Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

We now show that erlotinib does not affect survivin expression or induce apoptosis in *EGFR* mutation–positive NSCLC cells with PTEN loss. We further examined whether persistent survivin expression was associated with the sensitivity of cells to EGFR–TKIs and whether modulation of survivin expression might overcome resistance to these drugs in *EGFR* mutation–positive NSCLC cells with PTEN loss.

Materials and Methods

Cell culture and reagents

The human NSCLC cell lines HCC827 and H1650 were obtained from American Type Culture Collection. The NSCLC cell line PC9 was obtained as described previously (15). The NSCLC cell lines PC9/GEF1-1 and PC9/GEF2-1 were generated and characterized as described previously (12). We screened all cell lines for the presence of *EGFR* mutations by direct DNA sequencing of exons 18 to 21 as described previously (15) for this study. All cells were passaged for 3 months or less before the renewal from frozen, early-passage stocks obtained from the indicated sources. Cells were regularly screened for mycoplasma with the use of a MycoAlert Mycoplasma Detection Kit (Lonza). All cells were cultured under a humidified atmosphere of 5% CO_2 at 37°C in RPMI 1640 medium (Sigma) supplemented with 10% FBS. Erlotinib (Supplementary Fig. S1A) was obtained from Kemprotec, and YM155 (Supplementary Fig. S1B) was obtained from Astellas Pharma.

Immunoblot analysis

Cells were washed twice with ice-cold PBS and then lysed in a solution containing 20 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, and leupeptin (1 μg/mL). The protein concentration of the cell lysates was determined with the use of the Bradford reagent (Bio-Rad) and equal amounts of protein were subjected to SDS-PAGE on a 7.5% gel. The separated proteins were transferred to a nitrocellulose membrane, which was then exposed to 5% nonfat dried milk in PBS for 1 hour at room temperature before incubation overnight at 4°C with primary antibodies. Rabbit polyclonal antibodies to human phosphorylated EGFR (pY1068), to phosphorylated or total forms of AKT, to phosphorylated or total forms of extracellular signal-regulated kinase (ERK), to XIAP, to IAP-1, to Bcl-xl, to Bcl-2, to Mcl-1, to PARP, to BIM, and to PTEN were obtained from Cell Signaling Technology; those to survivin were from Santa Cruz Biotechnology; and those to β-actin were from Sigma. Mouse monoclonal antibodies to EGFR were obtained from Invitrogen. All antibodies were used at a 1:1,000 dilution, with the exception of those to β-actin (1:200). The nitrocellulose 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 (Sigma) or mouse (Santa Cruz Biotechnology) immunoglobulin G. Immune complexes were finally detected with chemiluminescence reagents (Perkin-Elmer Life Science).

Gene silencing

Cells were plated at 50% to 60% confluence in 6-well plates or 25 cm^2 flasks and then incubated for 24 hours before transient transfection for 24 hours with short interfering RNAs (siRNA) mixed with the Lipofectamine reagent (Invitrogen). Small interfering RNAs specific for PTEN (5′-UGAACCUGAUCAUUAUAGATT-3′) or survivin (5′-GAAGCAGUUUGAAGAAUUA-3′) mRNAs as well as a corresponding scrambled (control) siRNA were obtained from Nippon EGT.

Annexin V binding assay

The binding of Annexin V to cells was measured with the use of an Annexin V–FLUOS Staining kit (Roche). Cells were harvested by exposure to trypsin-EDTA, 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).

Cell growth inhibition assay

Cells were transferred to 96-well flat-bottomed plates and cultured for 24 hours before exposure for 72 hours to various concentrations of erlotinib. Tetra Color One (5 mmol/L tetrazolium monosodium salt and 0.2 mmol/L 1-methoxy-5-methyl phenazinium methylsulfate; Seikagaku Kogyo) was then added to each well, and the cells were incubated for 3 hours at 37°C before measurement of absorbance at 490 nm with a Multiskan Spectrum instrument (Thermo Labsystems). Absorbance values were expressed as a percentage of that for untreated cells.

Growth inhibition assay *in vivo*

All animal studies were carried out in accordance with the Recommendations for Handling of Laboratory Animals for Biomedical Research compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, Kinki University. The ethical procedures followed conformed to the guidelines of the United Kingdom Coordinating Committee on Cancer Prevention Research. Tumors cells (5×10^6) were injected subcutaneously into the axilla of 5- to 6-week-old female athymic nude mice (BALB/c nu/nu; CLEA Japan). Treatment was initiated when tumors in each group of 6 mice achieved an average volume of 300 to 600 mm^3. Treatment groups consisted of vehicle control, erlotinib alone (10 mg/kg), YM155 alone (5 mg/kg), and erlotinib plus YM155. Erlotinib was administered by oral gavage daily for 31 days, with control animals receiving a 0.5% (w/v) aqueous solution of hydroxypropylmethylcellulose as vehicle. Continuous infusion of YM155 has been found to induce tumor regression and intratumoral survivin suppression in established human hormone-refractory

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

prostate cancer, non-Hodgkin lymphoma, melanoma, and NSCLC xenografts (16–20). YM155 was thus administered over 7 consecutive days (days 1 to 7) with the use of an implanted micro-osmotic pump (Alzet model 1003D; DURECT Cupertino).

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.

Statistical analysis

Quantitative data are presented as means ± SE from 3 independent experiments or for 6 animals per group. The significance of differences in the percentage of Annexin V–positive cells or in tumor size was evaluated with the unpaired 2-tailed Student *t* test. $P < 0.05$ was considered statistically significant.

Results

Differential apoptotic responses of *EGFR* mutation–positive NSCLC cell lines to erlotinib

We first examined the effects of the EGFR-TKI erlotinib on apoptosis in *EGFR* mutation–positive NSCLC cell lines (HCC827, PC9, and H1650) by staining with Annexin V. HCC827, PC9, and H1650 cells harbor an *EGFR* allele with an activating mutation, whereas H1650 cells also show homozygous deletion of *PTEN*. Erlotinib induced a marked increase in the proportion of apoptotic cells among HCC827 and PC9 cells, whereas it was without effect in H1650 cells, despite these cells harboring an activating mutation in *EGFR* (Fig. 1A). Immunoblot analysis of the cleaved form of PARP confirmed that erlotinib did not induce apoptosis in H1650 cells (Fig. 1B). We and others previously showed that PI3K–AKT–survivin and mitogen-activated protein (MEK)–ERK–BIM signaling pathways play important roles in EGFR-TKI-induced apoptosis (13, 21–23). We therefore next compared the effects of erlotinib on the expression level or phosphorylation status of EGFR, AKT, ERK, PTEN, survivin, and BIM in the 3 NSCLC cell lines by immunoblot analysis. Erlotinib induced the dephosphorylation of EGFR as well as that of AKT and ERK in the erlotinib-sensitive cell lines HCC827 and PC9 (Fig. 1C). In addition, erlotinib induced downregulation of survivin expression and upregulation of BIM expression in HCC827 and PC9 cells, consistent with our previous observations (13).

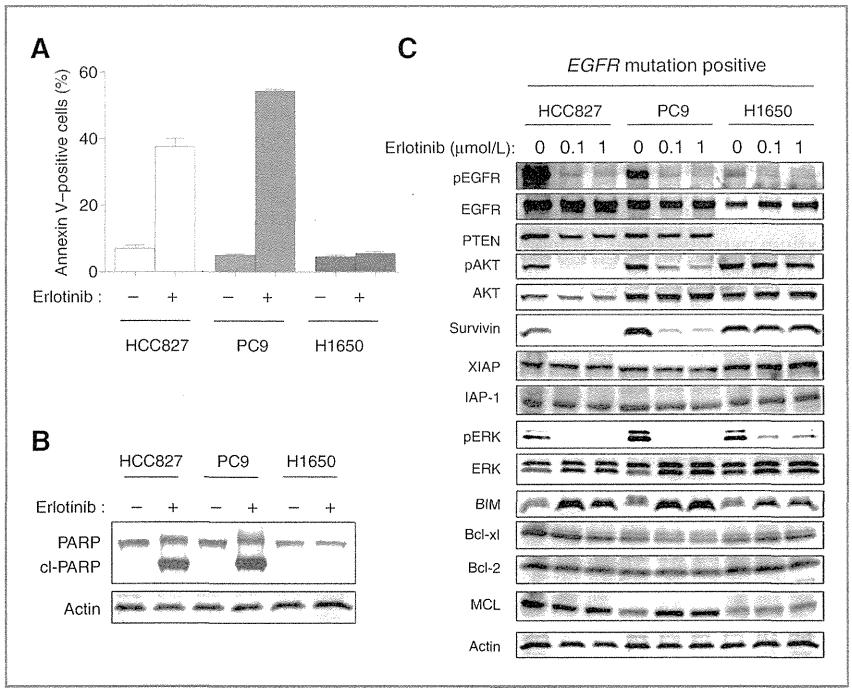

Figure 1. Differential apoptotic responses of *EGFR* mutation–positive NSCLC cell lines to erlotinib. A, HCC827, PC9, or H1650 cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. B, cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, lysed, and subjected to immunoblot analysis with antibodies to PARP or to β-actin (loading control). Bands corresponding to the intact and cleaved (cl) forms of PARP are indicated. C, cells were incubated in complete medium containing the indicated concentrations of erlotinib for 24 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to phosphorylated (p) or total forms of EGFR, AKT, or ERK or to the indicated proteins. Data in B and C are representative of 3 independent experiments.

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

However, whereas erlotinib induced the dephosphorylation of EGFR and ERK as well as the upregulation of BIM expression in H1650 cells, it had no effect of the levels of AKT phosphorylation or survivin expression (Fig. 1C). The expression of other IAP or Bcl family members, including XIAP, IAP-1, Bcl-2, Bcl-xl, and Mcl-1, was not substantially affected by erlotinib in any of the cell lines examined. Given that PTEN inhibits AKT activation by catalyzing the dephosphorylation of phosphatidylinositol 3,4,5-trisphosphate, the product of class I PI3K action, these results suggested that the resistance of some *EGFR* mutation–positive cells to erlotinib-induced apoptosis may be associated with persistent activation of the AKT–survivin pathway.

The effect of erlotinib on survivin expression correlates with that on apoptosis

We have previously established EGFR–TKI (gefitinib)-resistant sublines (PC9/GEF1-1 and PC9/GEF2-1) of PC9 cells that harbor the activating *EGFR* mutation of the parental cells but which have also lost PTEN expression, with the loss of PTEN contributing to their EGFR–TKI resistance (12). Both staining with Annexin V and immunoblot analysis of PARP revealed that erlotinib did not induce a substantial level of apoptosis in PC9/GEF1-1 or PC9/GEF2-1 cells (Fig. 2A and B), consistent with our previous observations (12). To investigate whether loss of PTEN expression affects the modulation of AKT–survivin signaling by erlotinib, we examined the effects of erlotinib on the expression level or phosphorylation status of EGFR, AKT, and survivin in these cell lines by immunoblot analysis. Similar to our observations with H1650 cells, erlotinib did not affect the levels of AKT phosphorylation or survivin expression in PC9/GEF1-1 and PC9/GEF2-1 cells, whereas it still induced the dephosphorylation of EGFR and ERK as well as the upregulation of BIM expression (Fig. 2C). These findings thus suggested that persistent expression of survivin in the presence of erlotinib contributes to the resistance to this drug associated with PTEN loss in *EGFR* mutation–positive NSCLC cells.

Knockdown of PTEN induces persistent survivin expression and reduces erlotinib sensitivity in *EGFR* mutation–positive cells

To investigate the contribution of PTEN to survivin expression and erlotinib resistance, we examined the effects of siRNA-mediated depletion of PTEN in PC9 and HCC827 cells. Transfection of these cells with PTEN siRNA attenuated the dephosphorylation of AKT and downregulation of survivin expression, without affecting the dephosphorylation of ERK and upregulation of BIM, induced by erlotinib (Fig. 3A). Staining with Annexin V also revealed that depletion of PTEN resulted in inhibition of erlotinib-induced apoptosis (Fig. 3B). Moreover, knockdown of PTEN reduced the sensitivity of HCC827 and PC9 cells to the inhibitory effect of erlotinib on cell growth (Fig. 3C). These results suggested that PTEN loss contributes to persistent survivin expression in the presence of erlotinib and to erlotinib resistance in *EGFR* mutation–positive cells.

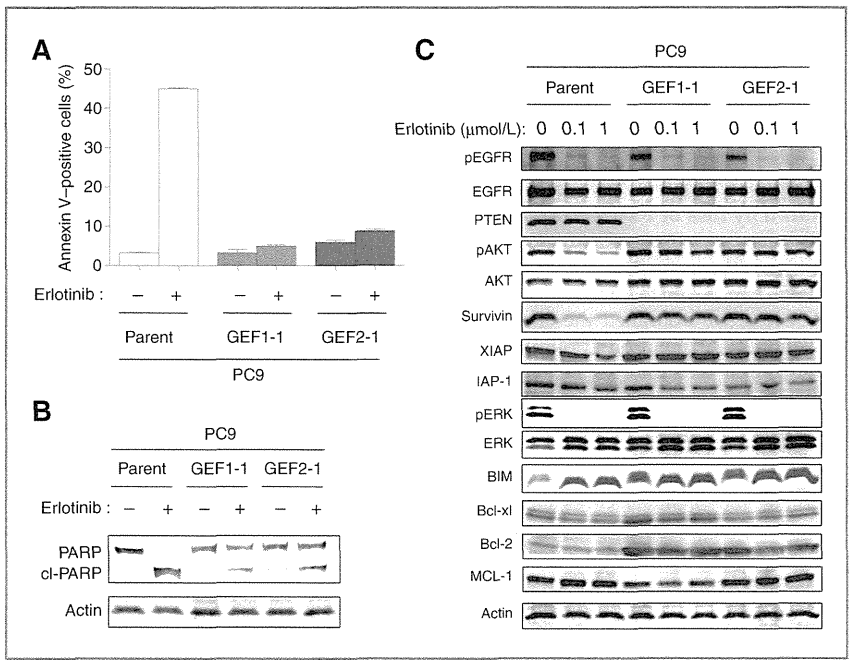

Figure 2. Effect of erlotinib on survivin expression correlates with sensitivity to erlotinib in *EGFR* mutation–positive NSCLC cells. A, PC9, PC9/GEF1-1, or PC9/GEF2-1 cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. B, cells were incubated in complete medium with or without erlotinib (100 nmol/L) for 48 hours, lysed, and subjected to immunoblot analysis with antibodies to PARP or to β-actin. C, cells were incubated in complete medium containing the indicated concentrations of erlotinib for 24 hours, after which cell lysates were subjected to immunoblot analysis with antibodies to the indicated proteins. Data in B and C are representative of 3 independent experiments.

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

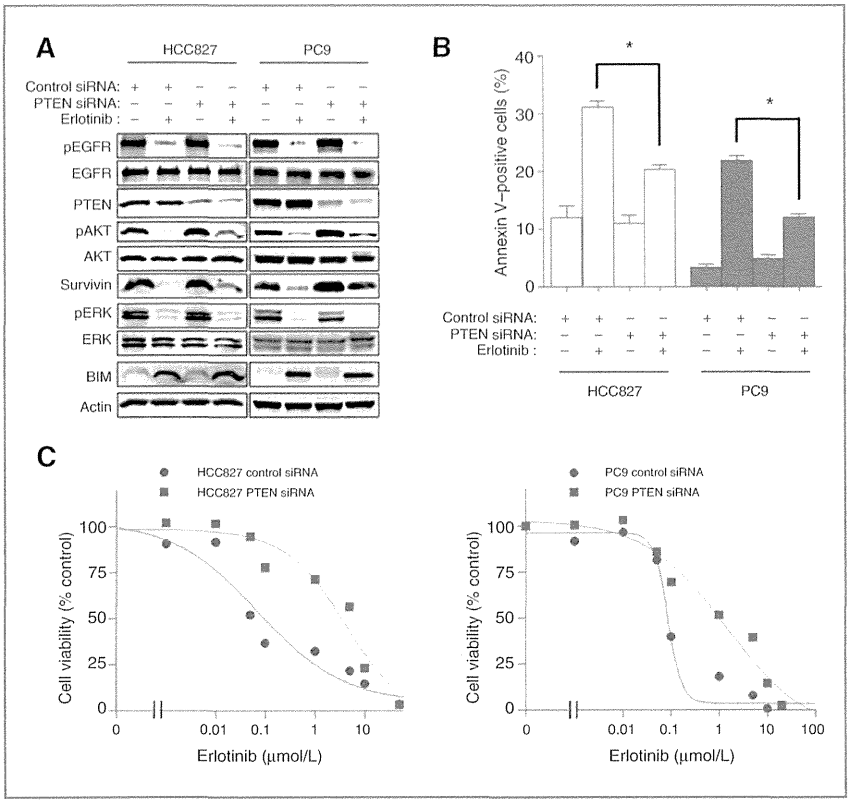

Figure 3. Knockdown of PTEN induces persistent survivin expression and reduces erlotinib sensitivity in *EGFR* mutation–positive cells. A, HCC827 or PC9 cells were transfected with PTEN or scrambled (control) siRNAs for 24 hours and then incubated in complete medium with or without erlotinib (100 nmol/L) for 24 hours. Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to the indicated proteins. Data are representative of 3 independent experiments. B, cells transfected as in A were incubated with or without erlotinib (100 nmol/L) for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. *, $P < 0.05$ for the indicated comparisons. C, cells transfected as in A were cultured in complete medium containing the indicated concentrations of erlotinib for 72 hours, after which cell viability was assessed. The number of viable cells is expressed as a percentage of the corresponding value for cells not exposed to erlotinib. Data are means from 3 independent experiments.

Knockdown of survivin reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive cells

To investigate further the contribution of persistent survivin expression to erlotinib resistance associated with PTEN loss, we examined the effect of siRNA-mediated depletion of survivin on erlotinib-induced apoptosis in parental PC9 cells and the EGFR–TKI-resistant sublines PC9/GEF1-1 and PC9/GEF2-1. Whereas erlotinib induced downregulation of survivin expression only in the parental PC9 cells, transfection with survivin siRNA resulted in specific depletion of survivin in both the parental and EGFR–TKI-resistant NSCLC cells (Fig. 4A). The erlotinib-induced upregulation of BIM expression was apparent in all 3 cell lines in the absence or presence of survivin siRNA (Fig. 4A). The survivin siRNA had no significant effect on erlotinib-induced apoptosis in PC9 cells, as revealed by staining with Annexin V (Fig. 4B). In contrast, depletion of survivin by transfection with the survivin siRNA sensitized PC9/GEF1-1 and PC9/GEF2-1 cells to the proapoptotic effect of erlotinib (Fig. 4B). A second siRNA targeted to a different region of survivin mRNA yielded similar results (data not shown). These observations thus suggested that persistent expression of survivin contributes to erlotinib resistance in *EGFR* mutation–positive cells with loss of PTEN.

YM155 reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive cells *in vitro*

We next examined the effect of the combination of erlotinib and YM155 on apoptosis in NSCLC cells with EGFR–TKI resistance due to loss of PTEN. YM155 is a small-molecule agent that specifically inhibits survivin expression in various types of cancer cell lines *in vitro* and *in vivo* (16–20). Treatment of PC9/GEF1-1 or PC9/GEF2-1 cells, or of parental PC9 cells, with YM155 resulted in downregulation of survivin expression (Fig. 5A). Erlotinib induced upregulation of BIM in all 3 cell lines in the

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

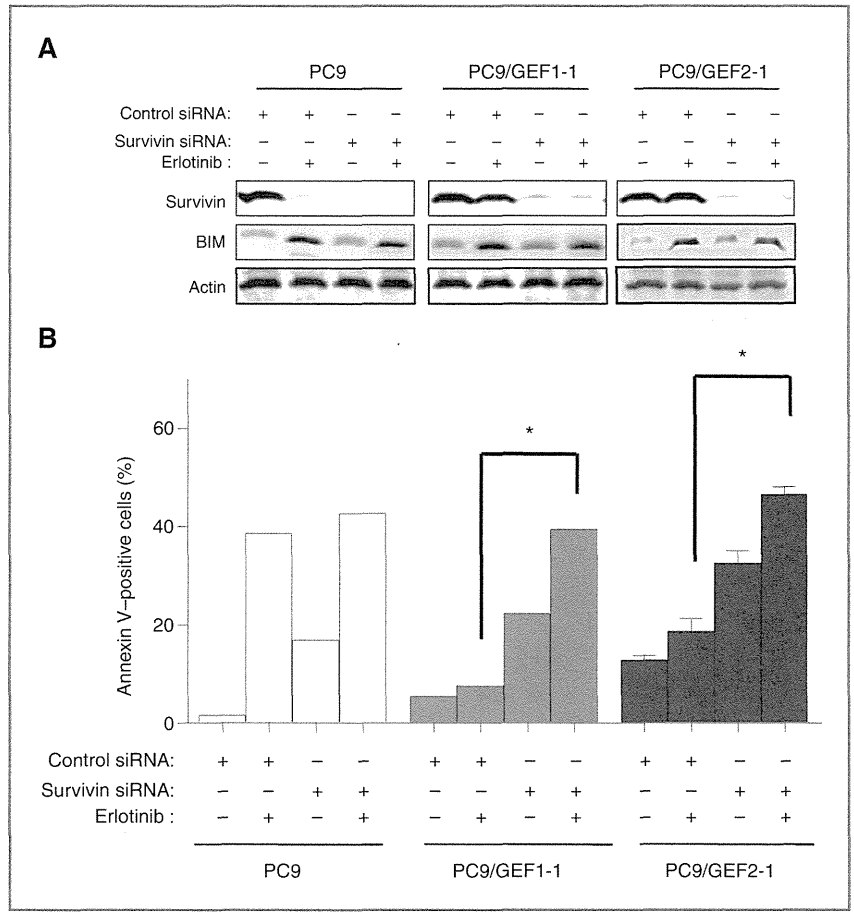

Figure 4. Knockdown of survivin expression reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive NSCLC cells. A, PC9, PC9/GEF1-1, or PC9/GEF2-1 cells were transfected with survivin or scrambled (control) siRNAs for 24 hours and then incubated in complete medium with or without erlotinib (100 nmol/L) for 24 hours. Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to survivin, BIM, or β-actin. Data are representative of 3 independent experiments. B, cells transfected as in A were incubated with or without erlotinib (100 nmol/L) for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. *, $P < 0.05$ for the indicated comparisons.

absence or presence of YM155 (Fig. 5A). Whereas YM155 had no significant effect on erlotinib-induced apoptosis in PC9 cells, it sensitized PC9/GEF1-1 and PC9/GEF2-1 cells to the proapoptotic effect of erlotinib (Fig. 5B). In addition, whereas YM155 did not affect the sensitivity of PC9 cells to the antiproliferative effect of erlotinib, it increased that of PC9/GEF1-1 and PC9/GEF2-1 cells to this action of erlotinib (Fig. 5C). These results thus suggested that downregulation of survivin expression by YM155 promoted the proapoptotic and antiproliferative effects of erlotinib in *EGFR* mutation–positive cells with loss of PTEN.

YM155 reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive cells *in vivo*

We also examined the effects of siRNA-mediated depletion of survivin and of YM155 on erlotinib-induced apoptosis in H1650 cells. Transfection with the survivin siRNA resulted in specific depletion of survivin and sensitized H1650 cells to the proapoptotic effect of erlotinib (Fig. 6A). YM155 also downregulated survivin expression and sensitized H1650 cells to the induction of apoptosis by erlotinib (Fig. 6B). In addition, YM155 increased the sensitivity of H1650 cells to the inhibitory effect of erlotinib on cell growth *in vitro* (Fig. 6C). Finally, to determine whether the enhancement of the proapoptotic effect of erlotinib by YM155 in EGFR–TKI-resistant cells observed *in vitro* might also be apparent *in vivo*, we injected H1650 cells into nude mice to elicit the formation of solid tumors. After tumor formation, the mice were treated with erlotinib, YM155, or both drugs. Combined treatment with erlotinib and YM155 inhibited tumor growth to a greater extent than did treatment with either drug alone (Fig. 6D and E). These data suggested that YM155 enhances the response of *EGFR* mutation–positive tumor cells with loss of PTEN to erlotinib both *in vitro* and *in vivo*.

Discussion

Tyrosine kinases have come to be recognized as key regulators of cancer cell proliferation and apoptosis as well as of tumor angiogenesis and they are therefore considered potential targets for anticancer therapies (24). Several strategies for targeting these kinases have

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

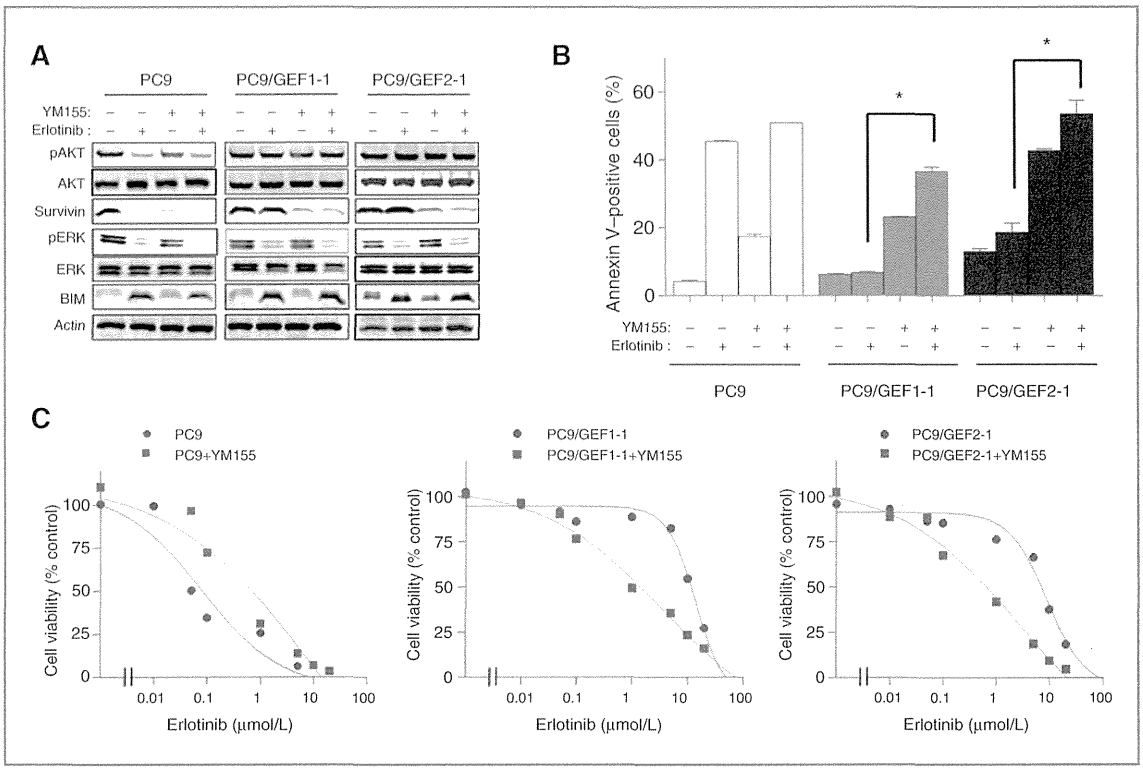

Figure 5. YM155 reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive NSCLC cells *in vitro*. A, PC9, PC9/GEF1-1, or PC9/GEF2-1 cells were incubated in complete medium with or without erlotinib (100 nmol/L), YM155 (10 nmol/L), or both drugs for 24 hours, after which cell lysates were prepared and subjected to immunoblot analysis with antibodies to the indicated proteins. Data are representative of 3 independent experiments. B, cells were incubated in complete medium with or without erlotinib (100 nmol/L), YM155 (10 nmol/L), or both drugs for 48 hours, after which the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry. Data are means ± SE from 3 independent experiments. *, $P < 0.05$ for the indicated comparisons. C, cells were cultured in complete medium containing the indicated concentrations of erlotinib with or without 10 nmol/L YM155 for 72 hours, after which cell viability was assessed. The number of viable cells is expressed as a percentage of the corresponding value for cells not exposed to erlotinib. Data are means from 3 independent experiments.

been pursued, the most successful of which has been the development of small-molecule TKIs (25–27). However, increasing evidence has shown the development of acquired resistance to these drugs, and extensive preclinical studies are ongoing to provide insight into the molecular mechanisms underlying such resistance (28). We and others have recently shown that loss of PTEN contributes to EGFR–TKI resistance in *EGFR* mutation–positive lung cancer through the activation of AKT (11, 12). Loss of PTEN was also found to reduce responsiveness to EGFR–TKIs in patients with recurrent glioblastoma expressing EGFR variant type III, a constitutively active mutant form of EGFR (29). In addition, restoration of PTEN expression was shown to increase the susceptibility to EGFR–TKI-induced apoptosis in *EGFR* mutation–positive NSCLC cells with PTEN loss (11). These observations thus support the notion that PTEN loss is associated with resistance to EGFR–TKIs in EGFR-driven tumors.

The precise molecular mechanism by which PTEN loss induces resistance to EGFR–TKIs has remained unclear, however. We have now shown that erlotinib did not induce apoptosis or downregulation of survivin expression in H1650 cells, which harbor an *EGFR* allele with an activating mutation and also no longer manifest PTEN expression. Similar results were obtained with EGFR–TKI-resistant PC9/GEF sublines of PC9 cells that have also lost PTEN expression and manifest persistent phosphorylation of AKT. We further found that an siRNA specific for PTEN mRNA inhibited the dephosphorylation of AKT and downregulation of survivin induced by erlotinib in *EGFR* mutation–positive NSCLC cells. These results are consistent with our previous observation that inhibition of the EGFR–PI3K–AKT pathway contributed to downregulation of survivin expression by EGFR–TKIs in *EGFR* mutation–positive NSCLC cells (13). Whereas the mechanism of survivin downregulation by EGFR–TKIs remains unclear, previous studies have suggested that the PI3K–AKT pathway regulates survivin expression through modulation of transcriptional factors (30, 31). We confirmed that erlotinib downregulated the amount of

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

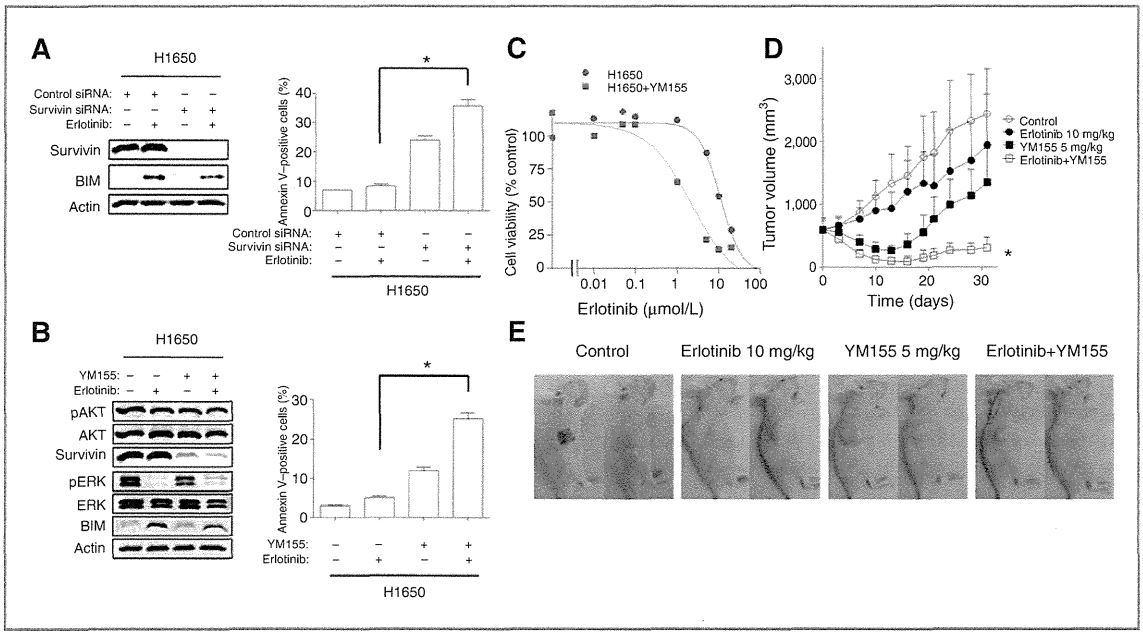

Figure 6. YM155 reverses erlotinib resistance induced by loss of PTEN in *EGFR* mutation–positive cells *in vivo*. A, H1650 cells were transfected with survivin or scrambled (control) siRNAs for 24 hours and then incubated in complete medium with or without erlotinib (2 μmol/L) for 24 hours (left) or 48 hours (right). Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to survivin, BIM, or β-actin (left), or the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry (right). B, cells were incubated in complete medium with or without erlotinib (2 μmol/L), YM155 (20 nmol/L), or both drugs for 24 hours (left) or 48 hours (right). Cell lysates were then prepared and subjected to immunoblot analysis with antibodies to the indicated proteins (left), or the proportion of apoptotic cells was determined by staining with fluorescein isothiocyanate–conjugated Annexin V and propidium iodide followed by flow cytometry (right). Data in A and B (left) are representative of or means ± SE (right) from 3 independent experiments. *, $P < 0.05$ for the indicated comparisons. C, cells were cultured in complete medium containing the indicated concentrations of erlotinib with or without 10 nmol/L YM155 for 72 hours, after which cell viability was assessed. The number of viable cells is expressed as a percentage of the corresponding value for cells not exposed to erlotinib. Data are means from 3 independent experiments. D, nude mice with tumor xenografts established by subcutaneous injection of H1650 cells were treated with vehicle (control), erlotinib (10 mg/kg), YM155 (5 mg/kg), or the combination of erlotinib and YM155 as described in Materials and Methods. Tumor volume was measured at the indicated times after the onset of treatment. Data are means ± SE for 6 mice per group. *, $P < 0.05$ for the combination of erlotinib plus YM155 versus control or either erlotinib or YM155 alone. E, representative mice treated as in D showing tumors at the end of the 31-day treatment period.

survivin mRNA in *EGFR* mutation–positive NSCLC cells without PTEN loss (data not shown), suggesting that survivin downregulation by erlotinib is mediated, at least in part, at the transcriptional level. These results suggest that the PI3K–AKT pathway and its downstream transcriptional factors are targeted by EGFR–TKIs in the regulation of survivin expression.

In this study, we also showed that downregulation of survivin expression either by transfection with survivin siRNA or by exposure to YM155 reversed *in vitro* or *in vivo* the resistance of *EGFR* mutation–positive NSCLC cells to erlotinib induced by PTEN loss. On the contrary, similar to *EGFR* mutation–positive cells without PTEN loss, the combination of erlotinib and YM155 did not manifest a synergistic antiproliferative effect in NSCLC cells harboring wild-type *EGFR* alleles (data not shown), suggesting that sensitization to EGFR–TKIs by YM155 is specific for EGFR–TKI-resistant cells with both an *EGFR* mutation and PTEN loss. Our observations thus show that PTEN loss activates AKT signaling and that this pathway contributes to the persistence of survivin expression in the presence of erlotinib and to EGFR–TKI resistance in *EGFR* mutation–positive NSCLC cells.

In addition to the PI3K–AKT–survivin pathway, we and others have previously shown that the induction of BIM expression through inhibition of the MEK–ERK pathway is important for EGFR–TKI-induced apoptosis (21–23, 32). Although erlotinib induced upregulation of the proapoptotic protein BIM in *EGFR* mutation–positive cells regardless of PTEN status, the proapoptotic effect of erlotinib was not observed in cells with PTEN loss. These results suggest that the PI3K–AKT–survivin signaling pathway might be more important than the MEK–ERK–BIM pathway in the regulation of survival in *EGFR* mutation–positive cells with loss of PTEN. However, we found that the addition of YM155 to erlotinib resulted in both the downregulation of survivin and the upregulation of BIM, leading to a greater increase in the number of apoptotic cells compared with that observed with either agent alone, in *EGFR* mutation–positive cells with PTEN loss. These

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

results suggest that, although the relative contributions of the PI3K–AKT–survivin and MEK–ERK–BIM signaling pathways to cell survival may vary among cells, concomitant downregulation of survivin and upregulation of BIM are necessary for the induction of a substantial level of apoptosis in *EGFR* mutation–positive cells. Nuclear factor-κB and FAS receptor signaling were also recently shown to regulate EGFR–TKI sensitivity in *EGFR* mutation–positive NSCLC cells (33), suggesting that multiple mechanisms underlie EGFR–TKI resistance.

We have found that the persistent activation of AKT–survivin signaling by PTEN loss represents a mechanism of primary or acquired resistance to erlotinib in *EGFR* mutation–positive cells. Our results show that erlotinib downregulates survivin expression in EGFR–TKI-sensitive NSCLC cells but not in EGFR–TKI-resistant NSCLC cells with PTEN loss. Although we previously showed that the expression of PTEN was reduced in tumor specimens from NSCLC patients showing acquired resistance to EGFR–TKIs (12), analysis of serial tumor samples obtained before and during treatment with these drugs will be required to determine whether survivin expression is affected by such treatment. Moreover, we found that YM155 significantly enhanced the antitumor effect of erlotinib in *EGFR* mutation–positive NSCLC cells with PTEN loss both *in vitro* and *in vivo*. YM155, a small-molecule inhibitor of the expression of the antiapoptotic protein survivin, is currently in clinical development as the first survivin suppressant (18, 19, 34–36). This drug was found to exhibit a favorable safety tolerability profile and moderate single-agent activity in a recent phase II trial with patients with advanced, refractory NSCLC (37). Our results now suggest that further studies of combination therapy with YM155 and erlotinib are warranted in NSCLC patients with EGFR–TKI resistance induced by PTEN loss.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported by KAKENHI (grants-in-aid for scientific research) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 17, 2011; revised November 2, 2011; accepted November 2, 2011; published OnlineFirst November 10, 2011.

References

1. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene 2009;28:S24–S31.
2. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 2008;14:2895–9.
3. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e225–35.
4. Koizumi F, Shimoyama T, Taguchi F, Saijo N, Nishio K. Establishment of a human non-small cell lung cancer cell line resistant to gefitinib. Int J Cancer 2005;116:36–44.
5. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
6. Okamoto W, Okamoto I, Tanaka K, Hatashita E, Yamada Y, Kuwata K, et al. TAK-701, a humanized monoclonal antibody to hepatocyte growth factor, reverses gefitinib resistance induced by tumor-derived HGF in non-small cell lung cancer with an EGFR mutation. Mol Cancer Ther 2011;9:2785–92.
7. Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res 2008;68:9479–87.
8. Takeda M, Okamoto I, Fujita Y, Arao T, Ito H, Fukuoka M, et al. *De novo* resistance to epidermal growth factor receptor-tyrosine kinase inhibitors in EGFR mutation-positive patients with non-small cell lung cancer. J Thorac Oncol 2010;5:399–400.
9. Li L, Ross AH. Why is PTEN an important tumor suppressor?J Cell Biochem 2007;102:1368–74.
10. Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 2006;6:184–92.
11. Sos ML, Koker M, Weir BA, Heynck S, Rabinovsky R, Zander T, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer Res 2009;69:3256–61.
12. Yamamoto C, Basaki Y, Kawahara A, Nakashima K, Kage M, Izumi H, et al. Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor-activating mutations. Cancer Res 2010;70:8715–25.
13. Okamoto K, Okamoto I, Okamoto W, Tanaka K, Takezawa K, Kuwata K, et al. Role of survivin in EGFR inhibitor-induced apoptosis in non-small cell lung cancers positive for EGFR mutations. Cancer Res 2010;70:10402–10.
14. Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anti-cancer therapy. Cancer Treat Rev 2009;35:553–62.
15. Okabe T, Okamoto I, Tamura K, Terashima M, Yoshida T, Satoh T, et al. Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification. Cancer Res 2007;67:2046–53.
16. Yamanaka K, Nakahara T, Yamauchi T, Kita A, Takeuchi M, Kiyonaga F, et al. Antitumor activity of YM155, a selective small-molecule survivin suppressant, alone and in combination with docetaxel in human malignant melanoma models. Clin Cancer Res 2011;17:5423–31.
17. Iwasa T, Okamoto I, Suzuki M, Nakahara T, Yamanaka K, Hatashita E, et al. Radiosensitizing effect of YM155, a novel small-molecule survivin suppressant, in non-small cell lung cancer cell lines. Clin Cancer Res 2008;14:6496–504.
18. Iwasa T, Okamoto I, Takezawa K, Yamanaka K, Nakahara T, Kita A, et al. Marked anti-tumour activity of the combination of YM155, a novel survivin suppressant, and platinum-based drugs. Br J Cancer 2010;103:36–42.
19. Nakahara T, Takeuchi M, Kinoyama I, Minematsu T, Shirasuna K, Matsuhisa A, et al. YM155, a novel small-molecule survivin

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

suppressant, induces regression of established human hormone-refractory prostate tumor xenografts. Cancer Res 2007;67:8014–21.
20. Kita A, Nakahara T, Yamanaka K, Nakano K, Nakata M, Mori M, et al. Antitumor effects of YM155, a novel survivin suppressant, against human aggressive non-Hodgkin lymphoma. Leuk Res 2011;35: 787–92.
21. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med 2007;4:1669–80.
22. Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med 2007;4: 1681–9.
23. Deng J, Shimamura T, Perera S, Carlson NE, Cai D, Shapiro GI, et al. Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. Cancer Res 2007;67:11867–75.
24. Robertson SC, Tynan J, Donoghue DJ. RTK mutations and human syndromes: when good receptors turn bad. Trends Genet 2000;16:368.
25. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996;2:561–6.
26. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
27. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–67.
28. Sierra JR, Cepero V, Giordano S. Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. Mol Cancer 2010;9:75.
29. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005;353:2012–24.
30. Guha M, Plescia J, Leav I, Li J, Languino LR, Altieri DC. Endogenous tumor suppression mediated by PTEN involves survivin gene silencing. Cancer Res 2009;69:4954–8.
31. Lim M, Zhong C, Yang S, Bell AM, Cohen MB, Roy-Burman P. Runx2 regulates survivin expression in prostate cancer cells. Lab Invest 2010;90:222–33.
32. Faber AC, Li D, Song Y, Liang MC, Yeap BY, Bronson RT, et al. Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. Proc Natl Acad Sci U S A 2009;106: 19503–8.
33. Bivona TG, Hieronymus H, Parker J, Chang K, Taron M, Rosell R, et al. FAS and NF-kappaB signalling modulate dependence of lung cancers on mutant EGFR. Nature 2011;471:523–6.
34. Nakahara T, Kita A, Yamanaka K, Mori M, Amino N, Takeuchi M, et al. Broad spectrum and potent antitumor activities of YM155, a novel small-molecule survivin suppressant, in a wide variety of human cancer cell lines and xenograft models. Cancer Sci 2011; 102:614–21.
35. Tolcher AW, Mita A, Lewis LD, Garrett CR, Till E, Daud AI, et al. Phase I and pharmacokinetic study of YM155, a small-molecule inhibitor of survivin. J Clin Oncol 2008;26:5198–203.
36. Satoh T, Okamoto I, Miyazaki M, Morinaga R, Tsuya A, Hasegawa Y, et al. Phase I study of YM155, a novel survivin suppressant, in patients with advanced solid tumors. Clin Cancer Res 2009;15: 3872–80.
37. Giaccone G, Zatloukal P, Roubec J, Floor K, Musil J, Kuta M, et al. Multicenter phase II trial of YM155, a small-molecule suppressor of survivin, in patients with advanced, refractory, non-small-cell lung cancer. J Clin Oncol 2009;27:4481–6.

Published OnlineFirst November 10, 2011; DOI: 10.1158/1535-7163.MCT-11-0638

AACR American Association for Cancer Research

Molecular Cancer Therapeutics

Overcoming Erlotinib Resistance in *EGFR* Mutation–Positive Non–Small Cell Lung Cancer Cells by Targeting Survivin

Kunio Okamoto, Isamu Okamoto, Erina Hatashita, et al.

Mol Cancer Ther 2012;11:204-213. Published OnlineFirst November 10, 2011.

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2012;42(6)528–533
doi:10.1093/jjco/hys042
Advance Access Publication 28 March 2012

Clinical Impact of Switching to a Second EGFR-TKI After a Severe AE Related to a First EGFR-TKI in *EGFR*-mutated NSCLC

Masayuki Takeda[1], Isamu Okamoto[1,*], Junji Tsurutani[1], Naoki Oiso[2], Akira Kawada[2] and Kazuhiko Nakagawa[1]

[1]Department of Medical Oncology, Kinki University Faculty of Medicine, Osaka and [2]Department of Dermatology, Kinki University Faculty of Medicine, Osaka, Japan

*For reprints and all correspondence: Isamu Okamoto, 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

Received January 23, 2012; accepted February 29, 2012

Objective: Somatic mutations in the epidermal growth factor receptor gene are associated with a therapeutic response to epidermal growth factor receptor tyrosine kinase inhibitors such as gefitinib and erlotinib in patients with non-small cell lung cancer. Although the safety profile of these drugs is favorable, a small proportion of patients with *EGFR* mutation-positive non-small cell lung cancer must discontinue treatment because of adverse events such as interstitial lung disease and hepatotoxicity. Subsequent chemotherapy has not been optimized in such patients.

Methods: We performed a retrospective analysis of *EGFR* mutation-positive non-small cell lung cancer patients who received both gefitinib and erlotinib at our institution. Patients received the second epidermal growth factor receptor-tyrosine kinase inhibitor after experiencing an adverse event or progressive disease on the first epidermal growth factor receptor-tyrosine kinase inhibitor.

Results: We identified 14 patients who received both gefitinib and erlotinib in the course of their treatment. Three patients initially treated with gefitinib and two with erlotinib discontinued epidermal growth factor receptor-tyrosine kinase inhibitor therapy because of severe non-hematologic toxicity (one because of gefitinib-induced interstitial lung disease, one because of erlotinib-induced lupus erythematosus-like eruption and three because of hepatotoxicity). All five of these patients were able successfully to continue therapy with the second epidermal growth factor receptor-tyrosine kinase inhibitor with no evidence of a recurrent adverse event. Progression-free survival was significantly longer in these five patients than in the nine patients who discontinued treatment with the first epidermal growth factor receptor-tyrosine kinase inhibitor because of disease progression.

Conclusions: *EGFR* mutation-positive non-small cell lung cancer patients who discontinue treatment with a first epidermal growth factor receptor-tyrosine kinase inhibitor because of an adverse event benefit substantially from switching to a second epidermal growth factor receptor-tyrosine kinase inhibitor before the development of drug resistance.

Key words: non-small cell lung cancer – epidermal growth factor receptor – tyrosine kinase inhibitor – adverse events

INTRODUCTION

Targeted therapies are undergoing active development as a means to improve treatment efficacy in selected patient populations. Small-molecule tyrosine kinase inhibitors (TKIs) that target the epidermal growth factor receptor (EGFR), such as gefitinib and erlotinib, are the first targeted drugs to enter the clinical use for the treatment of non-small cell lung cancer (NSCLC). Somatic mutations in the EGFR gene are associated with the therapeutic response to EGFR-TKIs in patients with advanced NSCLC. Indeed, retrospective and prospective trials have confirmed that the response rate to gefitinib or erlotinib in patients with *EGFR* mutations is ~70–80% (1,2). Moreover, recently completed randomized Phase III studies showed that first-line gefitinib treatment resulted in an improved progression-free survival (PFS) compared with standard chemotherapy in patients with advanced NSCLC who were selected on the basis of the presence of *EGFR* mutations (3,4), suggesting that more patients with *EGFR* mutation-positive tumors will now receive EGFR-TKIs.

Erlotinib and gefitinib share the same mechanism of action and exhibit highly similar side effect profiles, and a rule for drug selection between the two EGFR-TKIs has not been established. In the case of *EGFR* mutation-positive NSCLC, a study found no clinical evidence for the efficacy of erlotinib after disease progression on gefitinib (5), but the role for administration of a second EGFR-TKI after failure of treatment with a first such drug in *EGFR* mutation-positive patients warrants further investigation. EGFR-TKIs are generally well tolerated, with skin rash and diarrhea being the most common adverse events (AEs) of treatment. However, a small proportion (up to 14%) of NSCLC patients with *EGFR* mutations discontinue EGFR-TKI treatment as a result of more serious AEs such as interstitial lung disease (ILD) or hepatotoxicity (6–10). Moreover, subsequent chemotherapy has not been optimized in such patients. It would therefore be desirable if there were a role for treatment with a second EGFR-TKI after discontinuation of a first such drug as a result of a drug-related AE. We have now performed a retrospective study with the primary objective of assessing the efficacy of a second EGFR-TKI after failure of treatment with a first EGFR-TKI because of the development of a drug-related AE.

PATIENTS AND METHODS

Patients

All lung cancer patients diagnosed at Kinki University Hospital between September 2002 and April 2010 were reviewed. Criteria for the use of a patient's data included signed informed consent for *EGFR* mutation analysis, a diagnosis of Stage IIIb or IV or recurrent NSCLC with a proven *EGFR* mutation, and exposure to both gefitinib and erlotinib. Gefitinib at an initial dose of 250 mg/day or erlotinib at a starting dose of 150 mg/day also must have been given as the first EGFR-TKI therapy. Treatment response was determined on the basis of Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. All toxicities were graded according to National Cancer Institute common toxicity (NCI-CTC) criteria (v4.0). The institutional review board approved our study protocol with the condition that the study be disclosed publicly, according to the Ethical Guidelines for Human Genome Research published by the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Ministry of Economy, Trade and Industry of Japan.

EGFR Mutation Analysis

EGFR mutations that confer sensitivity to EGFR-TKIs were identified either by the Scorpion Amplified Refractory Mutation System (ARMS) method, by the PCR-Invader method (BML, Tokyo, Japan) or by the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan).

Statistical Analysis

Time to treatment failure was assessed from the first day of EGFR-TKI administration to the day of objective disease progression, death or withdrawal of treatment because of an AE. PFS was calculated from the date of EGFR-TKI treatment onset to that of radiographic tumor progression or death. Patients without documented disease progression at the time of the final analysis were evaluated on the basis of the date they were last known to be alive or of their last objective tumor assessment. The probability of survival as a function of time was estimated with the Kaplan–Meier method.

RESULTS

Patient Characteristics

Fifty-five patients with NSCLC who harbored *EGFR* mutations were treated with EGFR-TKIs during the study period. Five (9%) of the 55 patients discontinued EGFR-TKI therapy because of treatment-related AEs, and all five of these individuals subsequently received a second EGFR-TKI. The remaining 50 patients continued EGFR-TKI treatment until disease progression without discontinuation for unacceptable toxicity. Nine of these 50 individuals subsequently received a second EGFR-TKI based on the attending physician's decisions. We thus identified 14 *EGFR* mutation-positive patients who received both gefitinib and erlotinib in the course of their treatment. The patient characteristics and clinical outcome of those 14 patients are summarized in Table 1. Among 14 patients who discontinued first EGFR-TKI therapy, none of the patients except Patient 3 had interruption and resume of first EGFR-TKI administration. In the remaining 41 patients, none had interruption and resume of first EGFR-TKI administration.

Table 1. Characteristics and clinical course of *EGFR* mutation-positive non-small cell lung cancer patients receiving both gefitinib and erlotinib

No.	Sex	Age (years)	Histology	TKI sequence (lines of treatment)	*EGFR* mutation	Reasons for discontinuation (AEs are graded according to the CTCAE v4.0)	Best response to 1st TKI	TTF of 1st TKI (months)	Interval between TKIs (months)	Best response to 2nd TKI	PFS for 2nd TKI (months)
1	F	62	Ad	G (2) → E (3)	E19del	ILD (Gr. 4)	NE	0.8	4.0	SD	2.5
2	F	66	Ad	G (1) → E (2)	E19del	Hepatotoxicity (Gr. 4)	PR	7.5	1.6	PR	11.7
3	F	63	Ad	G (1) → E (2)	L858R	Hepatotoxicity (Gr. 3)	SD	13.4	0.9	SD	4.2[a]
4	F	53	Ad	E (2) → G (4)[b]	E19del	Hepatotoxicity (Gr. 4)	NE	1.4	16.7	SD	4.4
5	F	71	Ad	E (4) → G (5)	L858R	Atypical rash (Gr. 3)	NE	0.5	0.2	PR	10.0[a]
6	F	61	Ad	G (2) → E (3)	L858R	PD	CR	68.9	0.0	SD	4.5
7	F	76	Ad	G (2) → E (7)[c]	E19del	PD	PR	16.1	45.0	PD	0.9
8	F	70	Ad	G (1) → E (2)	L858R	PD	PR	20.4	0.2	PD	1.5
9	F	65	Ad	G (1) → E (2)	L858R	PD	PR	15.6	0.0	SD	4.2
10	F	58	Ad	G (2) → E (3)	L858R	PD	PR	4.2	0.0	SD	2.0
11	F	71	Ad	G (1) → E (2)	L858R	PD	NE	2.4	0.0	PD	1.1
12	F	71	Ad	G (3) → E (5)[d]	L858R	PD	PR	8.2	12.4	SD	4.0
13	F	61	AdSq	G (1) → E (2)	L858R	PD	SD	5.3	2.9	PD	2.2
14	M	63	Ad	E (4) → G (5)	E19del	PD	PR	14.0	0.8	PD	1.5

EGFR, epidermal growth factor receptor gene; TKI, tyrosine kinase inhibitor; AEs, adverse events; TTF, time to treatment failure; PFS, progression-free survival; Ad, adenocarcinoma; G, gefitinib; E, erlotinib; E19del, exon-19 deletion; ILD, interstitial lung disease; Gr., grade; NE, not evaluable; SD, stable disease; PR, partial response; PD, progressive disease; CR, complete response; AdSq, adenosquamous cell carcinoma.
[a]Patients have continued the second EGFR-TKI, and latest follow-up data were collected on 1 August 2011.
[b]Patient 4 received erlotinib followed by docetaxel.
[c]Patient 7 received several systemic chemotherapy regimens, including docetaxel, gefitinib, gemcitabine, S-1, gefitinib rechallenge, gemcitabine rechallenge and erlotinib.
[d]Patient 12 received gefitinib followed by docetaxel.

Clinical Course of Patients Who Developed Severe AEs Related to the First EGFR-TKI

A brief description of the five patients for whom an EGFR-TKI-related AE led to discontinuation of the treatment follows.

Patient 1 was treated with gefitinib (250 mg/day) as a second-line chemotherapy. After 3 weeks of gefitinib treatment, a chest computed tomography scan revealed extensive bilateral ground-glass opacities throughout both lungs, consistent with a diagnosis of gefitinib-induced Grade 4 ILD (Fig. 1). Discontinuation of gefitinib and initiation of high-dose methylprednisolone treatment resulted in improvement in the chest radiological findings. Four months after discontinuation of gefitinib, the patient received erlotinib (150 mg daily) and continued the treatment for 2.5 months until disease progression with no recurrence of ILD.

Patients 2 and 3 received gefitinib (250 mg daily) as a first-line therapy. Their serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased up to 599 U/l (Grade 3) and 1011 U/l (Grade 4) in Patient 2, and 226 U/l (Grade 3) and 519 U/l (Grade 3) in Patient 3, respectively. In Patient 3, reintroduction of gefitinib after its temporary discontinuation on two separate occasions resulted in an increase in AST and ALT levels. After improvement of the liver function on the withdrawal of gefitinib, the decision of subsequent treatment with erlotinib was made by the attending physician since gefitinib has demonstrated antitumor activity in Patients 2 and 3. Treatment with erlotinib (150 mg daily) was then initiated and was continued for 11.7 and 4.2 months in Patients 2 and 3, respectively, with no evidence of recurrent hepatic toxicity.

Patient 4 received erlotinib (150 mg daily) as a second-line treatment. At 6 weeks after the initiation of erlotinib administration, her serum transaminase levels had increased, reaching a pronounced high of 765 U/l (Grade 3) for AST and 1035 U/l (Grade 4) for ALT, and erlotinib was permanently discontinued. Treatment with gefitinib (250 mg/day) was started 16.7 months after the withdrawal of erlotinib, and the patient continued this treatment for 4.4 months until disease progression with no evidence of recurrent hepatic toxicity.

Finally, Patient 5 received erlotinib (150 mg daily) as a third-line therapy. After 2 weeks of erlotinib treatment, she manifested fever, multiple erythematous patches over her upper chest and upper limbs, and prominent butterfly-shaped plaque erythema over her malar eminences that was categorized as lupus erythematosus-like eruption (Grade 3). A skin biopsy specimen from the upper chest revealed superficial perivascular dermatitis with a vacuolar change consistent

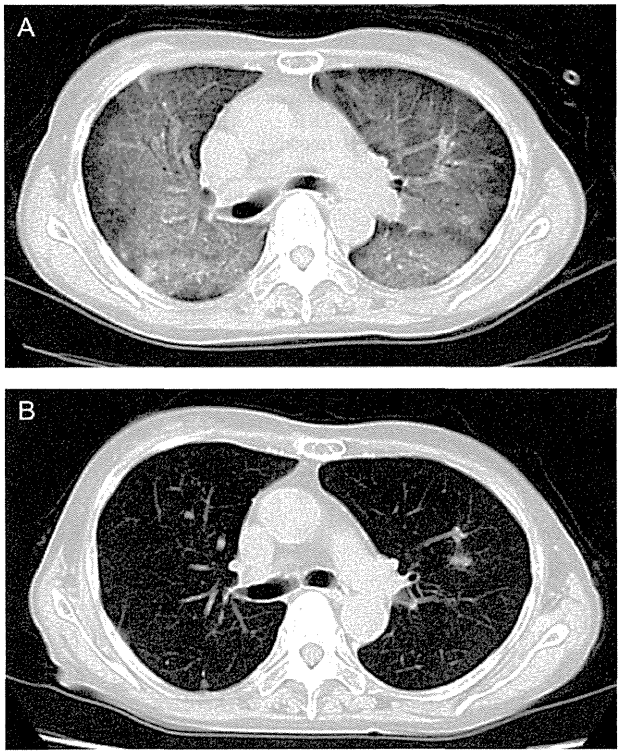

Figure 1. Chest computed tomography (CT) of Patient 1 showed bilateral pulmonary infiltrates on 24 days after initiation of treatment with gefitinib (A). After improvement of gefitinib-induced interstitial lung disease (ILD), chest CT scans demonstrated that no ILD was observed in the patients before treatment with erlotinib (B).

with interface dermatitis, including erythema multiforme, toxic epidermal necrolysis, fixed drug eruption, lupus erythematosus or graft-versus-host disease. A drug lymphocyte stimulation test yielded a strong positive result for erlotinib, suggesting that the atypical rashes were attributable to an allergic reaction to erlotinib rather than to dose-dependent toxicity. Erlotinib treatment was immediately discontinued, and the skin lesions resolved within 1 week with the application of topical corticosteroids. The patient was started on treatment with gefitinib (250 mg daily), which she has continued for 10.0 months with no evidence of disease progression or recurrent skin toxicity at her last follow-up.

Safety and Efficacy of Treatment with a Second EGFR-TKI

Responses could be evaluated in all 14 patients who received a second EGFR-TKI after the failure of treatment with the first EGFR-TKI (Table 1). No patient discontinued treatment with the second EGFR-TKI as the result of a drug-related AE. All five patients who discontinued treatment with the first EGFR-TKI because of an AE (AE group) achieved disease control [two with a partial response (PR) and three with stable disease (SD)] with the second EGFR-TKI. Of the nine patients who discontinued the first EGFR-TKI because of disease progression (PD group), none achieved a PR and four individuals had SD after treatment with the second EGFR-TKI. The Kaplan–Meier curves for PFS are shown in Fig. 2. The median PFS after the onset of treatment with the second EGFR-TKI was 11.7 and 2.0 months in the AE group and in the PD group, respectively (Fig. 2).

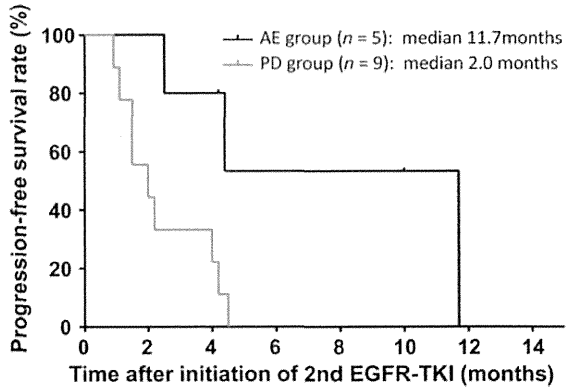

Figure 2. The Kaplan–Meier plots of progression-free survival after initiation of treatment with the second epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) in *EGFR* mutation-positive non-small cell lung cancer patients. Adverse event (AE) and progressive disease (PD) groups discontinued treatment with the first EGFR-TKI because of the development of drug-related AEs or PD, respectively.

DISCUSSION

Mutations of *EGFR* have been identified in tumor specimens from patients with NSCLC who respond to EGFR-TKI treatment. Several prospective studies of EGFR-TKI treatment in *EGFR* mutation-positive NSCLC patients have revealed that reasons for discontinuation of such treatment include non-hematologic AEs such as ILD and hepatitis, with the frequency of treatment withdrawal as a result of these events being 0–14% (6–10). However, subsequent chemotherapy has not been optimized for *EGFR* mutation-positive patients who discontinue EGFR-TKI treatment because of AEs, and limited information exists with regard to the long-term efficacy and safety of treatment with a second EGFR-TKI in such patients. In the present study, among 55 *EGFR* mutation-positive NSCLC patients who had received EGFR-TKIs, 5 individuals (9%) discontinued initial EGFR-TKI treatment as a result of the development of non-hematologic AEs, with the precise reasons for treatment discontinuation being similar to those described in previous studies. All five patients who discontinued treatment with the first EGFR-TKI because of drug-related toxicity were able to continue treatment with a second EGFR-TKI with no evidence of recurrent AEs.

The toxicity profile of erlotinib is highly similar to that of gefitinib, with acneiform rash and diarrhea being the most common side effects (11,12). These side effects are typically mild to moderate, easily managed and reversible. However,

previous studies have shown that ILD and hepatotoxicity are the major causes of permanent discontinuation of gefitinib treatment for patients harboring *EGFR* mutations (6–10). We have now shown that erlotinib is an effective and well-tolerated treatment option for *EGFR* mutation-positive NSCLC patients for whom gefitinib has been discontinued because of severe gefitinib-induced hepatotoxicity. We also found that it was safe to administer gefitinib after discontinuation of erlotinib because of drug-related hepatotoxicity, although no studies have described the safety and efficacy of gefitinib treatment after discontinuation of erlotinib. Erlotinib and gefitinib share a common chemical backbone including a 4-anilinoquinazoline base structure, but they differ in the substituents attached to the quinazoline and anilino rings. Minor differences in the chemical structures of these compounds may thus influence their associated AEs. There have been case reports of successful rechallenge with erlotinib after the development of gefitinib-induced ILD (13). Consistent with these cases, we were able to successfully manage patients who had previously developed gefitinib-induced ILD with a full dose of erlotinib. Given that EGFR-TKI-induced ILD has a high associated mortality, we cannot recommend the routine use of second EGFR-TKI in this setting.

A previous study of erlotinib administration after failure of gefitinib treatment in *EGFR* mutation-positive NSCLC patients found that most patients did not exhibit a radiographic response (5). Our data also show that none of the patients who discontinued treatment with the first EGFR-TKI because of disease progression achieved an objective tumor response to the second EGFR-TKI, with the median PFS after the onset of treatment with the second EGFR-TKI for these patients being only 2.0 months. These findings are consistent with preclinical data showing that the growth of *EGFR* mutation-positive NSCLC cells with a secondary T790M mutation of *EGFR* or with *MET* amplification, the two most common mechanisms of EGFR-TKI resistance, is not inhibited *in vitro* by clinically achievable concentrations of gefitinib or erlotinib (14–17). Together, these observations thus do not support the routine use of a second EGFR-TKI after disease progression during treatment with a first EGFR-TKI in *EGFR* mutation-positive NSCLC patients. In contrast, patients who discontinue treatment with a first EGFR-TKI because of a severe AE would still be expected to benefit substantially from 'switching' to a second EGFR-TKI before the development of resistance, assuming that such patients continue to receive the second EGFR-TKI with no evidence of recurrent toxicity. Although the number of patients with available data is small in the present study, the median PFS of 11.7 months after the onset of treatment with the second EGFR-TKI in the AE group would be considered promising.

In conclusion, treatment with a second EGFR-TKI is an effective and well-tolerated option for *EGFR* mutation-positive NSCLC patients for whom treatment with a first EGFR-TKI has been discontinued because of the development of a severe AE. Our results of this retrospective study are limited by the small sample at single institution. Therefore, further evidence from large cohort studies is warranted. Given the remaining potential for the development of adverse reactions, we suggest that a careful assessment of clinical symptoms and radiographic findings as well as informed consent are warranted in this setting.

Conflict of interest statement

Isamu Okamoto and Kazuhiko Nakagawa received honoraria from Chugai pharmaceuticals and AstraZeneca.

References

1. Morita S, Okamoto I, Kobayashi K, et al. Combined survival analysis of prospective clinical trials of gefitinib for non-small cell lung cancer with EGFR mutations. *Clin Cancer Res* 2009;15:4493–8.
2. Sequist LV, Bell DW, Lynch TJ, Haber DA. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. *J Clin Oncol* 2007;25:587–95.
3. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010;362:2380–8.
4. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010; 11:121–8.
5. Costa DB, Nguyen KS, Cho BC, et al. Effects of erlotinib in EGFR mutated non-small cell lung cancers with resistance to gefitinib. *Clin Cancer Res* 2008;14:7060–7.
6. 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.
7. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. *J Thorac Oncol* 2007;2:22–8.
8. Tamura K, Okamoto I, Kashii T, et al. Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403). *Br J Cancer* 2008;98:907–14.
9. Sunaga N, Tomizawa Y, Yanagitani N, et al. Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy. *Lung Cancer* 2007;56:383–9.
10. 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;26:2442–9.
11. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 2003;21:2237–46.
12. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *J Am Med Assoc* 2003;290:2149–58.
13. Chang SC, Chang CY, Chen CY, Yu CJ. Successful erlotinib rechallenge after gefitinib-induced acute interstitial pneumonia. *J Thorac Oncol* 2010;5:1105–6.

14. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007;104:20932–7.
15. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039–43.
16. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–92.
17. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; 2:e73.

Postmarketing Surveillance Study of Erlotinib in Japanese Patients With Non–Small-Cell Lung Cancer (NSCLC)

An Interim Analysis of 3488 Patients (POLARSTAR)

Kazuhiko Nakagawa, MD, PhD,† Shoji Kudoh, MD, PhD,†‡ Yuichiro Ohe, MD, PhD,§ Takeshi Johkoh, MD, PhD,†|| Masahiko Ando, MD, PhD,†¶ Naoya Yamazaki, MD, PhD,†# Akihiro Seki, MSc,** Shinya Takemoto, MSc,** and Masahiro Fukuoka, MD, PhD††*

Introduction: Interstitial lung disease (ILD) is an adverse drug reaction (ADR) of concern in Japanese patients with non–small-cell lung cancer (NSCLC) receiving erlotinib. To investigate erlotinib safety and efficacy in Japanese patients, a large-scale surveillance study was implemented.

Methods: All patients with recurrent/advanced NSCLC receiving erlotinib in Japan were enrolled (December 2007–October 2009). During the 12-month observation period, adverse-event data were collected; any adverse event where erlotinib could not be excluded as a causative factor was termed an ADR. An independent review committee assessed ILD-like events. Overall survival and progression-free survival were also assessed. Interim data were analyzed for patients registered before June 30, 2008.

Results: In total, 10,708 patients were enrolled, 3743 by June 30, 2008, with data available for 3488 patients. Overall ADR incidence was 81.8% (mostly grade 1/2); skin disorders (68.5%) including rash (63.0%) were most common. However, 81.8% of patients who experienced rash recovered or improved. ILD-like events, diagnosed by local physicians, were reported in 189 patients. The independent review committee confirmed ILD (all grades) in 158 patients (4.5% of interim population) with a mortality rate of 1.6% (55 patients). Significant ILD risk factors included concomitant or previous ILD, smoking history, concomitant or previous lung infection, and Eastern Cooperative Oncology Group performance status 2 to 4. Median overall survival and progression-free survival were 260 and 64 days, respectively.

Conclusions: These interim data support the clinical benefits of erlotinib in Japanese NSCLC patients with no new safety signals. The risk/benefit balance for erlotinib in recurrent/advanced NSCLC remains favorable.

Key Words: Erlotinib, Surveillance, Non–small-cell lung cancer, Safety, Interstitial lung disease (ILD).

(*J Thorac Oncol.* 2012;7: 1296–1303)

Erlotinib (Tarceva, Chugai Pharmaceutical Co. Ltd., Tokyo, Japan) is a highly potent, orally active, epidermal growth factor receptor (EGFR) tyrosine-kinase inhibitor (TKI). In the pivotal phase III BR.21 study, erlotinib significantly prolonged overall survival (OS) compared with placebo in patients with advanced non–small-cell lung cancer (NSCLC), who had received at least one line of chemotherapy.[1] Similar promising survival data were reported in two Japanese phase II trials of erlotinib in patients with advanced NSCLC,[2,3] which in 2007 led to the approval of erlotinib in Japan for the treatment of patients with recurrent/advanced NSCLC after failure of at least one prior chemotherapy regimen.

Erlotinib has been well tolerated as per the findings of Japanese phase I/II and pivotal BR.21 studies, with rash and diarrhea (generally mild or moderate) being the most common adverse events (AEs).[2–4] Among Japanese patients, interstitial lung disease (ILD), which is a heterogeneous group of parenchymal lung diseases, was reported in 4.9% of patients (6 of 123) with 2.4% (3 of 123 patients) being fatal.[2–4] Similar incidences of ILD have also been reported among Japanese patients with NSCLC treated with the EGFR TKI gefitinib (4.0%–5.8%).[5,6] Consequently, ILD was considered a major safety concern for erlotinib, and safety measures were implemented after its launch in Japan. The incidence of ILD, the risk factors for developing ILD, and erlotinib efficacy are

*Department of Medical Oncology, Kinki University School of Medicine, Osaka, Japan; †Independent Appropriate Use Advisory Board for Erlotinib; ‡Anti-Tuberculosis Association Fukujuji Hospital, Tokyo, Japan; §Division of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan; ||Department of Radiology, Kinki Central Hospital of Mutual Aid Association of Public School Teachers, Hyogo, Japan; ¶Center for Advanced Medicine and Clinical Research Nagoya University Graduate School of Medicine, Aichi, Japan; #Dermatology Division, National Cancer Center Hospital, Tokyo, Japan; **Pharmacovigilance Department, Chugai Pharmaceutical Co. Ltd, Tokyo, Japan; and ††Division of Cancer Center, Izumi Municipal Hospital, Osaka, Japan.

Disclosure: Drs. Nakagawa, Kudoh, Ohe, Johkoh, Ando, Yamazaki, and Fukuoka have all participated as advisory board members reimbursed by Chugai Pharmaceutical Co. Ltd. Dr. Ohe also has an immediate family member who is an employee of Chugai Pharmaceutical Co. Ltd. Akihiro Seki and Shinya Takemoto are full-time employees of Chugai Pharmaceutical Co. Ltd. This study was sponsored by Chugai Pharmaceutical Co. Ltd., Tokyo, Japan.

Address for correspondence: Kazuhiko Nakagawa, MD, PhD, Department of Medical Oncology, Kinki University School of Medicine, Osaka 589–8511, Japan. E-mail: nakagawa@med.kindai.ac.jp

ISSN: 1556-0864/12/0708-1296

being investigated in the real-world setting through a large-scale surveillance program that includes all Japanese patients with NSCLC treated with erlotinib (POst-Launch All-patient-Registration Surveillance in TARceva [POLARSTAR]-treated NSCLC patients). This is one of the largest surveillance studies in Japan, with more than 10,000 patients registered. This article reports interim safety and efficacy data from the first 6 months, and provides an insight into the treatment profile of erlotinib in a previously underevaluated population.

MATERIALS AND METHODS

Patients and Surveillance Study Design

Patients with unresectable, recurrent and/or advanced NSCLC, who were treated with erlotinib in Japan between December 2007 and October 2009 were enrolled. Eligible patients receiving oral erlotinib (150 mg once daily) from 1027 institutions that could prescribe erlotinib (up to October 12, 2009), were monitored until termination of erlotinib therapy or completion of 12 months of treatment.

Safety Assessments

Demographic and baseline data were collected for each patient, including information on age, sex, body mass index (BMI), tumor histology, tumor stage, Eastern Cooperative Oncology Group performance status (ECOG PS), treatment status, smoking history, metastatic disease, medical histories including hepatic dysfunction, renal dysfunction, cardiovascular disease, and lung disorder (emphysema, chronic obstructive pulmonary disease [COPD], lung infection, and ILD), which were diagnosed by local physicians, concomitant medications, and history of chemotherapy. Safety and efficacy data were collected at 1, 6, and 12 months after the start of erlotinib therapy. AEs were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (Japanese version JCOG/JSCO edition, October 2004) and were coded using Medical Dictionary for Regulatory Activities (MedDRA) version 12.1. An adverse drug reaction (ADR) was defined as an AE for which a causal relationship with erlotinib could not be ruled out.

AEs of special concern for erlotinib were captured (ILD, skin disorders, hepatic dysfunction, diarrhea, eye disorders, and hemorrhage). All reported ILD-like events were assessed individually by an independent ILD review committee of pulmonologists, chest radiologists and pathologists based on medical and pathologic findings and chest imaging. The outcome of ILD and time to ILD onset from the first erlotinib dose given during the observation period were analyzed. Patients without data for either the duration of observation or for the time from the start of erlotinib therapy were excluded from the analysis of time to ILD onset. The incidence of ILD was determined by dividing the number of patients who developed ILD during the specified observation period by the number of patient-days during the observation period (defined as the total duration [number of days] of observation for all patients receiving erlotinib during the specified observation period) and expressed per 100 patient-weeks. The incidence of fatal ILD was also expressed per 100 patient-weeks.

Efficacy Assessments

Efficacy was assessed by OS, defined as the time from the start of erlotinib treatment to death from any cause, and progression-free survival (PFS), defined as the time from the start of erlotinib administration to disease progression or death by any cause for patients without disease progression. Assessment of disease progression was performed according to treating physicians' standard clinical practices. No centralized independent assessment was done.

Statistical Analyses

Three thousand erlotinib-treated patients with NSCLC were to be enrolled so that an AE could be detected in one of 1000 patients, with a 95% confidence interval (95% CI). However, during enrollment, the target accrual was increased, and enrollment continued up to October 2009 after discussions with the Japanese Health Authority to further evaluate erlotinib safety and efficacy. Although patients were enrolled up to October 2009, an interim analysis of safety and efficacy was preplanned for the data available from patients enrolled up to June 30, 2008. The primary endpoint was the pattern of occurrence of ILD and risk factors for the onset of ILD among erlotinib-treated patients with NSCLC. Secondary endpoints included the pattern of ADRs other than ILD and factors that might affect the safety of erlotinib, and the overall safety and efficacy of erlotinib.

Multivariate Cox regression analysis with a stepwise model selection procedure was conducted to determine the risk factors for ILD. Occurrence or nonoccurrence of ILD was used as the dependent variable. Exploratory variables included sex; age; BMI; histology; time from the date of first diagnosis of NSCLC to the start of treatment; concomitant or previous emphysema or COPD; ILD or lung infection; concomitant hepatic dysfunction; renal dysfunction or cardiovascular disease; history of allergies; smoking history; ECOG PS; chest radiotherapy; pretreatment lactate dehydrogenase (LDH) levels; number of chemotherapy regimens for primary disease; and history of gemcitabine and gefitinib treatment. Exploratory variables with p value of 0.05 or more were eliminated from the final model. After the identification of any risk factors, additional multivariate analyses were conducted to investigate two-factor interactions, which included additional explanatory variables in the final model of the Cox regression analysis with each pair of risk factors. Statistical significance was set at p less than 0.05.

OS and PFS were estimated using Kaplan-Meier methodology. Patients without data for the duration of the observation period or from the time of treatment initiation were excluded from analyses of OS and PFS. OS and PFS data are presented as medians with 95% CI. Statistical analyses were performed using Statistical Analysis Software (version 9.1; SAS Institute, Cary, NC).

The safety population comprised all patients who received erlotinib and had case-report form data available. The efficacy population comprised all patients included in the safety population, except those for whom erlotinib therapy was prescribed off-label (first line) at the time of this study, or those patients whose therapeutic history was unknown.

RESULTS

Patient Demographics

A total of 10,708 patients were enrolled between December 18, 2007 and October 12, 2009 (Fig. 1). By June 30, 2008 (interim analysis data cutoff), 3743 patients had been enrolled, of whom 3488 were included in the interim safety population (255 patients were excluded from the safety analysis [Fig. 1]). The efficacy population comprised 3453 patients (35 patients were excluded from the interim efficacy analysis [Fig. 1]). One patient was excluded from OS analyses and seven patients were excluded from PFS analyses.

Baseline characteristics of the safety population are summarized in Table 1. Median age was 65 years. The majority of patients (83.1%) had adenocarcinoma, 51.7% had a history of smoking, 56.6% had received more than three lines of prior treatment at study enrollment, and 55.1% had a history of prior gefitinib therapy. Previous first-line chemotherapy included platinum-based doublets (73.2%), principally carboplatin-based (52.8%; predominantly carboplatin/paclitaxel, 39.1%), nonplatinum single agents (20.7%; predominantly gefitinib, 11.6%), and nonplatinum doublets (2.8%; predominantly vinorelbine/gemcitabine, 1.3%).

Safety

AEs and ADRs were reported in 2911 patients (83.5%) and 2852 patients (81.8%), respectively, and ADRs resulted in discontinuation of erlotinib therapy in 401 patients (14.1%). The majority of ADRs were grade 1 or 2 in severity (18.3% were grade ≥3) and the most common ADRs (incidence ≥ 10%) were skin and subcutaneous tissue disorders (68.5%), including rash (63.0%; 6.7% grade ≥ 3), and gastrointestinal disorders (32.0%), including diarrhea (23.5%; 1.3% grade ≥ 3) (Tables 2 and 3). For other ADRs of special concern, hepatic function disorder, eye disorder, and hemorrhage, the incidence of grade 3 or higher events was low (Table 3). However, for ILD, which occurred in 4.5% of patients, more than half the cases were grade 3 or higher (2.6%; Table 3).

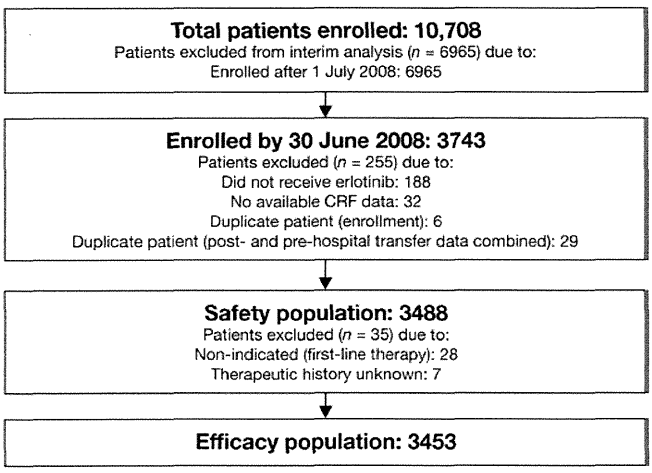

FIGURE 1. Disposition of patients included in the interim analysis. CRF, case-report form.

TABLE 1. Demographic and Baseline Characteristics (n = 3488)

Characteristic	Value[a] (Range or %)
Median age, years (range)	65 (15–92)
<65 yrs, *n* (%)	1676 (48.1)
≥65 yrs, *n* (%)	1811 (51.9)
Sex, *n* (%)	
Male	1792 (51.4)
Female	1696 (48.6)
BMI (kg/m^2), *n* (%)	
<18.5	595 (19.3)
18.5–25	2036 (66.1)
≥25	450 (14.6)
Histology, *n* (%)	
Adenocarcinoma	2891 (83.1)
Squamous cell	399 (11.5)
Large cell	53 (1.5)
Other	135 (3.9)
Stage, *n* (%)	
Recurrent	1268 (36.7)
IIIB	416 (12.0)
IV	1720 (49.8)
Other	52 (1.5)
ECOG PS, *n* (%)	
0	1008 (28.9)
1	1568 (45.0)
2	641 (18.4)
3	216 (6.2)
4	53 (1.5)
Treatment line, *n* (%)	
First	28 (0.8)
Second	666 (19.2)
Third	815 (23.5)
Fourth	760 (21.9)
≥Fifth	1205 (34.7)
History of smoking, *n* (%)	1777 (51.7)
History of gefitinib[b], *n* (%)	1905 (55.1)
History of platinum-based agent[b], *n* (%)	2976 (85.3)

BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status.
[a]Percentages do not include patients with unreported data.
[b]At any line before erlotinib treatment.

For the majority of ADRs, the median time to onset from the start of erlotinib therapy was within 15 days (notably rash and diarrhea occurred within 8 days) although paronychia occurred at 32 days. Of those who experienced skin disorders, diarrhea, and eye disorders, more than 70% continued erlotinib therapy, whereas for those experiencing hemorrhage, hepatitis, hepatic failure, or liver dysfunction, only around 50% of patients continued erlotinib therapy (Table 4). The majority of patients (72.6%–94.7%) who experienced an ADR either recovered or improved (Table 4).

Interstitial Lung Disease

Reported ILD-like events (n = 189) included ILD (n = 168), pneumonitis (n = 9), pulmonary fibrosis (n = 4), radiation

TABLE 2. Incidence of ADRs in Patients Receiving Erlotinib (n = 3488)

System Organ Class[a]	Any Grade[b], n (%)	Grade[b] ≥ 3, n (%)
Total	2852 (81.8)	637 (18.3)
Skin and subcutaneous tissue disorders	2388 (68.5)	256 (7.3)
Gastrointestinal disorders	1115 (32.0)	91 (2.6)
Infections and infestations	301 (8.6)	36 (1.0)
Respiratory, thoracic, and mediastinal disorders	264 (7.6)	118 (3.4)
Hepatobiliary disorders	256 (7.3)	49 (1.4)
Metabolism and nutrition disorders	242 (6.9)	45 (1.3)
Investigations	210 (6.0)	37 (1.1)
General disorders and administration-site conditions	161 (4.6)	27 (0.8)
Eye disorders	139 (4.0)	10 (0.3)
Nervous system disorders	87 (2.5)	14 (0.4)
Blood and lymphatic system disorders	27 (0.8)	15 (0.4)
Renal and urinary disorders	23 (0.7)	2 (0.1)
Psychiatric disorders	15 (0.4)	4 (0.1)
Musculoskeletal and connective-tissue disorders	13 (0.4)	2 (0.1)
Vascular disorders	10 (0.3)	2 (0.1)
Injury, poisoning, and procedural complications	7 (0.2)	1 (<0.1)
Cardiac disorders	5 (0.1)	2 (0.1)
Neoplasms benign, malignant and unspecified	2 (0.1)	0 (0.0)
Reproductive system and breast disorders	2 (0.1)	1 (<0.1)
Ear and labyrinth disorders	1 (<0.1)	0 (0.0)

ADR, adverse drug reaction.
[a]System Organ Class (SOC) as coded by the Medical Dictionary for Regulatory Activities (MedDRA).
[b]The highest grade with National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) was used for multiple same SOCs in one patient.

TABLE 3. Incidence and Severity of Major ADRs of Special Concern

Event	Any Grade[a], n (%)	Grade[a] ≥3, n (%)
Skin disorder		
Rash	2199 (63.0)	234 (6.7)
Dry skin	270 (7.7)	8 (0.2)
Pruritus	132 (3.8)	6 (0.2)
Paronychia	210 (6.0)	23 (0.7)
Diarrhea	819 (23.5)	44 (1.3)
Hepatic function disorder	374 (10.7)	64 (1.8)
ILD	158 (4.5)	90 (2.6)
Eye disorder	139 (4.0)	10 (0.3)
Hemorrhage	54 (1.5)	15 (0.4)

ADR, adverse drug reaction; ILD, interstitial lung disease.
[a]The highest grade with National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) was used for multiple same events in one patient.

pneumonitis (n = 3), pulmonary alveolar hemorrhage, organizing pneumonia (n = 2 each), and diffuse alveolar damage (n = 1). Of these 189 events, ILD was confirmed by the independent ILD review committee in 158 patients (4.5% of this interim population), of whom 86 (54.4%) recovered or experienced an improvement in their condition (Table 4). The majority of patients with ILD discontinued erlotinib (88.9%), and 55 patients died (34.8%); thus, the overall mortality rate resulting from ILD was 1.6%. A further 14 patients did not recover, one patient experienced sequelae and the outcome was unknown in two patients.

Time to onset of ILD was within 4 weeks (median 23 days; Table 4) of the start of erlotinib therapy in majority of the patients (n = 87, 55.0%). The highest incidence of ILD (0.82 per 100 patient-weeks) occurred within 2 weeks of the initiation of erlotinib therapy (Fig. 2). Within 2 to 8 weeks of the start of therapy, the incidence of ILD was 0.30 to 0.60 per 100 patient-weeks; notably, ILD also occurred after 8 weeks of treatment (0.11 per 100 patient-weeks) (Fig. 2). The incidence of fatal ILD was 0.16 to 0.29 within 4 weeks of the initiation of erlotinib therapy (Fig. 2).

Univariate analysis implied a higher risk of ILD in patients who were male or had a BMI less than 25 kg/m^2, with concomitant or previous emphysema or COPD, concomitant or previous ILD, concomitant or previous lung infection, a history of smoking, ECOG PS of 2 to 4, a history of chest radiotherapy, pretreatment lactate dehydrogenase (≤250 IU/l), and no previous treatment with gefitinib. Multivariate analysis showed that concomitant or previous ILD, smoking history, concomitant or previous lung infection, and ECOG PS of 2 to 4 were significant risk factors for the development of ILD (Table 5). Additional Cox regression analyses showed no significant pairs of risk factors and there were no two-factor interactions.

Efficacy

In the efficacy population, median OS was 260 days (95% CI: 239–279; Fig. 3*A*) and median PFS was 64 days (95% CI: 60–68; Fig. 3*B*). The 12-month rates for OS and PFS were 40.9% and 9.6%, respectively. In subgroup analyses, OS and PFS were numerically longer in women, nonsmokers, patients with nonsquamous-cell carcinoma, those with a PS of 0 to 1, and those experiencing rash of grade 2 or more (Fig. 4*A*, *B*).

DISCUSSION

The interim analysis of results from this surveillance study provides safety and efficacy data for 3488 Japanese patients with NSCLC treated with erlotinib during its first 6 months of approval. The development of ILD, a rare but potentially serious drug-related complication, was reported in 4.5% of patients with approximately one-third of cases proving fatal. These results are comparable with ILD-associated incidence rates of around 3% to 4% and mortality rates of 27.9% to 38.6% reported among Japanese patients with NSCLC treated with gefitinib or chemotherapy.[5,6] But these results are greater than those reported in the global Tarceva Lung Cancer Survival Treatment (TRUST) trial in pretreated NSCLC (<1% incidence).[7] The reasons for the higher incidence of ILD in