Strategies for Overcoming Acquired Resistance to Epidermal Growth Factor Receptor–Targeted Therapies in Lung Cancer

Geoffrey R. Oxnard, MD

Acquired resistance to targeted therapy in epidermal growth factor receptor (EGFR)–mutant lung cancer represents a valuable model for understanding strategies of overcoming different types of cellular resistance mechanisms. Using existing data on resistance in EGFR-mutant lung cancer, this review will discuss 3 basic approaches for overcoming resistance to EGFR-targeted therapies: intensification of EGFR inhibition, combination of EGFR inhibitors with other targeted therapies, and changing to anticancer therapies acting via alternate pathways.


Epidermal growth factor receptor (EGFR)–targeted therapy for non–small cell lung cancer represents a key model for understanding oncogene addiction and acquisition of resistance in oncology. Lung cancers harboring EGFR-activating mutations, with an incidence of more than 20,000 per year in the United States, are one of the most common cancers in a small group of oncogene-addicted neoplasms that now include chronic myelogenous leukemia, gastrointestinal stromal tumors, BRAF-mutant melanoma, and ALK-mutant lung cancer. Because of its relatively high prevalence, and because this is a uniquely genotype-defined disease (whereas morphology can be used to diagnose gastrointestinal stromal tumors and chronic myelogenous leukemia), EGFR-mutant lung cancer is a particularly valuable model for studying cellular mechanisms of resistance to targeted therapy.

Since acquired resistance to EGFR-targeted therapies was first described in 2005, several mechanisms of resistance to erlotinib and gefitinib have been described, motivating a variety of different therapeutic approaches aimed at overcoming resistance. The primary goal of this review will be to create a framework for understanding these different treatment strategies and the resistance mechanisms they target. Importantly, our understanding of acquired resistance to EGFR therapies in lung cancer is highly influenced by preclinical studies of a few well-described EGFR-mutant lung cancer cell lines. The challenge of obtaining clinical specimens from patients with acquired resistance has limited the types of questions that can be asked in vivo, though implementation of larger rebiopsy studies has created a new opportunity to improve our understanding of this condition.

A potentially helpful model for conceptualizing EGFR inhibition in EGFR-mutant lung cancer is that of hormone therapy for prostate cancer and for estrogen-dependent breast cancer. Androgen deprivation therapy for prostate cancer and estrogen deprivation therapy for breast cancer represent 2 of the earliest targeted therapies in solid tumor oncology. Both diseases have an initial stage wherein hormone therapy is commonly able to control the disease (Figure 1), either via through inhibition of endogenous hormone production (eg, gonadotropin-releasing hormone agonists like goserelin) or through inhibition of hormone receptor signaling (eg, tamoxifen and bicalutamide). After a period, these cancers both become insensitive to the effects of first-line hormone therapies. There then is a period during which further lines of hormonally acting therapies are used toward controlling the disease with minimal side effects. Eventually, chemotherapy is the only effective option (though chemical castration through gonadotropin-releasing hormone agonists is continued indefinitely in prostate cancer).

Such a treatment sequence has not yet been established in EGFR-mutant lung cancer, partly because EGFR mutation testing has only recently become a standard of care in the management of this disease. Still, we expect that a window will be found within which we will be able to treat these cancers with additional targeted therapies, though the lung cancers may eventually grow so anaplastic that cytotoxic therapy is the only effective option (Figure 1). Given how aggressively lung cancers can behave, part of the challenge for patients with acquired resistance to tyrosine kinase inhibitor (TKI) will be identifying where a given patient fits on this spectrum: that is, whether they deserve a specific type of targeted approach or whether they need immediate chemotherapy.

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OVERCOMING RESISTANCE THROUGH INTENSIFICATION OF EGFR INHIBITION

We know that lung cancer cells harboring EGFR mutations are highly addicted to this oncogene, such that blocking EGFR signaling leads to rapid and durable responses.\(^5\) Given that these cells’ growth and survival signals are so dependent upon EGFR activation, it makes sense that the cells would develop resistance mechanisms that reactivate EGFR despite the presence of an inhibitor. This is precisely what occurs when cells acquire a second-site mutation in the EGFR gene, the T790M mutation (Figure 2).

The T790M mutation was first reported as a secondary mutation occurring with L858R in a previously untreated patient.\(^6\) The biologic nature of T790M was made clear when it was identified as an acquired mutation in 3 of 6 lung cancers that had developed resistance to EGFR TKI after initial sensitivity.\(^1\) Aiming to decipher the role of T790M in cellular signaling, Godin-Heymann et al\(^7\) exposed EGFR-mutant lung cancer cell lines to a mutagen and cultured them in the presence of an EGFR TKI. In one group of resistant clones, resistance occurred despite effective suppression of EGFR phosphorylation; these cells must have developed a mechanism of survival that was independent of direct EGFR signaling (discussed more below). In the other group of clones, resistance was associated with persistent phosphorylation of EGFR; these clones harbored the T790M second-site mutation, whereas the first group did not. It has separately been found that T790M facilitates phosphorylation of EGFR despite the presence of a TKI through increasing the affinity of mutant EGFR for adenosine triphosphate.\(^8\)

Given this role of persistent EGFR signaling in causing resistance to TKI, many trials have studied intensification of EGFR inhibition through use of second-generation TKIs such as neratinib, afatinib, and dacomitinib.\(^2\) These inhibitors are different from erlotinib and gefitinib in 2 main ways: each forms a covalent, irreversible bond with the EGFR protein, and each also inhibits other members of the ERBB family of kinases. Although this broader activity leads to greater toxicity, it was hoped that it would also provide these drugs the power to overcome T790M-mediated acquired resistance to EGFR TKI. This was shown nicely in several preclinical studies;\(^2\) however, clinical studies have been disappointing: the phase III trial of afatinib versus placebo in patients with acquired resistance to EGFR TKI demonstrated a 2-month improvement in progression-free survival but no improvement in survival.\(^9\) Ongoing research is looking at higher doses of afatinib given intermittently in order to improve pharmacokinetics without increasing

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**Table 1.** Sequencing of targeted therapies. As with prostate cancer and breast cancer, it is likely that chemotherapy will eventually be needed if a cancer reaches a stage where its biology is no longer dependent upon the initial targeted oncogene. Abbreviations: EGFR, epidermal growth factor receptor; GNRH, gonadotropin-releasing hormone; TKI, tyrosine kinase inhibitor.

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**Figure 2.** Three distinct strategies toward overcoming acquired resistance. Intensified epidermal growth factor receptor (EGFR) inhibition (left) makes most sense when EGFR signaling is maintained. Adding other targeted therapies to EGFR inhibition (center) is needed when a parallel pathway is activated. In other circumstances the cancer may develop a growth mechanism entirely agnostic to EGFR (right) such that a different type of antineoplastic therapy is needed. Abbreviation: TKI, tyrosine kinase inhibitor.
toxicity. Additionally, a third generation of EGFR TKIs is now entering clinical trials; these compounds bind covalently to the adenosine triphosphate–binding cleft of mutant EGFR and appear to have selective activity against the T790M mutant. 10

A more recent strategy for intensification of EGFR inhibition has been the addition of monoclonal antibodies targeting EGFR, such as cetuximab, to EGFR TKIs. An initial trial of erlotinib plus cetuximab in patients with acquired resistance to erlotinib resulted in no objective responses, though some cases of meaningful tumor shrinkage were seen (Janjigian et al, CCR, 2011. PMID: 21248303). However, when cetuximab was added to afatinib in a more recent phase I/I trial, an impressive response rate of 36% was seen; responses were seen both in cancers with acquired T790M and in cancers without detectable T790M. 11 This combination of EGFR inhibitors, as might be expected, led to frequent skin toxicities. The mechanism by which a monoclonal antibody augments the activity of an irreversible TKI remains unclear, yet this type of combination may be a model worth studying in other cancers in which acquired resistance to a targeted therapy is a prevalent clinical problem.

OVERCOMING RESISTANCE THROUGH COMBINATION OF EGFR INHIBITORS WITH OTHER TARGETED THERAPIES

As described above, some lung cancers that acquire resistance to EGFR TKI are able to maintain growth despite adequate inhibition of EGFR phosphorylation by the TKI, and there are a number of possible explanations for this finding. One logical explanation is the acquisition of an oncogenic mutation in a protein downstream from EGFR leading to constitutive signaling independent of EGFR phosphorylation (Figure 2). This has been described in BRAF-mutant melanoma, where activating mutations of MEK have been identified in specimens with acquired resistance to vemurafenib. In lung cancer, it would make intuitive sense that EGFR-mutant cancers that acquired resistance to TKI might acquire KRAS mutations given the high prevalence of these mutations in non–small cell lung cancer; however, acquired KRAS mutations do not appear to be a resistance mechanism in these tumors.

One downstream mutation that has been described in lung cancers with acquired resistance to TKI is in PIK3CA, a gene encoding a protein in the PI3 kinase family. 12 PIK3CA mutations can be found in many different cancer types and have previously been described to lead to the development of lung adenocarcinoma when expressed in genetically engineered mouse models. 13 Sequist et al 12 performed multiplexed genotyping of specimens from 37 EGFR-mutant lung cancers with acquired resistance to erlotinib and found PIK3CA mutations in 2 cases (5%). It must be noted that when PIK3CA mutations are found in untreated lung adenocarcinoma they commonly occur with a concurrent driver mutation in EGFR, KRAS, or BRAF, consistent with the idea that these may be secondary resistance mutations (de novo or acquired) rather than independent driver mutations. 14 Several trials are studying the combination of EGFR TKIs and PI3 kinase inhibitors, though dosing these agents together can be difficult because of overlapping toxicities.

Another cellular mechanism that can lead to resistance in the setting of effective EGFR inhibition is the activation of a parallel signaling pathway, causing growth and survival by using the signaling cascade that previously was dependent upon mutant EGFR alone. This is believed to be how MET signaling is able to lead to acquired resistance to TKI in some lung cancers. MET is a receptor tyrosine kinase like EGFR and has been targeted as an oncogene in lung cancer for many years. The role of MET in acquired resistance was initially identified by 2 groups that screened for copy number alterations in lung cancer cells with acquired resistance, 15,16 both finding focal amplification of the region of chromosome 7 that harbors the MET gene. Using a variety of methods for assessing amplification, it was estimated that 20% of cases studied exhibited MET dependence explaining their resistance. MET was found to reactivate downstream activation of AKT through ERBB3 phosphorylation.

In the clinic, acquired resistance via MET amplification appears to occur in a smaller proportion of patients, with 4% of cases from 2 recent rebiopsy studies showing isolated MET amplification by fluorescence in situ hybridization. 2 The prevalence of MET-dependent resistance may depend upon the assay used; it could potentially be found more commonly using a broader assessment of overexpression of the MET kinase or of the MET ligand hepatocyte growth factor. The lack of a sensitive assay for identifying MET dependence in lung cancers with acquired resistance is an ongoing impediment to the clinical development of MET-targeted treatment strategies. Several clinical trials are currently underway combining EGFR TKI and MET inhibition, but these are studying unselected patients with acquired resistance to EGFR TKI. 2

A second receptor tyrosine kinase that is known to share downstream signaling targets with EGFR is the insulin-like growth factor 1 receptor. Several groups have previously published literature suggesting synergy between these 2 signaling pathways, leading to efforts to concurrently inhibit EGFR and insulin-like growth factor 1 receptor. In a study of EGFR-mutant lung cancer cell lines, there was found to be increased cell death when cells with baseline resistance to TKI received an insulin-like growth factor 1 receptor inhibitor along with gefitinib. 17 Specific findings of insulin-like growth factor 1 receptor–mediated resistance have not been well described in patient specimens, however, so the clinical relevance of this pathway remains unclear.

OVERCOMING RESISTANCE USING ANTICANCER THERAPIES ACTING VIA ALTERNATE PATHWAYS

The above sections describe EGFR-mutant cancers that, under the therapeutic stress of TKI therapy, find a way to restore the cascade of survival signaling downstream of EGFR. It also may be possible for a cancer cell to develop a growth mechanism that is entirely agnostic to EGFR pathway signaling (Figure 2). Such a resistance mechanism might parallel the point in the management of estrogen receptor–positive breast cancer where, after repeated rounds of hormone therapy, further inhibition of that pathway gains the patient no more benefit and chemotherapy must be started. As discussed previously, a similar point may occur in the management of patients with EGFR-mutant lung cancer, where inhibition of the EGFR signaling pathway will eventually achieve no further benefit and an anticancer therapy with a completely different action (such as chemotherapy) is needed.

One well-described biological subtype of acquired resistance where this approach makes much sense is when
EGFR-mutant adenocarcinoma transforms to small cell carcinoma morphology. Small cell morphology can be seen on rebiopsies in as many as 14% of cases in one series\(^1\) and as few as 3% in another larger series.\(^2\) Importantly, these small cell carcinomas continue to harbor the original EGFR-sensitizing mutation, but they have not been found to carry the T790M second-site mutation. Some of these cases have demonstrated aggressive behavior similar to that of small cell lung cancer, and one series found that 3 of 4 such patients treated with platinum and etoposide (the standard chemotherapy for small cell lung cancer) had a good response.\(^1\) Other approaches targeting epithelial-mesenchymal transformation may also make sense in this population of cancers.

Unfortunately, the effectiveness of chemotherapy in patients with acquired resistance to erlotinib is unclear. At initial presentation with advanced disease, EGFR-mutant lung cancer has been found to have a relatively high response rate to chemotherapy as compared with wild-type non–small cell lung cancer. But the activity of chemotherapy has not yet been prospectively studied in patients with acquired resistance. One retrospective series studied 41 patients with EGFR-mutant lung cancer who received chemotherapy after progressing on gefitinib: A Response Evaluation Criteria in Solid Tumors response was seen in 6 of 41 cases (15%) and progression-free survival was not assessed.\(^1\) Other novel treatment strategies being investigated in EGFR-mutant lung cancer with acquired resistance include inhibition of chaperone proteins (HSP90 inhibitors) and inhibition of the nuclear factor κB pathway\(^19,20\), such strategies would work outside the EGFR signaling pathway, exploiting other vulnerabilities of these cancer cells.

**THE HETEROGENEITY OF RESISTANCE**

One challenge to the task of selecting appropriate targeted therapies for patients with acquired resistance to EGFR TKI is the phenomenon of tumor heterogeneity. This seems to be a more pressing problem in cancers that have developed resistance to a targeted therapy than in cancers at diagnosis. In a landmark analysis of baseline tumor heterogeneity, Yatabe et al\(^21\) performed transsectional analysis of 55 early-stage resected tumors, separately genotyping different pieces of the tumor, and found that EGFR mutations were homogenously distributed throughout untreated tumors without any EGFR wild-type subpopulations. In fact, this is not surprising given that studies of acquired resistance specimens have found that EGFR mutations universally persist in the posttreatment specimens; if there were wild-type cells constituting a portion of the initial cancer, it would make sense that these cells would constitute the bulk of a resistant tumor given their lack of sensitivity to TKI.

Although cancers at diagnosis have reached a steady state with a relatively homogenous population of cells, acquired resistance is inherently a dynamic state, cultivated under the stress of a specific targeted therapy. This likely explains the finding that some cancers can first acquire the T790M resistance mutation after progressing on TKI, but this mutation may no longer be detectable after a period of treatment without TKI.\(^13\) This is thought to be due to an inefficient growth induced by the T790M mutation, such that the double mutant is not the preferred state except in the presence of TKI.\(^2\)

Such dynamic tumor heterogeneity makes it difficult to plan the proper therapy for a patient with acquired resistance. Even in a tumor that shows transformation to small cell carcinoma, there potentially remains a subpopulation of cells that are still sensitive to TKI. Switching completely to chemotherapy might allow these TKI-sensitive cells to regrow, likely causing the severe “flare” that has been described in 23% of patients a median of 8 days after stopping TKI.\(^23\) For this reason, some clinicians prefer to combine TKI with chemotherapy to simultaneously treat TKI-resistant and TKI-sensitive cells. This strategy is feasible given that chemotherapy plus TKI has been found to be only minimally more toxic than chemotherapy alone.\(^24\) Whether this strategy truly leads to better clinical outcomes requires further study.

**CONCLUSIONS**

This review has described 3 basic approaches being used toward the treatment of lung cancers with acquired resistance to EGFR TKI. Although there is tremendous enthusiasm toward leveraging our understanding of cancer biology into new treatments for this condition, it remains disappointing that the only approved therapy at this time is chemotherapy. Successful development of targeted therapies for these patients will require more than just new drugs; it will necessitate new techniques for tissue acquisition and comprehensive genomic analysis.

**References**


