Erlotinib at a Dose of 25 mg Daily for Non-small Cell Lung Cancers with *EGFR* Mutations

Wee-Lee Yeo, MD,† Gregory J. Riely, MD, PhD,‡ Beow Y. Yeap, ScD,§ Michelle W. Lau, MD,* Jeremy L. Warner, MD,* Kelly Bodio, MD,* Mark S. Huberman, MD,* Mark G. Kris, MD,‡ Daniel G. Tenen, MD,*† William Pao, MD, PhD,‖ Susumu Kobayashi, MD, PhD,* and Daniel B. Costa, MD, PhD**

Purpose: The tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib are effective in non-small cell lung cancers (NSCLCs) with *epidermal growth factor receptor* (*EGFR*) gene mutations. The usual clinical dose of gefitinib (250 mg/d) is only one third of its maximum tolerated dose, whereas the dose of erlotinib (150 mg/d) is at its maximum tolerated dose. In NSCLC cell lines, both TKIs have similar micromolar inhibitory concentrations. We explored whether erlotinib at 25 mg/d (trough serum concentration similar to gefitinib 250 mg/d) would be efficacious in *EGFR*-mutated NSCLC.

Methods: To study the inhibitory concentrations of gefitinib and erlotinib, we exposed *EGFR*-mutated cell lines (HCC827, H3255, PC-9, and H1975) to increasing concentrations of these TKIs. Further on, we performed a retrospective evaluation of seven patients with advanced *EGFR*-mutated (exon 19 deletions and L858R) NSCLC that were given erlotinib at 25 mg/d as their first EGFR TKI.

Results: Gefitinib and erlotinib generated similar inhibitory curves across our panel of *EGFR*-mutated NSCLC cell lines with overlapping mean 50% inhibitory concentration 95% confidence intervals for HCC827, PC-9, and H1975. Both drugs also displayed a high degree of correlation in mean 50% inhibitory concentration (Pearson's r = 0.99, $p = 0.0417$). Of the seven patients, five patients (71.5%) had partial responses to erlotinib 25 mg/d. Median progression-free survival was 17 months (95% confidence interval, 6–35 months). Toxicities were minimal, with only two (28.5%) patients having a rash and none experiencing (0%) diarrhea.

Conclusions: In NSCLC cell lines, gefitinib and erlotinib have similar inhibitory profiles. In patients with NSCLC and *EGFR*-activating mutations, a dose of erlotinib 25 mg/d (equivalent to gefitinib 250 mg/d) leads to impressive response rates and progression-free survival similar to the growing experience with the approved doses of gefitinib (250 mg/d) and erlotinib (150 mg/d). Identifying prospectively the lowest and clinically active dose ranges of erlotinib and gefitinib will help further to personalize care for patients with tumors harboring *EGFR* mutations.

Key Words: Epidermal growth factor receptor, EGFR, Mutation, Tyrosine kinase inhibitors, Gefitinib, Erlotinib, L858R, Exon 19 deletions, Lung cancer, Non-small cell lung cancer.

(*J Thorac Oncol.* 2010;5: 1048–1053)

Somatic mutations in the tyrosine kinase domain of the *epidermal growth factor receptor* (*EGFR*) gene were identified in patients with non-small cell lung cancer (NSCLC) in 2004.[1–3] These mutations are more prevalent in women, never or light smokers, patients with East Asian ancestry, and patients whose tumors demonstrate adenocarcinoma histology.[4] Most clinically relevant *EGFR* mutations consist of a single point mutation (L858R) in exon 21 and inframe deletions around the conserved LREA (leucine, arginine, glutamic acid, alanine) motif of exon 19 (residues 747–750).[5] *EGFR* mutations are oncogenic, alter the tyrosine kinase pocket of EGFR to a degree that enhances the sensitivity to ATP-competitive EGFR inhibitors, and make lung cancers with *EGFR* mutations dependent on EGFR signaling for their survival and proliferation.[6]

Two EGFR tyrosine kinase inhibitors (TKIs) have reached regulatory approval for NSCLC: gefitinib and erlotinib.[7–9] When given as first, second, or subsequent lines of therapies for patients with *EGFR* mutations, these agents lead to significant clinical and radiographic responses in most patients.[10] The reported response rates (RRs) exceed 70%, with median progression-free survival (PFS) intervals of approximately 6 to 12 months and overall survival times beyond 20 to 24 months.[11,12] Gefitinib improves RR and PFS when compared with standard platinum-doublet chemotherapy in therapy naive patients with metastatic *EGFR*-mutated NSCLC.[13] Erlotinib is equally efficacious in *EGFR*-mutated

*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; †Cancer Science Institute, National University of Singapore, Singapore; ‡Memorial Sloan-Kettering Cancer Center, New York, New York; §Massachusetts General Hospital, Boston, Massachusetts; and ‖Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, Tennessee.

Disclosure: W.P., G.J.R., and M.G.K. received consulting fees from Astra Zeneca. G.J.R. and M.G.K. received fees from Boehringer Ingelheim; M.G.K. also received fees from Pfizer and Novartis. The EGFR T790M patent is licensed to MolecularMD (W.P.).

Address for correspondence: Daniel B. Costa, MD, PhD, or Susumu Kobayashi, MD, PhD, Division of Hematology/Oncology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215. E-mail: dbcosta@bidmc.harvard.edu or skobayas@bidmc.harvard.edu

ISSN: 1556-0864/10/0507-1048

tumors when given as a first line therapy but has not been compared head-to-head with chemotherapy in the published reports.[14]

NSCLCs that harbor activating *EGFR* mutations are sensitive to gefitinib or erlotinib in equivalent submicromolar concentrations.[6] Despite the similar in vitro spectrum of action in NSCLC cells, the clinical development and therapeutic doses used in the clinic for these two TKIs, which were initially developed to target wild-type EGFR, differs. The dose of gefitinib (250 mg/d) that entered late stage clinical trials for NSCLC is only one third of its maximum tolerated dose (MTD), whereas the dose used for erlotinib (150 mg/d) is its MTD.[15,16] Data from phase I dose-escalation trials show that the expected mean trough steady state serum concentration of gefitinib at 250 mg/d is approximately 0.5 μM,[17] whereas erlotinib at 150 mg/d has mean trough steady state concentrations that exceed 2.5 μM.[18] This knowledge led us to hypothesize that erlotinib at 25 mg/d (its lowest available tablet size with presumed trough serum concentration around 0.5 μM) would be active in *EGFR*-mutated NSCLC.

Here, we confirm that both gefitinib and erlotinib have similar in vitro inhibitory concentrations in NSCLC cells with *EGFR* mutations and demonstrate that erlotinib 25 mg/d leads to a high RR and PFS in patients with NSCLC harboring *EGFR*-activating mutations.

MATERIALS AND METHODS

Reagents

Gefitinib and erlotinib were purchased from a commercial supplier. Stock solutions for gefitinib and erlotinib were prepared as described previously.[19]

Cell Culture

The human lung cancer–derived cells lines NCI-H1975 (H1975), NCI-H3255 (H3255), PC-9, and HCC827 were maintained in Roswell Park Memorial Institute culture medium supplemented with 10% fetal bovine serum.

Cell Proliferation Assay

Growth inhibition was assessed by CellTiter 96 AQueous One solution proliferation kit (Promega, Madison, WI) as previously described.[20,21] Briefly, cells were transferred to wells at 1500 to 5000 cells/well (for H1975, 1500; for HCC827, 2,500; for PC-9, 3,500, and for H3255, 5000) in 96-well flat-bottom plates with various concentrations of inhibitors and incubated for 72 hours before photometric analysis. Experiments were repeated for three times.

Patient Selection

Patients were identified retrospectively from the clinical databases of two academic medical centers: (1) Beth Israel Deaconess Medical Center and (2) Memorial Sloan-Kettering Cancer Center. Both centers have institutional-approved protocols for chart review and genomic analysis of stored tumor tissues. Inclusion criteria to use the patient's data included a diagnosis of stage IV metastatic NSCLC with a proven *EGFR* mutation and exposure to erlotinib at a dose of 25 mg daily. Data were collected from the patients' medical records for clinical, demographic, and pathologic characteristics and toxicity. Radiographic data was reviewed by each center. Treatment decisions were made by each treating physician.

EGFR Genotype in the Identified Patients

DNA isolation from paraffin-embedded tissue and *EGFR* genotype followed protocols described previously.[15] In all cases, either exons 18 to 21 of the *EGFR* gene were sequenced or sensitive polymerase chain reaction amplification techniques identified L858R and deletions in exon 19.

Treatment Schedules, Response, Progression-Free Survival Assessment, and Statistical Analysis in the Identified Patients

All retrospectively identified patients had the same initial treatment schedule for erlotinib. This medication was given orally at a dose of 25 mg/d, and erlotinib was used until tumor progression, unacceptable toxicity, or at the physician's discretion. Because the patients identified had been treated as a part of routine clinical care, the investigators had no control of the dosing schedules and follow-up proposed by each treating physician. Objective tumor response was determined retrospectively by RECIST version 1.0.[22] PFS was calculated from the date of starting erlotinib until the date of radiographic tumor progression. All patients had adverse reactions determined, by chart extraction, while receiving erlotinib according to the Common Terminology Criteria for Adverse Events, version 3.0. The data cutoff for analysis of PFS and other endpoints was February 1, 2010.

Statistical Analysis

The inhibitory proliferation curves and the 50% inhibitory concentration (IC_{50}) in the aforementioned proliferation assays were generated and calculated, respectively, using the GraphPad Prism version 5 software (GraphPad Software Inc, La Jolla, CA). Pearson's correlation coefficient (r) was used as a measure of association between IC_{50}s for gefitinib and erlotinib. A log transformation was applied to the dose data, and the exact two-sided *p* value was calculated using StatXact (Cytel Software Corporation, Cambridge, MA). PFS estimates were made using the Kaplan-Meier method,[23] and the 95% confidence interval (CI) for the median was based on the sign test. Analysis was conducted with SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

In Vitro Inhibitory Concentration of Gefitinib and Erlotinib in NSCLC Cells with *EGFR*-Activating Mutations

We selected a set of NSCLC cell lines to identify the sensitivity of *EGFR*-mutated cells to the effects of increasing concentrations of gefitinib and erlotinib. HCC827 and PC-9 have an identical exon 19 deletion (delE746_A750).[20] H3255 carries the L858R *EGFR* exon 21 point mutation.[2] H1975 carries the L858R mutation in association with the T790M EGFR TKI-resistant mutation.[24]

After exposure to increasing concentrations of gefitinib or erlotinib, HCC827, PC-9, and H3255 had their proliferation inhibited by these EGFR TKIs in sub 0.5 μM concentrations (Figure 1). H1975 cells were not significantly inhibited up to 5 μM of exposure (Figure 1).

As indicated in Figure 1, both gefitinib and erlotinib led to similar inhibitory curves across our sample of *EGFR*-mutated cells. At concentrations between 0.001 and 1 μM, the curves overlapped for HCC827, PC-9, and H1975.

The calculated mean IC_{50} for gefitinib was 0.002686 μM (95% CI, 0.001649–0.004376) for HCC827, 0.02632 μM (95% CI, 0.01748–0.03963) for PC-9, 0.03843 μM (95% CI, 0.03149–0.04690) for H3255, and 11.580 μM (95% CI, 9.827–13.660) for H1975. The mean IC_{50} for erlotinib was 0.002142 μM (95% CI, 0.001307–0.003510) for HCC827, 0.03136 μM (95% CI, 0.02082–0.04724) for PC-9, 0.08898 μM (95% CI, 0.07294–0.1085) for H3255, and 9.183 μM (95% CI, 7.640–11.040) for H1975.

The mean IC_{50} values for both gefitinib and erlotinib in three of four *EGFR*-mutated cell lines had overlapping 95% CIs (HCC827, PC-9, and H1975). Gefitinib and erlotinib also had a high degree of correlation in mean IC_{50} (Pearson's r = 0.99, $p = 0.0417$). These results indicate that both EGFR inhibitors have similar inhibitory patterns in *EGFR*-mutated cells and that they are strongly correlated.

Patient Characteristics

Records of *EGFR* genotyped patients from our centers during the periods of 2004 to 2010 were reviewed. We retrospectively identified seven *EGFR*-mutated patients that had received erlotinib at a dose of 25 mg daily as their first EGFR TKI.

Clinical, demographic, pathologic, and molecular characteristics of this cohort are displayed in Table 1. Medical record extraction and direct confirmation with the treating physician identified that in all cases, the treating physician offered the dose of 25 mg daily of erlotinib because of its supposed equivalence to gefitinib 250 mg daily and that patients were informed that the 25 mg dose was not the standard dose of erlotinib for NSCLC. In one of the cases (patient 4, Table 2), notes from the treating physician also reported a previous history of treated hepatitis B as a reason to offer erlotinib at 25 mg daily.

Response and PFS to Erlotinib 25 mg/d

Five of the seven patients (71.5%) had partial radiographic responses as defined by RECIST to erlotinib (Table 2), a number that is compatible with retrospective and prospective data for *EGFR*-mutated patients treated with gefitinib 250 mg/d or erlotinib 150 mg/d.[25] Two patients (28.5%) had stable disease, and none of the patients had de

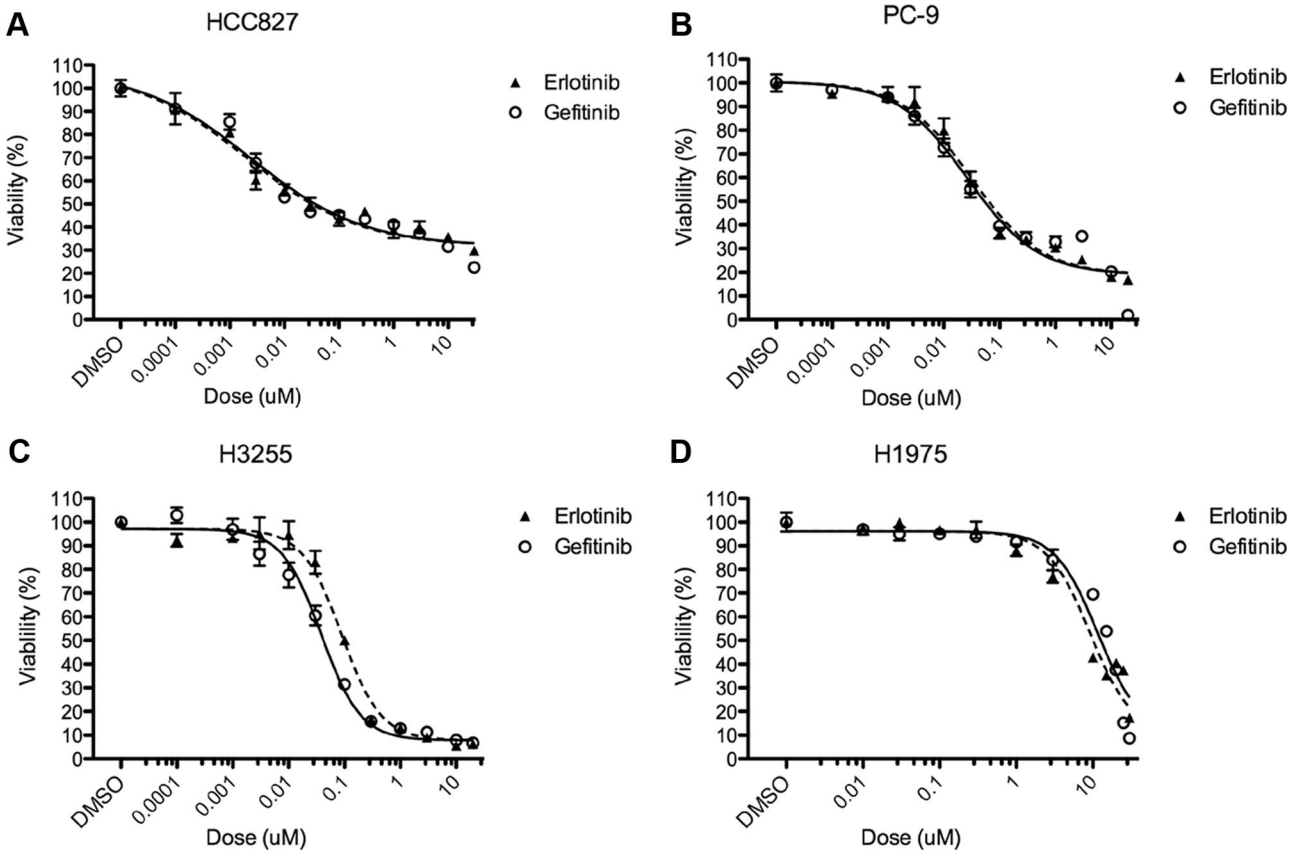

FIGURE 1. Proliferation assays of non-small cell lung cancer cells exposed to gefitinib (—) and erlotinib (---) for 72 hours. *A,* HCC827 (n = 3). *B,* PC-9 (n = 3). *C,* H3255 (n = 3). *D,* H1975 (n = 3).

TABLE 1. Clinical, Pathologic, Demographic, and Molecular Characteristics of the Studied *EGFR*-Mutated Patients

Characteristic	Patients, N (%)
Age (yr), median (range)	65 (55–86)
Sex	
Female	5 (71.5)
Male	2 (28.5)
Smoking history	
Never smoker	1 (14.2)
Former smoker	5 (71.5)
Current smoker	1 (14.2)
Histology	
Adenocarcinoma	6 (85.7)
NSCLC-NOS	1 (14.2)
EGFR mutation	
Exon 19 deletion[a]	5 (71.5)
L858R	2 (28.5)
Therapy before erlotinib	
Platinum-based chemotherapy	2 (28.5)
No previous therapy	5 (71.5)

[a] Specific *EGFR* sequences of the exon 19 deletions are detailed in Table 2.

EGFR, epidermal growth factor receptor; NSCLC-NOS, non-small cell lung cancer-not otherwise specified.

novo resistance to erlotinib with progressive disease as best response.

The median PFS calculated by the Kaplan-Meier method was 17 months (95% CI, 6–35 months) as shown in Figure 2. Individual PFS and the patients' dose schedules while using erlotinib 25 mg/d are detailed in Table 2.

Adverse Reactions to Erlotinib 25 mg/d

There were no grade 3 or 4 adverse events recorded during the treatment period, and no patients discontinued erlotinib because of unacceptable toxicity. Only two of seven (29%) patients developed a grade 1 rash while on erlotinib, and none (0%) developed diarrhea (Table 2). The only other toxicity recorded was related to liver test abnormalities in one of the patients (Table 2) that improved with use of erlotinib at 25 mg every other day. Clinical or radiographic signs of interstitial pulmonary fibrosis or pneumonia were not seen in these patients.

DISCUSSION

Gefitinib and erlotinib are orally available anilinoquinazoline small molecule ATP-mimetic TKIs most specific against EGFR. The molecular weight of gefitinib is 446.90 g/mol, and gefitinib's clinical dose for NSCLC of 250 mg/d is far less than its MTD of 700 to 1000 mg/d.[16,17,26,27] Based on a phase I dose-escalation trial, the mean steady state trough serum concentration of gefitinib following 225 mg/d averaged 0.16 μg/ml or a calculated 0.358 μM.[17] The mean trough concentration increased to 0.24 μg/ml or 0.537 μM at 300 mg/d and to 1.1 μg/ml or 2.461 μM at 1000 mg/d of gefitinib.[17] The molecular weight of erlotinib is 429.90 g/mol, and its clinical dose of 150 mg/d is its MTD.[7,18] In a phase I dose-escalation trial, erlotinib's steady state trough concentrations at 150 mg/d ranged from 0.33 to 2.64 μg/ml,[18] with a median of 1.26 μg/mL or the equivalent to 2.930 μM. In the same trial, a dose of 25 mg/d led to a median trough less than 0.22 μg/ml or the equivalent to less than 0.511 μM.[18] Therefore, erlotinib is given at a higher biologically active dose than gefitinib in routine clinical practice.[16] In agreement with the pharmacokinetic data, in the larger phase III randomized trials of these TKIs versus placebo for NSCLC,[7,9] the rates of rash and diarrhea (the two most common and dose-dependent side effects of these drugs) were higher (almost double) with erlotinib 150 mg/d[7] than gefitinib 250 mg/d.[9]

Despite their different clinical development paths for unselected NSCLCs, both gefitinib and erlotinib have a similar spectrum of activity in vitro against EGFR mutant proteins or *EGFR*-mutated tumors.[2,28,29] Gefitinib 250 mg/d has proven highly effective in prospective and retrospective series of patients with *EGFR*-activating mutations consistently yielding RRs that exceed 70% is chemotherapy-naive patients and PFS times that can reach more than 9 months.[12,13] Erlotinib 150 mg/d is equally effective in *EGFR*-mutated NSCLC.[5,14] However, both drugs have never been compared head-to-head in a clinical trial of patients with *EGFR* mutations, and until now, it was unknown whether a "lower" dose of erlotinib could generate similar responses to gefitinib 250 mg/d. Based on the pharmacokinetic data of phase I trials for both compounds and their similar in vitro inhibitory profile, a dose of erlotinib 25 mg/d seemed to best represent a dose of gefitinib 250 mg/d.[17,18] At these doses, both drugs are expected to have average trough concentrations of approximately 0.5 μM, a concentration that far exceeds the IC_{50} of most *EGFR*-mutated cells lines treated with these EGFR TKIs. Serum trough concentrations are only surrogate markers of doses of these EGFR TKIs. Gefitinib has a higher distribution volume than erlotinib, and drug concentrations in the tumor may be higher with gefitinib than erlotinib.[16] We and others[16,30] were able to determine that gefitinib and erlotinib inhibit *EGFR*-mutated cell lines with similar inhibitory curves and remarkable correlation in IC_{50} values. Therefore, at similar molar concentrations, both EGFR inhibitors are expected to have a comparable spectrum of activity. This hypothesis drove our rationale to evaluate the clinical outcomes obtained with erlotinib 25 mg/d in patients with advanced NSCLC and *EGFR* mutations.

Our compiled retrospective clinical data of seven patients treated with erlotinib at its lowest available tablet size of 25 mg/d reinforces our assumptions. The radiographic RR was extraordinarily high at 71.5% in this small cohort of patients. Despite the retrospective nature of the analysis, the PFS values observed are similar to those reported previously with gefitinib 250 mg/d[12] based on a lower bound of 6 months for the 95% CI for median PFS in our cohort. The lack of significant adverse reactions in most of our treated patients is also consistent with a lower trough concentration of erlotinib when this drug is given at 25 mg instead of 150 mg. Rash and diarrhea, hallmark and dose-dependent side effects of EGFR inhibitors, were not frequent in our patients. Indeed, only two patients (28.5%) had a minor rash to erlotinib 25 mg/d. None

TABLE 2. Clinical, Pathologic, Demographic, Molecular Characteristics, Response to Therapy, and Progression-Free Survival in the Studied Patients

	Clinical, Pathologic, and Molecular Characteristics					Efficacy			Toxicity (Grade-CTCAE)		
Patient	Age (yr)	Sex	Smoking History	Histology	*EGFR* Mutation	Daily Dose Erlotinib (Line of Therapy)	Response (RECIST)	PFS (mo)	Rash	Diarrhea	Other
1	55	M	Former (60 py)	Adenocarcinoma	delE746_A750	25 mg (first line)	PR	6	None (0)	None (0)	None
2	64	F	Never	Adenocarcinoma	delE746_A750	25 mg (first line)	PR	8+	None (0)	None (0)	None
3	65	F	Former (30 py)	Adenocarcinoma	delL747_T751 + R776S	25 mg (first line)[a]	PR	11+	None (0)	None (0)	LFT (2)
4	46	M	Former (30 py)	NSCLC, poorly differentiated	L858R	25 mg (second line)	SD	4	Yes (1)	None (0)	None
5	67	F	Current (1.5 py)	Adenocarcinoma	Exon 19 deletion[b]	25 mg (first line)	PR	10	Yes (1)	None (0)	None
6	69	F	Former (0.5 py)	Adenocarcinoma	Exon 19 deletion[b]	25 mg (second line)	SD	17	None (0)	None (0)	None
7	86	F	Former (30 py)	Adenocarcinoma	L858R	25 mg (first line)[c]	PR	35	None (0)	None (0)	None

[a] Patient 3 had her dose of erlotinib reduced to 25 mg every other day after LFT abnormalities were noted. All other patients remained on erlotinib daily until disease progression. Patients 1–4 were followed up at Beth Israel Deaconess Medical Center and patients 5–7 at Memorial Sloan-Kettering Cancer Center.

[b] Exon 19 deletions were detected using length analysis of fluorescently labeled PCR in patients 5 and 6, and no further characterization of the mutation by sequencing was performed.

[c] Patient 6 had her dose of erlotinib increased to 50 mg/d after initial response to erlotinib 25 mg/d.

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; PFS, progression-free survival; py, pack-years; CTCAE, common terminology criteria for adverse events version 3.0; M, male; F, female; PR, partial response; SD, stable disease; +, ongoing response; LFT, liver function test abnormalities.

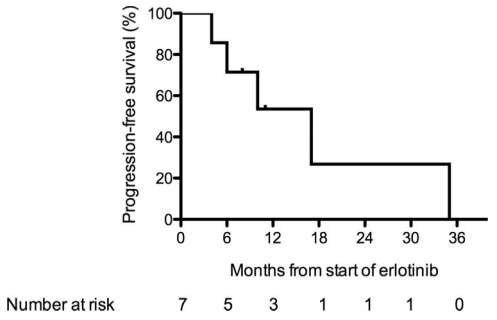

FIGURE 2. Kaplan-Meier progression-free survival curve for *epidermal growth factor receptor*–mutated patients treated with erlotinib 25 mg/d.

of our patients experienced interstitial pulmonary fibrosis, a known and serious side effect of EGFR TKIs.[31] The only other toxicity noted in one patient was liver function test abnormalities, which are well-described idiosyncratic side effects of EGFR TKIs.[7,9]

EGFR-mutated patients treated with gefitinib or erlotinib invariably develop acquired resistance to TKI therapy.[25] The *EGFR*-T790M mutation[19,24] accounts for half of cases, other secondary resistance mutations (L747S, D761Y, T854A) have been reported infrequently,[20,32,33] and *hepatocyte growth factor receptor (MET)* amplification[34,35] or expression of hepatocyte growth factor[36] are detected in more than one fifth of patients with *EGFR*-mutated TKI-resistant NSCLC. The pattern of resistance seems similar between patients treated with gefitinib 250 mg/d or erlotinib 150 mg/d.[25,37] Cells with *EGFR*-T790M and *MET* amplification cannot be inhibited with the use of gefitinib or erlotinib by in vitro doses equivalent to their MTDs.[21,34,35] In patient 1 (Table 2) of this study, an increase of erlotinib to 150 mg/d after progression on erlotinib 25 mg/d was attempted without changing the progressive nature of the tumor's radiographic disease (data not shown). Dose escalations to 150 mg/d were not made by treating physicians in the other six patients analyzed. Therefore, it is difficult to speculate whether erlotinib at 25 mg/d or at other dose ranges less than 150 mg/d[38] will alter the course and type of acquired resistance that treated patients will experience.

Our small retrospective study is hypothesis generating and will need to be confirmed in larger prospective series of NSCLC patients. Future prospective clinical trials of erlotinib at 25 mg daily and randomized trials of 25 mg versus 150 mg daily doses of erlotinib or of gefitinib 250 mg versus erlotinib 150 mg daily in advanced *EGFR*-mutated NSCLC may shed light on the differential efficacy, time to acquisition of resistance, safety, and quality of life attained by these different dosing approaches of approved EGFR TKIs.

In summary, our data indicate that *EGFR*-mutated NSCLC lines with activating sensitive mutations are inhibited by similar concentrations of gefitinib and erlotinib in vitro (<0.5 μM) and that erlotinib 25 mg/d (a concentration expected to be similar to gefitinib 250 mg/d) may be an alternative dosing scheme for patients with *EGFR* mutations. Future identification of the lowest and clinically active dose ranges of erlotinib and gefitinib in patients with tumors harboring *EGFR* mutations will help further to personalize their care.

ACKNOWLEDGMNTS

Supported in part by National Institutes of Health (NIH) grants R00CA126026-03 (to S.K.) and 2PA50-CA090578-07 (to

D.B.C., D.G.T., and B.Y.Y.); the American Association for Cancer Research 07-40-12-COST (to D.B.C.); a Career Development Award by the American Society of Clinical Oncology Cancer Foundation CDA-15431 (to D.B.C.); and a Research Fellowship Award by the National Medical Research Council, Ministry of Health, Singapore (to W.Y.).

REFERENCES

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.
2. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;304: 1497–1500.
3. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci USA* 2004;101:13306–13311.
4. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–346.
5. Riely GJ, Politi KA, Miller VA, et al. Update on epidermal growth factor receptor mutations in non-small cell lung cancer. *Clin Cancer Res* 2006;12:7232–7241.
6. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–181.
7. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–132.
8. Kim ES, Hirsh V, Mok T, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* 2008;372:1809–1818.
9. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005;366: 1527–1537.
10. Sequist LV, Bell DW, Lynch TJ, et al. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. *J Clin Oncol* 2007;25:587–595.
11. 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.
12. Costa DB, Kobayashi S, Tenen DG, et al. Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. *Lung Cancer* 2007;58:95–103.
13. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;361:947–957.
14. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. *N Engl J Med* 2009;361:958–967.
15. 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;14:7060–7067.
16. Rukazenkov Y, Speake G, Marshall G, et al. Epidermal growth factor receptor tyrosine kinase inhibitors: similar but different? *Anticancer Drugs* 2009;20:856–866.
17. Baselga J, Rischin D, Ranson M, et al. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J Clin Oncol* 2002;20:4292–4302.
18. Hidalgo M, Siu LL, Nemunaitis J, et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol* 2001; 19:3267–3279.
19. 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;352:786–792.
20. 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.
21. Kobayashi S, Ji H, Yuza Y, et al. An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. *Cancer Res* 2005;65:7096–7101.
22. 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.
23. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–481.
24. 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.
25. Nguyen KS, Kobayashi S, Costa DB. Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancers dependent on the epidermal growth factor receptor pathway. *Clin Lung Cancer* 2009;10:281–289.
26. Ranson M, Hammond LA, Ferry D, et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. *J Clin Oncol* 2002;20:2240–2250.
27. 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. *JAMA* 2003;290:2149–2158.
28. Politi K, Zakowski MF, Fan PD, et al. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. *Genes Dev* 2006;20:1496–1510.
29. Ji H, Li D, Chen L, et al. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* 2006;9:485–495.
30. Gandhi J, Zhang J, Xie Y, et al. Alterations in genes of the EGFR signaling pathway and their relationship to EGFR tyrosine kinase inhibitor sensitivity in lung cancer cell lines. *PLoS One* 2009;4:e4576.
31. Ando M, Okamoto I, Yamamoto N, et al. Predictive factors for interstitial lung disease, antitumor response, and survival in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2006;24:2549–2556.
32. Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clin Cancer Res* 2006;12:6494–6501.
33. Bean J, Riely GJ, Balak M, et al. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. *Clin Cancer Res* 2008;14:7519–7525.
34. 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: 20932–20937.
35. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039–1043.
36. Yano S, Wang W, Li Q, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res* 2008;68:9479–9487.
37. Kaira K, Naito T, Takahashi T, et al. Pooled analysis of the reports of erlotinib after failure of gefitinib for non-small cell lung cancer. *Lung Cancer* 2010;68:99–104.
38. Lind JS, Postmus PE, Heideman DA, et al. Dramatic response to low-dose erlotinib of epidermal growth factor receptor mutation-positive recurrent non-small cell lung cancer after severe cutaneous toxicity. *J Thorac Oncol* 2009;4:1585–1586.

