REVIEW ARTICLE

Driven by Mutations: The Predictive Value of Mutation Subtype in *EGFR*-Mutated Non–Small Cell Lung Cancer

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ABSTRACT

EGFR-mutated NSCLC is a genetically heterogeneous disease that includes more than 200 distinct mutations. The implications of mutational subtype for both prognostic and predictive value are being increasingly understood. Although the most common *EGFR* mutations—exon 19 deletions or L858R mutations—predict sensitivity to EGFR tyrosine kinase inhibitors (TKIs), it is now being recognized that outcomes may be improved in patients with exon 19 deletions. Additionally, 10% of patients will have an uncommon *EGFR* mutation, and response to EGFR TKI therapy is highly variable depending on the mutation. Given the growing recognition of the genetic and clinical variation seen in this disease, the development of comprehensive bioinformatics-driven tools to both analyze response in uncommon mutation subtypes and inform clinical decision making will be increasingly important. Clinical trials of novel EGFR TKIs should prospectively account for the presence of uncommon mutation subtypes in study design.

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Keywords: Lung cancer; Targeted therapy; EGFR; Mutation subtype

Introduction

The advent of targeted therapy has revolutionized treatment for a subset of patients with NSCLC, and testing patients with newly diagnosed NSCLC for the presence of driver mutations is now considered standard of care.[1] The EGFR belongs to a family of receptor tyrosine kinases that include EGFR/erb-b2 receptor tyrosine kinase 1 (ERBB1), erb-b2 receptor tyrosine kinase 2 (HER2/ERBB2), erb-b2 receptor tyrosine kinase 3 (HER3/ERBB3), and erb-b2 receptor tyrosine kinase 4 (HER4/ERBB4).[2] When stimulated, the transmembrane receptors trigger a cascade of intracellular signaling that affects cellular proliferation, angiogenesis, and apoptosis.[2] Sensitizing *EGFR* mutations are the most common actionable driver mutations found in patients with NSCLC, and they occur in approximately 10% of white patients and up to 50% of Asian patients.[3–5]

Mutations occur within the *EGFR* exons 18 to 21, which encode a portion of the EGFR kinase domain. Although approximately 90% of patients with *EGFR*-mutated NSCLC will have either deletions in exon 19 or substitutions of leucine for arginine (L858R) in exon 21 of the *EGFR* gene (the "common mutations"),[6–9] numerous other mutations with varying sensitivity to EGFR tyrosine kinase inhibitors (TKIs) have been identified. With some exception, most mutations involving exons 18, 19, and 21 are considered predictive of sensitivity to EGFR TKI therapy, whereas mutations in exon 20 are typically resistant.[10–19] This review focuses on the prognostic and predictive implications of *EGFR* mutation subtype.

Development of EGFR TKIs: A Brief History

The first EGFR TKIs, erlotinib and gefitinib, were developed before the identification of *EGFR* somatic gene mutations and were first studied in an unselected patient population.[20,21] Erlotinib received U.S. Food and Drug Administration approval for the treatment of patients with disease progression after initial chemotherapy on the basis of the National Cancer Institute of

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Canada Clinical Trials Group study BR.21, which demonstrated that compared with placebo, erlotinib improved overall survival (OS) (6.7 versus 4.7 months, respectively); response rate (8.9% versus <1%, respectively); and tumor-related symptoms of pain, cough, and dyspnea.[20] A similar study in an unselected patient population, ISEL, compared gefitinib with placebo and showed nonstatistically significant trends toward improved OS and time to treatment failure (TTF) that favored the gefitinib arm,[21] leading to the approval of gefitinib outside of the United States.

Although these initial trials were performed before the development of molecular testing, certain clinical features, including female sex, never or light smoking status, Asian ethnicity, and adenocarcinoma histologic subtype, were noted to be associated with increased chance of response and prolonged survival.[20,21] On the basis of these data, the IRESSA Pan-Asia Study was designed to compare first-line gefitinib to carboplatin and paclitaxel in a clinically enriched patient population with metastatic NSCLC; tumor samples were retrospectively analyzed for the presence of *EGFR* mutation.[6] The IRESSA Pan-Asia Study was the first trial to demonstrate an improvement in response rate and median progression-free survival (PFS) in patients with *EGFR* mutation–positive NSCLC treated with an EGFR TKI compared with chemotherapy (71.2% versus 47.3% and 9.5 months versus 6.3 months, respectively, with a hazard ratio [HR] for progression of 0.48).[22] Subsequent studies confirmed the *EGFR* mutation to be the most important predictor of benefit of response to therapy with an EGFR TKI.[22–28]

Options for first-line therapy were expanded with the approval of afatinib, an oral, irreversible ErbB-family blocker for first-line treatment of patients with metastatic NSCLC whose tumors have exon 19 deletion or L858R *EGFR* mutations (as detected by a U.S. Food and Drug Administration–approved test)[29] on the basis of the results of two randomized phase III studies (LUX-Lung 3 and LUX-Lung 6).[8,9] More recently, it has also been indicated for patients with locally advanced or metastatic NSCLC of the squamous histologic subtype progressing during or after platinum-based chemotherapy.[29] Preclinical data demonstrated that afatinib was active against *EGFR* T790M,[30] an acquired resistance mutation seen in approximately 50% of patients after progression during first-line EGFR TKI therapy.[31] In studies comparing erlotinib, gefitinib, and afatinib with chemotherapy in molecularly selected populations, all three of these agents have consistently demonstrated an improvement in PFS (although not in OS in the intention-to-treat population), with PFS ranging from 9.2 to 16.4 months; the lack of improvement in OS has been attributed to high rates of crossover to an EGFR TKI after progression with chemotherapy.[6–9,32–35]

Table 1 presents a summary of EGFR TKI trials in patients with exon 19 deletions or L858R mutations; Table 2 presents a summary of EGFR TKI trials in patients with uncommon mutations.

Major *EGFR* Mutations

Multiple studies have confirmed that upward of 80% to 90% of patients with *EGFR*-mutated NSCLC will have either an exon 19 deletion or an L858R point mutation.[4,40,45] Individual trials of erlotinib and gefitinib have been underpowered to detect differences in outcome by mutation subtype. In most clinical trials of EGFR TKIs, patients with uncommon mutations are either excluded or molecular stratification is simplified into common (exon 19 deletions and exon 21 L858R substitutions) and uncommon mutations.[7,35,46] There are, however, data suggesting that patients with exon 19 deletions have improved outcomes when treated with first-generation EGFR TKIs as compared with L858R substitutions, although whether this benefit translates into an OS difference between the two mutations has not been proved.[47,48] Both OPTIMAL and ENSURE, phase III studies of erlotinib compared with platinum doublets, showed a trend toward improved PFS in patients with exon 19 deletions when compared with L858R mutations.[26,27] An early small prospective study of 36 patients treated with either gefitinib or erlotinib found improved OS among patients with exon 19 deletions as compared with L858R substitutions (38 versus 17 months; $p = 0.04$), as well as trends toward higher response rates (73% versus 50%) and PFS (24 versus 10 months).[47] Similar results were found in a single-institution retrospective study of patients treated with first-generation EGFR TKIs.[48] Of the 34 evaluable patients with *EGFR* mutations, those with *EGFR* exon 19 deletions had better outcomes than patients with L858R mutations, with PFS of 12 months versus 5 months ($p = 0.01$) and OS of 34 months versus 8 months ($p = 0.01$), respectively. The reasons for this possible clinical benefit have not been determined, although kinetic analysis of these two mutations found that exon 19 deletions appeared to be more sensitive to erlotinib inhibition than tumors harboring the L858R substitution.[49] Additionally, when outcomes were examined in larger prospective studies, patients with exon 19 deletions demonstrated improvements in OS and PFS compared with those harboring L858R mutations after treatment with first-generation EGFR TKIs.[33,50]

In contrast to studies of first-generation TKIs, several studies of afatinib have examined outcomes by *EGFR* mutation subtype, and both common and uncommon

Table 1. EGFR TKI Trials in Patients with Exon 19 Deletions or L858R Mutations

Study	Study Type	Patient Population	ORR (%)	Median PFS (mo)	Median OS (mo)
LUX-Lung 2[36]	Phase II trial	129 *EGFR* mutation-positive Pts treated with afatinib	61	10.1	24.8
		Pts with exon 19 del: 52	69 (Pts with exon 19 del)	13.7 (Pts with exon 19 del)	38.7 (Pts with exon 19 del)
		Pts with L858R mutation: 54	63 (Pts with L858R mutations)	13.7 (Pts with L858R mutations)	31.5 (Pts with L858R mutations)
LUX-Lung 3[8,37]	Phase III trial	345 *EGFR* mutation-positive Pts treated with afatinib vs. cisplatin/pemetrexed	56 vs. 23	11.1 vs. 6.9 (HR = 0.58; 95% CI: 0.43-0.78; $p = 0.001$)	28.2 vs. 28.2 (HR = 0.88; 95% CI: 0.66-1.17; $p = 0.39$)
		Pts with exon 19 del: 170		13.6 vs. 6.9 (for exon 19 del and L858R Pts only) (HR = 0.47; 95% CI: 0.34-0.65; $p = 0.001$)	33.3 vs. 21.1 (exon 19 del) (HR = 0.54; 95% CI: 0.36-0.79; $p = 0.0015$)
		Pts with L858R mutation: 138			27.6 vs. 40.3 (L858R mutation) (HR = 1.30; 95% CI: 0.80-2.11; $p = 0.29$)
LUX-Lung 6[9,37]	Phase III trial	364 *EGFR* mutation-positive Pts treated with afatinib vs. cisplatin/gemcitabine	66.9 vs. 23 ($p < 0.0001$)	11.0 vs. 5.6 (HR = 0.28; 95% CI: 0.20-0.39; $p < 0.0001$)	23.1 vs. 23.5 (HR = 0.93; 95% CI: 0.72-1.22; $p = 0.61$)
		Pts with exon 19 del: 186			31.4 vs. 18.4 (exon 19 del) (HR = 0.64; 95% CI: 0.44-0.94; $p = 0.023$)
		Pts with L858R mutation: 138			19.6 vs. 24.3 (L858R mutation) (HR = 1.22; 95% CI: 0.81-1.83; $p = 0.34$)
IPASS[6,22]	Phase III trial	261 *EGFR* mutation-positive Pts treated with gefitinib vs. carboplatin/paclitaxel	71.2 vs. 47.3 (Pts with *EGFR* mutations) ($p < 0.001$)	9.5 vs. 6.3 (Pts with *EGFR* mutations) (HR = 0.48; 95% CI: 0.36-0.64; $p < 0.001$)	21.6 vs. 21.9 (Pts with *EGFR* mutations) (HR = 1.0; 95% CI: 0.76-1.33; $p = 0.99$)
		Pts with exon 19 del: 140 Pts with L858R mutation: 111	84.8 vs. 60.9 (exon 19 del vs. L858R mutation)		
NEJ002[7,38]	Phase III trial	230 sensitizing *EGFR* mutation-positive Pts treated with gefitinib vs. carboplatin/paclitaxel	73.7 vs. 30.7 ($p < 0.001$)	10.8 vs. 5.4 (HR = 0.30; 95% CI: 0.22-0.41; $p < 0.001$)	27.7 vs. 26.6 (HR = 0.89; 95% CI: 0.63-1.24; $p = 0.48$)
		Pts with exon 19 del: 117 Pts with L858R mutation: 97	82.8 vs. 67.3 (exon 19 del vs. L858R mutation)	11.5 vs. 10.8 (exon 19 del vs. L858R mutation)	
WJTOG3405[32,39]	Phase III trial	172 activating *EGFR* mutation-positive Pts treated with gefitinib vs. cisplatin/docetaxel	62.1 vs. 32.2 ($p < 0.0001$)	9.2 vs. 6.3 (HR = 0.49; 95% CI: 0.34-0.71; $p < 0.0001$)	34.8 vs. 37.3 (HR = 1.25; 95% CI: 0.883-1.78)
		Pts with exon 19 del: 87 Pts with L858R mutation: 85		9.0 vs. 9.6 mo (exon 19 del vs. L858R mutation) (HR = 1.13; 95% CI: 0.63-2.0; $p = 0.68$)	

(continued)

Table 1. Continued

Study	Study Type	Patient Population	ORR (%)	Median PFS (mo)	Median OS (mo)
EURTAC[40]	Phase III trial	173 activating *EGFR* mutation-positive Pts treated with erlotinib vs. cisplatin/docetaxel/gemcitabine	64 vs. 18	9.7 vs. 5.2 (HR = 0.37; 95% CI: 0.25-0.54; $p < 0.0001$)	19.3 vs. 19.5 (HR = 1.04; 95% CI: 0.65-1.68; $p = 0.87$)
		Pts with exon 19 del: 115		11.0 vs. 8.4 (exon 19 del vs. L858R mutation)	
		Pts with L858R mutation: 58		11.0 vs. 4.6 (exon 19 del) (HR = 0.30; 95% CI: 0.18-0.50; $p < 0.0001$)	
				8.4 vs. 6.0 (L858R mutation) (HR = 0.55; 95% CI: 0.29-1.02; $p = 0.054$)	
First-Signal[25]	Phase III trial	42 activating *EGFR*-mutation-positive Pts treated with gefitinib vs. gemcitabine/cisplatin	84.6 vs. 37.5 ($p = 0.002$)	8.0 vs. 6.3 (HR = 0.54; 95% CI: 0.27-1.10; $p = 0.086$)	27.2 vs. 25.6 (HR = 1.04; 95% CI: 0.50-2.18)
		Pts with exon 19 del: 27			
		Pts with L858R mutation: 15			
OPTIMAL[26,41]	Phase III trial	154 activating *EGFR* mutation-positive Pts treated with erlotinib vs. gemcitabine/carboplatin	83 vs. 36 ($p < 0.0001$)	13.1 vs. 4.6 (HR = 0.16; 95% CI: 0.10-0.26; $p < 0.0001$)	22.8 vs. 27.2 (HR = 1.19; 95% CI: 0.83-1.71; $p = 0.27$)
		Pts with exon 19 del: 82			
		Pts with L858R mutation: 72			
ENSURE[27]	Phase III trial	217 activating *EGFR* mutation-positive Pts treated with erlotinib vs. gemcitabine/cisplatin	62.7 vs. 33.6	11.0 vs. 5.6 (HR = 0.42; 95% CI: 0.27-0.66; $p = 0.0001$)	26.3 vs. 25.5 (HR = 0.91; 95% CI: 0.63-1.31; $p = 0.61$)
		Pts with exon 19 del: 118		11.1 vs. 8.3 (exon 19 del vs. L858R mutation)	
		Pts with L858R mutation: 98			

TKI, tyrosine kinase inhibitor; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; Pts, patients; del, deletion; HR, hazard ratio; CI, confidence interval.

Table 2. EGFR TKI Trials in Patients with Uncommon Mutations

Study	Study Type	Patient Population	ORR (%)	Median PFS (mo)	Median OS (mo)
Yang et al.[14]	Post hoc analysis of LUX-Lung 2, 3, and 6	75 Pts with uncommon *EGFR* mutations receiving afatinib			
		Group 1: point mutations or duplications in exons 18–21 (38 Pts)	Group 1: 71.1	Group 1: 10.7	Group 1: 19.4
		Group 2: de novo T790M mutations in exon 20 alone or in combination with other mutations (14 Pts)	Group 2: 14.3	Group 2: 2.9	Group 2: 14.9
		Group 3: exon 20 insertions (23 Pts)	Group 3: 8.7%	Group 3: 2.7	Group 3: 9.2
Baek et al.[10]	Retrospective analysis	54 Pts with uncommon *EGFR* mutations treated with gefitinib (35) or erlotinib (19)	20.4	2.6	12.7
Chiu et al.[11]	Retrospective analysis	161 Pts with uncommon *EGFR* mutations; 478 Pts with common *EGFR* mutations	41.6 vs. 66.5 (uncommon vs. common mutations) ($p < 0.001$)	7.7 vs. 11.4 (uncommon vs. common mutations) ($p < 0.001$)	24.0 vs. 29.7 (uncommon vs. common mutations) ($p = 0.005$)
Arrieta et al.[42]	Observational prospective cohort	38 Pts with uncommon *EGFR* mutations; 150 Pts with common *EGFR* mutations	32.4 vs. 63.8 (uncommon vs. common mutations) ($p < 0.001$)	3.9 vs. 15.5 (uncommon vs. common mutations) ($p < 0.001$)	17.4 vs. 37.3 (uncommon vs. common mutations) ($p < 0.001$)
Watanabe et al.[43]	Post hoc analysis of NEJ002 trial	10 Pts with uncommon *EGFR* mutations (only G719X and L861Q); 215 Pts with common *EGFR* mutations	20 vs. 76 (gefitinib group: uncommon vs. common mutations) ($p = 0.017$) 20 vs. 32 (carboplatin-paclitaxel group: uncommon vs. common mutations) ($p = 0.34$)	2.2 vs. 11.4 (gefitinib group: uncommon vs. common mutations) ($p < 0.001$) 5.9 vs. 5.4 (carboplatin-paclitaxel group: uncommon vs. common mutations) ($p = 0.85$)	11.9 vs. 29.3 (gefitinib group: uncommon vs. common mutations) ($p < 0.001$) 22.8 vs. 28.0 (carboplatin-paclitaxel group: uncommon vs. common mutations) ($p = 0.36$)
Lohinai et al.[44]	Retrospective analysis	49 Pts with uncommon *EGFR* mutations; 42 Pts with common *EGFR* mutations	37 vs. 71 (uncommon vs. common mutations) ($p = 0.039$)	6.2 vs. 12 (uncommon vs. common mutations) ($p = 0.048$)	7.4 vs. 20.5 (uncommon vs. common mutations)

TKI, tyrosine kinase inhibitor; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; Pts, patients.

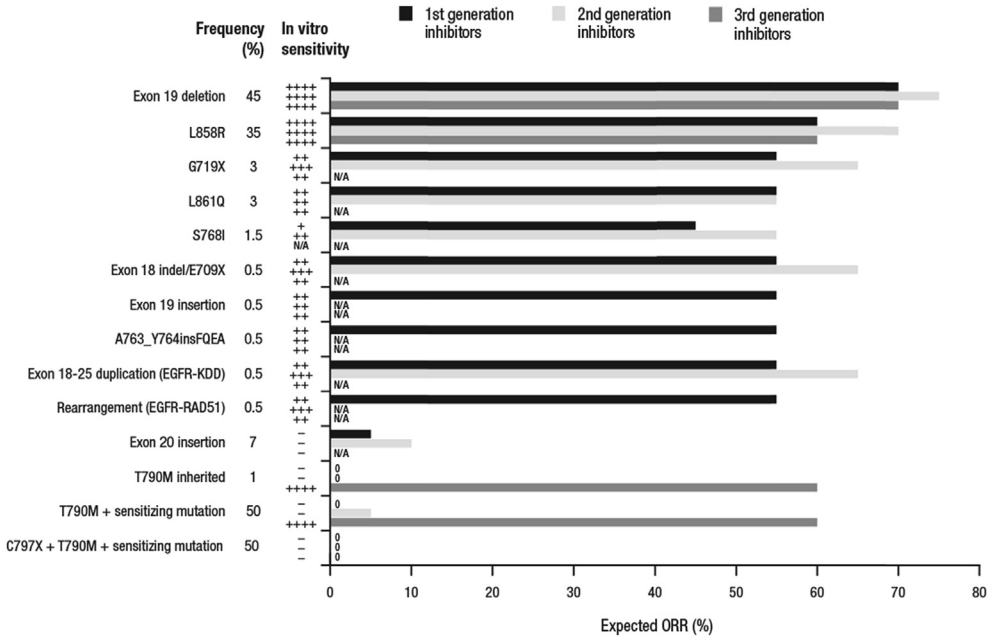

Figure 1. Frequency of *EGFR* mutations and in vitro sensitivity to EGFR TKIs.[52] TKI, tyrosine kinase inhibitor; KDD, kinase domain duplication; ORR, objective response rate.

mutations have been included in different trials. LUX-Lung 2 was a phase II study of afatinib in a population of 129 EGFR TKI–naive patients who were treated with two different doses of afatinib, with the primary end point of response.[36] Ninety-nine patients received a starting dose of 50 mg daily, and 30 patients received a starting dose of 40 mg daily. Of the 129 patients, 47% (61) received afatinib as a first-line therapy and 53% (68) had received at least one prior line of chemotherapy. Although the objective response rate (ORR) was 61%, patients with L858R mutations or exon 19 deletions were the most sensitive to afatinib, with 66% of those patients demonstrating a response in comparison with 39% of patients harboring other types of *EGFR* mutations. Additionally, patients with exon 19 deletions or L858R mutations had a median PFS of 13.7 months, whereas patients with uncommon mutations had a shorter PFS (3.7 months). Two large, randomized phase III trials, LUX-Lung 3 and LUX-Lung 6, compared afatinib with standard first-line chemotherapy consisting of cisplatin and pemetrexed (LUX-Lung 3) or cisplatin and gemcitabine (LUX-Lung 6) in patients with advanced-stage NSCLC who were positive for the *EGFR* mutations.[8,9] Both trials reported a significant improvement in PFS, with no significant improvement in OS for the intention-to-treat populations. However, a preplanned analysis of both trials independently demonstrated a significant improvement in OS in patients with exon 19 deletions who were treated with first-line afatinib versus with chemotherapy (LUX-Lung 3: median OS = 33.3 months versus 21.1 months, respectively, HR = 0.54, 95% confidence interval [CI]: 0.36–0.79, $p = 0.0015$, and LUX-Lung 6: median OS = 31.4 months versus 18.4 months, respectively, HR = 0.64, 95% CI: 0.44–0.94, $p = 0.023$).[37] This benefit was not seen in patients who were L858R mutation–positive.

A recent meta-analysis using randomized trial data from studies of patients undergoing first-line treatment with first- and second-generation EGFR TKIs examined the impact of mutation subtype and clinical characteristics on PFS outcome.[50] A total of seven studies involving 1649 patients treated with gefitinib, erlotinib, or afatinib were included. A total of 950 patients were assigned to EGFR TKIs, and 699 were assigned to chemotherapy. Across all *EGFR* mutation subtypes, treatment with an EGFR TKI showed a 63% reduction in the risk for disease progression or death as compared with treatment with chemotherapy (HR = 0.37, 95% CI: 0.32–0.42, $p < 0.001$). The vast majority of patients harbored common *EGFR* mutations: 872 patients had exon 19 deletions, and 686 patients had exon 21 L858R substitutions. Subgroup analyses demonstrated that patients with exon 19 deletions showed a 50% greater PFS benefit when treated with an EGFR TKI than did patients with exon 21 L858R substitutions (interaction $p < 0.001$).

Rare *EGFR* Mutations

Because most patients with *EGFR*-mutated NSCLC harbor either an L858R substitution or an exon 19 deletion, randomized trial data of other uncommon mutations are lacking. However, insight into the

behavior and clinical responsiveness of uncommon *EGFR* mutations has been gained from various case series, retrospective studies, and pooled analyses.[10–19,51] Available information regarding mutation frequency, including in vitro sensitivity and rate of response to first-, second-, and third-generation EGFR TKIs according to mutation type, is illustrated in Figure 1.[52] Generally, mutations involving exons 18 to 21 are considered sensitive to EGFR TKIs, with the exception being mutations involving exon 20, including T790M and exon 20 insertions.

Studies in which patients with uncommon *EGFR* mutations have been analyzed as a single group often find these patients to be less responsive to EGFR TKI therapy than patients with either of the common mutations alone.[10,11,42,43] However, when analyses are performed on individual mutations or smaller, selective subsets, it is clear that significant clinical heterogeneity exists.

Mutations in exon 18 are typically considered sensitizing to EGFR TKI therapy. The most frequently detected exon 18 mutation is the G719X mutation, followed by the E709X mutation.[12,13] The G719X mutation is associated with a 10-fold increase in EGFR activation compared with wild-type *EGFR*,[53] but in vitro studies have suggested that it is not as sensitive to gefitinib as NSCLC cell lines with L858R mutations.[54] Although patients with G719X mutations do respond to EGFR TKI therapy, responses have not always been as prolonged as those seen with the more common mutations. For example, a retrospective analysis of patients treated with afatinib after progression during one line of prior EGFR TKI treatment and chemotherapy as part of the Afatinib Compassionate-Use Program included 10 patients with G719X mutations, and median TTF was only 2.6 months.[55] In contrast, patients with E709X (exon 18 indel) mutations had a median TTF of 12.2 months with afatinib therapy. Interestingly, complex mutations within exon 18 may be associated with a better prognosis than point mutations.[10,13] For example, the L861Q mutation is associated with sensitivity to EGFR TKIs.[44,56] A post hoc analysis of 838 patients from LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6 found uncommon *EGFR* mutations in 100 study patients (12%), 16 of which were L861Q substitutions.[14] The ORR associated with afatinib for patients with L861Q mutations was 56.3%, with median PFS and OS of 8.2 months and 17.1 months, respectively. Additional sensitizing *EGFR* aberrations, occurring at frequencies of less than 0.5%, include exon 19 insertions, exon 18 to 25 kinase domain duplications, and gene rearrangements.[52] Clinical responses have been reported with EGFR TKIs in individual patients with exon 19 insertions,[57] and case reports of patients with the *EGFR* kinase domain duplication have shown responses to afatinib and gefitinib/erlotinib.[58,59] Although gene fusions of *EGFR* with the RAD51 recombinase or with purine-rich element binding protein B have been discovered only recently, antitumor responses have been observed in patients with lung cancer harboring these alterations.[60]

EGFR mutations that are associated with resistance to EGFR TKI therapy remain a clinical challenge. Exon 20 mutations exist at a prevalence of approximately 2% among patients with stage IV lung adenocarcinoma.[15] Although it appears that exon 20 mutations are more likely to occur in patients with a phenotype similar to the common *EGFR* mutations (i.e., female sex, Asian ethnicity, and never-smoker status), multiple studies have confirmed that this mutation confers primary resistance to EGFR TKI therapy, with an ORR of approximately 10% and PFS of approximately 2.5 months.[12,14,16–18,61,62] Structural and molecular analyses have shown that although these mutations may activate EGFR, unlike the common sensitizing *EGFR* mutations, they activate EGFR without increasing receptor affinity for EGFR TKIs.[61] One exception may be the A763_Y764insFQEA variant, which appears to have sensitivity to EGFR TKIs both in vitro and in vivo, although the reported sample sizes are small.[15,61] Another mutation of note is the S768I mutation, which is associated with poor responses in some studies[19,63] and did show good clinical outcomes from treatment with afatinib, with median PFS of 14.7 months and median OS not yet reported (95% CI: 3.4 months–not estimable).[14]

The T790M mutation is well characterized as the most common mechanism of acquired resistance to EGFR TKI therapy,[64,65] and it has been identified as a de novo T790M mutation, as a germline mutation, and in combination with other genetic aberrations. This "gatekeeper" mutation, which involves a threonine-to-methionine substitution in exon 20, increases the affinity of mutant EGFR for adenosine triphosphate, thereby competitively inhibiting the binding ability of reversible EGFR TKIs.[66] Third-generation EGFR TKIs that are highly selective for mutant *EGFR* have demonstrated efficacy in patients with lung cancer with acquired T790M mutations after progression during treatment with an EGFR TKI,[67,68] with the first approval of a third-generation EGFR TKI (osimertinib) occurring in 2015. T790M germline mutations occur in approximately 1% of patients with NSCLC; however, these patients may also have a second activating *EGFR* mutation.[69–71] Familial studies have found that patients carrying a germline T790M mutation have a high lifetime risk for development of lung cancer (up to 31% among never-smoking genetic carriers).[69,72] De novo baseline EGFR T790M mutations occur in less than 1% of patients with

NSCLC; like patients with acquired resistance from T790M mutations, this population does not respond to first-generation EGFR TKIs.[73,74] However, a retrospective analysis of 60 patients with an uncommon *EGFR* mutation who were treated with afatinib after progression during one line of prior EGFR TKI treatment and chemotherapy suggested that patients with T790M mutations, as well as with exon 20 insertions, may derive benefit from this agent.[55] The median TTF with afatinib for patients with T790M mutation and exon 20 insertions was 4.4 months (range 0.4–21.8 months) and 6.5 months (range 3.6–9.1 months), respectively. However, this positive finding may have been related to positive selection, as only patients who had previously responded to EGFR TKI therapy were eligible for the compassionate-use program.

Although the rate of germline and de novo T790M mutations is low, the frequency of T790M with another activating mutation ranges from 0.32% to 79%, with variation based on detection method.[75] In a meta-analysis of three randomized controlled trials and 15 observational studies, pretreatment T790M mutations were more likely to be present with L858R mutations than with exon 19 deletions.[75] This association may underlie the observations of improved PFS in patients with exon 19 deletions compared with L858R mutations.[37,47,48] T790M is most often seen in the *cis* position with a L858R mutation or exon 19 deletion; however, it can occur in the *trans* position as well.[65] Interestingly, in a study of acquired resistance to third-generation EGFR inhibitors, when C797S and T790M mutations were present in *trans*, cells were resistant to third-generation TKIs but sensitive to combined treatment with first- and third-generation inhibitors. When these mutations were in *cis* conformation, no TKIs were able to suppress EGFR activity.[76]

Although the EGFR T790M mutation can be a mechanism of acquired resistance, adaptive resistance also plays an important role in response to targeted therapy. Unlike acquired resistance, adaptive resistance is a process whereby tumor cells can rapidly respond to oncogene inhibition by altering signaling pathways to promote cell proliferation and survival.[77] One proposed mechanism of adaptive resistance in NSCLC involves inhibition of mitogen-activated protein kinase kinase, a downstream signaling molecule of EGFR, leading to activation of signal transducer and activator of transcription 3 and interleukin-6, which promotes cell survival and ultimately resistance.[78] Additional research has demonstrated that therapies targeting EGFR can immediately activate nuclear factor kappa B to induce an antiapoptotic signaling cascade.[77] These findings suggest that in addition to targeting EGFR, upfront combined inhibition of nuclear factor kappa B, interleukin-6, and signal transducer and activator of transcription 3 may be necessary to subvert mechanisms of adaptive resistance and prevent tumor cell survival.

As the cost and convenience of comprehensive molecular testing improve, it is expected that comprehensive tumor genome profiling will become common practice,[79,80] increasing the likelihood that oncologists will encounter rare *EGFR* mutations for which little or no trial data are available. For instance, a recent analysis of next-generation sequencing data from a commercial entity identified rare *EGFR* mutations in approximately 20% of samples,[52] a higher proportion than had been previously estimated.[45,46] More than 200 known combinations of unique *EGFR* mutations have been identified to date in patients treated with TKIs,[81] and comprehensive data are available on only a select group of these, as described previously. Thus, development of accessible tools to support medical decision making will be key to making the increasing burden of molecular data clinically useful. One such tool, the DNA-Mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT), is a comprehensive electronic catalog of reported *EGFR* NSCLC mutations paired with clinical outcome.[81] Information was pulled by using a retrospective PubMed medical subject heading search to identify patient-level, mutation-specific, drug response data from different studies in NSCLC with *EGFR*-mutant tumors. At last report, DIRECT cataloged 188 unique *EGFR* mutations that occurred in 207 different combinations, including 149 mutation combinations associated with disease control and 42 associated with disease progression.[81] Electronic queries of DIRECT will result in a customized report of patient-level, mutation-specific, drug response data. A similar approach was taken in My Cancer Genome, a searchable database that matches specific tumor mutations to a clinical significance report, including pertinent active clinical trials.[80] These data are continuously updated for public use. A growing number of genomic profiling services that include pertinent clinical relevance data are becoming available for community use. For example, FoundationOne utilizes massively parallel DNA sequencing to characterize clinically actionable mutations across a wide array of cancer-related genes from routine formalin-fixed and paraffin-embedded tissue specimens.[82] This information is provided in conjunction with an interpretive report that highlights the genomic alterations related to available targeted therapies or clinical trials, and it has the potential to identify new mutations or genetic fusions not been previously described.[60] Finally, novel biopsy-free techniques such as digital droplet polymerase chain reaction, in which genomic testing can be performed on circulating tumor DNA, may make mutational testing at diagnosis and at progression more convenient,

which has the potential to increase use not only at diagnosis but also at progression.[83,84] As identification of rare or combination mutations becomes routine clinical practice, the use of dynamic, evidence-based databases to guide clinical treatment will likely become an essential component of oncology practice.

Clinical Characteristics

Although certain clinical characteristics have been associated with *EGFR*-mutated NSCLC, it has also been demonstrated that not all patients with *EGFR* mutations will fit this phenotype.

Although patients with *EGFR*-mutated NSCLC and a history of heavy smoking do show response to EGFR TKI therapy, outcomes may not be as good as in patients with a history of never or light smoking.[20,21] Prior study of the pharmacokinetics of erlotinib found that erlotinib had an increased metabolic clearance in current smokers as compared with in never-smokers.[85] A multivariate analysis of data from four large trials of first-generation EGFR TKIs found that, compared with chemotherapy, EGFR TKI treatment demonstrated a 27% greater benefit in women than in men (interaction $p = 0.02$), with a pooled HR for PFS of 0.33 in women and 0.45 in men.[50] Similar findings were demonstrated when the pooled HR for PFS in never-smokers was compared with that in smokers. Although both never-smokers and smokers showed significant improvement in PFS with EGFR TKI treatment as compared with chemotherapy (pooled HRs for PFS of 0.32 and 0.50, respectively), treatment with an EGFR TKI resulted in a 36% greater benefit to never-smokers than to current or former smokers (interaction $p = 0.002$). However, there was no difference in improvement to PFS with EGFR TKI treatment compared with chemotherapy with respect to ethnicity, age, tumor histologic subtype, or performance status in this meta-analysis.

Conclusions

EGFR-mutated NSCLC is a heterogeneous entity in which response to EGFR TKIs depends on the mutational subtype of the tumor. The vast majority of patients with *EGFR*-mutated NSCLC will have either an exon 19 deletion or an L858R substitution. Increasingly, it appears that these two mutations may exhibit different biology when treated with EGFR TKI therapy, with improved outcomes in patients harboring exon 19 deletions. Uncommon mutations are a clinically heterogeneous group comprising approximately 10% to 20% of *EGFR*-mutated NSCLCs.

Thus, analyses of EGFR TKI sensitivity in uncommon mutations should be performed on individual mutations or in appropriately selected subgroups so that sensitivity to certain mutations is not masked by resistance in others. Patients with primary resistance, such as those with baseline T790M mutations or exon 20 insertions, remain a therapeutic challenge. As the number of identified mutations continues to grow, the development of comprehensive, dynamic, data-driven tools to support clinical decision making will become increasingly useful. *EGFR* mutation subtype has important prognostic and predictive implications and should inform both future drug development and interpretation of clinical trial outcomes.

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