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Clinical Cancer Research

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A Randomized Phase II Study of Gefitinib Plus Simvastatin Versus Gefitinib Alone in Previously Treated Patients with Advanced Non–Small Cell Lung Cancer

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Abstract

Purpose: To evaluate the efficacy and safety of gefitinib plus simvastatin (GS) versus gefitinib alone (G) in previously treated patients with advanced non–small cell lung cancer (NSCLC).

Experimental Design: Between May 2006 and September 2008, 106 patients (51% men, 75% adenocarcinoma, 50% never smoker) were randomly assigned to G alone (250 mg/d, $n = 54$) or GS (250 and 40 mg/d, respectively, $n = 52$). One cycle was 4 weeks of treatment. Therapy was continued until disease progression or intolerable toxicity was observed. The primary endpoint was response rate (RR). Secondary endpoints included toxicity, progression-free survival (PFS), and overall survival (OS).

Results: The RR was 38.5% (95% CI, 25.3–51.7) for GS and 31.5% (95% CI, 19.1–43.9) for G. The median PFS was 3.3 months [M] (95% CI, 1.4–5.2M) for GS and 1.9M (95% CI, 1.0–2.8M) for G. The median OS was 13.6M (95% CI, 7.1–20.1M) for GS and 12.0M (95% CI, 7.8–16.2M) for G. In exploratory subgroup analysis, GS showed higher RR (40% vs. 0%, $P = 0.043$) and longer PFS (3.6M vs. 1.7M, $P = 0.027$) compared with G alone in patients with wild-type epidermal growth factor receptor (*EGFR*) nonadenocarcinomas. Adverse events in both arms were generally mild and mainly consisted of skin rashes.

Conclusions: Although no superiority of GS to G was demonstrated in this unselected NSCLC population, GS showed higher RR and longer PFS compared with G alone in patients with wild-type *EGFR* nonadenocarcinomas. Simvastatin may improve the efficacy of gefitinib in that subgroup of gefitinib-resistant NSCLC patients. *Clin Cancer Res; 17(6); 1553–60. ©2011 AACR.*

Introduction

The epidermal growth factor receptor (EGFR) is a key regulator of proliferation, differentiation, and survival of epithelial cells and has been implicated in the oncogenesis of epithelial cancers, including lung cancer (1). Because 50% and 80% of non–small cell lung cancer (NSCLC) cases reportedly express the EGFR protein, it is expected that an efficient EGFR inhibitor would be effective for the majority of patients with NSCLC (2). However, both gefitinib and erlotinib, orally active EGFR tyrosine kinase inhibitors (TKIs), produce durable treatment responses in a surprisingly small fraction of patients with NSCLC (3–6). DNA sequencing studies in primary lung tumor samples have shown a convincing association between responses to EGFR–TKIs and the presence of somatic mutations in the *EGFR* gene (7–9). Recently, several randomized phase III studies have demonstrated that gefitinib is superior to conventional chemotherapy in patients with *EGFR*-mutant tumors (10–12). Patients treated with gefitinib have displayed significantly longer progression-free survival (PFS) and higher response rates compared with chemotherapy among those genetically selected patients. Thus, activating somatic mutations in the *EGFR* gene are regarded as the strongest predictors of better response to gefitinib. However, the overall frequency of *EGFR* mutations is 5% to 20%, depending on geographic location (13). The remaining majority of NSCLC patients have wild-type *EGFR* and hardly benefit from gefitinib. To date, several studies have reported efficacy data for relapsed patients with wild-type *EGFR* tumors in the subgroup analysis of phase III trials. In those patients treated with gefitinib, the response rate, median progression-free and overall survival (OS) times were less than 10%, around 2 and 6 months, respectively (14–16). Although these results are similar to those observed with docetaxel monotherapy (14), several questions remain to be answered to improve the efficacy of gefitinib in those genetically unselected patients.

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Translational Relevance

The impact of statins on epidermal growth factor receptor (EGFR) function and signaling and *in vivo* activity against tumor cells has generated interest in studying statins as a potential EGFR-targeted therapeutic intervention. This is the first randomized phase II study comparing the efficacy of gefitinib plus simvastatin versus gefitinib alone in previously treated patients with advanced non–small cell lung cancer. The results show that in unselected patients, no significant difference in response rate and survival was observed between the 2 arms. However, gefitinib plus simvastatin improved the response rate and PFS compared with gefitinib alone in the exploratory subgroup analysis of the patients with wild-type *EGFR* nonadenocarcinomas. This finding suggests that inhibiting the mevalonate pathway using simvastatin may enhance the efficacy of gefitinib in this relatively gefitinib-resistant subpopulation.

Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the rate-limiting enzyme of the mevalonate pathway and is required for the synthesis of cholesterol and isoprenoids such as farnesylpyrophosphate and geranylgeranylpyrophosphate (17). The impact of statins on cholesterol and isoprenoid synthesis may have anticancer effects through at least 2 primary mechanisms, which include impairment of protein prenylation and interference with the formation of cholesterol-rich lipid microdomains called lipid rafts within the cell membrane (18). Both of these processes are critical for the function of EGFR and the activity of numerous proteins important for EGFR signaling, such as Ras (18, 19). The impact of statins on EGFR function and signaling and *in vivo* activity against tumor cells has generated interest in studying statins as a potential EGFR-targeted therapeutic intervention. Some *in vitro* studies have reported that the combination of gefitinib and lovastatin has synergistic cytotoxicity and enhances EGFR inhibition in squamous cell head and neck carcinoma, NSCLC, and colon carcinoma cell lines (20–22). Interestingly, not all of the studied cell lines possess *EGFR* mutations, which confer increased sensitivity to gefitinib. We also showed in a previous study that the combination of gefitinib and lovastatin induces synergistic cytotoxicity in gefitinib-resistant NSCLC cells (23). Lovastatin effectively downregulates Ras protein and suppresses the phosphorylation of RAF, ERK1/2, AKT, and EGFR in NSCLC cells, which enhances gefitinib-induced apoptosis. These promising preclinical data led to the present randomized phase II study.

Methods

Patients

The main eligibility criteria were histologic or cytologic confirmation of locally advanced or metastatic (stage IIIB/IV) NSCLC after failure of at least 1 platinum-based chemotherapy, age ≥18 years, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of less than 3, and a life expectancy ≥12 weeks. Patients were required to have measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST, ref. 24). Patients with brain metastases were permitted if clinically stable without steroid treatment. Exclusion criteria included significant hematologic, hepatic, renal, or cardiac dysfunction and any previous treatment with EGFR signaling inhibitors and statins.

Study design and treatment

This randomized, open-label, phase II study of gefitinib and simvastatin (CJ Pharmaceutical Co.) versus gefitinib alone was conducted at a single institution (National Cancer Center) from May 2006 to September 2008. In both study arms, patients received once-daily oral doses of 250 mg gefitinib alone (G) or in combination with 40 mg/d simvastatin (GS) in 28-day cycles. Study treatment continued until disease progression (PD) or until another termination criterion was met: unacceptable toxicity, consent withdrawal, loss to follow-up, death, major protocol violation, or noncompliance. The protocol was approved by an independent ethics committee/institutional review board and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice. Each patient provided written informed consent.

Assessment

The primary endpoint was tumor response rate (complete response [CR] + partial response [PR] using RECIST). Disease assessment was performed by investigators every 8 weeks until PD. Secondary endpoints included PFS, OS, and safety. Adverse events (AEs) were assessed throughout treatment and for 30 days after the last treatment dose and were graded using NCI CTCAE 3.0.

Detection of *EGFR* mutations

Whole-blood and tissue samples were collected immediately before treatment. Plasma was separated within 2 hours after the sample collection and stored at −80°C until used. Genomic DNA was extracted from plasma and paraffin-embedded tissues by using the QIAamp DNA mini kit (Qiagen). We used peptide nucleic acid (PNA) clamping-based asymmetric PCR with melting curve analysis using unlabeled probes (25). A capillary PCR machine (Light Cycler; Roche) was used instead of plate PCR, and the melting carve analysis for the probe peak was performed in the same machine. Forward and reverse primers were designed to amplify the commonly mutated portions of exon fragments 19 and 21, and the amplicon sizes were 91 and 89 base pairs, respectively. Locked nucleic acids were incorporated into the forward primer of exon 19 to increase the annealing temperature. The forward primer for exon 19 antisense PNAs and sense mutation probes were designed to span the mutation sites of exons 19 and 21 of the *EGFR* gene. The antisense PNA complementary to the wild-type sequence was used to clamp PCR for wild type but not mutant alleles. The sense mutation probes that were complementary to mutant alleles were used to detect

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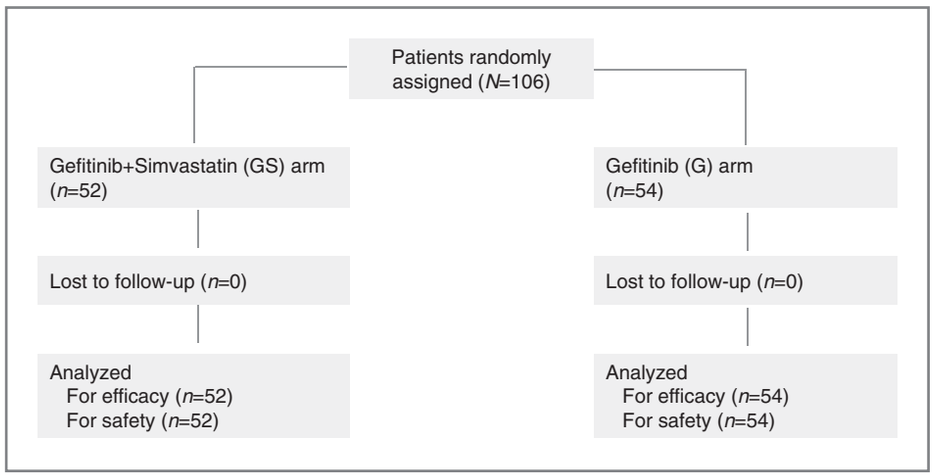

Figure 1. CONSORT diagram.

both wild-type and mutant alleles. The mutation probe for exon 19 was complementary to E746-A750del type 1 (2235-2249del) and was used to detect wild-type and E746-A750del type 2 (2236-2250del) mutant as well as E746-A750del type 1. The mutation probe for exon 21 is complementary to L858R (T2573G) and was used to detect both wild-type and L858R mutant alleles.

Statistical analysis

The primary endpoint was objective tumor response rate. This study employed a "pick up the winner" design based on the randomized phase II clinical trial approach proposed by Simon and colleagues, which gives a 90% chance of selecting the better treatment if the difference is at least 15% and the smaller response rate is assumed to be 10% (26). To ensure that at least 84 patients (42 per arm) enrolled, approximately 106 patients overall were planned for. Patients were stratified based on sex (female vs. male), performance status (PS) by ECOG (0/1 vs. 2/3), and the number of prior regimens (1 vs. 2 or more). Secondary endpoints included toxicity profile, PFS, and OS. PFS was defined as the interval between the start date of treatment and the date of occurrence of progressive disease or death. OS was measured from the date of study entry until the date of death. If a patient was lost to follow-up, the patient was censored on the date of last contact. PFS and OS were evaluated by using the Kaplan-Meier method. All treated patients were included in the analysis of efficacy and safety. Although no formal statistical comparison between the 2 arms was planned, the χ^2 or Fisher's exact test was performed on the response rate, and the log-rank test was applied to survival curves for exploratory purpose. Cox proportional hazards regression models were used to estimate hazard ratios (HRs). All expressed *P* values are 2-sided.

Results

Patient characteristics

Between May 2006 and September 2008, 106 patients were randomly assigned and received treatment with G alone ($n = 54$) or GS ($n = 52$). All patients were assessable for efficacy and safety (Fig. 1). There were 82 deaths (77%) at the time of data cutoff (July 5, 2010): 43 patients randomly assigned to G and 39 randomly assigned to GS.

Patient characteristics and baseline demographics were generally similar in both arms (Table 1). The median age was 60 years (range, 20–84), and 51% of the patients were men. Forty-nine percent of patients were never smokers, 31% were former smokers, and 20% were current smokers. Among ever (current or former) smokers, the median pack-year was 32 (range, 0.5–127.5). The majority of patients (75%) had adenocarcinoma.

Safety

The safety profiles of both arms were similar (Table 2). The most commonly observed AE was rash. Overall, the grade 3 or 4 toxicity rates were low in both arms. There were no treatment-related deaths.

Response and survival

The response rate was 31.5% (95% CI, 19.1–43.9%) for G and 38.5% (95% CI, 25.3–51.7%) for GS (Table 3). Median PFS was 1.9 months (95% CI, 1.0–2.8 months) for G and 3.3 months (95% CI, 1.4–5.2 months) for GS (Fig. 2A). The estimated HR was 0.891 (95% CI, 0.604–1.315, $P = 0.549$). After a median follow-up of 30 months, the median OS was 12 months (95% CI, 7.8–16.2 months) for G and 13.6 months (95% CI, 7.1–20.1 months) for GS (Fig. 2B). The 1-year survival rate was 50% for G and 56% for GS; the 2-year survival rates were 30% and 28%, respectively. The estimated HR was 0.876 (95% CI, 0.567–1.354, $P = 0.491$).

EGFR mutation analysis and exploratory subgroup analyses

Among a total of 106 patients, 94 plasma DNA samples were adequate for *EGFR* mutation analysis. Activating mutations were detected in 26 of 94 (28%) cases (23 exon 19 deletions and 3 exon 21 L858R mutations, Table 1). To validate the plasma *EGFR* mutation results, we tested the

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Table 1. Demographic characteristics of patients

Characteristics	Gefitinib alone ($n = 54$)		Gefitinib and simvastatin ($n = 52$)	
	N	(%)	*N*	(%)
Median age, y	60		58	
Range	(32–84)		(20–76)	
Sex				
Male	29	(54)	25	(48)
Female	25	(46)	27	(52)
ECOG performance status				
0	16	(30)	19	(36)
1	32	(59)	30	(58)
2	6	(11)	2	(4)
3	0	(0)	1	(2)
Stage				
IIIB	6	(11)	3	(6)
IV	48	(89)	49	(94)
Prior chemotherapy				
1	24	(44)	25	(48)
2	30	(56)	25	(48)
3	0	(0)	2	(4)
Histology				
Adenocarcinoma	41	(76)	39	(75)
Squamous	8	(15)	9	(17)
NOS[a]	5	(9)	4	(8)
Smoking status				
Never smoker	27	(50)	26	(50)
Former smoker	16	(30)	16	(31)
Current smoker	11	(20)	10	(19)
Pack-year, median (range)	33	(2–60)	32.5	(0.5–127.5)
EGFR mutations ($n = 94$)				
Negative	31	(69)	37	(76)
Positive	14	(31)	12	(25)

[a]Non–small cell lung cancer, not otherwise specified.

2 major *EGFR* mutations, the exon 19 deletion and exon 21 (L858R), in the paired tumor tissues. In the 15 paired specimens of plasma and tumor tissues, 11 (73%) revealed concordant results. The comparison between *EGFR* mutation status in plasma and tumor samples is summarized in Table 4. Although they were not statistically significant, trends toward higher frequency of *EGFR* mutations were observed in adenocarcinoma (32% [23/71] vs. 13% [3/23]

Table 2. Common adverse events

	Gefitinib alone ($n = 54$)								Gefitinib+Simvastatin ($n = 52$)							
	Grade 1		Grade 2		Grade 3		Grade 4		Grade 1		Grade 2		Grade 3		Grade 4	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Rash	21	(39)	17	(32)	1	(2)	0	(0)	14	(27)	21	(40)	2	(4)	0	(0)
Dry skin	30	(56)	2	(4)	0	(0)	0	(0)	26	(50)	6	(12)	0	(0)	0	(0)
Stomatitis	11	(20)	1	(2)	0	(0)	0	(0)	21	(40)	1	(2)	0	(0)	0	(0)
Anorexia	20	(37)	5	(9)	0	(0)	0	(0)	16	(31)	5	(7)	0	(0)	0	(0)
Diarrhea	18	(33)	3	(6)	0	(0)	0	(0)	17	(33)	3	(8)	0	(0)	0	(0)
Asthenia	7	(13)	2	(4)	0	(0)	0	(0)	7	(13)	2	(4)	0	(0)	0	(0)
Nausea	5	(9)	0	(0)	0	(0)	0	(0)	6	(12)	0	(0)	0	(0)	0	(0)

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Table 3. Overall and subset analysis of response according to treatment

	Response	Gefitinib alone ($n = 54$)		Gefitinib and simvastatin ($n = 52$)		*P*
		N	(%)	*N*	(%)	
Overall	PR	17	(31.5)	20	(38.5)	.666
	SD	10	(18.5)	7	(13.5)	
	PD	27	(50.0)	25	(48)	
Subset analysis		RR	(*N*)	RR	(*N*)	*P*
Histology	Adenocarcinoma	39%	(16/41)	39%	(15/39)	.959
	Nonadenocarcinoma	8%	(1/13)	39%	(5/13)	.063
Smoking status	Never-smoker	44%	(12/27)	62%	(16/26)	.213
	Ever-smoker	19%	(5/27)	15%	(4/26)	.761
Sex	Male	21%	(6/29)	16%	(4/25)	.658
	Female	44%	(11/25)	59%	(16/27)	.271
EGFR mutations	Positive	64%	(9/14)	75%	(9/12)	.555
	Negative	13%	(4/31)	27%	(10/37)	.151
Adenocarcinoma	EGFR mutation-positive	62%	(8/13)	80%	(8/10)	.340
	EGFR mutation-negative	19%	(4/21)	22%	(6/27)	.788
Nonadenocarcinoma	EGFR mutation-positive	100%	(1/1)	50%	(1/2)	.667
	EGFR mutation-negative	0%	(0/10)	40%	(4/10)	.043

Abbreviations: PR, partial response; PD, progressive disease by RECIST1.0; RR, response rate; SD, stable disease.

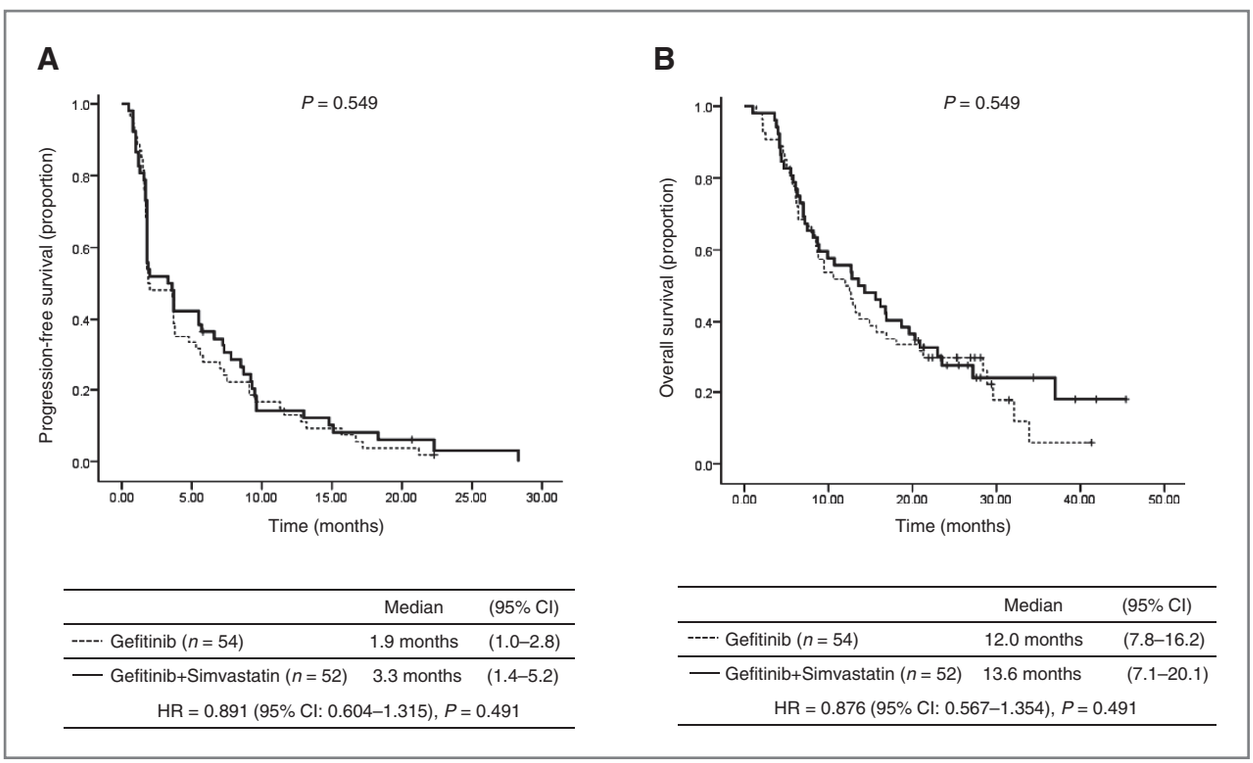

Figure 2. Progression-free survival (A) and overall survival (B).

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Table 4. Comparison of EGFR mutation status in the paired specimens of plasma and tumor samples ($n = 15$)

Tumor tissue	Plasma sample	
	Positive	Negative
Positive	2	3
Negative	1	9

for nonadenocarcinoma, $P = 0.107$), women (36% [16/45] vs. 20% [10/49] for men, $P = 0.101$), and never smokers (33% [15/46] vs. 23% [11/48] for ever smokers, $P = 0.294$). Among the entire population, patients with *EGFR*-mutant tumors showed significantly higher response rates (69% [18/26] vs. 21% [14/68] for wild-type *EGFR*, $P < 0.0001$), and longer PFS (HR = 0.386 [95% CI, 0.238–0.627], $P < 0.0001$) and OS (HR = 0.540 [95% CI, 0.316–922], $P = 0.024$) compared with those with wild-type *EGFR* tumors.

Exploratory subgroup analyses (Table 3) suggested a trend toward an improved response rate in the GS group compared with the G group among patients with nonadenocarcinoma histology (39% vs. 8%, respectively, $P = 0.063$) and wild-type *EGFR* (27% vs. 13%, respectively, $P = 0.151$). We therefore subdivided tumor histology according to *EGFR* mutation status and compared the efficacy of G versus GS. Among patients with nonadenocarcinomas, those with wild-type *EGFR* showed a higher response rate (40% vs. 0%, respectively, $P = 0.043$) and longer median PFS (3.7 months vs. 1.7 months, respectively, $P = 0.027$; Fig. 3) when treated with GS compared with G alone.

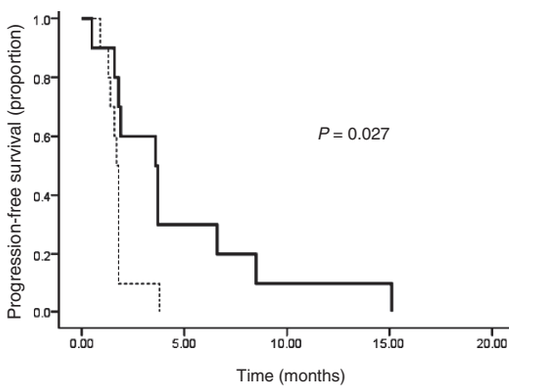

Figure 3. Progression-free survival in nonadenocarcinoma with *EGFR* wild type.

Discussion

This is the first randomized phase II study comparing the efficacy of GS versus G in previously treated patients with advanced NSCLC. The results show that in unselected patients, no significant difference in response rate and survival was observed between the 2 arms. However, GS improved the response rate and PFS compared with G in the exploratory subgroup analysis of the patients with wild-type *EGFR* nonadenocarcinomas. This finding suggests that inhibiting the mevalonate pathway using simvastatin may enhance the efficacy of gefitinib in this relatively gefitinib-resistant subpopulation.

To date, several preclinical studies have shown that the combination of statins with gefitinib induces a potent synergistic cytotoxicity in a variety of tumors without *EGFR* mutations (20, 21). Recently, Zhao and colleagues reported that lovastatin inhibits ligand-induced EGFR dimerization in squamous cell head and neck carcinoma cells, which results in inhibition of AKT activation along with its downstream targets that regulate protein translation initiation. Lovastatin induces actin cytoskeletal disorganization and increases the expression of inactive rhoA, which also inhibits EGFR dimerization and activation. Furthermore, they retrospectively evaluated the effect of statin on the OS and disease-specific survival (DSS) of NSCLC patients enrolled in the NCIC Clinical Trials Group phase III clinical trial BR21 (erlotinib vs. placebo). Although it was not statistically significant, erlotinib-treated patients with statin use showed a trend toward improved OS and DSS compared with patients without statin use (22). In our study, patients with wild-type *EGFR* nonadenocarcinomas treated with GS showed higher response rates and longer PFS compared with G. Although the number of these patients was small, these clinical observations may support the preclinical evidence of the effective EGFR-inhibitory activity of statins in wild-type *EGFR* non-adenocarcinoma tumors. Moreover, the combination treatment was generally well tolerated, and the AEs observed were similar to those reported with gefitinib alone.

In our study, some inconsistency of EGFR mutation results was found between tissue and plasma DNA. The significance of a negative result for an *EGFR* mutation is highly dependent upon samples tested as well as methods performed. Direct DNA sequencing is a common detection method but has well-known sensitivity limitations depending on the proportion of tumor cells present in the material available for DNA extraction (27). The peptide nucleic acid-locked nucleic acid (PNA–LNA) PCR clamp is capable of detecting EGFR mutations in the presence of 100-fold background levels of wild-type EGFR from normal cells. Because of its high sensitivity and specificity, PNA–LNA PCR clamp is considered suitable to detect EGFR mutations in cytology samples (28). However, this method uses

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mutation-specific primers and therefore can miss rare mutations (e.g., L861Q or exon 18 mutations). In addition, the rate of detection of L858R in our study was very low compared with the rate of E746_A750del. Similar results were also reported previously using Scorpion Amplified Refractory Mutation System technology detect EGFR mutations in serum DNA (29). Further analyses in much larger groups of patients will be necessary to clarify the low-frequency L858R mutation could be due to assay-related false-negative findings.

Although this study did not demonstrate the superiority of gefitinib plus simvastatin as a salvage treatment in unselected NSCLC patients, the improved efficacy of this combination in the patients with wild-type *EGFR* nonadenocarcinomas may provide a novel, promising therapeutic approach using gefitinib as a salvage treatment in those gefitinib-resistant patients. Further studies of this combination as a salvage treatment for patients with wild-type *EGFR* nonadenocarcinomas are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Mendelsohn J, Baselga J. The EGF receptor family as targets for cancer therapy. Oncogene 2000;19:6550–65.
2. Gazdar AF. Epidermal growth factor receptor inhibition in lung cancer: the evolving role of individualized therapy. Cancer Metastasis Rev 2010;29:37–48.
3. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, 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). J Clin Oncol 2003;21:2237–46.
4. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, 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;290:2149–58.
5. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, 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;366:1527–37.
6. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, et al. National Cancer Institute of Canada Clinical Trials Group. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123–32.
7. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, 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;350:2129–39.
8. Paez JG, Jänne 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.
9. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, 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 U S A 2004;101:13306–11.
10. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.
11. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
12. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, 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.
13. 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 (Suppl 1):S24–31.
14. Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008;372:1809–18.
15. Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, Liu N, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2008;26:4268–75.
16. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Franklin WA, Dziadziuszko R, Thatcher N, et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol 2006;24:5034–42.
17. Endo A. The discovery and development of HMG-CoA reductase inhibitors. J Lipid Res 1992;33:1562–82.
18. Grzegorz S., Nowakowski GS, Maurer MJ, et al. Statin use and prognosis in patients with diffuse large b-cell lymphoma and follicular lymphoma in the rituximab era. J Clin Oncol 2010;28:412–7.
19. Jakobisiak M, Golab J. Potential antitumor effects of statins. Int J Oncol 2003;23:1055–69.
20. Mantha AJ, Hanson JE, Goss G, Lagarde AE, Lorimer IA, Dimitroulakos J. Targeting the mevalonate pathway inhibits the function of the epidermal growth factor receptor. Clin Cancer Res 2005;11:2398–407.
21. Dimitroulakos J, Lorimer IA, Goss G. Strategies to enhance epidermal growth factor inhibition: targeting the mevalonate pathway. Clin Cancer Res 2006;12:4426s–31s.
22. Zhao TT, Le Francois BG, Goss G, Ding K, Bradbury PA, Dimitroulakos J. Lovastatin inhibits EGFR dimerization and AKT activation in squamous cell carcinoma cells: potential regulation by targeting rho proteins. Oncogene 2010;29:4682–92.
23. Park IH, Kim JY, Jung JI, Han JY. Lovastatin overcomes gefitinib resistance in human non-small cell lung cancer cells with K-Ras mutations. Invest New Drugs 2009 Sep 17. [Epub ahead of print].
24. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, 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;92:205–16.
25. Oh JE, Lim HS, An CH, Jeong EG, Han JY, Lee SH, et al. Detection of low-level KRAS mutations using PNA-mediated asymmetric PCR clamping and melting curve analysis with unlabeled probes. J Mol Diagn 2010;12:418–24.

DOI:10.1158/1078-0432.CCR-10-2525

26. Simon R, Wittes RE, Ellenberg SS. Randomized phase II clinical trials. Cancer Treat Rep 1985;69:1375–81.

27. Pao W, Ladanyi M. Epidermal growth factor receptor mutation testing in lung cancer: searching for the ideal method. Clin Cancer Res 2007;13:4954–5.

28. Sutani A, Nagai Y, Udagawa K, Uchida Y, Koyama N, Murayama Y, et al. Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. Br J Cancer 2006;95:1483–9.

29. Kimura H, Kasahara K, Kawaishi M, Kunitoh H, Tamura T, Holloway B, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res 2006;12:3915–21.