TOPIC HIGHLIGHT

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Management of tyrosine kinase inhibitor resistance in lung cancer with EGFR mutation

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Abstract

The identification of driver mutations and drugs that inhibit their activity has been a major therapeutic advance for patients with advanced lung adenocarcinoma. Unfortunately, the success of these drugs is limited by the universal development of resistance. Treatment failure can result from inadequate drug exposure or selection of resistant malignant clones. Clinically distinct mechanisms of disease progression have been identified and can inform treatment decisions. Investigations into the biochemical mechanisms of tyrosine kinase inhibitor resistance may provide additional therapeutic targets by which the efficacy of targeted therapy can be improved.

Key words: Epidermal growth factor receptor mutation; Tyrosine kinase inhibitor; Lung cancer; Adenocarcinoma; Resistance; Targeted therapy

Core tip: The causes of epidermal growth factor receptor tyrosine kinase inhibitor (TKI) treatment failure including pharmacokinetic failure, intrinsic resistance and acquired resistance are discussed. We review the molecular mechanisms of resistance and the options for clinical management of disease progression. Promising investigational strategies for overcoming TKI resistance are highlighted.

INTRODUCTION

In the treatment of lung adenocarcinoma, drugs that inhibit unique driver mutations have proven superior to conventional chemotherapy in molecularly-defined subgroups, altering treatment paradigms and research agendas. The observation that dramatic responses to erlotinib or gefitinib occurred in patients with epidermal growth factor receptor (EGFR) mutations affirmed the concept of "oncogene addiction[1]" in non-small cell lung cancer: despite the complexity of genetic and epigenetic changes in malignant cells, interfering with the activity of a single dominant oncogene can induce tumor regression. Translating this concept into clinical benefit required identification of the driver mutations to which the cancers are "addicted" and the development of drugs capable of selectively blocking their activity. Mutually exclusive driver mutations can be detected in approximately 60% of lung adenocarcinomas through multiplexed testing techniques[2]. Single agent anti-tumor activity has been reported with drugs that inhibit the kinase activity of EGFR, EML4-ALK, ROS1, HER-2, BRAF, RET and MET[3-9]. Moreover, targeted therapy with afatinib, gefitinib or erlotinib in EGFR-mutated lung cancer and crizotinib in lung cancer harboring EML4-ALK translocations have dem-

Table 1 Drugs that may lower serum levels of targeted therapies	
Erlotinib/gefitinib	**Afatinib**
Rifampin, rifabutin, rifapentine, phenytoin, phenobarbital, carbamazepine, St. John's wort, proton pump inhibitors, H2-blockers, antacids, tobacco	Rifampin, phenytoin, carbamazepine, St. John's wort, primidone, tipranavir

onstrated clinically significant improvements in response rates, progression-free survival (PFS) and quality of life when compared with standard chemotherapy[3,4,10-12]. In the LUX-Lung 3 trial, for example, afatinib produced longer PFS (11.1 mo *vs* 6.9 mo) and higher response rates (56% *vs* 23%) compared with pemetrexed and cisplatin in the first-line treatment of patients with EGFR mutations[3]. Based on these results, practice guidelines recommend targeted therapy as first-line treatment for lung cancers with EGFR mutations or ALK translocations[13]. Unfortunately, this therapeutic success is invariably temporary as all patients ultimately develop resistance to currently available targeted therapies. The goals of this review are therefore to (1) examine the mechanisms of failure of tyrosine kinase inhibitors (TKIs); and (2) discuss the strategies for preventing or overcoming resistance that are currently in development. We will focus on resistance to targeted therapy in lung cancers harboring EGFR mutations where these concepts are best characterized, though some concepts may be applicable to targeted therapy in general.

DIFFERENTIATING DRUG RESISTANCE FROM PHARMACOKINETIC FAILURE

The first step in identifying the mechanism of treatment failure is to differentiate pharmacokinetic failure from true drug resistance. Pharmacokinetic failure refers to disease progression due to inadequate drug exposure. True drug resistance occurs when malignant cells survive and divide in the presence of therapeutic drug levels and can be further characterized as intrinsic or acquired. In cases of pharmacokinetic failure, interventions to achieve therapeutic drug levels may effectively halt or prevent disease progression.

Interactions
Gefitinib and erlotinib are metabolized by the cytochrome p450 system and therefore have the potential for numerous interactions (Table 1). Concurrent medications and homeopathic remedies that induce p450 enzymes may lower systemic drug levels of these targeted therapies. The clinical significance of such interactions is demonstrated in the published case of a patient with advanced EGFR-mutated lung cancer that did not respond to initial treatment with erlotinib while concurrent medications included fenofibrate, a CYP3A4 inducer[14]. Serum trough levels of erlotinib were sub-therapeutic and disease regression was achieved after dose escalation re-

sulting in therapeutic drug concentrations. Furthermore, current smokers have increased clearance of erlotinib, likely due to induction of CYP1A2 and CYP1A1[15] and similar interactions are possible with gefitinib based on pharmacokinetic studies[16]. In a study to determine the maximum tolerated dose of erlotinib in patients currently smoking at least 10 cigarettes daily, the trough plasma concentration and toxicity profile at 300 mg daily was similar to the standard dose of 150 mg in non-smokers[15]. In patients taking erlotinib who cannot achieve smoking cessation, dose escalation to 300 mg daily as tolerated is recommended[17]. Afatinib undergoes minimal metabolism by the cytochrome P450 system but is a substrate of p-glycoprotein. P-glycoprotein inducers may therefore lower systemic drug concentrations of afatinib[18]. As oral drugs, gastric contents and pH may also impact bioavailability. Afatinib absorption is reduced when taken with a high fat meal whereas erlotinib absorption is increased and patients are directed to take both medications on an empty stomach. Drugs that increase gastric pH can reduce absorption of erlotinib and gefitinib, and have been shown to lower drug levels[17,19]. When patients require antacid therapy, twice-daily histamine receptor blockers are recommended over proton pump inhibitors when possible and patients are advised to take erlotinib ten hours after the last dose and two hours prior to the next dose to minimize the effect on absorption[17].

Blood-brain barrier
Central nervous system (CNS) involvement in the form of brain or leptomeningeal metastases is common in patients with advanced non-small cell lung cancer, either at the time of diagnosis or as a site of disease progression. The blood-brain barrier restricts most large and hydrophilic substances from passing from the circulation into the CNS. The cerebrospinal fluid (CSF) to plasma concentration ratios for erlotinib and gefitinib have each been shown to be less than 0.01[20,21]. While confirming the reduced penetration of these drugs into the CNS, these measurements likely underestimate the exposure of brain metastases to each of these drugs due to local disruption of the blood-brain barrier in abnormal tumor vasculature. Despite CSF concentrations that would predict subtherapeutic drug exposure, radiographic responses have been observed in brain metastases treated with erlotinib, gefitinib and afatinib at standard doses[22,23]. Moreover, dose escalation of gefitinib or high-dose weekly erlotinib can increase drug levels in the CSF and reverse CNS disease progression that occurred during standard dosing. In one case report, a patient with an exon 19 mutation exhibited progressive brain and leptomeningeal metastases despite systemic disease control on gefitinib. The gefitinib concentration in the CSF was found to be less than that required to inhibit the growth of a cell line derived from the patient's tumor. After dose escalation to 1000 mg daily, the CSF concentration of gefitinib exceeded the half maximal inhibitory concentration and the patient experienced radiographic and symptomatic improvement

with clearing of malignant cells from the CSF[20]. Since sustained escalated doses of erlotinib are poorly tolerated, high-dose weekly administration was investigated as a strategy to improve erlotinib CNS penetration. In a retrospective series of nine patients with CNS progression while on standard dose erlotinib, the CNS response rate to 1500 mg once weekly was 67%[24].

DEFINING MECHANISMS OF DRUG RESISTANCE

True drug resistance, the survival and proliferation of malignant cells in the presence of therapeutic drug levels, is observed to varying degrees in all EGFR-mutated lung cancers. The complete response rates for currently available agents are less than 5% suggesting the presence of intrinsic resistance in a population of tumor cells of most patients at the time of treatment initiation. Furthermore, disease progression after initial response occurs due to emergence of acquired resistance with median PFS ranging from 9 to 13 mo in clinical trials. Given that some factors present at the time of diagnosis can predict both reduced probability of response and shorter duration of response, there is clearly overlap between the mechanisms of intrinsic and acquired resistance.

The response to EGFR-targeted therapy varies according to EGFR mutation
Both the probability and the duration of response to EGFR-targeted therapy vary according to the specific EGFR mutation. Activating EGFR mutations occur within exons 18-21 of the tyrosine kinase binding domain. Exon 19 deletions and L858R point mutations in exon 21 comprise 85%-90% of EGFR mutations and most reliably predict response to EGFR-targeted therapy[25]. For this reason, FDA approval for first-line therapy with erlotinib or afatinib is limited to cancers harboring these mutations. However, differential sensitivity within this group has been observed: patients harboring exon 19 deletions show longer progression free survival compared with patients with L858R mutations[26,27], despite similar *in vitro* activity[28]. The reasons for the differences in clinical activity observed are not clear.

The efficacy of EGFR TKIs in the treatment of uncommon EGFR mutations is less predictable, in part due to their relative rarity. This is a heterogeneous group that includes exon 20 mutations, exon 19 insertions, exon 21 missense mutations (other than L858R), and exon 18 point mutations[25]. The data on TKI efficacy in these cancers is limited to subset analyses of larger trials, small series and case reports. No partial responses were observed in patients with uncommon EGFR mutations in a phase II trial of first-line gefitinib, although some patients achieved prolonged stable disease[29]. In the LUX-lung 3 trial of first-line treatment with afatinib *vs* pemetrexed and cisplatin, the progression free survival of patients treated with afatinib was improved when less common mutations were excluded from the analysis, sug-

gesting a higher prevalence of intrinsic or acquired resistance in this group[3]. Exon 20 mutations in particular are generally associated with clinical resistance to all currently available EGFR TKIs. However, even within the group of tumors bearing exon 20 mutations there is heterogeneity and responses to EGFR TKIs have been observed with selected mutations[30].

EGFR mutation abundance and heterogeneity
EGFR activating mutations occur *de novo* during tumor development and heterogeneity with regard to EGFR mutation status in a particular tumor nodule has been reported[31,32]. While conventional DNA sequencing can detect an EGFR mutation present in at least 10% of tumor cells, a more sensitive method, the Scorpion amplification refractory mutation system (ARMS; DxS, Manchester, United Kingdom) uses unimolecular fluorescent probes to detect mutations present in 1%-10% of cells[33]. In a retrospective study of 100 randomly selected archived cases, treatment with EGFR TKIs achieved a longer progression free survival of 11.3 mo in patients whose tumor demonstrated high EGFR mutation abundance (more than 10%) than those with low EGFR mutation abundance (1%-10%, PFS 6.9 mo) and the PFS in both cases were longer than that in patients with wild type tumors (PFS 2.1 mo)[34]. This notion that higher EGFR mutation abundance in the tumor correlates with treatment effect in prolonging tumor control requires prospective validation. The heterogeneity of EGFR mutation status is not only observed in the primary tumor, but also between the primary and the metastatic lung nodules, with a discordance rate as high as 24%[35]. The cases with discordance appear to show mixed response to EGFR TKIs[35]. Therefore, genetic heterogeneity could be another mechanism for apparent TKI resistance at tumor progression.

Additional pathways that modulate response to targeted therapy
Several additional pathways appear to influence the depth and duration of response to TKIs in patients with EGFR-mutated tumors and provide new targets for improving therapeutic efficacy. The pro-apoptotic protein BIM has been shown to be necessary for EGFR TKI-induced apoptosis and tumor regression in EGFR-mutated cell lines and xenograft models[36,37]. In a small retrospective series, treatment with an EGFR TKI was associated with a higher response rate (57% *vs* 29%, *P* = 0.04) and longer PFS (13.7 mo *vs* 4.7 mo, *P* = 0.007) in patients whose tumors showed higher pre-treatment levels of BIM RNA[38]. An intronic deletion polymorphism of BIM found in 12% of East Asian patients and associated with reduced anti-apoptotic activity correlated with inferior response to EGFR TKIs[39]. In cell lines and xenografts with this polymorphism, restoration of BIM function with BH3-mimetic drugs or HDAC inhibition overcame TKI resistance[39,40]. This suggests that therapeutic strategies to augment BIM function, particularly in low-BIM expressing tumors, may reduce the problem

of TKI resistance in oncogene-addicted tumors. In addition, activation of the NF-κB pathway has been shown to confer in vitro resistance to erlotinib in EGFR-mutant cell lines. Patients whose tumors showed high expression of the NF-κB inhibitor I-κB were more likely to respond to treatment with erlotinib and had longer progression free and overall survival, suggesting that NF-κB signaling may have a clinically significant role in EGFR TKI resistance[41]. Therefore, combined EGFR and NF-κB inhibition presents another potential opportunity for improving the efficacy of targeted therapy.

Acquisition of secondary EGFR mutations
An important strategy for defining mechanisms of resistance to EGFR TKIs has been to re-biopsy the tumor at the time of disease progression. In two reported series comprising 192 patients treated with erlotinib or gefitinib, a distinct histologic change or molecular mechanism of resistance could be identified in the majority of cases[42,43]. Importantly, all TKI-resistant tumors retained the original EGFR mutation. In over half of tumors analyzed, a second EGFR point mutation, T790M in exon 20, was newly detected. T790M mutations are thought to reactivate EGFR signaling by increasing the receptor's affinity for ATP over TKIs[44]. Though a systematic analysis of the mechanisms of resistance to afatinib has not yet been published, resistance due to emergence of T790M has been reported[45].

EGFR-independent mechanisms of acquired resistance
Signaling through alternate oncogenic kinases can bypass EGFR inhibition to re-activate proliferation and survival pathways in EGFR-mutated cells. Amplifications of MET and HER2 were identified in a minority of resistant tumors examined in the two re-biopsy series mentioned above[42,43,46]. In one of the studies, PIK3CA mutations were also identified in two patients[42]. Furthermore, an analysis of a large tissue database identified BRAF mutations as a possible mechanism of resistance in 2% of specimens[47]. In addition, histologic changes such as transformation to small cell histology and epithelial to mesenchymal transition have also been observed, though the mechanisms by which they develop and lead to resistance are incompletely understood[42,43]. So far, mechanisms of resistance have been studied in a limited number of tumors and therefore the prevalence of each resistance mechanism is likely to change as more data accrues.

CLINICAL MANAGEMENT OF TKI-RESISTANT DISEASE

When a patient who previously responded to a TKI develops progression of disease, acquired TKI resistance occurs due to the various mechanisms described above. Although its clinical utility is debated, re-biopsy at progression in selected cases could be critical to understanding the mechanism of TKI resistance and hence guide

management decisions. While it is standard practice to discontinue chemotherapy at the time of disease progression, there is definitely a rationale for TKI continuation as discussed below. Treatment options include adding local therapy or conventional chemotherapy, or TKI continuation alone. Two hypotheses guide current strategies for treatment of TKI-resistant disease: (1) a population of TKI-sensitive clones remains at the time of disease progression; and (2) Resistant clones may be detected radiographically before widespread dissemination occurs.

As discussed above, the T790M secondary mutation is by far the most common mechanism of resistance, responsible for disease progression in more than half of patients treated with erlotinib or gefitinib. In vitro data has demonstrated that cells that acquire T790M second site mutations grow at a slower rate than parental cells without the mutations[48]. Furthermore, the same study suggested that in the presence of TKIs, resistant cell populations are heterogeneous and consist of slow-growing cells harboring T790M along with quiescent cells without the secondary mutation. Clinical observations support these in vitro results. In two patients with acquired TKI resistance and T790M mutations, serial biopsies during treatment with and without TKIs showed that T790M becomes undetectable after a period without TKI treatment[42]. Moreover, patients with T790M mutations identified at the time of disease progression have longer post-progression survival than those without the mutation[49]. Presumably, continuation of the original TKI exerts selective pressure that inhibits more aggressive TKI-sensitive clones and allows only the indolent T790M-harboring cells to proliferate. Therefore, in patients with T790M-mediated resistance or asymptomatic patients with radiographic evidence of progression and limited overall increase in disease burden, immediate change of systemic therapy may not be necessary and continuation of targeted therapy may still provide some measure of disease control.

It is conceivable that TKI-resistant clones develop in a single site of disease and can be detected on imaging before widespread dissemination. Patients who initially achieve disease control with EGFR-targeted therapy may subsequently show signs of disease progression in only one or a few sites of disease while other sites remain suppressed. Several groups have described their experience with the use of local therapies such as radiation or surgery to these sites of limited disease progression and have observed prolonged disease control after local therapy without a change in systemic therapy[50-52]. Progression-free-survival after local therapy of 6-10 mo[50,51] has been reported and the time until change in systemic therapy in one study was 22 mo[50]. Clearly, patient selection is key to the success of this strategy. Factors that might predict prolonged stable disease after local therapy include EGFR exon 19 deletions and longer duration of initial disease control on targeted therapy[52]. These observational studies suggest that local therapy may be of benefit, though prospective trials are needed to determine

whether local therapy can truly alter disease course.

In patients with symptomatic or rapid radiographic progression, re-biopsy of a rapidly growing lesion should be considered. If transformation to small cell histology is discovered, small cell chemotherapy regimens are appropriate for those patients. The remaining majority of patients are generally treated with chemotherapy. In this group of patients, the question of whether the original TKI should be continued is under investigation. Although no benefit was observed with the addition of TKIs to chemotherapy in unselected non-small cell lung cancer populations[53,54], several observations suggest benefit in the treatment of patients with EGFR mutations and acquired TKI resistance. A retrospective series reported higher response rates in patients who continued the original TKI after initiating chemotherapy, though no difference in progression-free survival was observed[55]. Furthermore, accelerated disease progression or "disease flair," defined by hospitalization or death attributable to disease progression, was observed in the short wash out period in some patients who stopped TKIs awaiting further chemotherapy[56]. These results suggest that some clones remain sensitive to EGFR blockade at the time of disease progression and that maintaining EGFR suppression is beneficial. Clinical trials are underway to prospectively assess the benefit of continuing TKI when chemotherapy is initiated (NCT01544179, NCT01928160 clinicaltrials.gov). If the TKI is not continued during chemotherapy, re-responses to erlotinib can be seen after post-progression "drug holidays[57]."

FUTURE DIRECTIONS

The identification of common recurring mechanisms of resistance to TKIs provides the opportunity for rationally designed treatment of resistant disease. One strategy is the development of new TKIs with activity against secondary resistance mutations. Though there was initial optimism based on preclinical data that afatinib, an irreversible TKI, would overcome resistance to erlotinib or gefitinib including T790M, the response rate was only 7% in a phase Ⅱb/Ⅲ trial in patients with disease progression after initial disease control on erlotinib or gefitinib[58]. CO-1686, a third-generation EGFR TKI, has shown *in vitro* and *in vivo* activity against cells and tumors harboring T790M mutations and is currently being studied in a phase 1/2 clinical trial of EGFR TKI-resistant disease[59]. In addition, an alternate dosing regimen incorporating intermittent high-dose afatinib showed *in vitro* activity against T790M and is being studied in a phase Ib clinical trial (NCT01647711 clinicaltrials.gov). Furthermore, combination therapy with afatinib and cetuximab showed promising activity in erlotinib resistant disease including cancers harboring the T790M mutation[60]. Other strategies to prevent or treat TKI-resistant disease include the addition of an inhibitor of one of the bypass pathways (MET, AKT, PI3K, IGFR) and HSP-90 inhibitors, which may decrease signaling through EGFR by decreasing the stability of the protein[61].

The identification of driver mutations in lung adenocarcinoma and the subsequent development of drugs that inhibit their oncogenic activity has been a major therapeutic advance benefitting patients with advanced disease. An understanding of the reasons for drug failure enables the optimal use of currently available EGFR targeted TKIs and maximizes their clinical benefit. Current evidence to guide management of TKI-resistant disease is limited but suggests that new principles may apply in the era of targeted therapy. The field of targeted therapy of lung cancer is rapidly evolving and the full potential of this treatment strategy is yet to be realized.

ACKNOWLEDGMENTS

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