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Impact of *EGFR* mutations on treatment of non-small cell lung cancer

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Abstract Somatic mutations in the epidermal growth factor receptor (*EGFR*) have been identified in advanced non-small cell lung cancer (NSCLC) patients who achieve dramatic clinical and radiographic responses to treatment with the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Retrospective studies comparing outcomes of patients with and without *EGFR* mutations treated with EGFR-TKIs demonstrate that patients with *EGFR* mutations live significantly longer than those without mutations. In addition, patients with NSCLC and a somatic deletion mutation of exon 19 exhibit longer survival than patients with point mutations of exon 21. Secondary resistance mutations have also been identified. Patients exhibiting a somatic sensitizing mutation of *EGFR* who achieve partial response to gefitinib or erlotinib therapy eventually develop clinical resistance to treatment with EGFR-TKI. Approximately half of these resistant patients develop a detectable secondary acquired resistance mutation (T790M) in their tumor. New irreversible EGFR inhibitors have in vitro and in vivo evidence of antitumor activity against lung cancer cells harboring both the sensitizing and resistance mutations. These findings suggest that patients with advanced NSCLC bearing somatic *EGFR* mutations should receive treatment with an EGFR-TKI included as at least part of their initial therapy. Trials are starting to test the irreversible EGFR inhibitors in patients with NSCLC after they develop resistance to their initial treatment with gefitinib or erlotinib.

Keywords Receptor · Epidermal growth factor · Lung neoplasm · Carcinoma · Non-small cell lung cancer

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Clinical predictors of response to and survival on EGFR-TKIs

Initial studies of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) provided proof-of-principle demonstrations that inhibition of a growth factor receptor, the EGFR, can lead to dramatic tumor regression in 10–15% of patients with relapsed non-small cell lung cancer (NSCLC) [3, 12]. However, not all patients appeared to benefit from these treatments. Patients most likely to achieve radiographic responses to EGFR-TKIs were women, never-smokers, patients with adenocarcinomas, and Japanese patients [1, 3, 9, 12, 16]. Cigarette smoking status appears the strongest predictor; patients who are non-smokers have the greatest likelihood of achieving clinical response to EGFR-TKI therapy [15]. These clinical

predictors of response have been confirmed in randomized clinical trials of gefitinib and erlotinib that prospectively collected smoking information [21, 24]. Patients with relapsed NSCLC who had never smoked cigarettes had the highest clinical response rates and longest survival when treated with these two agents [21, 24].

EGFR mutations in NSCLC

The relationship between sensitizing mutations in the *EGFR* kinase domain and clinical response to gefitinib was reported in the spring of 2004 [14, 18]. Thirteen of 14 gefitinib-responsive patients were found to have somatic activating mutations in the *EGFR* kinase domain, while none of the 11 patients whose disease progressed on gefitinib had these *EGFR* mutations. *EGFR* mutations are found in the first 4 exons (18–21) of the tyrosine kinase domain of *EGFR*. Three types of mutations have been described: deletions in exon 19 accounting for about 60% of all mutations; a common missense mutation in exon 21 (L858R) accounting for another 25%; and rare point mutations in exons 18, 20, and 21 and insertion/duplications in exon 20 accounting for the remainder [8].

Outcome in patients with *EGFR* mutations treated with EGFR-TKIs

Clinical outcomes in patients with *EGFR* mutations undergoing treatment with EGFR-TKIs have been examined in several retrospective series (Table 1), although no prospective studies have yet been reported. These retrospective studies are limited by their small sample sizes and potential bias because tumor specimens were available from fewer than half of the patients. However, survival of patients with *EGFR* mutations on gefitinib appears longer than in those without *EGFR* mutations (Table 1). Response rates ranged between 60 and 94%, and median survival was approximately 2 years in patients with somatic *EGFR* mutations in their tumors. To date, no other treatment for NSCLC in any selected subgroup of individuals has demonstrated such impressive findings. In contrast, patients without detectable *EGFR* mutations exhibited response rates between 9 and 14%, and median survival was approximately 5–14 months in patients with wild-type *EGFR* in their tumors. These observations have prompted clinical trials that are ongoing to attempt prospectively to document favorable outcome in patients with NSCLC and somatic *EGFR* mutations undergoing treatment with either erlotinib or gefitinib. On the other hand, not all retrospective studies have demonstrated improved outcomes in patients with *EGFR* mutations receiving EGFR-TKIs. Two published studies have demonstrated no significant improvement of survival in patients with *EGFR* mutations receiving either gefitinib or erlotinib compared with those with wild-type *EGFR* [1, 25].

Table 1 Outcomes in patients with and without *EGFR* mutations treated with gefitinib

	Mutant *EGFR*	Wild type	*P* value
Han et al. [5]			
Response rate (%)	65.0	13.7	<0.001
Median survival (months)	30.5	6.6	<0.001
Mitsudomi et al. [17]			
Response rate (%)	83	10	<0.0001
Median survival (months)	>20	13.0	0.0053
Takano et al. [22]			
Response rate (%)	82	11	<0.0001
Median survival (months)	20.0	7.0	0.0001
Cortes-Funes et al. [2]			
Response rate (%)	60	9	0.001
Median survival (months)	13.0	4.9	0.02
Taron et al. [23]			
Response rate (%)	94	13	<0.0001
Median survival (months)	>20	9.9	0.001

There is also some correlation between specific somatic mutations of *EGFR* and outcomes in patients with NSCLC treated with gefitinib or erlotinib. This has been studied in patients with the two most common types of EGFR mutations. The exon 19 deletion and L858R missense mutations make up approximately 85% of reported sensitizing somatic mutations of the *EGFR* [8]. NSCLC patients with exon 19 deletion mutations of *EGFR* have >2-fold longer time to progression and two-fold longer survival than those with L858R missense mutations (Table 2). Therefore there is a relationship between these two different somatic mutations of *EGFR* and outcome in patients with NSCLC treated with gefitinib and erlotinib.

EGFR mutations associated with resistance to gefitinib and erlotinib

Specific mutations of the *EGFR* are emerging as predictors of resistance. These include T790M and the exon 20 insertion mutations of *EGFR*. There is more information on the T790M mutation, initially identified in patients with dramatic responses to gefitinib who developed disease progression. The T790M mutation is associated with both in vitro and in vivo resistance to

Table 2 Outcomes in NSCLC patients with *EGFR* exon 19 deletion mutations or exon 21 point mutations receiving gefitinib and erlotinib

	Exon 19 deletion	Exon 20	*P* value
Riely et al. [20]			
TTP (months)	12	5	0.01
Median survival (months)	34	8	0.01
Jackman et al. [7]			
Response rate (%)	73	50	>0.05
TTP (months)	24	10	0.04
Median survival (months)	38	20	0.04

TTP time to progression

gefitinib and erlotinib. Three reports identified eight NSCLC patients who initially had dramatic clinical responses to gefitinib, then developed progressive NSCLC [10, 13, 19]. Six of these eight patients had both a sensitizing deletion mutation or missense mutation of the *EGFR* as well as an acquired secondary resistance mutation in exon 20, the T790M mutation. The lung cancer cell line NCI-H1975 has a sensitizing L858R exon 21 missense mutation and the T790M exon 20 secondary resistance mutation. NCI-H1975 is >100-fold more resistant to gefitinib than the lung cancer cell line with just the sensitizing mutation in *EGFR*, NCI-H3255 [19]. Investigators have studied the effects of sensitizing and resistance mutations by transfecting a sensitizing mutation of *EGFR* and/or the T790M mutation into a murine proB cell line (Ba/F3), human embryonic kidney cells (293T cells), and a mouse fibroblast cell line (NIH 3T3) [4, 11, 13, 19]. These different cell lines transfected with DNA containing the deletion or missense sensitizing *EGFR* mutations were 100-fold more sensitive to treatment with gefitinib than cell lines transfected with both the sensitizing and resistance (T790M) mutation. A new class of drugs, the irreversible EGFR inhibitors, covalently binds to a cysteine in the EGFR or ERBB2 receptor. These irreversible inhibitors, HKI-357, HKI-272, and CL-387, 785, are effective against the NCI-H1975 lung cancer cell line with both the sensitizing missense mutation in *EGFR* and the secondary resistance mutation T790M. This class of drugs is also effective against host cell lines transfected with both the sensitizing and resistance mutations of *EGFR*. This in vitro evidence of antitumor activity in lung cancer cell lines as well as in transfected cell lines has prompted a clinical trial of the irreversible inhibitor HKI-272 in NSCLC patients who have become resistant to EGFR-TKIs.

The insertion 20 mutation has recently been demonstrated to be associated with in vitro resistance to gefitinib and erlotinib [4]. NIH-3T3 cells transfected with the insertion 20 mutations (D770-N771insNPG) were 100-fold less sensitive to gefitinib and erlotinib treatment than exon 19 *EGFR* deletion mutants and exon 21 missense point mutants. Furthermore, three patients with somatic insertion mutations of exon 20 reportedly progressed while undergoing treatment with erlotinib or erlotinib plus chemotherapy [4]. Since irreversible EGFR inhibitors have been shown effective against the insertion mutant, they may be a useful strategy for managing patients with NSCLC and an insertion mutation if gefitinib or erlotinib are not clinically effective [4].

Conclusions and future directions

Epidermal growth factor receptor mutations are a strong predictive marker for radiographic and clinical responses to the EGFR-TKIs gefitinib and erlotinib. Since the initial identification of *EGFR* mutations, additional potential molecular markers including increased *EGFR* copy number and tumor EGFR expression detected by immunohistochemistry have also emerged as predictors of efficacy of EGFR-TKIs. The next generation of clinical trials is prospectively evaluating the efficacy of EGFR-TKIs in clinically or molecularly enriched patient populations. A relationship between genotype, different types of somatic mutations of EGFR, and outcome in patients with NSCLC treated with gefitinib and erlotinib has been demonstrated in two studies. Patients with exon 19 deletion mutations exhibit longer time to progression and survival than patients with exon 21 missense mutations. Further prospective studies are needed to confirm this observation. Secondary acquired resistance mutations in the *EGFR* have been identified. In vitro studies of NSCLC cell lines and transfected cell lines have identified irreversible EGFR inhibitors that are effective in these cell lines with both sensitizing EGFR and resistance mutations. These agents have entered clinical trials for patients who develop resistance to treatment with gefitinib or erlotinib. These studies will help further refine the population of patients with NSCLC who are likely to derive the greatest benefit from EGFR-TKIs and generate new treatments for those who relapse after treatment with gefitinib or erlotinib.

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