

Repeated Favorable Responses to Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in a Case of Advanced Lung Adenocarcinoma

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The presence of epidermal growth factor receptor (*EGFR*) mutation is a prognostic and predictive marker for *EGFR*-tyrosine kinase inhibitor (TKI) therapy. However, inevitably, relapse occurs due to the development of acquired resistance, such as T790M mutation. We report a case of repeated responses to *EGFR*-TKIs in a never-smoked woman with adenocarcinoma. After six cycles of gemcitabine and cisplatin, the patient was treated by gefitinib for 4 months until progression. Following the six cycles of third-line pemetrexed, gefitinib retreatment was initiated and continued with a partial response for 6 months. After progression, she was recruited for an irreversible *EGFR* inhibitor trial, and the time to progression was 11 months. Although *EGFR* direct sequencing on the initial diagnostic specimen revealed a wild-type, we performed a rebiopsy from the progressed subcarinal node at the end of the trial. The result of peptide nucleic acid clamping showed L858R/L861Q.

Key Words: Adenocarcinoma; Receptor, Epidermal Growth Factor; Gefitinib

Introduction

Epidermal growth factor receptor-tyrosine kinase inhibitor (*EGFR*-TKI) has improved the outcome of advanced non-small cell lung cancer (NSCLC). *EGFR*-TKIs have higher response rates in Asian patients, females, non-smokers, and those with adenocarcinoma histology¹⁻³. The efficacy of *EGFR*-TKIs is associated with the activation of *EGFR* mutations^{2,3}. However, most patients who respond to *EGFR*-TKIs eventually experience progression after approximately 10–14 months⁴.

Here, we report a case of three repeated favorable responses to *EGFR*-TKIs including gefitinib retreatment and irreversible *EGFR* inhibitor in a patient with stage IV adenocarcinoma.

Case Report

In December 2008, a 43-year-old Korean woman was admitted to our hospital with a 3-month history of dry cough and dyspnea. The patient had never smoked and had no recent history of travel or use of medications. Physical examination revealed some crackles on the left upper lung. Laboratory findings were within normal range, except for elevations of white blood cell count (26,600/mm³) and C-reactive protein (7.6 mg/dL). Chest radiograph (Figure 1) and computed tomography (CT) scans showed diffuse consolidations and ground glass opacities mixed with multiple hematogenous metastases in the left upper lobe (LUL) and left lower lobe. Pathologic results of transbronchial lung biopsy from the

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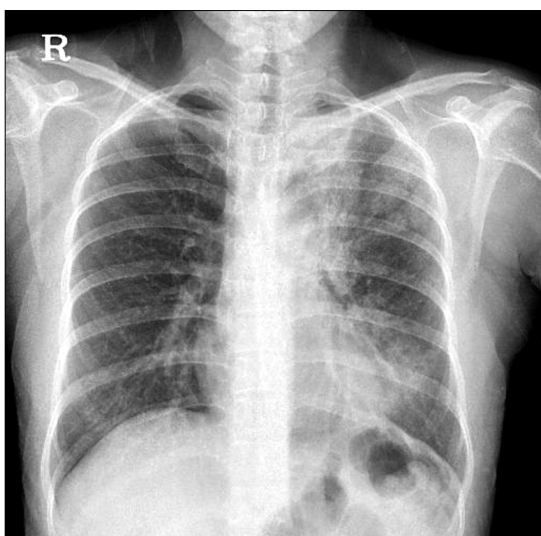


Figure 1. Initial chest radiography shows diffuse patchy consolidations with geographic ground glass opacities in the left upper and lower lung fields.

LUL showed a well-differentiated adenocarcinoma. We performed direct DNA sequencing⁵ for *EGFR* mutations in exon 18, 19, 20, and 21 with this specimen. The result indicated wild-type *EGFR*. First-line chemotherapy with gemcitabine and cisplatin was started on December 2008. After 6 cycles, second-line treatment with gefitinib 250 mg/day was initiated in May 2009 because of progressive disease. A follow-up CT showed partial response (Figure 2A, B). But, the time-to-progression of gefitinib was only 4 months (Figure 2C), so she received third-line pemetrexed for 6 cycles. After that the CT scan in July 2010 revealed aggravated pulmonary metastatic nodules, and we enrolled the patient in an ongoing clinical trial⁶ for evaluation of gefitinib retreatment in advanced NSCLC patients who were controlled previously with gefitinib. The patient displayed marked improvement in symptoms and radiologic findings (Figure 2D, E). We attempted to obtain a gefitinib-resistant tumor biopsy sample at the end of this trial, but could not perform *EGFR* mutation analysis because of insufficient specimen. After 6 months, there were more progressed LUL nodule and hematogenous metastases (Figure 2F). On the suspicion of clinically defined acquired resistance to EGFR-TKI, we recruited the patient to a phase II trial with PF-00299804 (PF-00299804 in

treating patients with stage IIIB or stage IV NSCLC that has not responded to standard therapy for advanced or metastatic cancer, ClinicalTrials.gov number, NCT0100-0025). In April 2011, the patient was started on PF-00299804 as fifth-line therapy. A radiologically evident partial response occurred once more (Figure 2G, H). The patient continued the experimental drug for 11 months before newly developed subcarinal lymphadenopathy was detected by CT imaging in March 2012 (Figure 2I). Endobronchial ultrasonography-guided transbronchial needle aspiration biopsy confirmed metastatic adenocarcinoma. An *EGFR* mutation test with this specimen using the peptide nucleic acid (PNA) clamping method^{5,6} revealed two mutations in exon 21 (L858R/L861Q) and exon 20 (T790M) (Figure 3). With the progressive disease, sixth-line docetaxel chemotherapy was started beginning in April 2012.

Discussion

To the best of our knowledge, this is the first case report of repeated partial responses to gefitinib retreatment and subsequent irreversible EGFR inhibitor in a patient with advanced adenocarcinoma in Korea. Recent retrospective and prospective reports have suggested the possibility that retreatment of EGFR-TKI might be useful for initial responders following a drug holiday^{6,8}. Oh et al.⁶ reported a disease control rate of 65.2% in advanced NSCLC patients with retreatment of gefitinib after prior gefitinib treatment evidenced partial response or stable disease. However, as in the present case, despite repetitive responses to EGFR-TKIs, the development of secondary resistance inevitably leads to treatment failure. Recently, several second-generation irreversible EGFR inhibitors have been developed with a specific focus on T790M to overcome acquired resistance of EGFR-TKI. Clinical studies have addressed the benefits of irreversible EGFR inhibitors such as afatinib or PF-00299804⁹⁻¹¹. PF-00299804 is an oral, irreversible small-molecule inhibitor of HER1, HER2, and HER4. Our case also showed clinical favorable response to PF-00299804 with duration of 11 months.

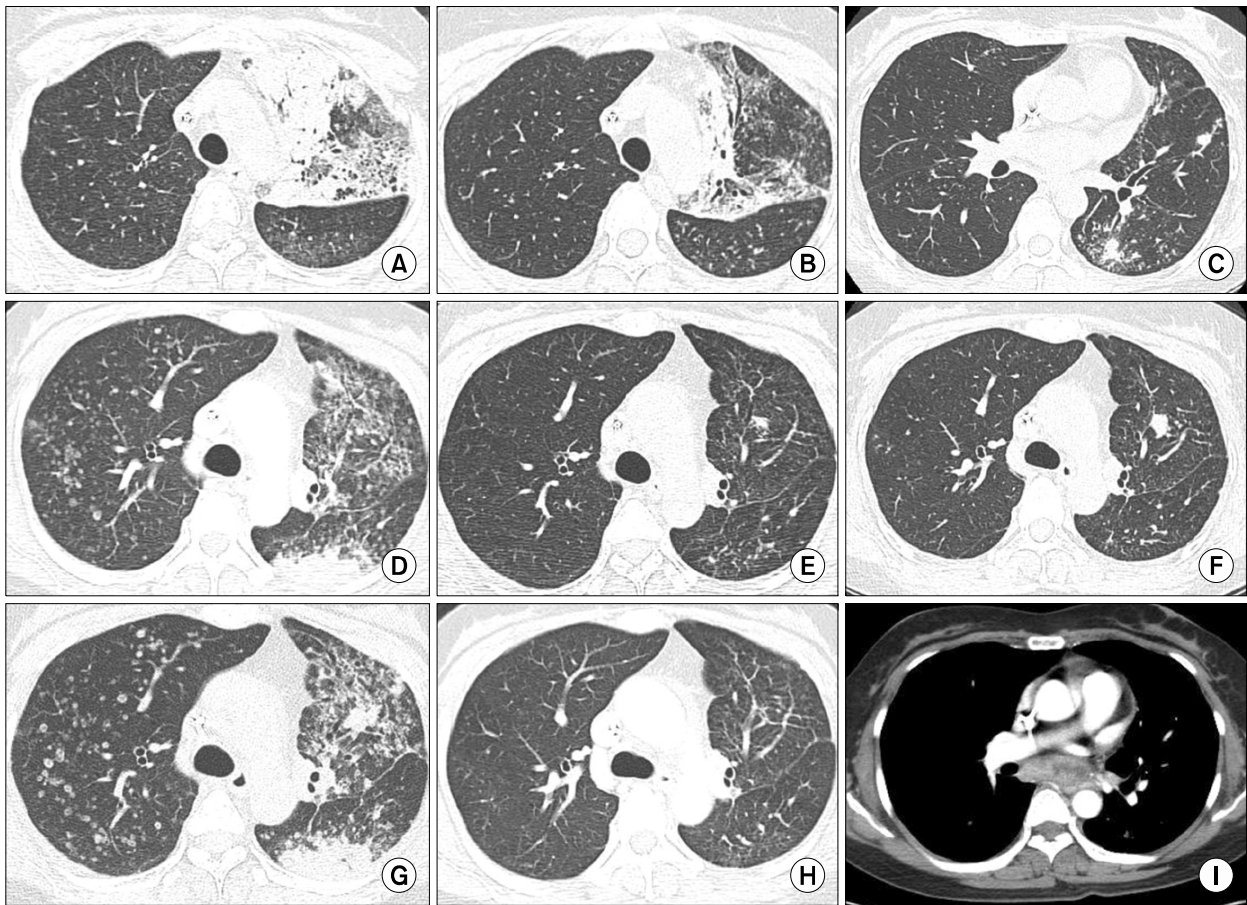
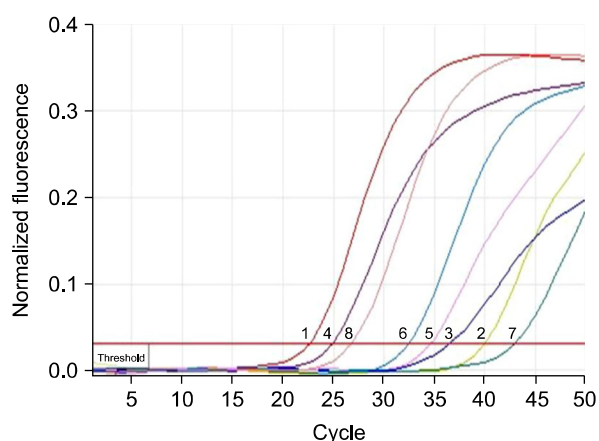


Figure 2. Computed tomography scans are displayed as the timings of (A, D, G) baseline before treatments, (B, E, H) 1-month follow-up after treatments, and (C, F, I) end of treatments. After sixth cycle of gemcitabine and cisplatin, (A) multiple consolidations were developed in left upper lobe and (B) were improved by initial gefitinib treatment. (C) Newly appeared metastatic nodules were shown in left lower lobe after 4 months. Followed by sixth cycle of pemetrexed, (D) multiple metastatic nodules were prominent in both lungs. (E) These lesions were improved by gefitinib retreatment, but (F) residual left upper lobar nodule and tiny metastases were progressed 6 months later. (G) Both lung nodules were markedly aggravated 1 month after gefitinib retreatment. (H) Dramatic response was shown after PF-00299804 trial, but metastatic subcarinal lymphadenopathy was noted in 11-month follow-up scan.

However, as in our patient, other mechanisms can lead to eventually relapse under irreversible EGFR inhibitor therapy. Ercan et al.¹² suggested that the amplification of T790M causes resistance to an irreversible EGFR inhibitor both *in vitro* and using a xenograft model *in vivo*. They demonstrated that although irreversible EGFR inhibitors may be transiently effective against cancers harboring *EGFR* T790M, clones harboring amplified T790M will rapidly emerge *in vitro* and *in vivo* through selection of pre-existing T790M amplified or high-expressing clones leading to clinical drug resistance. This

findings show that *EGFR* T790M is a common resistance mechanism to both reversible, and when amplified, the irreversible EGFR inhibitors. In our case, the response duration of irreversible EGFR inhibitor was 11 months which was unusually longer than those of initial gefitinib (for 4 months) and second round gefitinib (for 6 months) treatments. Recently an alternative approach is to develop novel strategies, instead of irreversible EGFR inhibitors, to inhibit EGFR and/or EGFR signaling, including the use of inhibitors of HSP90 and/or EGFR downstream signaling (such as phosphatidylinositol



Curve #	Exon 18	Exon 19	Exon 20				Exon 21	
	G719X	Deletion	T790M	S768I	Ins. 3 dup	Ins.3	L858R/L861Q	Non-PNA
Curve #	2	3	4	5	6	7	8	1
H12-4187	39.95	36.53	24.85	34.59	32.44	42.93	26.80	22.62
Standard Ct	38.40	34.93	35.60	34.48	32.57	32.95	35.12	32.95
ΔCt-1	17.33	13.91	2.23	11.97	9.82	20.31	4.18	
ΔCt	-1.55	-1.60	10.75	-0.11	0.13	-9.98	8.32	
Result	wild	wild	mutant	wild	wild	wild	mutant	

Figure 3. The epidermal growth factor receptor (*EGFR*) status detected by peptide nucleic acid (PNA) clamping after treatment with EGFR-tyrosine kinase inhibitor indicate two mutations in exon 21 (L858R/L861Q) and exon 20 (T790M). ΔCt: standard Ct - sample Ct; ΔCt-1: sample Ct - (non-PNA Ct).

3-kinase and MEK inhibitors) as these resistant cancers still remain dependent on EGFR signaling for their growth¹². And multiple target therapy, such as multiple kinase inhibition (e.g., EGFR and c-met) or a combination of EGFR-TKI with chemotherapy or vertical inhibition by small molecules and antibodies will be needed to overcome secondary EGFR-TKI resistance for the near future.

Another interesting point of the present case is the suggestion that PNA clamping is a more sensitive method for the detection of *EGFR* mutations, especially with a specimen containing a low proportion of tumor cells. Some clinical data have suggested that PNA clamping is a simple procedure that is more sensitive to direct sequencing for the detection of *EGFR* mutations^{5,13}. Although *EGFR* gene mutation analysis by direct sequencing on initial bronchoscopic lung biopsy specimen revealed wild-type EGFR, rebiopsy specimen by

the PNA clamping method after failure of irreversible EGFR inhibitor therapy revealed L858R/L861Q and T790M mutations. Because of repeated favorable responses to initial gefitinib and second round gefitinib, we suggest that the result of the initial *EGFR* mutation test was a false negative due to insufficient specimen. Although we could not perform an *EGFR* mutation test at the time of progression after gefitinib retreatment, the patient had another partial response with irreversible EGFR inhibitor, which is believed to overcome secondary resistance to reversible EGFR-TKI related with T790M mutation. So, we suggest that the patient had acquired T790M mutation in addition to the original L858R/L861Q after gefitinib retreatment.

We report an adenocarcinoma case responded three times to reversible and irreversible EGFR-TKIs. This case show that scheduled 'drug holidays' or intermittent therapy may benefit for prolonged lung cancer control.

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