative for *MET* amplification or mutation.

**Methods:** The antitumor action of crizotinib was evaluated on the basis of signal transduction, cell proliferation, apoptosis, and progression of tumor xenografts.

**Results:** Inhibition of MET signaling by crizotinib or by RNA interference-mediated MET depletion resulted in the induction of apoptosis accompanied by inhibition of AKT and extracellular signal-regulated kinase phosphorylation in lung cancer cells with *MET* amplification but not in cells with a *MET* mutation or in those without amplification or mutation of *MET*. These results suggest that MET signaling is essential for the survival of cells with *MET* amplification but not for that of cells without this genetic change, including those with a *MET* mutation. Crizotinib up-regulated the expression of BIM, a proapoptotic member of the Bcl-2 family, and down-regulated that of survivin, a member of the inhibitor of apoptosis protein family, in cells with *MET* amplification. Forced depletion of BIM and expression of survivin each inhibited crizotinib-induced apoptosis, suggesting that both up-regulation of BIM and down-regulation of survivin contribute to the proapoptotic effect of crizotinib.

**Conclusions:** Crizotinib shows a marked antitumor action in *MET* amplification-positive lung cancer cells but not in cells without *MET* amplification, including those with a *MET* mutation.

**Key Words:** MET, Lung cancer, Crizotinib, BIM, Survivin.

(*J Thorac Oncol.* 2011;6: 1624–1631)

Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Isamu Okamoto, MD, PhD, Department of Medical Oncology, Kinki University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-Sayama, Osaka 589-8511, Japan. E-mail: chi-okamoto@dotd.med.kindai.ac.jp



ISSN: 1556-0864/11/0610-1624

Activation of protein tyrosine kinases (PTKs) plays a key role in oncogenesis, as exemplified by the role of the *BCR-ABL* fusion gene in chronic myeloid leukemia and by that of epidermal growth factor receptor (*EGFR*) gene mutation and the *EML4-ALK* fusion gene in non-small cell lung cancer (NSCLC). Tyrosine kinase inhibitors (TKIs) that target activated PTKs have exhibited marked therapeutic efficacy in patients with these specific molecular alterations.[1–5] The identification of other target kinases or kinase gene alterations would thus be expected to facilitate the development of new molecularly targeted therapies.

Lung cancer is the leading cause of cancer death worldwide. Despite the successful development of EGFR- or EML4-ALK-targeted TKIs, treatment options remain limited for patients with advanced lung cancer, making the identification of new therapeutic targets an important goal. The tyrosine kinase MET is one such potential therapeutic target. Amplification of *MET* occurs in ~5% of lung cancer cases,[6–9] and *MET* mutations have recently been detected in ~10% of patients with this condition.[10,11] Nevertheless, the relationship between the efficacy of MET-TKIs and *MET* status, such as amplification or mutation, has not been well established. We have, therefore, now investigated the effects of the MET-TKI crizotinib (PF-02341066),[12,13] which is currently undergoing clinical studies, on cell survival and signal transduction in lung cancer cells with or without amplification or mutation of *MET*. We further examined the molecular mechanism underlying the antitumor action of this agent.

## MATERIALS AND METHODS

### Cell Culture and Reagents

The human NSCLC cell lines H1993, H2122, H1437, A549, H1299, PC9, HCC827, and H596 were obtained from American Type Culture Collection (Manassas, VA). The human NSCLC cell line EBC-1 was obtained from the Health Science Research Resources Bank (Tokyo, Japan). The human NSCLC cell line H3122 was obtained as described previously.[14] All cells were maintained under a humidified atmosphere of 5% $CO_2$ at 37°C in RPMI 1640 medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine

serum. Crizotinib was kindly provided by Pfizer Global Research & Development (Groton, CT). EBC-1 and H1993 cell lines were previously shown to manifest high-level amplification of *MET* (copy number, >10).[15] Sequencing of all 21 coding exons of *MET* also previously revealed that H2122 cells contain an N375S mutation and that H1437 and H596 cells harbor a deletion of exon 14 of this gene.[10,11]

### Growth Inhibition Assay In Vitro

Cells were plated in 96-well flat-bottomed plates and cultured for 24 hours before exposure to various concentrations of crizotinib for 72 hours. Cell viability was then assessed with the use of a Cell Titer-Glo Luminescent Cell Viability Assay (Promega, Madison, WI). Absorbance values were expressed as a percentage of that for untreated cells, and the concentration of crizotinib resulting in 50% growth inhibition ($IC_{50}$) was calculated.

### Immunoblot Analysis

Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and then lysed in a solution containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, and leupeptin (1 μg/ml). The protein concentration of cell lysates was determined with a BCA protein assay kit (Thermo Fischer Scientific, Waltham, MA), and equal amounts of lysate protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5 or 12% gel (Bio-Rad, Hercules, CA). The separated proteins were transferred to a nitrocellulose membrane, which was then incubated with Blocking One solution (Nacalai Tesque, Kyoto, Japan) for 20 minutes at room temperature before incubation overnight at 4°C with primary antibodies. Rabbit polyclonal antibodies to phosphorylated human MET (pY1234/pY1235), to AKT, to phosphorylated AKT, to poly(ADP-ribose) polymerase (PARP), to BIM, to Bcl-$x_L$, to Mcl-1, and to XIAP were obtained from Cell Signaling Technology (Danvers, MA); those to extracellular signal-regulated kinase (ERK) and to phosphorylated ERK were from Santa Cruz Biotechnology (Santa Cruz, CA); those to MET were from Zymed (South San Francisco, CA); those to survivin were from Novus (Littleton, CO); and those to β-actin were from Sigma. All antibodies were used at a 1:1000 dilution, with the exception of those to β-actin (1:200). The membrane was then washed with PBS containing 0.05% Tween 20 before incubation for 1 hour at room temperature with horseradish peroxidase-conjugated goat antibodies to rabbit immunoglobulin G (Sigma). Immune complexes were finally detected with chemiluminescence reagents (GE Healthcare, Little Chalfont, UK).

### Annexin V Binding Assay

Binding of annexin V to cells was measured with the use of an Annexin-V-FLUOS Staining Kit (Roche, Basel, Switzerland). Cells were harvested by exposure to trypsin ethylenediaminetetraacetic acid, washed with PBS, and centrifuged at 200 *g* for 5 minutes. The cell pellets were resuspended in 100 μl of Annexin-V-FLUOS labeling solution, incubated for 10 to 15 minutes at 15 to 25°C, and then analyzed for fluorescence with a flow cytometer (FACSCalibur) and Cell Quest software (Becton Dickinson, Franklin Lakes, NJ).

### Gene Silencing

Cells were plated at 50 to 60% confluence in six-well plates or 25-$cm^2$ flasks and then incubated for 24 hours before transient transfection for the indicated times with small interfering RNAs (siRNAs) mixed with the Lipofectamine reagent (Invitrogen, Carlsbad, CA). The siRNAs specific for MET (MET-1, 5′-ACAAGAUCGUCAACAAAAA-3′; MET-2, 5′-CUACAGAAAUGGUUUCAAA-3′) or BIM (BIM-1, 5′-GGAGGGUAUUUUUGAAUAA-3′; BIM-2, 5′-AGGAGGGUAUUUUUGAAUA-3′) messenger RNAs (mRNAs); and nonspecific (control) siRNAs were obtained from Nippon EGT (Toyama, Japan). The data presented for the effects of BIM depletion were obtained with the BIM-1 siRNA, but similar results were obtained with the BIM-2 siRNA.

### Forced Expression of Survivin

The pQCXIH-survivin vector was constructed as described previously.[16] The expression vector was introduced into EBC-1 or H1993 cells by transfection for 48 hours with the use of the Lipofectamine 2000 reagent (Invitrogen).

### Growth Inhibition Assay In Vivo

Tumor cells ($5 \times 10^6$) were injected subcutaneously into the axilla of 5- to 6-week-old male athymic nude mice. Treatment was initiated when tumors in each group achieved an average volume of 200 or 600 $mm^3$. Treatment groups ($n = 5$ mice each) consisted of control and crizotinib (25 or 50 mg/kg of body weight). Crizotinib was administered by oral gavage daily for 28 days; control animals received a 0.5% (wt/vol) aqueous solution of hydroxypropylmethylcellulose as vehicle. Tumor volume was determined from caliper measurements of tumor length ($L$) and width ($W$) according to the formula $LW^2/2$. Both tumor size and body weight were measured twice per week. The in vivo experiments were approved by the appropriate ethics committee.

### Statistical Analysis

Unless indicated otherwise, quantitative data are presented as means ± SE from three independent experiments and were analyzed with the unpaired two-tailed Student's $t$ test. A $p$ value of less than 0.05 was considered statistically significant.

## RESULTS

### Crizotinib Inhibits the Proliferation of Lung Cancer Cells with *MET* Amplification

We first examined the effect of the MET-TKI crizotinib on the proliferation in vitro of lung cancer cells positive or negative for *MET* amplification. Both of two cell lines with *MET* amplification, EBC-1 and H1993, were sensitive to crizotinib, with $IC_{50}$ values of ≤10 nM (Table 1). In contrast, crizotinib did not substantially inhibit the proliferation of lung cancer cells with a *MET* mutation (H2122, H1437, and H596), with an *EGFR* mutation (PC9 and HCC827) or without such gene amplification or mutation (A549 and H1299) (Table 1). These data suggested that crizotinib has a marked antiproliferative effect in lung cancer cells with *MET*

TABLE 1. $IC_{50}$ Values of Crizotinib for Inhibition of the Growth of NSCLC Cells In Vitro

| *MET* and *EGFR* Status | Cell Line | Crizotinib $IC_{50}$ (nM) |
|---|---|---|
| *MET* amplification (+) | EBC-1 | 5 |
| | H1993 | 10 |
| *MET* amplification (−) | | |
| *MET* mutation (+) | H2122 | 472 |
| | H1437 | 1284 |
| | H596 | 752 |
| *EGFR* mutation (−) | A549 | 646 |
| | H1299 | 642 |
| *EGFR* mutation (+) | PC9 | 787 |
| | HCC827 | 767 |

Data are means of triplicates from representative experiments that were repeated a total of three times.
EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

amplification but not in those without *MET* amplification, including those with a *MET* mutation.

## Effects of Crizotinib on Downstream Signaling of MET and on Apoptosis in Lung Cancer Cells with or without *MET* Amplification

We next examined the effects of crizotinib on phosphorylation of AKT and ERK in lung cancer cell lines. Crizotinib markedly inhibited the phosphorylation of AKT and ERK and that of MET in cells with *MET* amplification (Figure 1*A*). Lung cancer cells with a *MET* mutation manifested a low level of MET phosphorylation that was completely inhibited by crizotinib, whereas this agent had little effect on the phosphorylation of AKT or ERK in these cells (Figure 1*A*). In addition, crizotinib did not inhibit AKT or ERK phosphorylation in lung cancer cells with an *EGFR* mutation or in those without amplification of *MET* or mutation of *MET* or *EGFR* (Figure 1*A*). We further investigated the effect of crizotinib on apoptosis in lung cancer cells. An annexin V binding assay revealed that crizotinib induced a substantial level of apoptosis in *MET* amplification-positive cells but was largely without effect in the other cell lines studied (Figure 1*B*). Consistent with these results, immunoblot analysis showed that crizotinib triggered the generation of the cleaved form of PARP in cells with *MET* amplification but not in those with a *MET* mutation and in those without amplification or mutation of *MET* (Figure 1*C*, data not shown). These data thus suggested that crizotinib inhibits the phosphorylation of AKT and ERK, resulting in induction of apoptosis, in lung cancer cells with *MET* amplification, whereas such effects were not observed in cells without *MET* amplification, including those with a *MET* mutation.

## Effects of Depletion of MET in Lung Cancer Cells with *MET* Amplification or a *MET* Mutation

To verify that the antitumor action of crizotinib in *MET* amplification-positive lung cancer cells is indeed mediated by MET inhibition rather than by nonspecific inhibition of other kinases, we transfected lung cancer cells with two independent siRNAs specific for MET mRNA. Transfection with each MET siRNA resulted in a marked decrease in the expression of MET in both cells with *MET* amplification and those with a *MET* mutation (Figure 2*A*). Transfection of *MET* amplification-positive cells with the MET siRNAs also resulted in pronounced inhibition of AKT and ERK phosphorylation, whereas transfection of *MET* mutation-positive cells had little such effect (Figure 2*A*). Depletion of MET markedly increased the proportion of apoptotic cells (Figure 2*B*) and induced generation of the cleaved form of PARP (Figure 2*C*) in cells with *MET* amplification but not in those with a *MET* mutation. These data thus indicated that the antitumor action of crizotinib in lung cancer cells is mediated by inhibition of MET, and they also suggested that the survival of lung cancer cells with a *MET* mutation is not predominantly dependent on MET signaling.

## Effects of Crizotinib on the Expression of Apoptosis-Related Proteins in *MET* Amplification-Positive Lung Cancer Cells

Given that crizotinib induced apoptosis in *MET* amplification-positive lung cancer cells, we examined the effects of this drug on the expression of apoptosis-related proteins in such cells. Crizotinib up-regulated the expression of BIM, a proapoptotic member of the Bcl-2 family of proteins, in both of the *MET* amplification-positive cell lines examined, whereas it had little effect on the expression of other Bcl-2 family members including Mcl-1 and Bcl-$x_L$ (Figure 3*A*). Furthermore, crizotinib down-regulated the expression of survivin, a member of the inhibitor of apoptosis protein (IAP) family, in cells with *MET* amplification, whereas the expression of XIAP, another IAP family member, remained unaffected (Figure 3*A*).

## Role of BIM Induction and Survivin Down-Regulation in Crizotinib-Induced Apoptosis in Cells with *MET* Amplification

To investigate further whether the up-regulation of BIM is related to the induction of apoptosis by crizotinib, we transfected *MET* amplification-positive lung cancer cells with a siRNA specific for BIM mRNA. Transfection with the BIM siRNA markedly suppressed the up-regulation of BIM by crizotinib without affecting the down-regulation of survivin (Figure 3*B*). The annexin V binding assay revealed that such transfection resulted in inhibition of crizotinib-induced apoptosis (Figure 3*B*), indicating that BIM induction contributes to the proapoptotic effect of crizotinib in lung cancer cells with *MET* amplification. We obtained similar results with a second siRNA targeted to a different sequence within BIM mRNA (data not shown). Given that crizotinib induced down-regulation of survivin and up-regulation of BIM, we next examined the role of survivin down-regulation in crizotinib-induced apoptosis. Transfection of *MET* amplification-positive cells with an expression vector for human survivin resulted in inhibition of the crizotinib-induced down-regulation of survivin (Figure 3*C*). Such overexpression of survivin also inhibited the induction of apoptosis by crizotinib (Figure 3*C*), indicating that down-regulation of survivin by crizotinib

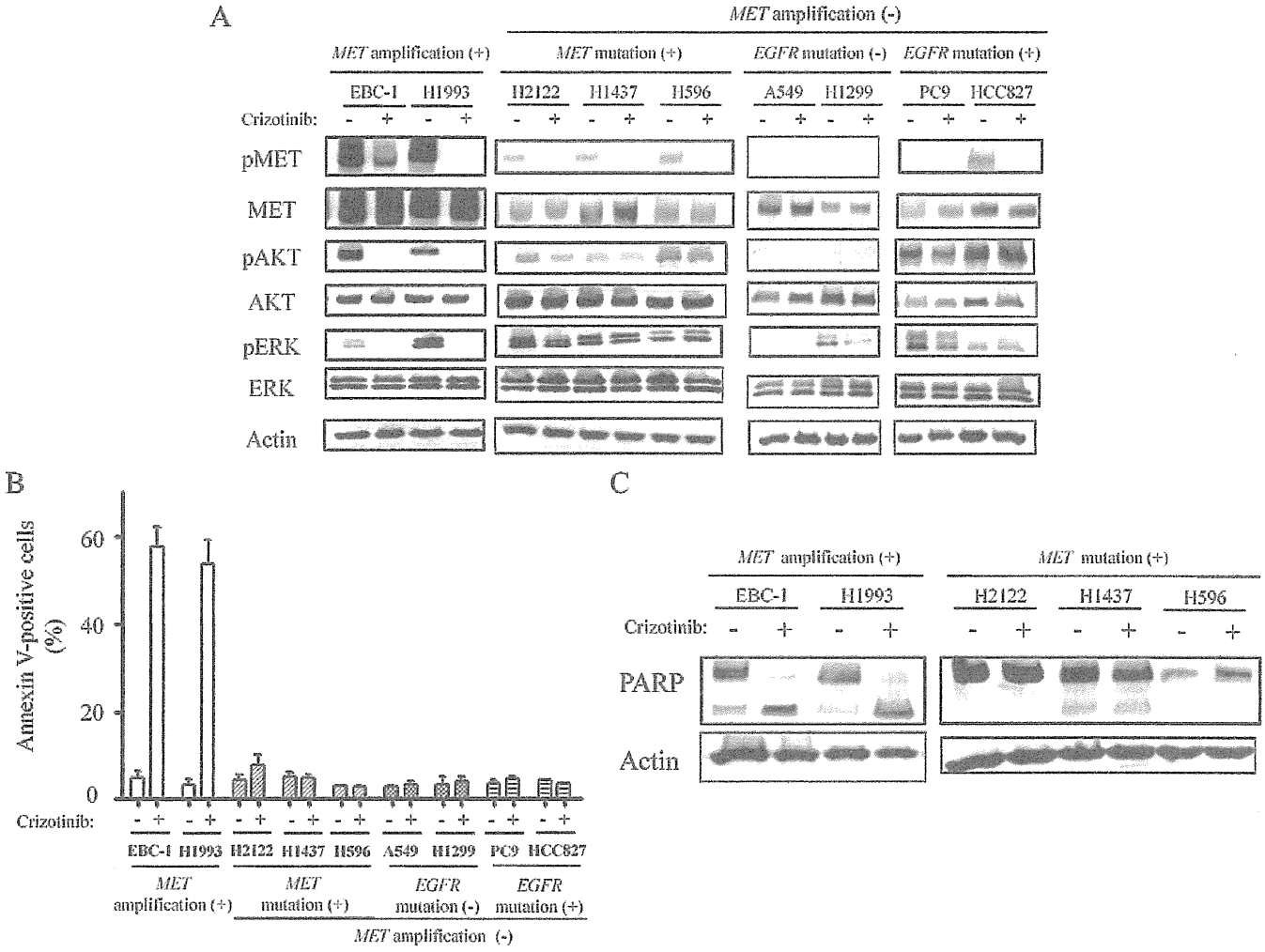


**FIGURE 1.** Effects of crizotinib on MET, AKT, and extracellular signal-regulated kinase (ERK) phosphorylation and on apoptosis in lung cancer cell lines. *A*, The indicated cell lines were incubated with or without crizotinib (100 nM) for 24 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of MET, AKT, or ERK or to β-actin (loading control). *B*, Cells were incubated for 72 hours with or without crizotinib (100 nM), after which the number of apoptotic cells was determined by staining with annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from three independent experiments. *C*, Cells incubated as in (*B*) were lysed and subjected to immunoblot analysis with antibodies to poly(ADP-ribose) polymerase (PARP) or to β-actin.

contributes to the proapoptotic effect of this agent in lung cancer cells with *MET* amplification. Together, these data thus suggested that the induction of apoptosis by crizotinib in lung cancer cells with *MET* amplification is mediated, at least in part, by BIM up-regulation and survivin down-regulation.

## Effect of Crizotinib on the Growth of Lung Cancer Cells In Vivo

To determine whether the antitumor action of crizotinib observed in vitro might also be apparent in vivo, we injected EBC-1 cells (positive for *MET* amplification), H1437 cells (positive for a *MET* mutation), or A549 cells (negative for *MET* amplification and mutation) into nude mice to elicit the formation of solid tumors. After tumor formation, the mice were treated with vehicle (control) or crizotinib at a daily dose of 25 or 50 mg/kg by oral gavage for 4 weeks. Crizotinib at either dose eradicated tumors in mice injected with EBC-1 cells (Figure 4*A*). In contrast, tumors in mice injected with H1437 or A549 cells were not affected by crizotinib treatment even at the dose of 50 mg/kg/d (Figures 4*B*, *C*). Treatment with crizotinib at either dose was well tolerated by the mice, with no signs of toxicity or weight loss during therapy (data not shown). Crizotinib thus exhibited a marked antitumor effect in lung cancer xenografts positive for *MET* amplification, whereas it had little effect on those negative for *MET* amplification, including those with a *MET* mutation, consistent with our results obtained in vitro.

## DISCUSSION

Aberrant activation of PTK signaling pathways contributes to the development of various types of cancer. Small-

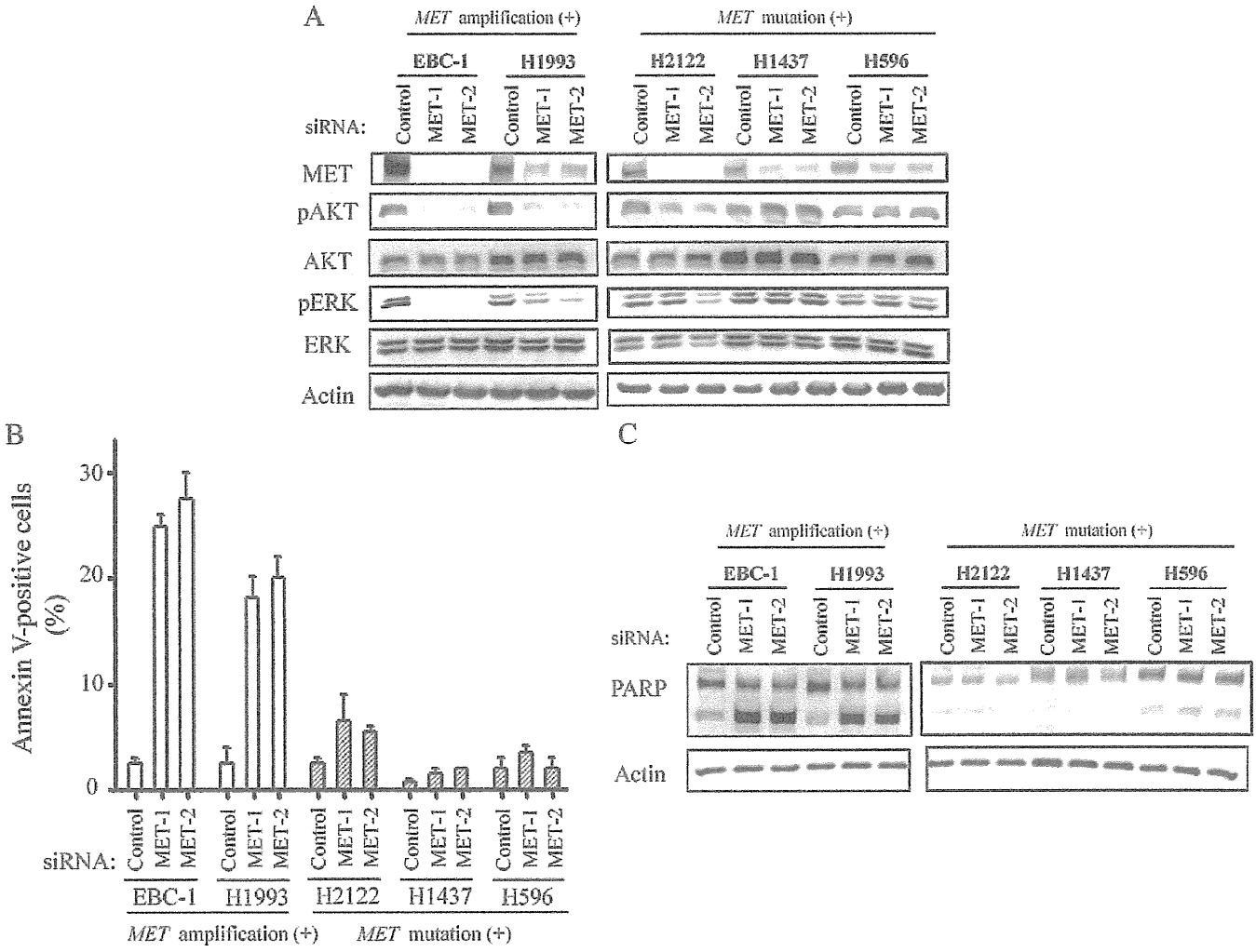


**FIGURE 2.** Effects of depletion of MET on signal transduction and apoptosis in lung cancer cells. *A*, The indicated cell lines were transfected with nonspecific (control) or MET small interfering RNAs (siRNAs) for 48 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of MET, AKT, or ERK or to β-actin. *B*, Cells were transfected with nonspecific (control) or MET siRNAs for 72 hours, after which the number of apoptotic cells was determined by staining with annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from three independent experiments. *C*, Cells transfected as in *B* were lysed and subjected to immunoblot analysis with antibodies to poly(ADP-ribose) polymerase (PARP) or to β-actin.

molecule inhibitors that target these activated kinases have been developed and have shown substantial efficacy in clinical trials. The receptor tyrosine kinase MET is considered one such potential target in cancer, and several MET-TKIs are currently undergoing clinical trials in humans. The identification of patient subgroups that might actually benefit from treatment with such drugs would be expected to optimize their efficacy. We have now shown that the MET-TKI crizotinib, which is presently undergoing clinical evaluation, exerted a marked antitumor action in lung cancer cells with *MET* amplification but not in those without this genetic change, including those with a *MET* mutation. In lung cancer cells with *MET* amplification, inhibition of MET by either crizotinib or siRNAs specific for MET mRNA resulted in down-regulation of AKT and ERK signaling and the induction of apoptosis. We also found that the secretion of hepatocyte growth factor (HGF), a MET ligand, did not differ substantially between lung cancer cells with or without *MET* amplification (see Figure, Supplemental Digital Content 1, http://links.lww.com/JTO/A98). In addition, exogenous HGF had little effect on the viability of lung cancer cells with *MET* amplification (see Figure, Supplemental Digital Content 1, http://links.lww.com/JTO/A98). Together, these results suggest that *MET* amplification itself results in constitutive activation of MET downstream signaling and that tumors with *MET* amplification are dependent on such signaling for their growth and survival. Targeting of MET signaling by MET-TKIs is thus a potentially valuable therapeutic strategy for patients with lung cancer with *MET* amplification, who account for ~5% of all lung cancer cases.[6–8]

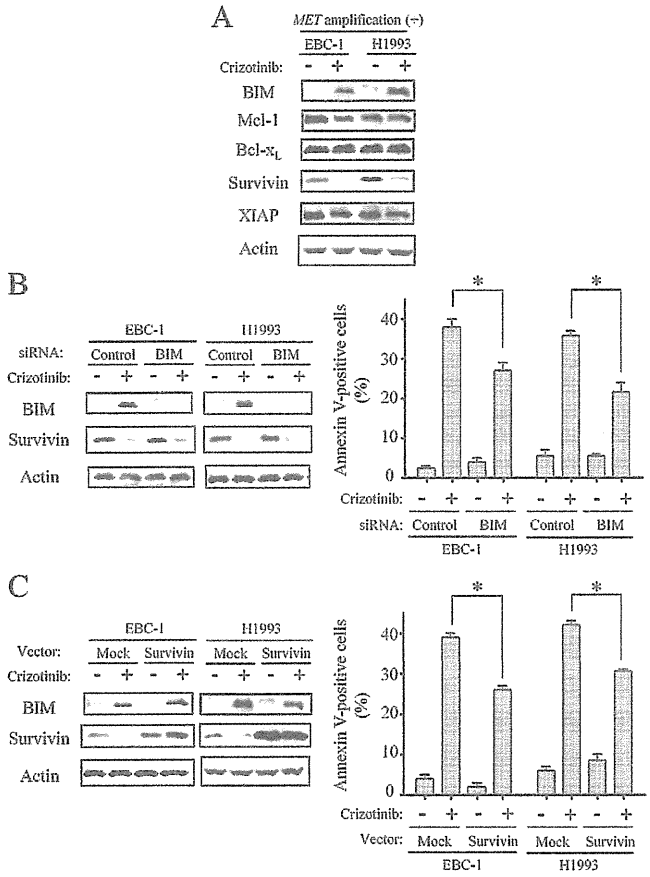


**FIGURE 3.** Effects of BIM depletion and forced expression of survivin on apoptosis induced by crizotinib in lung cancer cells with *MET* amplification. *A*, EBC-1 and H1993 cells were incubated with or without crizotinib (100 nM) for 48 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to the indicated proteins. The position of the band corresponding to $BIM_{EL}$ is indicated. *B*, Cells were transfected with BIM or nonspecific small interfering RNAs (siRNAs) for 24 hours and then incubated for 72 hours with or without crizotinib (100 nM). The cells were then lysed and subjected to immunoblot analysis with antibodies to the indicated proteins (left panel), or they were evaluated for apoptosis by staining with annexin V and propidium iodide followed by flow cytometry (right panel). *C*, Cells were transfected with an expression vector for survivin or with the corresponding empty vector (Mock) for 24 hours, incubated with or without 100 nM crizotinib for 72 hours, and then analyzed as in (*B*). Quantitative data in (*B*) and (*C*) are means ± SE from three independent experiments. $*p < 0.05$ for the indicated comparisons.

Given that crizotinib showed a proapoptotic effect in *MET* amplification-positive lung cancer cells, we further investigated the mediators of crizotinib-induced apoptosis in these cells. We found that crizotinib induced up-regulation of BIM, a key proapoptotic member of the Bcl-2 family of proteins that initiates apoptosis signaling by binding and antagonizing the function of prosurvival Bcl-2 family members.[17] Furthermore, depletion of BIM by RNA interference resulted in inhibition of crizotinib-induced apoptosis in lung cancer cells with *MET* amplification, suggesting that up-regulation of BIM contributes to the induction of apoptosis by crizotinib in such cells. We also found that crizotinib induced down-regulation of survivin, a member of the IAP family that protects cells against apoptosis by either directly or indirectly inhibiting the activation of effector caspases.[18] Moreover, forced expression of survivin suppressed the induction of apoptosis by crizotinib, suggesting that inhibition of survivin expression plays a key role in the proapoptotic effect of this agent in lung cancer cells with *MET* amplification. Our present data thus suggest that MET inhibitor-induced apoptosis is mediated both by up-regulation of BIM and by down-regulation of survivin in lung cancer cells with *MET* amplification. We and others have previously shown that EGFR-TKIs induce the up-regulation of BIM through inhibition of the MEK-ERK signaling pathway in lung cancer cells with an *EGFR* mutation.[16,19–21] Furthermore, we recently demonstrated that EGFR-TKIs induce down-regulation of survivin expression through inhibition of the phosphoinositide 3-kinase (PI3K)-AKT signaling pathway and that the MEK-ERK-BIM and PI3K-AKT-survivin pathways independently contribute to EGFR-TKI-induced apoptosis in *EGFR* mutation-positive lung cancer cells.[16] Given that the MET-TKI crizotinib inhibited phosphorylation of both ERK and AKT and induced BIM up-regulation and survivin down-regulation in *MET* amplification-positive lung cancer cells, MEK-ERK-BIM and PI3K-AKT-survivin pathways likely also mediate crizotinib-induced apoptosis in these cells.

Several *MET* mutations, including those that affect the kinase domain or other domains (extracellular semaphorin and juxtamembrane domains) of the protein, have been identified in tumors. MET-TKIs have been shown to be active against MET with mutations in the kinase domain,[22] whereas the relationship between the efficacy of such agents and nonkinase domain mutants of MET has been unclear. *MET* mutations occur in ~10% of patients with lung cancer and they cluster in nonkinase domain regions of MET.[10,23] The NSCLC cell line H2122 harbors the N375S mutation of *MET*, which is the most frequent mutation of this gene in lung cancer. This mutation is localized to the ligand-binding semaphorin domain of MET and was found to be associated with loss of affinity of the receptor for HGF,[10] although its biological consequences remain largely unclear. We have now shown that crizotinib had little effect on signal transduction or cell survival in H2122 cells, consistent with the previous observation that transient expression of the N375S mutant of MET did not result in an increased susceptibility of the transfected cells to a MET-TKI compared with that of cells expressing wild-type MET.[10] We also examined the effects of crizotinib in lung cancer cells (H1437 and H596 cells) with another *MET* mutation, deletion of exon 14. Loss of the juxtamembrane domain encoded by this exon was found to result in a reduced level of MET ubiquitination and an increase in the half-life of MET, leading to enhancement of ligand-dependent cell proliferation.[11] Nevertheless, the antitumor effects of MET-TKIs in lung cancers with deletion of exon 14 of *MET* have not been well characterized. We have

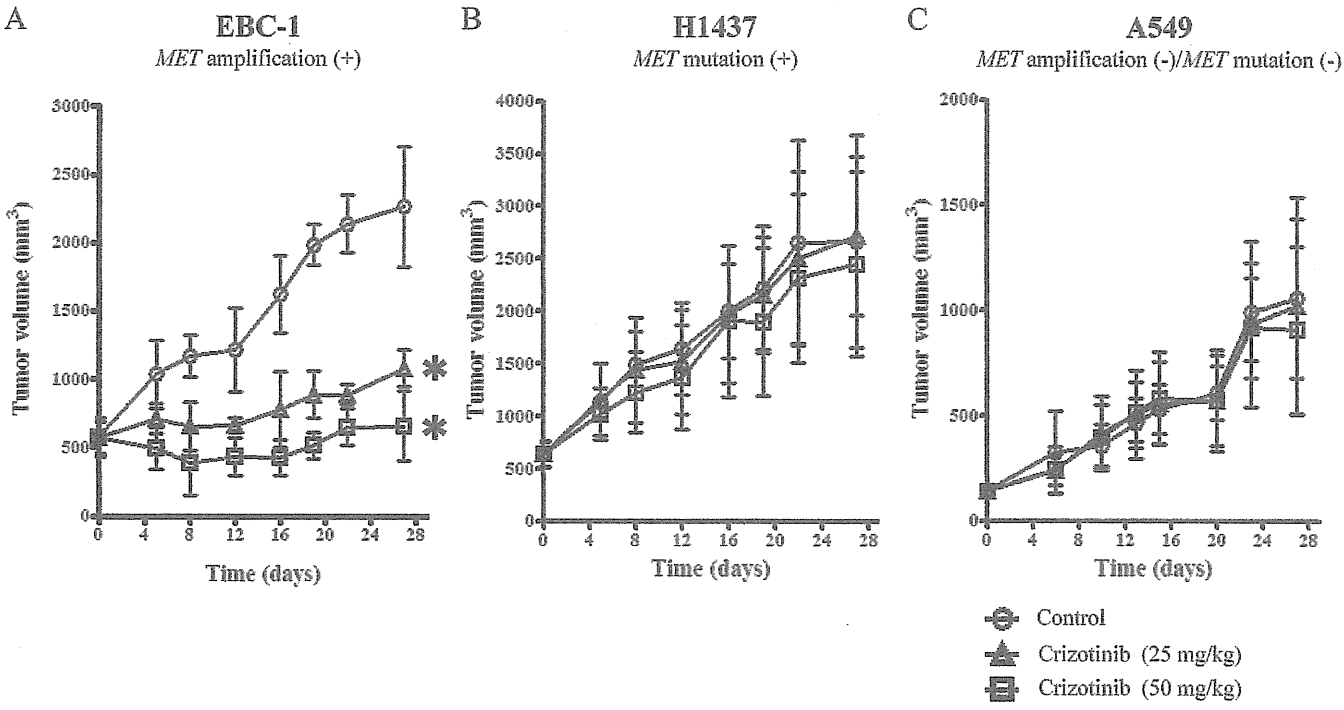


**FIGURE 4.** Effect of crizotinib on the growth of lung cancer cells in vivo. Nude mice with tumor xenografts established by subcutaneous injection of EBC-1 (*A*), H1437 (*B*), or A549 (*C*) cells were treated daily for 4 weeks with vehicle (control) or crizotinib (25 or 50 mg/kg). Tumor volume was determined at the indicated times after the onset of treatment. Data are means ± SE for 5 mice/group. $*p < 0.05$ for crizotinib (25 or 50 mg/kg) at 28 days versus the corresponding control value.

now shown that inhibition of MET by crizotinib had no substantial antitumor effect on lung cancer cells with an exon 14 deletion either in vitro or in vivo. In contrast to its effects in lung cancer cells with *MET* amplification, depletion of MET by RNA interference did not substantially affect the phosphorylation of AKT or ERK or induce apoptosis in lung cancer cells with either of the *MET* mutations studied. Our present study thus suggests that MET-TKIs may have little clinical efficacy in patients with lung cancer with a *MET* mutation.

Our analysis of the effects of crizotinib on the growth of lung cancer cells in vivo revealed that this agent had a pronounced antitumor effect in cells with *MET* amplification but not in those with a *MET* mutation or in those without amplification or mutation of *MET*. These in vivo data are consistent with our results obtained in vitro. In addition to amplification of *MET*, the MET pathway is also activated by HGF stimulation. Although lung cancer cells with *MET* mutations did not produce substantial amounts of HGF, we found that exogenous HGF stimulation tended to enhance the growth of such cells and that of cells without amplification or mutation of *MET* in vitro (see Figure, Supplemental Digital Content 1, http://links.lww.com/JTO/A98). Furthermore, this HGF-induced growth enhancement was inhibited by crizotinib. These data suggest that crizotinib may also exert antitumor activity in lung cancer without *MET* amplification under conditions of paracrine HGF-induced tumor growth. Our in vivo observations should, therefore, be interpreted with caution given the limitation that the local stromal environment of subcutaneous xenograft tumors in animals differs, at least in part, from that of human cancers in situ.

Crizotinib also inhibits oncogenic fusion variants of the tyrosine kinase ALK in addition to MET.[24] We found that crizotinib markedly inhibited the survival of H3122 lung cancer cells, which are positive for EML4-ALK, in addition to that of *MET* amplification-positive cells (see Figure, Supplemental Digital Content 2, http://links.lww.com/JTO/A99). Given that crizotinib has recently shown marked therapeutic efficacy in patients with lung cancer positive for EML4-ALK,[25] this agent may also have substantial clinical activity and be an attractive therapeutic option for patients with lung cancer with *MET* amplification.

In conclusion, our results have shown that crizotinib has pronounced effects on signal transduction and survival in lung cancer cells with *MET* amplification but not in those without *MET* amplification, including those with a *MET* mutation. Furthermore, both BIM induction and survivin down-regulation were found to mediate the proapoptotic effect of crizotinib in lung cancer cells with *MET* amplification. Our observations thus provide a rationale for clinical evaluation of MET-TKIs in patients with lung cancer with *MET* amplification.

## ACKNOWLEDGMENTS

*The authors thank E. Hatashita for technical assistance.*

## REFERENCES

1. Asahina H, Yamazaki K, Kinoshita I, et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with

epidermal growth factor receptor mutations. *Br J Cancer* 2006;95:998–1004.
2. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J Clin Oncol* 2008;26:2442–2449.
3. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344:1031–1037.
4. Okamoto I. Epidermal growth factor receptor in relation to tumor development: EGFR-targeted anticancer therapy. *FEBS J* 2010;277:309–315.
5. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363:1693–1703.
6. Masuya D, Huang C, Liu D, et al. The tumour-stromal interaction between intratumoral c-Met and stromal hepatocyte growth factor associated with tumour growth and prognosis in non-small-cell lung cancer patients. *Br J Cancer* 2004;90:1555–1562.
7. Nakamura Y, Niki T, Goto A, et al. c-Met activation in lung adenocarcinoma tissues: an immunohistochemical analysis. *Cancer Sci* 2007;98:1006–1013.
8. Zhao X, Weir BA, LaFramboise T, et al. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. *Cancer Res* 2005;65:5561–5570.
9. Go H, Jeon YK, Park HJ, et al. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. *J Thorac Oncol* 2010;5:305–313.
10. Krishnaswamy S, Kanteti R, Duke-Cohan JS, et al. Ethnic differences and functional analysis of MET mutations in lung cancer. *Clin Cancer Res* 2009;15:5714–5723.
11. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res* 2006;66:283–289.
12. Zillhardt M, Christensen JG, Lengyel E. An orally available small-molecule inhibitor of c-Met, PF-2341066, reduces tumor burden and metastasis in a preclinical model of ovarian cancer metastasis. *Neoplasia* 2010;12:1–10.
13. Zou HY, Li Q, Lee JH, et al. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res* 2007;67:4408–4417.
14. Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* 2008;14:4275–4283.
15. Lutterbach B, Zeng Q, Davis LJ, et al. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. *Cancer Res* 2007;67:2081–2088.
16. Okamoto K, Okamoto I, Okamoto W, et al. Role of survivin in EGFR inhibitor-induced apoptosis in non-small cell lung cancers positive for EGFR mutations. *Cancer Res* 2010;70:10402–10410.
17. Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. *Mol Cell* 2005;17:393–403.
18. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–776.
19. Costa DB, Halmos B, Kumar A, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med* 2007;4:1669–1679, discussion 1680.
20. Cragg MS, Kuroda J, Puthalakath H, et al. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLoS Med* 2007;4:1681–1689, discussion 1690.
21. Gong Y, Somwar R, Politi K, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLoS Med* 2007;4:e294.
22. Timofeevski SL, McTigue MA, Ryan K, et al. Enzymatic characterization of c-Met receptor tyrosine kinase oncogenic mutants and kinetic studies with aminopyridine and triazolopyrazine inhibitors. *Biochemistry* 2009;48:5339–5349.
23. Ma PC, Jagadeeswaran R, Jagadeesh S, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res* 2005;65:1479–1488.
24. Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol Cancer Ther* 2007;6:3314–3322.
25. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010;363:1693–1703.

 

# S-1 Plus Cisplatin with Concurrent Radiotherapy for Locally Advanced Non-small Cell Lung Cancer

## *A Multi-Institutional Phase II Trial (West Japan Thoracic Oncology Group 3706)*

*Yukito Ichinose, MD,* Takashi Seto, MD,* Tomonari Sasaki, MD,† Takeharu Yamanaka, PhD,‡ Isamu Okamoto, MD,§ Koji Takeda, MD,‖ Masahiro Tanaka, MD,¶ Nobuyuki Katakami, MD,# Toshiyuki Sawa, MD,** Shinzoh Kudoh, MD,†† Hideo Saka, MD,‡‡ Yasumasa Nishimura, MD,§§ Kazuhiko Nakagawa, MD,‖‖ and Masahiro Fukuoka, MD¶¶*

**Purpose:** To evaluate the combination chemotherapy using oral antimetabolite S-1 plus cisplatin (SP) with concurrent thoracic radiotherapy (RT) followed by the consolidation SP for locally advanced non-small cell lung cancer.

**Patients and Methods:** Patients with stage III non-small cell lung cancer, 20 to 74 years of age, and Eastern Cooperative Oncology Group performance status 0 to 1 were eligible. The concurrent phase consisted of full dose S-1 (orally at 40 mg/m$^2$/dose twice daily, on days 1–14) and cisplatin (60 mg/m$^2$ on day 1) repeated every 4 weeks for two cycles with RT delivered beginning on day 1 (60 Gy/30 fractions over 6 weeks). After SP-RT, patients received an additional two cycles of SP as the consolidation phase.

**Results:** Fifty-five patients were registered between November 2006 and December 2007. Of the 50 patients for efficacy analysis, the median age was 64 years; male/female 40/10; Eastern Cooperative Oncology Group performance status 0/1, 21/29; clinical stage IIIA/IIIB 18/32; and adenocarcinoma/others 20/30. There were 42 clinical responses including one complete response with an objective response rate of 84% (95% confidence interval [CI], 71–93%). The 1- and 2-year overall survival rates were 88% (95% CI, 75–94%) and 70% (95% CI, 55–81%), respectively. The median progression-free survival was 20 months. Of the 54 patients for safety analysis, common toxicities in the concurrent phase included grade 3/4 neutropenia (26%), thrombocytopenia (9%), and grade 3 esophagitis (9%) and febrile neutropenia (9%). In one patient, grade 3 pneumonitis was observed in the consolidation phase. There were two treatment-related deaths caused by infection in the concurrent phase.

**Conclusions:** SP-RT showed a promising efficacy against locally advanced NCSLC with acceptable toxicity.

**Key Words:** Concurrent chemoradiotherapy, Non-small cell lung cancer, Phase II trial, S-1, Cisplatin.

(*J Thorac Oncol.* 2011;6: 2069–2075)

The standard treatment modality in patients with unresectable stage III non-small cell lung cancer (NSCLC) is concurrent chemoradiotherapy.[1] Nevertheless, this combined treatment is associated with greater acute toxicity, including bone marrow[2] suppression, pneumonitis, and esophagitis,[2] compared with the sequential combination of chemotherapy and radiotherapy (RT). About a decade ago, we developed a concurrent chemoradiotherapy regimen using uracil-tegafur (UFT, Taiho Pharmaceutical Co., Ltd, Tokyo, Japan) plus cisplatin (UP) with concurrent thoracic RT (2 Gy per fraction, total 60 Gy) (UP-RT).[3] The response rate and median survival time of locally advanced unresectable stage III (IIIA 20%, IIIB 80%) patients treated with the UP-RT were 80% and 16.5 months, respectively, and these figures are similar to those reported in other concurrent chemoradiotherapy trials.[4,5] Nevertheless, the incidence of leukopenia and esophagitis of grade 3 or 4 was 16% and 3% of the patients, respectively,[3] and these figures are far lower than those of other trials.

S-1 (TS-1, Taiho Pharmaceutical Co., Ltd) is a second-generation oral anticancer agent based on uracil-tegafur, which has a dihydropyrimidine dehydrogenase (DPD) inhibitory fluoropyrimidine. S-1 comprises tegafur (a 5-FU Pro-

*Department of Thoracic Oncology, National Kyushu Cancer Center, Fukuoka; †Department of Radiation Oncology, National Kyushu Cancer Center, Fukuoka; ‡Institute for Clinical Research, National Kyushu Cancer Center, Fukuoka; §Department of Medical Oncology, Kinki University Faculty of Medicine, Osakasayama; ‖Department of Clinical Oncology, Osaka City General Hospital, Osaka; ¶Department of Radiation Oncology, Osaka City General Hospital, Osaka; #Department of Integrated Oncology, Institute of Biomedical Research and Innovation, Kobe; **Division of Respiratory Medicine and Medical Oncology, Gifu Municipal Hospital, Gifu; ††Department of Respiratory Medicine, Osaka City University Medical School, Osaka; ‡‡Department of Medical Oncology, Nagoya Medical Center, Nagoya; §§Department of Radiation Oncology, Kinki University Faculty of Medicine, Osakasayama; ‖‖Department of Medical Oncology, Kinki University Faculty of Medicine, Osakasayama; and ¶¶Izumi City Hospital, Izumi, Japan.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Yukito Ichinose, Department of Thoracic Oncology, National Kyushu Cancer Center, 3-1-1, Notame, Minami-ku, Fukuoka 811-1395, Japan. E-mail: yichinos@nk-cc.go.jp



ISSN: 1556-0864/11/0612-2069

drug), 5-chloro-2, 4-dihydroxypyridine (an inhibitor of DPD), and potassium oxonate (an inhibitor of phosphoribosyl transferase), in a molar ratio of 1:0.4:1 and has been shown to induce a comparable response to the other single agents for metastatic NSCLC.[6] Furthermore, combination chemotherapy using S-1 plus cisplatin (SP) for advanced NSCLC has been reported to show a response rate of 33 to 47% and a median survival time of 11 to 16 months.[7,8] Those data were better than the usual response rate of 29 to 38% and the median survival time of 8 to 13 months for the combination chemotherapeutic regimens using UP,[9,10] whereas the frequency of severe hematological and nonhematological adverse events induced by both UP and SP was lower than that of other platinum-based combination regimens such as carboplatin plus paclitaxel (CP), cisplatin plus docetaxel, and so on.[11–13] In addition, West Japan Oncology Group (WJOG) recently demonstrated that chemotherapy using S-1 plus carboplatin was noninferior in terms of overall survival (OS) compared with CP in advanced NSCLC.[14]

The above-mentioned observations indicated that it might be possible to use the same dose of SP as is used for metastatic advanced NSCLC for the treatment of locally advanced NSCLC with concurrent thoracic RT, similar to UP-RT. If this is possible, SP and concurrent thoracic RT (SP-RT) would be expected to provide several advantages over UP-RT. First, SP could have stronger antitumor activity for both locally advanced NSCLC and micrometastatic lesions than UP. Second, although both cisplatin and 5-FU have been reported to have a radiosensitizing effect,[15,16] the level of the latter in the blood by SP could not only be maintained at a higher level than by UP[17,18] but also 5-chloro-2, 4-dihydroxypyridine in S-1 has been recently reported to have a radiosensitizing effect as well as a strong DPD activity.[19,20] A single-institutional experience with SP-RT in 11 patients was reported showing that all the patients had a partial response, with acceptable hematological and nonhematological toxicities. On the basis of these findings, the WJOG (formally, West Japan Thoracic Oncology Group) conducted a multiinstitutional phase II trial to confirm the antitumor effects and safety of SP-RT.

## PATIENTS AND METHODS

### Eligibility Criteria

The eligibility requirements for enrollment in this phase II trial were cytologically or histologically confirmed, unresectable stage III NSCLC, for which radical dose RT could be prescribed. The staging was performed according to the 6th edition of tumor, node, metastasis (TNM). All patients were required to meet the following criteria: measurable disease; an Eastern Cooperative Oncology Group performance status of 0 or 1; a projected life expectancy of more than 3 months; a leukocyte count of $\geq$4000/$\mu$L; a platelet count of $\geq$100,000/$\mu$L; a blood gas oxygen level of $\geq$70 torr; a serum bilirubin level below 1.5 mg/dL; serum glutamic oxaloacetic transaminase/glutamic pyruvic transaminase levels of no more than 100 IU/ml; a creatinine level of $\leq$1.2 mg/dL; and a creatinine clearance level of $\geq$60 mL/min. Other eligibility criteria included no prior treatment and an age <75 years. All eligible patients underwent computed tomography (CT) scans of the thorax and upper abdomen and a radioisotope bone scan. Patients who had malignant pleural effusion, malignant pericardial effusion, a concomitant malignancy, or serious concomitant diseases were excluded from the study. Written informed consent was required from all patients, and the protocol was approved by the institutional ethics committee of each participating institute. All data were centrally monitored by the WJOG datacenter. This study is registered with the University Hospital Medical Information Network in Japan (number 000001370).

### Treatment Schedule

#### Chemotherapy with SP

S-1 (40 mg/m$^2$/dose) in the form of 20 and 25 mg capsules containing 20 and 25 mg of tegafur, respectively, were taken orally twice a day after meals between days 1 and 14 as follows: in a patient with a body surface area (BSA) <1.25 m$^2$, 40 mg twice daily; for those with BSA 1.25 m$^2$, but <1.5 m$^2$, 50 mg twice daily; and BSA >1.5 m$^2$, 60 mg twice daily. Cisplatin (60 mg/m$^2$) was administered as a $\geq$120-minute infusion on day 1. The patients were also hydrated with 1000 to 2000 mL saline by infusion before cisplatin was administered. An antiemetic agent was administered at the discretion of each patient's physician.

The combination chemotherapy with SP was repeated twice, with a 4-week interval, concurrently with thoracic RT (SP-RT). At 2 to 4 weeks after the completion of the concurrent chemoradiotherapy, two further cycles of the same SP regimen were administered as a consolidation chemotherapy as shown in Figure 1.

A leukocyte count of 3000/$\mu$L or greater and the entry eligibility criteria regarding organ functions had to be satisfied for the patients to start the next cycle. If these criteria were satisfied 4 weeks after day 1 of each cycle of chemotherapy, the next cycle was administered. The doses of S-1 were adjusted according to the degree of hematological and nonhematological toxicity. The dose was reduced by one level (20 mg day) in patients whose BSA was 1.25 m$^2$ or more if there was evidence of grade 4 hematologic toxicity or grade 3 or more nonhematological toxicity during any cycle

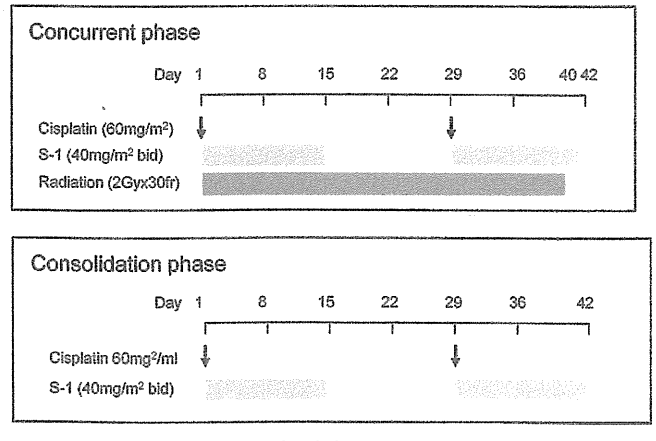


**FIGURE 1.** Treatment schedule.

of administration. If recovery from such toxicities was confirmed at a reduced dose, administration at the reduced dose was continued. If a patient with a BSA less than 1.25 $m^2$ experienced the above toxicities, then no further treatment with S-1 was performed. If a rest period of more than 4 weeks between two chemotherapy cycles of concurrent and consolidation phases was required or if the consolidation chemotherapy could not start within 6 weeks after SP-RT, then the SP treatment was discontinued.

### Radiotherapy

All patients were treated with a linear accelerator photon beam of 6 MV or more from day 1. The primary tumor and involved nodal disease received 60 Gy in 2-Gy fractions over a period of 6 weeks. In this protocol, both 2- and 3-dimensional (D) treatment planning systems were allowed. The radiation doses were specified at the center of the target volume. The doses were calculated assuming tissue homogeneity without correcting for lung tissues for both 2- and 3-D treatment planning. Among the 54 patients assessable for toxicity, 2- and 3-D treatment planning were performed for 7 and 47 patients, respectively. The initial 40 Gy/20 fractions were delivered to clinical target volume 1 (CTV1), and the final 20 Gy/10 fractions were delivered to a reduced volume defined as clinical target volume 2 (CTV2). CTV1 included the primary tumor, ipsilateral hilum, and mediastinal nodal areas from the paratracheal (no. 2) to subcarinal lymph nodes (no. 7). For the primary tumors and the involved lymph nodes of 1 cm or more larger in the shortest diameter, a margin of at least 0.5 cm was added. The contralateral hilum was not included in CTV1. The supraclavicular areas were not treated routinely but were treated when the supraclavicular nodes were involved. CTV2 included only the primary tumor and the involved lymph nodes, with a margin of 0.5 to 1 cm. The spinal cord was excluded from the fields for CTV2 by appropriate methods, such as the oblique opposing method. The appropriate planning target volume margin and leaf margin were added for CTV1 and CTV2. When grade 4 hematologic toxicity, grade 3 to 4 esophagitis or dermatitis, pyrexia of ≥38°C, or a decrease in the partial pressure of arterial oxygen of 10 torr or more were compared with that before RT occurred, RT was interrupted. If a rest period of more than 2 weeks was required, then the patient was withdrawn from the study.

### Evaluation of the Response and Toxicity

All registered patients, excluding those withdrawn from the study, received the following evaluations. Chest x-rays, complete blood cells, and blood chemistry studies were repeated once a week during the treatment period. Thoracic CT was performed every 1 or 2 months during the treatment period. After the treatment, a thoracic CT was obtained every 6 months, and other imaging examinations were obtained when recurrence was suspected. The response was evaluated in accordance with the RECIST version 1.0 guidelines.[21] In this study, the results of the response which an investigator determined were not used, and all responses were confirmed by the board members of the independent response review.

During the evaluation of both the initial staging and the antitumor effects, an extramural review was conducted. Only patients whose initial clinical stage was judged to be stage IIIA and IIIB and who were eligible for the study were analyzed for the response to treatment. The toxicity for all patients who received any treatment was assessed and graded by using the National Cancer Institute Common Terminology Criteria for Adverse Event version 3.

### Statistical Analysis

The primary end point of this study was the objective tumor response rate. On the basis of the assumption that a response rate of higher than 80% would be expected from the combined modality treatment, while a rate below 60% would make a further investigation unnecessary, a sample size of 49 patients was required by the exact binomial test with a one-sided alpha error of 0.05 and a beta error of 0.1. Therefore, a total of 55 patients was the planned accrual size in view of possibly including ineligible patients. For determining the response rate, the exact binomial confidence interval (CI) was calculated. OS was defined as the time from registration until death from any cause. Progression-free survival (PFS) was defined as the time between registration and disease progression or death. The Kaplan-Meier method was used to estimate OS and PFS curves. All statistical analyses were done with SAS version 9.1.

## RESULTS

### Characteristics of Patients

Between November 2006 and December 2007, a total of 55 patients were enrolled from 18 institutes. One patient withdrew his consent and four patients were found to be ineligible by the extramural review (one malignant effusion, one carcinomatous lymphangitis, and 2 stage IV diseases). Therefore, the efficacy analyses were performed for the 50 remaining eligible patients. Safety analyses were performed for 54 patients who underwent SP-RT. Table 1 shows that 80% of the 50 eligible patients were male, with a mean age of 63 years (range, 40–74 years). Squamous cell carcinoma was the most common histological diagnosis, including 48% of the patients, and most patients had clinical stage IIIB disease (IIIA versus IIIB; 36% versus 64%). The most frequently classified TNM categories were T1-3N2 (36%), T1-3N3M0 (28%), and T4N0-1M0 (18%).

### Adverse Events

The major adverse events (grade 3 and 4 toxicities) of SP-RT are listed in Table 2. Among the hematologic toxicities of the concurrent phase, grade 3 or higher leukopenia and neutropenia was observed in 17 patients (32%) and 14 patients (26%), respectively. Five patients (9%) developed grade 3 or higher thrombocytopenia. Among the nonhematologic toxicities, grade 3 and 4 febrile neutropenia was observed in four (7%) and one (2%) patient, respectively, whereas grade 3 esophagitis occurred in 4 patients (7%). Although no cases of severe pneumonitis occurred in the concurrent phase, two patients had a treatment-related death: one patient died of sepsis soon after the completion of the

**TABLE 1. Patient Characteristics**

| | |
|---|---|
| No. of eligible patients | 50 |
| Age, yrs | |
| Mean (range) | 63 (40–74) |
| Gender | |
| Male | 40 (80%) |
| Female | 10 (20%) |
| ECOG PS | |
| 0 | 21 (42%) |
| 1 | 29 (48%) |
| Smoking history | |
| Absent | 2 (4%) |
| Present | 48 (96%) |
| Histology | |
| Squamous cell carcinoma | 24 (48%) |
| Adenocarcinoma | 20 (40%) |
| Others | 6 (12%) |
| cTNM | |
| Stage IIIA | 18 (36%) |
| T1-3N2 | 18 (36%) |
| Stage IIIB | 32 (64%) |
| T1-3N3M0 | 14 (28%) |
| T4N0-1M0 | 9 (18%) |
| T4N2M0 | 7 (14%) |
| T4N3M0 | 2 (4%) |
| Location of primary site | |
| Upper lobe | 36 (72%) |
| Middle lobe | 4 (8%) |
| Lower lobe | 8 (16%) |
| Others | 2 (4%) |

ECOG, Eastern Cooperative Oncology Group; PS, performance status; TNM, tumor, node, metastasis.

**TABLE 2. Hematological and Nonhematological Major Adverse Events**

| | Concurrent Chemoradiotherapy ($n$ = 54) | | | Consolidation Chemotherapy ($n$ = 39) | | |
|---|---|---|---|---|---|---|
| | Grade | | | Grade | | |
| Toxicities | 3 | 4 | Frequency of 3 or 4 (%) | 3 | 4 | Frequency of 3 or 4 (%) |
| Hematological | | | | | | |
| Leukopenia | 12 | 5 | 31.5 | 6 | 0 | 15.4 |
| Neutropenia | 10 | 4 | 25.9 | 4 | 0 | 10.3 |
| Thrombocytopenia | 1 | 4 | 9.3 | 4 | 0 | 10.3 |
| Anemia | 4 | 2 | 11.1 | 7 | 1 | 20.5 |
| Nonhematological | | | | | | |
| Febrile neutropenia | 4 | 1 | 9.3 | 0 | 0 | |
| Nausea | 1 | 1 | 3.7 | 0 | 0 | |
| Vomiting | 2 | 0 | 3.7 | 0 | 0 | |
| Anorexia | 6 | 1 | 13.0 | 0 | 0 | |
| Creatinine | 0 | 0 | | 0 | 0 | |
| AST/ALT | 1 | 1 | 3.7 | 0 | 0 | |
| Diarrhea | 2 | 0 | 3.7 | 0 | 0 | |
| Stomatitis | 1 | 0 | 1.9 | 0 | 0 | |
| Pneumonitis | 0 | 0 | | 1 | 0 | 2.6 |
| Esophagitis | 4 | 0 | 7.4 | 1 | 0 | 2.6 |

AST, aspartate transaminase; ALT, alanine aminotransferase.

**TABLE 3. Radiation Delivered ($N$ = 50)**

| | |
|---|---|
| Radiation dose (Gy) | |
| Median (range) | 60.0 (28–60) |
| Average | 58.4 |
| Reasons for interruption, $N$ (%) | |
| Adverse events | 7 (14.0) |
| Other | 2 (4.0) |
| Rate of completion of treatment with 60 Gy, $N$ (%) | 47 (94.0) |

**TABLE 4. Chemotherapy Delivered ($N$ = 50)**

| | $N$ (%) |
|---|---|
| Concurrent chemotherapy | |
| Chemotherapy cycles | |
| 1 | 50 (100) |
| 2 | 46 (92.0) |
| Reasons for discontinuation | |
| Adverse event | 2 (4.0) |
| Patient decision | 2 (4.0) |
| Reasons for not proceeding to consolidation chemotherapy | |
| Adverse event | 8 (16.0)[a] |
| Other | 1 (2.0) |
| Consolidation chemotherapy | |
| Chemotherapy cycles | |
| 1 | 37 (74.0) |
| 2 | 31 (62.0) |
| Reasons for discontinuation | |
| Adverse event | 5 (10.0) |
| Disease progression | 1 (4.0) |
| Rate of completion of 4 cycles of treatment (95% CI) | 62% (47.2–75.3) |

[a] Two treatment-related deaths were included after completion of concurrent chemotherapy.
CI, confidence interval.

concurrent phase and the other patient died of pneumonia after the recovery from the bone marrow suppression because of that phase.

Thirty-nine (72%) of the 54 patients proceeded to consolidation chemotherapy. As shown in Table 2, the frequency of grade 3 or 4 in any major toxicity caused by consolidation chemotherapy was lower than that in concurrent chemoradiotherapy, except for anemia and pneumonitis. It was of note that no febrile neutropenia was observed.

## Treatments Delivered to Eligible Patients

Tables 3 and 4 show RT and chemotherapy delivered to 50 eligible patients, respectively. Forty-six patients (92%) completed two cycles of SP concurrent with thoracic RT of 60 Gy. Two patients refused further protocol treatment after one cycle of chemotherapy because of adverse events. The other two patients did not meet the criteria to start the second cycle of SP because of prolonged neutropenia. Although 46 patients completed the concurrent phase of the SP-RT, seven patients could not proceed to the consolidation phase because of mainly prolonged hematological toxicity, and two patients

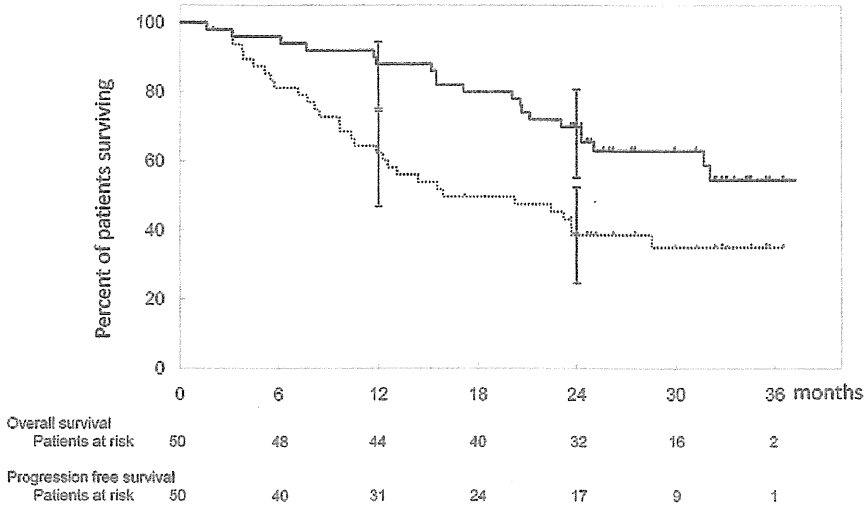


**FIGURE 2.** Overall survival (——) and progression-free survival (·····). Each *tick* represents one patient who is alive with/without recurrence. The *bars* represent the 95% confidence intervals of the survival rate at 1 and 2 years after treatment.

were lost to treatment-related death. Of 37 patients, one and five patients received only one cycle of the consolidation chemotherapy because of disease progression and adverse events, respectively. Thus, a total of 31 (62%) of the 50 eligible patients received all four cycles of SP chemotherapy.

### Response

Of the 50 patients eligible for efficacy analysis, 42 patients had responses (84%; 95% CI, 71–93%; $p < 0.0001$), including 1 patient with a complete response, and 2 patients with stable disease. Only one patient showed progressive disease. Five patients were unevaluable for a response. There were no differences in the response rate by histology (88% in squamous cell carcinoma versus 81% in others, $p = 0.704$ by the exact binomial test).

### Survival

The overall median follow-up time for the 29 patients who were still alive as of January 2010 was 28 months (range, 24–37 months). As shown in Figure 2, the median PFS and OS was 20 months and not reached, respectively, and the OS rates at 1 and 2 years were 88% (95% CI, 75–94%) and 70% (95% CI, 55–81%), respectively.

### Sites of First Failures

With respect to the sites of first failure among the 28 (56%) patients with disease progression of the 50 eligible patients, 19 (38%), 6 (21%), and 3 (6%) patients had distant metastases, intrathoracic local diseases, and both, respectively. Those nine occurred in the irradiated field. The frequently observed initial distant metastases were observed in bone in eight patients and in brain and lung in four each. Only four patients (8%) developed a brain metastasis alone as the initial failure site.

## DISCUSSION

The purpose of concurrent chemoradiotherapy for NSCLC patients with stage III disease is to achieve local control, for which RT plays the main role, and also to eradicate occult distant metastases by chemotherapy. For the latter purpose, the development of regimens that can allow administration of the systemic (full) doses of chemotherapy during RT is necessary. Although the so-called "third generation" agents such as paclitaxel, vinorelbine, docetaxel, and gemcitabine have been evaluated in several concurrent studies in combination with platinum compounds, a lower dose of that agent plus the platinum compound has generally been used due to toxicities. Therefore, induction chemotherapy with sufficient systemic doses of the agents was considered a potentially effective addition to the concurrent chemoradiotherapy.[22] Nevertheless, a recent randomized trial (CALGB 39801) showed that two cycles of induction chemotherapy with full doses of CP did not provide a survival benefit over concurrent chemoradiotherapy alone, using weekly CP at lower doses.[23] Furthermore, the randomized phase III trial conducted by the Hoosier Oncology Group and U.S. Oncology Group demonstrated that the addition of consolidation chemotherapy using docetaxel after full-dose chemotherapy using cisplatin plus etoposide with concurrent RT (PE-RT) failed to achieve the primary end point of improved survival compared with PE-RT alone.[24] On the basis of these randomized trials, concurrent chemotherapy alone is recommended for the treatment of locally advanced-NSCLC. However, the optimal chemotherapy regimen remains to be determined.

In this study, SP-RT using systemic doses had the advantage of eradicating occult distant metastases. In addition, 5-FU has been reported to have a radiosensitizing effect in preclinical and clinical studies of various cancers, including NSCLC,[15,16] and S-1 is orally administered for 14 consecutive days in each course of chemotherapy, providing long-term potential radiosensitization. The antitumor effects of SP-RT might explain the high response rate of 82% and the prolonged median PFS of 20 months, as well as the median OS, which was not reached when follow-up time ranged from 24 to 37 months. Another SP-RT phase II trial with a similar schedule and dose, which was conducted during almost the same period as the present trial, also demonstrated a good overall response rate of 88%, median PFS of 12 months, and a median OS of 33 months, whereas the median follow-up

time was 25 months, ranging from 12 to 38 months.[25] Nevertheless, these data cannot be directly compared with our data. In this trial, the extraordinarily good results may not be only because of the chemotherapy regimen but also to the high frequency of the primary site being within the upper lobe. In completely resected NSCLC with N2 disease, the 5-year survival rate in patients with their primary site in the upper lobe is well known to be significantly better than that of patients with the primary tumor in the lower lobe.[26] In addition, the tumors in the upper lobe with upper mediastinal nodal metastases are easier to treat with RT than the tumors in the lower lobe in terms of the irradiation field.

Two additional cycles of the same chemotherapy after concurrent chemoradiotherapy were called consolidation chemotherapy in this trial. Although the original PE-RT regimen used two additional cycles of the same PE after PE (two cycles)-RT, the above-mentioned randomized trial did not use consolidation PE in both control and experimental groups.[24] Similarly, the original mitomycin, vindesine plus cisplatin (MVP)-RT regimen had the two additional cycles of the same MVP[5] after MVP (two cycles)-RT, whereas a recent randomized trial used MVP (two cycles)-RT alone as a control arm.[27] The median OS of PE (2 cycles)-RT and MVP (2 cycles)-RT was 23.2 and 23.7 months, respectively.[24,27] In addition, only 41% of the patients could complete four cycles of MVP in the MVP-RT group of a recent WJTOG phase III trial (WJTOG0105), which had a median OS of 20.5 months.[28] In this trial, 62% of the patients completed four cycles of SP despite a low frequency of severe toxicities, whereas the WJOG phase III trial showed that the safest regimen with concurrent RT was CP among MVP, CP, and carboplatin plus CPT-11, although the completion rate of two cycles in the consolidation chemotherapy of CP arm was only 50%.[28] These observations suggest that a phase III trial is necessary to clarify whether or not a total of four cycles of chemotherapy in this setting provides a better result than two cycles of chemoradiotherapy.

The irradiated dose of 60 Gy in 30 fractions with concurrent chemotherapy is currently used in the majority of institutes in Japan, whereas that of 66 Gy in 33 fractions in combination with chemotherapy in the United States seems to be the most common treatment regimen. Because PET/CT scan and 3-D planning were not used in all patients, it would therefore be interesting to elucidate whether or not the present survival of such patients can be prolonged by these techniques, including a total irradiated doses of 66 Gy.

Although the present treatment with SP-RT should be acceptably safe in terms of the frequency of grade 3 and 4 adverse events, the treatment-related death of two patients was observed. Therefore, it is necessary to keep in mind that there is no totally safe regimen for concurrent chemoradiotherapy. At present, the WJOG is conducting a randomized phase II trial comparing SP-RT to combination chemotherapy using cisplatin plus vinorelbine with concurrent RT.

## REFERENCES


1. Pfister DG, Johnson DH, Azzoli CG, et al; American Society of Clinical Oncology. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. *J Clin Oncol* 2004;22:330–353.
2. Auperin A, Le Pechoux C, Rolland E, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. *J Clin Oncol* 2010;28:2181–2190.
3. Ichinose Y, Nakai Y, Kudoh S, et al. Uracil/tegafur plus cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer: a multi-institutional phase II trial. *Clin Cancer Res* 2004;10:4369–4373.
4. Curran W, Scott C, Langer C. Phase III comparison of sequential vs concurrent chemoradiation for pts with unresected stage III non-small cell lung cancer (NSCLC): Initial report of Radiation Therapy Oncology Group (RTOG) 9410. *Lung Cancer* 2000;29(93s, suppl):abstr 303.
5. Furuse K, Fukuoka M, Kawahara M, et al. Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small-cell lung cancer. *J Clin Oncol* 1999;17:2692–2699.
6. Kawahara M, Furuse K, Segawa Y, et al; S-1 Cooperative Study Group (Lung Cancer Working Group). Phase II study of S-1, a novel oral fluorouracil, in advanced non-small-cell lung cancer. *Br J Cancer* 2001;85:939–943.
7. Ichinose Y, Yoshimori K, Sakai H, et al. S-1 plus cisplatin combination chemotherapy in patients with advanced non-small cell lung cancer: a multi-institutional phase II trial. *Clin Cancer Res* 2004;10:7860–7864.
8. Kubota K, Sakai H, Yamamoto N, et al. A multi-institution phase I/II trial of triweekly regimen with S-1 plus cisplatin in patients with advanced non-small cell lung cancer. *J Thorac Oncol* 2010;5:702–706.
9. Ichinose Y, Takanashi N, Yano T, et al. A phase II trial of oral tegafur and uracil plus cisplatin in patients with inoperable nonsmall cell lung cancer. *Cancer* 1995;75:2677–2680.
10. Ichinose Y, Yosimori K, Yoneda S, et al. UFT plus cisplatin combination chemotherapy in the treatment of patients with advanced nonsmall cell lung carcinoma: a multiinstitutional phase II trial. *Cancer* 2000;88:318–323.
11. Kubota K, Watanabe K, Kunitoh H, et al; Japanese Taxotere Lung Cancer Study Group. Phase III randomized trial of docetaxel plus cisplatin versus vindesine plus cisplatin in patients with stage IV non-small-cell lung cancer: the Japanese Taxotere Lung Cancer Study Group. *J Clin Oncol* 2004;22:254–261.
12. Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. *Ann Oncol* 2007; 18:317–323.
13. Schiller JH, Harrington D, Belani CP, et al; Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92–98.
14. Okamoto I, Yoshioka H, Morita S, et al. Phase III trial comparing oral S-1 plus carboplatin with paclitaxel plus carboplatin in chemotherapy-naive patients with advanced non-small-cell lung cancer: results of a west Japan oncology group study. *J Clin Oncol* 2010;28:5240–5246.
15. Byfield JE, Calabro-Jones P, Klisak I, et al. Pharmacologic requirements for obtaining sensitization of human tumor cells in vitro to combined 5-Fluorouracil or ftorafur and x rays. *Int J Radiat Oncol Biol Phys* 1982;8:1923–1933.
16. Douple EB, Richmond RC. Enhancement of the potentiation of radiotherapy by platinum drugs in a mouse tumor. *Int J Radiat Oncol Biol Phys* 1982;8:501–503.
17. Gemma A, Kudoh S, Yoshimura A, et al. Pilot trial of a combination comprising of consecutive oral administration of UFT, and two-divided administration of CDDP in non-small cell lung cancer. *Anticancer Res* 1995;15:2691–2695.
18. Koizumi W, Tanabe S, Saigenji K, et al. Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. *Br J Cancer* 2003;89:2207–2212.
19. Fukushima M, Sakamoto K, Sakata M, et al. Gimeracil, a component of S-1, may enhance the antitumor activity of X-ray irradiation in human cancer xenograft models in vivo. *Oncol Rep* 2010;24:1307–1313.
20. Takagi M, Sakata K, Someya M, et al. Gimeracil sensitizes cells to radiation via inhibition of homologous recombination. *Radiother Oncol* 2010;96:259–266.
21. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of

the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.
22. Vokes EE, Herndon JE II, Crawford J, et al. Randomized phase II study of cisplatin with gemcitabine or paclitaxel or vinorelbine as induction chemotherapy followed by concomitant chemoradiotherapy for stage IIIB non-small-cell lung cancer: cancer and leukemia group B study 9431. *J Clin Oncol* 2002;20:4191–4198.
23. Vokes EE, Herndon JE II, Kelley MJ, et al; Cancer and Leukemia Group B. Induction chemotherapy followed by chemoradiotherapy compared with chemoradiotherapy alone for regionally advanced unresectable stage III Non-small-cell lung cancer: Cancer and Leukemia Group B. *J Clin Oncol* 2007;25:1698–1704.
24. Hanna N, Neubauer M, Yiannoutsos C, et al; Hoosier Oncology Group; US Oncology. Phase III study of cisplatin, etoposide, and concurrent chest radiation with or without consolidation docetaxel in patients with inoperable stage III non-small-cell lung cancer: the Hoosier Oncology Group and U.S. Oncology. *J Clin Oncol* 2008;26:5755–5760.
25. Ohyanagi F, Yamamoto N, Horiike A, et al. Phase II trial of S-1 and cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer. *Br J Cancer* 2009;101:225–231.
26. Ichinose Y, Kato H, Koike T, et al; Japanese Clinical Oncology Group. Completely resected stage IIIA non-small cell lung cancer: the significance of primary tumor location and N2 station. *J Thorac Cardiovasc Surg* 2001;122:803–808.
27. Segawa Y, Kiura K, Takigawa N, et al. Phase III trial comparing docetaxel and cisplatin combination chemotherapy with mitomycin, vindesine, and cisplatin combination chemotherapy with concurrent thoracic radiotherapy in locally advanced non-small-cell lung cancer: OLCSG 0007. *J Clin Oncol* 2010;28:3299–3306.
28. Yamamoto N, Nakagawa K, Nishimura Y, et al. Phase III study comparing second- and third-generation regimens with concurrent thoracic radiotherapy in patients with unresectable stage III non-small-cell lung cancer: West Japan Thoracic Oncology Group WJTOG0105. *J Clin Oncol* 2010;28:3739–3745.

 

International Journal of
Radiation Oncology
biology • physics

www.redjournal.org

Clinical Investigation

# A Phase I Study of Chemoradiotherapy with Use of Involved-Field Conformal Radiotherapy and Accelerated Hyperfractionation for Stage III Non—Small-Cell Lung Cancer: WJTOG 3305

**Takuhito Tada, M.D.,[*,†] Yasutaka Chiba, M.D.,[‡] Kayoko Tsujino, M.D.,[§] Haruyuki Fukuda, M.D.,[‖] Yasumasa Nishimura, M.D.,[¶] Masaki Kokubo, M.D., Ph.D.,[**] Shunichi Negoro, M.D.,[††] Shinzoh Kudoh, M.D.,[‡‡] Masahiro Fukuoka, M.D.,[§§] Kazuhiko Nakagawa, M.D., Ph.D.,[‖‖] and Yoichi Nakanishi, M.D.[¶¶]**

*Departments of [*]Radiology and [‡‡]Respiratory Medicine, Osaka City University Graduate School of Medicine, Osaka; Departments of [†]Radiology and [§§]Medical Oncology, Izumi Municipal Hospital, Izumi; Departments of [‡]Environmental Medicine and Behavioural Science, [¶]Radiation Oncology, and [‖‖]Medical Oncology, Kinki University Faculty of Medicine, Osaka-sayama; Departments of [§]Radiation Oncology and [††]Medical Oncology, Hyogo Cancer Center, Akashi; [‖]Department of Radiology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino; [**]Division of Radiation Oncology, Institute of Biomedical Research and Innovation, Kobe; and [¶¶]Research Institute for Disease of the Chest, Graduate School of Medical Science, Kyusyu University, Fukuoka, Japan*

Received Jan 21, 2011, and in revised form May 19, 2011. Accepted for publication Jun 12, 2011

**Summary**

This phase I study of chemoradiotherapy used involved-field conformal radiotherapy with accelerated Twice-daily hyperfractionation in patients with stage III non-small cell lung cancer. Although the dose of radiation was escalated to 72 Gy in 48 fractions, the maximum tolerated dose was not reached.

**Purpose:** A Phase I study to determine a recommended dose of thoracic radiotherapy using accelerated hyperfractionation for unresectable non—small-cell lung cancer was conducted.
**Methods and Materials:** Patients with unresectable Stage III non—small-cell lung cancer were treated intravenously with carboplatin (area under the concentration curve 2) and paclitaxel (40 mg/m$^2$) on Days 1, 8, 15, and 22 with concurrent twice-daily thoracic radiotherapy (1.5 Gy per fraction) beginning on Day 1 followed by two cycles of consolidation chemotherapy using carboplatin (area under the concentration curve 5) and paclitaxel (200 mg/m$^2$). Total doses were 54 Gy in 36 fractions, 60 Gy in 40 fractions, 66 Gy in 44 fractions, and 72 Gy in 48 fractions at Levels 1 to 4. The dose-limiting toxicity, defined as Grade $\geq$4 esophagitis and neutropenic fever and Grade $\geq$3 other nonhematologic toxicities, was monitored for 90 days.
**Results:** Of 26 patients enrolled, 22 patients were assessable for response and toxicity. When 4 patients entered Level 4, enrollment was closed to avoid severe late toxicities. Dose-limiting toxicities occurred in 3 patients. They were Grade 3 neuropathy at Level 1 and Level 3 and Grade 3 infection at Level 1. However, the maximum tolerated dose was not reached. The median survival time was 28.6 months for all patients.

Reprint requests to: Takuhito Tada, M.D., Department of Radiology, Izumi Municipal Hospital, 4-10-10 Fuchucho, Izumi City, 594-0071, Japan. Tel: (+81) 725411331; Fax: (+81) 725433350; E-mail: tada@msic.med.osaka-cu.ac.jp

Presented in part at the 52nd Annual Meeting of the American Society of Radiation Oncology, Oct 31 to Nov 4, 2010, San Diego, CA.

Conflict of interest: M. Kokubo is an advisor to Mitsubishi Heavy Industries Ltd. M. Fukuoka receives honoraria from Astrozeneca, Chugai Pharmaceutical Co., Boehringer Ingelheim, and Daiichisankyo. Y. Nakanishi receives research funding from Bristol-Myers. The authors declare no other conflict of interest.


0360-3016/$ - see front matter 
doi:10.1016/j.ijrobp.2011.06.1991

**Conclusions:** The maximum tolerated dose was not reached, although the dose of radiation was escalated to 72 Gy in 48 fractions. However, a dose of 66 Gy in 44 fractions was adopted for this study because late toxicity data were insufficient. © 2011 Elsevier Inc.

**Keywords:** Non–small-cell lung cancer, Chemoradiation, Three-dimensional conformal radiotherapy, Accelerated hyperfractionation, Dose escalation

## Introduction

For the treatment of locally advanced inoperable non–small-cell lung cancer (NSCLC), concurrent chemoradiotherapy has shown significantly better survival than sequential therapy (1–4). Even in concurrent chemoradiotherapy, however, locoregional control is unsatisfactory at a standard dose of 56 to 60 Gy (3, 5–7). To improve locoregional control, several dose escalation trials have been performed using three-dimensional (3D) planning techniques, and it has been suggested that 74 Gy is tolerable with concurrent or sequential chemotherapy (8–11).

In conventional fractionation, the benefits of dose escalation are considered limited. Irradiation at a dose of 74 Gy in conventional fractionation requires more than 7 weeks. Even at standard doses, accelerated repopulation is induced during the later part of radiation therapy and is a cause of radiation failure (12). When the treatment time is prolonged, the influence of accelerated repopulation becomes more evident.

Therefore, dose escalation without prolonged treatment time is supposed to bring better outcome, and accelerated hyperfractionation seems to be an effective strategy for shortening treatment time. As the first step to verify the hypothesis, the West Japan Thoracic Oncology Group designed a Phase I trial to define the maximum tolerated dose (MTD) of 3D conformal radiotherapy (CRT) using accelerated hyperfractionation in NSCLC patients.

## Methods and Materials

### Eligibility

The patient eligibility was as follows: histologic or cytologic diagnosis of NSCLC, unresectable Stage IIIA or IIIB disease, age less than 75 years, Eastern Cooperative Oncology Group performance score of 0 to 1, and function as shown by laboratory determinations including leukocyte count of at least 4,000/$mm^3$, hemoglobin concentration of at least 9.5 g/dL, platelet count of at least 100,000/$mm^3$, aspartate aminotransferase and alanine aminotransferase of 2.0 times the upper limit of normal range or less, serum total bilirubin of 1.5 mg/dL or less, serum creatinine of 1.5 mg/dL or less, and $PaO_2$ at rest of at least 70 mm Hg.

The patients were ineligible if they met any of the following criteria: supraclavicular nodal metastases, interstitial pneumonitis or pulmonary fibrosis, prior thoracic radiation therapy, malignant pleural effusion or malignant pericardial effusion, active concomitant malignancy or recent (<3 years) history of any malignancy, or other serious concomitant medical conditions. The study protocol was approved by each institutional review board for clinical use. All patients gave written informed consent before enrollment.

### Patient assessment

All patients underwent a complete medical history and physical examination. Imaging studies, including chest X-ray, computed tomography of the chest and upper abdomen, computed tomography or magnetic resonance imaging of the brain, and positron emission tomography, were required.

### Treatment schedule

The patients received concurrent chemoradiotherapy using accelerated hyperfractionation. On Days 1, 8, 15, and 22, carboplatin (area under the concentration curve 2 using the Calvert equation) and paclitaxel (40 mg/$m^2$) were administered intravenously.

After the concurrent chemoradiotherapy, the patients received two cycles of consolidation chemotherapy consisting of carboplatin (area under the concentration curve 5) and paclitaxel (200 mg/$m^2$) with an interval of 3 weeks. The first cycle of consolidation chemotherapy was begun 4 weeks after the concurrent chemoradiotherapy, if leukocyte count was at least 4,000/$mm^3$, platelet count at least 100,000/$mm^3$, aspartate aminotransferase and alanine aminotransferase 2.0 times the upper limit of normal range or less, serum total bilirubin 1.5 mg/dL or less, serum creatinine 1.5 mg/dL or less, and Eastern Cooperative Oncology Group performance score of 0 to 2. The subsequent cycle of consolidation chemotherapy was repeated if leukocyte count was at least 3,000/$mm^3$, neutrophil count at least 1,500/$mm^3$, platelet count at least 100,000/$mm^3$, serum creatinine 1.5 mg/dL or less, and body temperature not exceeding 38°C.

The 3D CRT began on Day 1. Irradiation was performed with 4-MV or higher photons from a linear accelerator. Patients received 1.5 Gy per fraction twice daily with at least a 6-hour interval between each fraction.

### Target volume definitions

Elective nodal irradiation was not performed. The gross tumor volume (GTV) was defined as the volume occupied by visible disease. The GTV included the primary tumor and involved lymph nodes measuring larger than 1.0 cm (short axis measurement) or lymph nodes with a diameter of 5 mm or more as shown by positron emission tomography. The clinical target volume (CTV) was defined as the GTV of the primary tumor plus a margin of 5 mm for all borders and GTV of the lymph nodes without a margin. The planning target volume (PTV) was the CTV plus an adequate margin added to compensate for variability in treatment setup, breathing, or motion during treatment. In

general, the PTV included the CTV plus 1.0 cm of expansion at all borders.

Tissue inhomogeneity corrections were used. The volume of both lungs that received more than 20 Gy should not exceed 35% of the total lung, and the maximum dose to the spinal cord could not exceed 45 Gy. It was desirable but not required that the PTV receive more than 93% but less than 107% of its prescribed dose. After the dose of 36 Gy was reached, the PTV could be reduced after shrinkage of the GTV.

### Dose escalation

The MTD was defined as the dose at which 3 or more of 6 patients experienced a dose-limiting toxicity (DLT). The DLT was defined as Grade 4 or more esophagitis, neutropenic fever, dermatitis, or nausea/vomiting and other Grade 3 or more nonhematologic toxicity. Furthermore, interruption of irradiation for more than 2 weeks was also defined as a DLT. The DLT was monitored for 90 days.

Irradiation was performed for 5 days per week. The prescribed doses were 54 Gy in 36 fractions over 3.6 weeks, 60 Gy in 40 fractions over 4.0 weeks, 66 Gy in 44 fractions over 4.4 weeks, and 72 Gy in 48 fractions over 4.8 weeks (Levels 1–4). When the DLT was observed in 0 of 4 patients, in $\leq$1 of 5 patients, or in $\leq$2 of 6 patients at each level, the radiation dose was to be escalated.

### Evaluation

The Response Evaluation Criteria in Solid Tumors were used for response assessment (13). Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria (version 3.0). An extramural review was conducted to validate the eligibility of the patients and staging.

The duration of survival was counted from the day of entry to the study, and the overall survival was calculated according to the Kaplan-Meier method (14).

## Results

### Patients' characteristics

Between April 2006 and April 2008, 26 patients were enrolled in this study. Four patients were excluded because of allergic reactions to paclitaxel on Day 1 ($n$ = 1), cerebral infarction on Day 2 ($n$ = 1), and supraclavicular nodal metastases ($n$ = 2). The remaining 22 patients were included in the analysis. They were 6, 7, 5, and 4 patients at Levels 1 through 4, respectively. Although, as a rule, 4 to 6 patients were enrolled in each level, 1 patient was increased at Level 2 because the sixth and seventh patients enrolled at the same time. The baseline characteristics of the 22 patients are summarized in Table 1.

When 4 patients entered Level 4, enrollment was closed to avoid severe late toxicities in the esophagus and the bronchia.

### Treatment administration

All patients received full doses of radiation therapy, and interruption of radiation therapy was required in only 4 patients.

**Table 1** Patient characteristics ($n$ = 22)

| Characteristics | $n$ | % |
|---|---|---|
| Age (y) | | |
| Median | | 63 |
| Range | | 45–70 |
| Sex | | |
| M | 19 | 86 |
| F | 3 | 14 |
| ECOG performance status | | |
| 0 | 7 | 32 |
| 1 | 15 | 68 |
| Histology | | |
| Squamous cell carcinoma | 10 | 45 |
| Adeno carcinoma | 10 | 45 |
| Large cell carcinoma | 0 | 0 |
| Others | 2 | 10 |
| Stage | | |
| IIIA | 11 | 50 |
| IIIB | 11 | 50 |

*Abbreviation:* ECOG = Eastern Cooperative Oncology Group.

Interruptions ranged from 1 day to 7 days. All patients received four cycles of concurrent chemotherapy, and 19 patients (86%) received two cycles of consolidation chemotherapy.

### Toxicity

The major toxicities are summarized in Table 2.

The DLTs occurred in 3 patients. Two cases of Grade 3 neuropathy were observed, one at Level 1 and the other at Level 3, and one case of Grade 3 infection occurred at Level 1. Furthermore, Grade 5 radiation pneumonitis was observed at Level 1; however, it was not treated as a DLT because the event occurred after the observation period of 90 days. Grade 3 esophagitis was observed in 3 patients, 1 at Level 3 and the others at Level 4. Grade 3 esophagitis and nausea were not defined as DLTs.

At Level 4, no DLT occurred in the 4 patients. Therefore, the MTD was not reached in the present study.

### Response and survival

The figure shows the overall survival. The median survival time was 28.6 months for all patients and 30.2 months for patients who received more than 60 Gy. The response rate was 77% for all patients.

### Patterns of relapse

Table 3 shows the first sites of relapse. Of 11 patients with locoregional relapse, 1 had upper mediastinal lymph node metastasis, which was located out of the radiation portal. Of 5 patients with distant metastasis, 3 had lung metastasis.

## Discussion

With the use of 3D planning techniques, several dose escalation trials have been performed. Kong *et al.* reported that doses of CRT

**Table 2** Major toxicities ($n$ = 22)

| Toxicity | Grade 3 | | Grade 4 | | Grade 5 | |
|---|---|---|---|---|---|---|
| | $n$ | % | $n$ | % | $n$ | % |
| Hematologic | | | | | | |
| Leukopenia | 16 | 73 | 1 | 5 | | |
| Neutropenia | 5 | 23 | 12 | 55 | | |
| Anemia | 2 | 9 | 0 | 0 | | |
| Thrombocytopenia | 0 | 0 | 0 | 0 | | |
| Nonhematologic | | | | | | |
| Neuropathy | 2 | 9 | 0 | 0 | 0 | 0 |
| Infection | 1 | 5 | 0 | 0 | 0 | 0 |
| Pneumonitis | 0 | 0 | 0 | 0 | 1 | 5 |
| Esophagitis | 3 | 14 | 0 | 0 | 0 | 0 |
| Nausea | 1 | 5 | 0 | 0 | 0 | 0 |

could be escalated up to 103 Gy for smaller tumors (15). However, the 5-year local control rates were only 49% even at 92 to 103 Gy and 35% at 74 to 84 Gy. The insufficient local control indicated limitation of dose escalation in conventional fractionation and warranted further exploration for different strategies.

A risk of severe late toxicities, such as esophageal stenosis and bronchial occlusion, was predicted from the beginning of the study. After that, experience with Levels 1 through 3 indicated that prescription of high doses in the esophagus or the main bronchi was inevitable in most patients. Therefore, enrollment was closed in the middle of Level 4 to avoid severe late toxicities. Emami *et al.* reported that in treatment of the esophagus, the tolerance dose that would result in a 50% probability of complications within 5 years of treatment was 72 Gy (16). However, data on tolerance doses by accelerated hyperfractionation are lacking. Therefore, careful long-term follow-up of the present study is required. Recently, Atsumi *et al.* reported that the severity and frequency of esophageal stenosis after radiation therapy were greater in patients with esophageal cancer with full circumference involvement and increased with esophageal wall thickness (17). The tolerance dose for the esophagus might be higher in patients without esophageal cancer than in those with esophageal cancer.

In radiation therapy using accelerated hyperfractionation, acute esophagitis is a toxicity of particular concern. In the present study, 3 patients experienced Grade 3 esophagitis: 2 of 4 patients at Level 4, but only 1 patient at Levels 1 through 3 ($n$ = 18). The

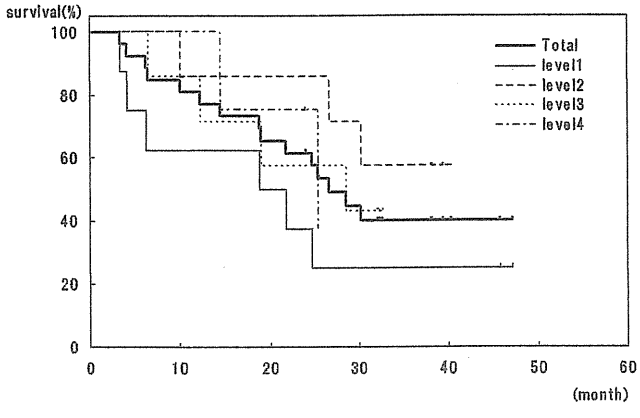


**Fig.** Kaplan-Meier survival curves for all patients and for patients in Levels 1–4. The median survival time was 28.6 months for all patients.

**Table 3** Site of first failure ($n$ = 22)

| Site | $n$ | % |
|---|---|---|
| Progression free | 9 | 41 |
| Locoregional alone | 8 | 36 |
| Locoregional and distant | 3 | 14 |
| Distant* | 5 | 23 |
| Lung | 3 | 14 |
| Brain | 1 | 5 |
| Small intestine | 1 | 5 |

* Distant includes locoregional and distant, and distant alone.

low frequency of esophagitis has often been observed in other Japanese trials (18, 19). The causes of this phenomenon are not well known. One possible explanation is differences in ethnic background. Twice-daily CRT with a dose of 1.3 to 1.45 Gy per fraction could be recommended in patients with other ethnic backgrounds, if this regimen is shown to bring a better outcome.

Another toxicity of concern is radiation pneumonitis. In the present study, Grade 5 radiation pneumonitis was observed in 1 patient. In other patients, however, Grade 3 or more radiation pneumonitis was not observed. Some radiation pneumonitis is inevitable to some degree, and the frequency of Grade 3 or more pneumonitis was rather low in the present study. Tsujino *et al.* reported a decreased incidence of radiation pneumonitis by accelerated hyperfractionation in the treatment of limited-stage small-cell lung cancer, although initially they expected that accelerated hyperfractionation would increase the incidence and severity of radiation pneumonitis (20). The results in the present study are consistent with those reported by Tsujino *et al.*

The DLTs observed in the current study were Grade 3 neuropathy and Grade 3 infection. They were mainly caused by chemotherapy. By contrast, Grade 5 radiation pneumonitis was not treated as a DLT because it occurred after the observation period of 90 days. The definition of DLT used in this study was probably inadequate for a radiation dose escalation study. Chemotherapy-induced toxicities should be given less consideration, and those caused by radiation therapy should be more strictly weighed.

The cutoff of 90 days for the observation period of DLTs was considered not sufficient. However, the observation period could not be more prolonged because the Phase I study had to be completed within a suitable time. Toxicities were evaluated after the observation period. In recent Phase I studies, the observation period was defined similarly (21–23).

Although the number of patients was relatively small in the present study, the method of assigning 6 patients to each dose bin is an option in a Phase I study (21). However, the data about survival were not reliable because of the small sample size. The data are to be verified in the following Phase II study.

In the present study, the dose of CRT was escalated to 72 Gy in 48 fractions, and MTD was not reached. In principle, 72 Gy should be the recommended dose. However, late toxicity data are insufficient, and enrollment was closed in the middle of Level 4. Furthermore, on the basis of our experience with the treatment of small-cell lung cancer, CRT with a dose of 66 Gy in accelerated hyperfractionation brings better local control than 74 Gy in conventional fractionation, which was defined as a recommended dose in several trials. The favorable median survival time of 30.2 months for patients who received 60 to 72 Gy in the present study is consistent with our experience. Therefore, a dose of 66 Gy in 44 fractions was adopted in the present study. On the basis

of the results presented here, we are currently preparing a Phase II study.

In conclusion, the MTD was not reached in the present study, although the dose of radiation was escalated to 72 Gy in 48 fractions. Acute toxicities were relatively mild. However, a dose of 66 Gy in 44 fractions was adopted for the present study because late toxicity data were insufficient.

## References


1. Furuse K, Fukuoka M, Kawahara M, *et al.* Phase III study of concurrent versus sequential thoracic radiotherapy in combination with mitomycin, vindesine, and cisplatin in unresectable stage III non-small cell lung cancer. *J Clin Oncol* 1996;17:2692–2699.
2. Fournel P, Robinet G, Thomas P, *et al.* Randomized phase III trial of sequential chemoradiotherapy compared with concurrent chemo-radiotherapy in locally advanced non-small-cell lung cancer: Group Lyon-Saint-Etienne d'Oncologie Thoracique-Groupe Francais de Pneumo-Cancerogie NPC 95-01 Study. *J Clin Oncol* 2005;23: 5910–5917.
3. Zatloukal P, Petruzelka L, Zemanova M, *et al.* Concurrent versus sequential chemoradiotherapy with cisplatin and vinorelbine in locally advanced non-small cell lung cancer: A randomized study. *Lung Cancer* 2004;46:87–98.
4. Curran W, Scott C, Langer C, *et al.* Long term benefit is observed in a phase III comparison of sequential vs concurrent chemo-radiation for patients with unresectable stage III NSCLC: RTOG 9410. *Proc Am Soc Clin Oncol* 2003;22:621 (abstr 2499).
5. Schaake-Koning C, Vand den Bogaert W, Dalesio O, *et al.* Effect of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med* 1992;326:524–530.
6. Tada T, Minakuchi K, Matsui K, *et al.* A single institutional subset analysis of the WJLCG study comparing concurrent and sequential chemoradiotherapy for stage III non-small-cell lung cancer. *Radiat Med* 2004;22:163–167.
7. Le Chevalier T, Arriagada R, Tarayre M, *et al.* Significant effect of adjuvant chemotherapy on survival in locally advanced non-small-cell lung carcinoma [Letter]. *J Natl Cancer Inst* 1992;84:58.
8. Socinski MA, Rosenmann JG, Halle JS, *et al.* Induction carboplatin/paclitaxel followed by concurrent carboplatin/paclitaxel and dose-escalating conformal thoracic radiation therapy in unresectable stage IIIA/B non-small cell lung cancer: A modified phase I trial. *Cancer* 2000;89:534–542.
9. Rosenmann JG, Halle JS, Socinski MA, *et al.* High dose conformal radiotherapy for treatment of stage IIIA/IIIB non-small-cell lung cancer: Technical issues and results of a phase I/II trial. *Int J Radiat Oncol Biol Phys* 2002;54:348–356.
10. Blackstock AW, Lesser GJ, Fletcher-Steede J, *et al.* Phase I study of twice-weekly gemcitabin and concurrent thoracic radiation for patients with locally advanced non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2001;51:1281–1289.
11. Blackstock AW, Ho C, Butler J, *et al.* Phase Ia/Ib chemo-radiation trial of gemcitabin and dose-escalated thoracic radiation in patients with stage III A/B non-small cell lung cancer. *J Thorac Oncol* 2006; 1:434–440.
12. Withers HR, Taylor JMG, Maciewjewski B, *et al.* The hazard of accelerated tumor clonogen repopulation during radiotherapy. *Acta Oncol* 1988;27:131–146.
13. Therasse P, Arbuck SG, Eisenhauer EA, *et al.* New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;92:205–216.
14. Kaplan EL, Meier P. Nonparametric estimation from incomplete evaluations. *J Am Stat Assoc* 1958;53:457–481.
15. Kong FM, Haken RKT, Schipper MJ, *et al.* High dose radiation improved local tumor control and overall survival in patients with inoperable/unresectable non-small-cell lung cancer: Long-term results of a radiation dose escalation study. *Int J Radiat Oncol Biol Phys* 2005;63:324–333.
16. Emami B, Lyman J, Brown A, *et al.* Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991;21: 109–122.
17. Atsumi K, Shioyama Y, Nakamura K, *et al.* Predictive factors of esophageal stenosis associated with tumor regression in radiation therapy for locally advanced esophageal cancer. *J Radiat Res* 2010;51: 9–14.
18. Saito H, Takada Y, Ichinose Y, *et al.* Phase II study of etoposide and cisplatin with concurrent twice-daily thoracic radiotherapy followed by irinotecan and cisplatin in patients with limited-disease small-cell lung cancer: West Japan Thoracic Oncology Group 9902. *J Clin Oncol* 2006;24:5247–5252.
19. Takada M, Fukuoka M, Kawahara M, *et al.* Phase III study of concurrent versus sequential thoracic radiotherapy in combination with cisplatin and etoposide for limited-stage small-cell lung cancer: Results of the Japan Clinical Oncology Group Study 9104. *J Clin Oncol* 2002;20:3054–3060.
20. Tsujino K, Hirota S, Kotani Y, *et al.* Radiation pneumonitis following concurrent accelerated hyperfractionated radiotherapy and chemotherapy for limited-stage small-cell lung cancer: Dose-volume histogram analysis and comparing with conventional chemoradiation. *Int J Radiat Oncol Biol Phys* 2006;64:1100–1105.
21. Schild SE, McGinnis WL, Graham D, *et al.* Results of a phase I trial of concurrent chemotherapy and escalating doses of radiation for unresectable non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2006;65:1106–1111.
22. Nishimura Y, Nakagawa K, Takeda K, *et al.* Phase I/II trial of sequential chemoradiotherapy using novel hypoxic cell radiosensitizer, doranidazole (PR-350), in patients with locally advanced non-small-cell lung cancer (WJTOG-0002). *Int J Radiat Oncol Biol Phys* 2007;69: 786–792.
23. Marks LB, Garst J, Socinsky MA, *et al.* Carboplatin/paclitaxel or carboplatin/vinorelbine followed by accelerated hyperfractionated conformal radiation therapy: Report of a prospective phase I dose escalation trial from the Carolina conformal therapy consortium. *Clin Oncol* 2004;22:4329–4340.