EGFR: The Paradigm of an Oncogene-Driven Lung Cancer

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Abstract

Somatic, activating mutations in Epidermal Growth Factor Receptor (*EGFR*) identify a significant minority of patients with non-small cell lung cancer (NSCLC). While these mutations are associated with an ~70% response rate to some EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, and afatinib), patients develop resistance (i.e. "acquired resistance") after a median of 9–12 months. In patients with clinical acquired resistance, repeat biopsy of tumors has identified a number of relevant mechanisms of resistance, but by far the most frequent event is the acquisition of EGFR T790M, a mutation in the "gatekeeper" residue that confers resistance to gefitinib, erlotinib, and afatinib. This emphasizes the critical dependence upon EGFR signaling for some tumors, a property that has been exploited therapeutically. Dual EGFR blockade using afatinib and cetuximab led to a 29% radiographic response rate. More recently, drugs which target EGFR T790M (e.g. rociletinib, AZD9291, and others) have entered clinical trials, with impressive results observed in phase 1 clinical trials. The development of these newer drugs, with efficacy after resistance to first line EGFR TKI, has led to exploration of these strategies in multiple disease settings: at resistance, in the first line, and adjuvant treatment of those with completely resected early stage disease who would otherwise die of recurrent/metastatic disease. This example of translational research that identifies mechanisms of resistance to first generation drugs, and then targets those mechanisms yielding clinical benefit is a paradigm for how targeted therapies can be developed.

Introduction

Drugs targeting the epidermal growth factor receptor (EGFR) began initial development in the late 1990s and were hypothesized to be effective since a variety of epithelial malignancies, including non-small cell lung cancer (especially squamous cell lung cancer (1)) overexpressed EGFR protein. The earliest molecules to reach the clinic were cetuximab, gefitinib, and erlotinib. While the anti-EGFR antibody cetuximab did not show significant clinical activity (as a single-agent or in combination with chemotherapy), in large clinical trials both gefitinib and erlotinib had single-agent activity, with response rates <10% (2–5).

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This modest single agent activity led to initial regulatory approvals for both erlotinib and gefitinib in patients with previously treated advanced non-small cell lung cancer (Table 1). The US FDA approval for erlotinib was based on improvement in overall survival, compared to placebo, for an unselected group of patients with previously treated lung cancer (5). Gefitinib received an accelerated approval based upon response rate in single-arm trials of pretreated patients (2, 3), contingent upon the results of subsequent randomized trials. After a randomized trial comparing gefitinib and best supportive care to best supportive care alone in patients with previously treated lung cancer (analgous to (5) with erlotinib) failed to show an improvement in overall survival (6), in 2005, the US label for gefitinib was changed to effectively withdraw its approval.

Despite the low frequency of overall response rates, these clinical trials provided an opportunity to observe dramatic radiographic and clinical responses in a small proportion of patients treated with erlotinib or gefitinib. Initial trials noted higher response rates for patients from Asia, those who were never smokers, and those patients with adenocarcinoma histology (2, 7). Molecular analysis of tumors from patients with radiographic responses led to the identification of somatic activating mutations in the *EGFR* gene that were present more frequently in patients with response to erlotinib or gefitinib (8–10). These seminal papers identified the activating characteristics of these mutations and their association with response to erlotinib and gefitinib.

While the data regarding *EGFR* mutations and their association with response was clear, *EGFR* mutations occurred in just 10–20% of patients and in the early development of EGFR TKIs, some investigators explored the role of other predictive biomarkers, including *EGFR* copy number (not frank amplification but rather increased copy number). The IPASS trial was the single trial which best clarified the predictive nature of *EGFR* mutations (11). This trial randomized patients with clinical factors predictive of response to EGFR TKI (East Asian patients, never smokers, adenocarcinoma) to either gefitinib or paclitaxel and carboplatin. In the ensuing biomarker analysis, despite analyzing EGFR IHC, *EGFR* copy number, and clinical factors, the best predictor of response was *EGFR* mutations and any predictive effect of IHC or *EGFR* copy number was driven by their association with *EGFR* mutation. This led to the European approval of gefitinib as first-line treatment of *EGFR* mutant lung adenocarcinoma. Arguments have been made that a similar approval would be appropriate in the United States as well (12).

Subsequent to IPASS, multiple randomized phase 3 trials explored the use of erlotinib, gefitinib, and aftatinib (an irreversible kinase inhibitor that blocks both EGFR and HER2) as first line treatment, in comparison with conventional platinum-based chemotherapy doublets (13–16). In each of these trials, which prospectively enrolled only patients with *EGFR* mutant lung cancer, the EGFR TKI improved the progression-free survival as compared to chemotherapy. Taken together these trials made clear that *EGFR* mutations were the predictor of choice and that EGFR TKI were the standard of care first line treatment.

The initial translational work to identify somatic activating *EGFR* mutations and their association with responsiveness to EGFR TKI, followed by rigorous randomized clinical trials have established that *EGFR* mutant lung cancer is a distinct clinical entity. These

discoveries have led to a new paradigm in how we understand lung cancer, how we identify new drug targets, and how we treat all types of lung cancer.

Differences Among EGFR Mutations

With the initial discovery of *EGFR* mutations, the frequency of individual sensitizing *EGFR* mutations (exon 19 deletion L858R, G719X, etc.) was typically described but no additional significance was related to these individual genotypes. The two most common *EGFR* mutations, *EGFR* exon 19 deletion and L858R, typically represent the vast majority of patients identified. However, individual uncommon mutations, including G719, L861, and rarer mutations, are identified. The clinical relevance of these mutations is not clear and, many randomized trials have excluded such patients. More recently, larger retrospective series (17, 18) have been reported which suggest that many of these mutations are associated with good response rates to EGFR TKI (~50%) and there is a clear need for online resources that allow collation of results for uncommon mutations (such as My Cancer Genome [19]).

While some preliminary data with erlotinib and gefitinib (20, 21) suggested that the two most common *EGFR* genotypes (*EGFR* exon 19 deletion and *EGFR* L858R) may predict different outcomes, the dramatic sensitivities of all EGFR mutations to EGFR TKI were the focus. More recently, additional data have explored the difference between *EGFR* exon 19 deletion and *EGFR* L858R, the two most common EGFR mutation genotypes. In a combined analysis of randomized afatinib versus chemotherapy clinical trials (Lux Lung 3 and 6), investigators found that patients with *EGFR* exon 19 deletion randomized to initial chemotherapy had shorter overall survival compared to those patients allocated to afatinib (22). In contrast, patients with *EGFR* L858R who had been assigned to chemotherapy had a similar overall survival to those patients with initially assigned to afatinib, suggesting that patients with *EGFR* L858R had less of a benefit with afatinib than those with exon 19 deletion. More recently, circulating tumor DNA data from the EURTAC trial (a randomized trial of erlotinib vs chemotherapy) demonstrated significant difference in outcome based upon *EGFR* genotype, with a poorer outcome for patients with *EGFR* L858R (23). These data have re-emphasized the notion that there may be a differential effect of EGFR tyrosine kinase inhibitors for the two most common genotypes of *EGFR* mutation. While initial data supported this distinction based on findings with erlotinib and gefitinib, these new data extend these findings to afatinib.

Mechanisms of Acquired Resistance to EGFR Tyrosine Kinase Inhibitors

After initial response to EGFR tyrosine kinase inhibitors, patients typically develop progression of disease after 9–12 months. Understanding how resistance develops in such patients remains a key question. Multiple pre-clinical and clinical approaches have been used to understand mechanisms of resistance to tyrosine kinase inhibitors with a broad list of pathways implicated (Table 2). Initial focused sequencing analysis of biopsy specimens from patients with acquired resistance looking for secondary mutations (built upon the identification of gatekeeper mutations in the BCR-ABL fusion oncogene in patients who had become resistant to imatinib) led to the identification of EGFR T790M as a secondary mutation in EGFR that was associated with acquired resistance (24, 25). In the laboratory,

investigators have developed cell lines and in vivo tumor xenografts that that are resistant to EGFR TKIs. Analysis of such cell lines has helped to identify such findings as *MET* amplification (26, 27), AXL overexpression (28), and epithelial to mesenchymal transition (29). Similarly, analysis of genetically engineered mouse models of *EGFR* mutant cancers (mice with inducible expression of various mutant *EGFR*s that develop lung cancers which mimic the clinical responsiveness of human tumors to EGFR TKI) has been used, with resulting observations including the upregulation of the gene for PDL-1 (30). These data implicate development of an immunosuppressive environment in tumors with resistance to EGFR TKI. Immunotherapies currently in development may play a role in the treatment of EGFR mutant lung cancers (31).

In multiple biopsy series with analysis of a number of the previously reported mechanisms of resistance for the frequency of these events (though there has been little comprehensive analysis of samples for all reported mechanisms of resistance), it has become apparent that the most frequently identified mechanism of acquired resistance is the secondary mutation in EGFR T790M, occurring in >60% of tumors (32–34). This secondary mutation in EGFR is thought to alter kinase ATP affinity above that of gefitinib or erlotinib (35). The secondary mutations in EGFR emphasize the continued dependence upon EGFR signaling which is required for patients with *EGFR* mutant lung cancer. Without a complete understanding of mechanisms of acquired resistance to EGFR TKI and prior to development of drugs that target EGFR T790M, investigators explored a broad array of single-agent and combination therapies in the setting of acquired resistance to erlotinib, gefitinib, and afatinib with generally dismal results (reviewed in Yu et al (36)).

While understanding the molecular mechanisms of acquired resistance to EGFR TKIs has been important in developing new therapies, some clinical resistance occurs in individual sites (such as isolated central nervous system [CNS] lesions as well as visceral sites). A variety of approaches have been developed to deal with resistance to EGFR TKIs clinically, prior to development of new therapies. Primarily, these strategies revolve around the use of local therapies. Two groups have reported interesting retrospective series which suggest that local therapies (radiation or surgery) for CNS progression or extra-CNS progression may delay the time until an additional change in therapy is required (37, 38).

Targeting Acquired Resistance to Erlotinib and Gefitinib with Dual Blockade of EGFR

The high frequency of EGFR T790M mutation in the acquired resistance setting (and other, less common secondary mutations such as T854A and D761Y), emphasized that continued signaling through EGFR was critical to survival of *EGFR* mutant lung cancer cells (34, 39, 40). This observation led to the hypothesis that dual EGFR blockade using an EGFR tyrosine kinase coupled with an antibody to EGFR (i.e. cetuximab) could sufficiently dampen EGFR signaling (even in the context of EGFR T790M) thus leading to cancer cell apoptosis (41). While an initial trial of erlotinib combined with cetuximab showed only modest activity and no RECIST-defined partial responses, there was significantly greater activity with the combination of afatinib and cetuximab (42, 43). After an initial dose escalation which defined the tolerability of full doses of afatinib and cetuximab in

combination, a broader study of efficacy was reported (42). In this study of both patients with and without EGFR T790M, but with clinically proven resistance to erlotinib or gefitinib, there was a response rate of 29% and a median progression-free survival of 5 months. The response rate understates the evidence of tumor shrinkage in the majority of patients treated with this combination therapy. Enthusiasm for the efficacy of this combination has led to a clinical trial that compares the efficacy and tolerability of the combination of afatinib and cetuximab to afatinib alone as initial therapy for *EGFR* mutant lung cancers.

EGFR T790M-Directed Therapies

As the primary mechanism of acquired resistance, EGFR T790M was a clear target for drug development to address this important medical need. Moreover, a drug which preferentially targeted mutant EGFR (T790M along with the activating mutations) would likely reduce WT EGFR related adverse events often observed (including rash and diarrhea). There was a sea change in drug development for patients with acquired resistance to first generation EGFR TKI with the first described compound directed at EGFR T790M, WZ4002 (44). This drug, identified by screening an irreversible kinase inhibitor library for drugs that bound EGFR T790M, was 100 fold less potent against WT EGFR and 30–100 fold more potent against EGFR T790M. This compound binds irreversibly to mutant *EGFR* at the C797 residue. While this compound was not taken forward into clinical development, multiple other drugs with similar characteristics have begun early phase clinical trials (see Table 1). The first reported clinical data came with rociletinib (CO-1686, Clovis Oncology) (45) with clear evidence of single-agent clinical activity in patients with acquired resistance to erlotinib or gefitinib (46). Subsequently, AZD9291 (AstraZeneca) (47) entered phase 1 clinical trials in patients with *EGFR* mutant lung cancer and acquired resistance to erlotinib, gefitinib, or afatinib (48). Both of these drugs have been explored in single-arm studies with relatively large numbers of patients. A number of other compounds have more recently entered clinical trials with early clinical data anticipated in 2015 (Table 1).

AZD9291 and rociletinib, the EGFR T790M-directed drugs with the most data, have reported similar high response rates > 50%, but they appear to have unique patterns of adverse events which may distinguish some members of this class of drugs. Rociletinib has an 22% rate of observed grade 3 hyperglycemia and grade 3 QTc prolongation has been reported in 7%, but the worst rash reported was grade 1 (46). AZD9291 has been reported to have grade 3 rash and diarrhea in 2–5% (48). In early reports, there were no grade 3 events of QTc prolongation or hyperglycemia with AZD9291. These differences in adverse events may allow for preferential combination therapies with other classes of drugs.

While AZD9291 and rociletinib have shown impressive response rates as single-agent kinase inhibitors, it is likely that clinically meaningful drug resistance will occur for this class of drugs after a relatively short time, emphasizing the importance of beginning to understand mechanisms of resistance to these drugs. Potential mechanisms of resistance to EGFR T790M-specific kinase inhibitors have been identified in pre-clinical work. In the initial presentation of the WZ4002 compound, Zhou and colleagues identified the C797 binding site as a potential source of resistance and went on to show that a C797S mutation

would lead to a 100 fold increase in IC50 for cell lines with this mutation (44). Similarly, development of a cell line resistant to WZ4002 implicated amplification of the *MAPK1* gene and upregulation of MAPK signaling as mechanisms of acquired resistance to EGFR TKI treatment (49). Importantly, treatment with a MEK inhibitor restored EGFR-TKI sensitivity in their models. Combining the EGFR T790M specific drug with a MEK inhibitor significantly delayed the emergence of resistance *in vitro*, suggesting one of many rational combinations that will be explored as development of T790M specific drugs moves forward. In addition, in a cell line that was developed to be resistant to rociletinib, elevated levels of phosphorylated AKT were observed (50). Moreover, addition of an AKT inhibitor made the cell line sensitive to rociletinib. The impressive clinical activity observed with 3rd-generation, mutant specific EGFR TKI has led to a rapid move to compare these drugs to erlotinib, gefitinib, and afatinib, in the first line treatment of patients with *EGFR* mutant NSCLC.

Can EGFR TKIs Be Used to Cure Some *EGFR* Mutant Lung Cancers?

Since the identification of *EGFR* mutations and their association with response to EGFR TKI in 2004, there have been intermittent calls for a targeted trial of EGFR TKI in the adjuvant setting for patients with early stage *EGFR* mutant lung cancer to build upon the palliative results see in the advanced stage setting. While retrospective data support the use of EGFR TKI in the adjuvant setting, with likely improved disease-free and overall survival, only modest prospective randomized data exist (51). During the same time period, investigators have demonstrated that imatinib prolongs overall survival when given as adjuvant therapy for patients with resected gastrointestinal stromal tumors (GIST) (52). GIST investigators have gone on to show that 3 years of adjuvant imatinib is better than 1 year of adjuvant imatinib (53). With an annual incidence of 4-6000 cases per year in the US, these trials were completed in relatively short order.

Three clinical trials have been reported where the prospective study of adjuvant EGFR TKI was explored in patients with *EGFR* mutant lung cancer, all with significant methodological limitations. The BR.19 trial was a placebo-controlled trial of adjuvant gefitinib for patients with completely resected lung cancers that was stopped early after a trial of gefitinib in unselected patients with advanced lung cancer failed to improve overall survival (54). While an unselected patient population was included in this trial, EGFR mutation testing was done on available specimens and just 15 patients had *EGFR* mutant lung cancer, prohibiting meaningful analysis of the results. Similarly, the RADIANT study explored the value of adjuvant erlotinib in patients with completely resected lung cancer who had over-expression or increased gene copy number of EGFR (55). Subset analysis of the small proportion of patients with *EGFR* mutant lung cancer showed a disease-free survival benefit (that, due to hierarchical testing, was not deemed statistically significant), but was underpowered to detect a survival advantage. Finally, a randomized trial of adjuvant gefitinib after all patients with stage IIIA *EGFR* mutant lung cancer were treated with pemetrexed and carboplatin showed improved disease-free survival, and a trend toward improved OS, but, with just 60 patients, the study was underpowered to show statistically significant overall survival differences (56). Fortunately, there are multiple ongoing randomized studies that have enrolled patients with EGFR mutant lung cancer to treatment with erlotinib or gefitinib

(Table 3). In North America, the most prominent trial is the NCI-sponsored ALCHEMIST EGFR trial that seeks to enroll 410 people with resected, Stage IB–III, *EGFR* mutant lung cancers previously treated with standard chemotherapy to either erlotinib or placebo (57). The investigators are seeking to show a Hazard Ratio of 0.67 for overall survival, its primary endpoint. Similar trials are enrolling patients in Asia.

While there are ongoing trials of erlotinib or gefitinib in the adjuvant setting and retrospective data suggest that this strategy will improve overall survival, there are questions about whether these studies use a duration of therapy which is likely to show benefit and whether the optimal drug is being used. As described above, in GIST, there is evidence that 3 years of imatinib is better than 1 year. Similarly, in women with estrogen receptor expressing breast cancers, the use of adjuvant hormonal therapy is now recommended for 10 years for some patients. The early impressive clinical data for patients with the EGFR inhibitors which target EGFR T790M (i.e. rociletinib and AZD9291) in conjunction with a suggestion of better tolerability (lower rates of grade 3 rash, grade 3 diarrhea), would seem to make them better candidates for testing in the adjuvant setting. With these caveats, it is likely that optimization of duration of therapy and identification of the right drug can be accomplished and that the use of EGFR TKI may cure some patients with early stage resected *EGFR* mutant lung cancers who might otherwise die from recurrent or metastatic disease.

Acknowledgments

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Table 1

Representative EGFR TKIs currently in use or development

First generation (target WT EGFR)	Second generation (irreversible inhibitors of EGFR and HER2)	Third generation (EGFR mutant-specific, irreversible inhibitors)
Erlotinib[*]	Neratinib	Rociletinib (Clovis)
Gefitinib	Afatinib[*]	AZD9291 (AstraZeneca)
Icotinib	Dacomitinib	HM61713 (Hanmi) EGF816 (Novartis) ASP8273 (Astellas)

[*] FDA-approved in the US for treatment of lung cancer

Table 2

A partial list of mechanisms implicated in acquired resistance to EGFR TKI

Secondary Mutations	Activation of alternate pathways	Other pathways
EGFR T790M (24, 25)	*HER2* amplification (58)	Activation of PD-1 (30)
EGFR D761Y (39)	*MET* amplification (26, 27)	Epithelial to mesenchymal transition (29, 33, 59)
EGFR T854A (40)	mTORC1 (60)	Small Cell Transformation (61, 62)
BRAF V600E (63)	AXL (64)	
NRAS mutation (65)	IGF1-R (66, 67)	
	FGFR activation (68, 69)	

Table 3

Ongoing adjuvant EGFR TKI trials

Trial	EGFR TKI	Comparison Arm	Population
NCT0140214	erlotinib x 2 years	cisplatin/vinorelbine for 4 cycles	Stage III, EGFR mutations in exon 19 or 21
NCT01405079	gefitinib x 2 years	cisplatin/vinorelbine for 4 cycles	Stage II–III, EGFR exon 19 deletion or L858R
NCT01683175	erlotinib x 2 years	cisplatin/vinorelbine for 4 cycles	Stage III, EGFR exon 19 deletion or L858R
NCT02125240	icotinib x 2 years	placebo	Stage II–III, EGFR exon 19 deletion or L858R, treated with 4 cycles of platinum-based chemotherapy
NCT01746251	afatinib x 2 years	afatinib x 3 months	Stage I–III, EGFR mutation, prior chemotherapy allowed
NCT01929200	icotinib x 2 years	icotinib x 1 year	Stage II–III, EGFR mutation in exon 19 or 21
NCT01996098	chemotherapy followed by icotinib x 6 or 12 months	chemotherapy alone	Stage II–III, EGFR mutation in exon 19 or 21
NCT02194738	erlotinib x 2 years	placebo	Stage IB–III, EGFR mutation
NCT02264210	icotinib x 12 months	observation	Stage IB, EGFR mutation in exon 19 or 21
WJOG6410L	gefitinib x 2 years	cisplatin/vinorelbine	Stage II–III, EGFR exon 19 deletion or L858R