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Emerging Paradigms in the Development of Resistance to Tyrosine Kinase Inhibitors in Lung Cancer

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ABSTRACT

The success of tyrosine kinase inhibitors (TKIs) in select patients with non–small-cell lung cancer (NSCLC) has transformed management of the disease, placing new emphasis on understanding the molecular characteristics of tumor specimens. It is now recognized that genetic alterations in the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) define two unique subtypes of NSCLC that are highly responsive to genotype-directed TKIs. Despite this initial sensitivity, however, the long-term effectiveness of such therapies is universally limited by the development of resistance. Identifying the mechanisms underlying this resistance is an area of intense, ongoing investigation. In this review, we provide an overview of recent experience in the field, focusing on results from preclinical resistance models and studies of patient-derived, TKI-resistant tumor specimens. Although diverse TKI resistance mechanisms have been identified within *EGFR*-mutant and *ALK*-positive patients, we highlight common principles of resistance shared between these groups. These include the development of secondary mutations in the kinase target, gene amplification of the primary oncogene, and upregulation of bypass signaling tracts. In *EGFR*-mutant and *ALK*-positive patients alike, acquired resistance may also be a dynamic and multifactorial process that may necessitate the use of treatment combinations. We believe that insights into the mechanisms of TKI resistance in patients with *EGFR* mutations or *ALK* rearrangements may inform the development of novel treatment strategies in NSCLC, which may also be generalizable to other kinase-driven malignancies.

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INTRODUCTION

Advances in molecular biology have highlighted the genomic complexity of cancer cells. Within this diverse genetic landscape, however, certain cancers are dependent on single oncogenic pathways for survival. This state of "oncogene addiction" commonly involves aberrant kinase activation, providing a therapeutic basis for agents directed against the corresponding dysregulated kinases.[1] The success of the tyrosine kinase inhibitor (TKI) imatinib in chronic myeloid leukemia (CML) originally validated this treatment paradigm.[2] Subsequently, this approach has been translated to other oncogene-driven malignancies, including gastrointestinal stromal tumors (GIST), *BRAF*-mutant melanoma, and epidermal growth factor receptor (*EGFR*) –mutant non–small-cell lung cancer (NSCLC).[3-7] Although selective kinase inhibitors produce high objective response rates (ORRs) in these molecularly defined populations, the long-term impact of such therapies are limited to variable degrees by the development of resistance.[8-10]

NSCLC provides an instructive conceptual framework for examining kinase-directed therapies and the mechanisms that ultimately limit their effectiveness. In the last decade, NSCLC management has evolved toward stratification of patients based on genetic alterations within "driver" oncogenes, such as *EGFR* and anaplastic lymphoma kinase (*ALK*). Somatic mutations in *EGFR* are identified in 10% to 30% of patients with NSCLC.[6,7,11] Common *EGFR* alterations include the L858R point mutation and exon 19 deletions.[12] These mutations result in enhanced EGFR signaling and confer sensitivity to the EGFR TKIs gefitinib and erlotinib.[6,7,11] In first-line treatment, EGFR inhibitors produce ORRs nearing 75% in patients with typical *EGFR* mutations.[12] Randomized trials have also demonstrated improved progression-free survival (PFS) for *EGFR*-mutant patients receiving TKIs compared with chemotherapy.[13-15]

Like *EGFR* mutations, *ALK* rearrangements define a unique molecular subset of NSCLC. Most *ALK* rearrangements arise from chromosomal inversions that generate novel *ALK* fusion transcripts,

commonly involving echinoderm microtubule-associated protein-like 4 (*EML4*) as the 5′ fusion partner (*EML4-ALK*).[16,17] Identified in 4% to 6% of patients with NSCLC,[16,18-20] *ALK* rearrangements are associated with unique clinicopathologic features and sensitivity to the ALK TKI crizotinib.[20] Initial clinical studies of crizotinib demonstrated ORRs of 60% and a median PFS of 8 to 10 months.[21-23] Given its high response rate, the US Food and Drug Administration (FDA) granted accelerated approval of crizotinib in 2011.

Despite the success of genotype-directed therapies in *EGFR*-mutant and *ALK*-positive patients, resistance inevitably develops. Indeed, the median PFS after treatment with EGFR or ALK inhibitors in target populations is generally less than 1 year.[13-15,21-23] Thereafter, standard management usually consists of cytotoxic chemotherapy. It is therefore critical to develop new insights into the mechanisms of TKI resistance to develop more effective treatment strategies.

DEFINITIONS OF RESISTANCE

Resistance to targeted therapies is generally classified as either primary (ie, intrinsic) or secondary (ie, acquired). Primary resistance describes a de novo lack of treatment response, whereas acquired resistance denotes disease progression after an initial response. Like CML and GIST,[24,25] criteria for acquired resistance were recently proposed for *EGFR*-mutant NSCLC (Table 1).[26] Although similar criteria have not been established for *ALK*-positive NSCLC, our definition will largely mirror that of *EGFR* for this review.

PRIMARY RESISTANCE

EGFR

Although ORRs to EGFR TKIs are high among *EGFR*-mutant patients, some patients exhibit intrinsic resistance. The mechanistic basis for these observations is largely unknown. Primary resistance may be due in part to differential TKI sensitivities across various *EGFR* mutations. "Classic" *EGFR* mutations, namely exon 19 deletions and L858R, are associated with marked sensitivity to TKIs.[27] Conversely, exon 20 insertions or duplications (~4% of *EGFR* mutations) seem to be resistant to EGFR inhibitors despite in vitro evidence suggesting that these alterations result in aberrant kinase activation.[28-30]

Table 1. Criteria for Acquired Resistance to EGFR Tyrosine Kinase Inhibitors

1. Patient has received prior therapy with an EGFR TKI (monotherapy).
2. Tumor genotyping confirms the presence of a typical *EGFR* mutation that is associated with sensitivity to EGFR TKIs. Examples include exon 19 deletions, L858R, and G719X.
OR
Patient achieves either a documented partial or complete response OR prolonged stable disease (≥ 6 months) based on RECIST or WHO criteria.
3. Disease progression occurs despite uninterrupted exposure to an EGFR TKI within 30 days.
4. Patient has not received additional systemic therapy since discontinuation of EGFR TKIs.

Adapted from Jackman et al.[26]
Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.

Intrinsic resistance to EGFR inhibitors may also be due to secondary genetic alterations that co-occur with sensitizing *EGFR* mutations. For instance, a T790M mutation within *EGFR* has been occasionally identified as a minor clone within treatment-naive tumor specimens containing classic *EGFR* mutations.[31-33] Similarly, *MET* amplification has been reported in *EGFR*-mutant tumors before TKI exposure.[33-35] As is discussed later, *MET* amplification and T790M are common mechanisms of acquired resistance. When present de novo, it has been suggested that these genetic alterations may also promote intrinsic resistance if present at sufficiently high allelic frequencies. Alternatively, selective pressure from TKIs may permit cells containing T790M or *MET* amplification to emerge as dominant clones early during therapy.

ALK

A small number of *ALK*-positive patients experience disease progression immediately after starting crizotinib. Recent preclinical data suggest that differences in specific *ALK* fusion gene products may partially account for heterogeneous treatment responses.[36] A number of different 5′ *ALK* fusion partners have been identified.[37] Additionally, multiple different *EML4-ALK* variants exist, all of which preserve the *ALK* kinase domain but differ with respect to the *EML4* breakpoint. In one cell line model, differences in crizotinib sensitivity were observed between different *EML4-ALK* fusion variants and *ALK* fusion partners.[36] Despite these in vitro observations, subgroup analysis from a phase I trial of crizotinib showed no correlation between *EML4-ALK* variant type and response.[21]

Another explanation for primary resistance to crizotinib may be false-positive genotyping. *ALK* rearrangements may be detected by various techniques, but only ALK fluorescence in situ hybridization (FISH) testing is currently approved by the FDA.[38] This assay is technically challenging because *EML4* and *ALK* both map to chromosome 2 and are normally separated by only ~12 megabases.[16,38] False-positive results may occur as a result of sectioning artifact, poor nucleus morphology, aberrant probe hybridization, or misinterpretation at pathologic review.[39] It is therefore possible that rare cases of "primary resistance" to crizotinib may be due to technical factors rather than intrinsic biology. Lastly, ALK FISH may identify true-positive *ALK* translocations, but these may not generate functional rearrangements in all patients.

Heterogeneity of TKI Response

ALK and EGFR TKIs can produce wide spectrums of response, even among those with identical genetic alterations. One intriguing explanation for this heterogeneity involves differences within the cellular apoptotic machinery. In particular, recent data have suggested that the pro-apoptotic protein BIM is a biomarker and mediator of TKI-induced apoptosis in several oncogene-driven malignancies.[40-46] In *EGFR*-mutant cell lines, BIM is upregulated in response to EGFR TKIs, and BIM levels correlate with the degree of apoptotic response.[42-45] Likewise, inhibition of BIM expression promotes intrinsic resistance to EGFR TKIs. Consistent with these preclinical findings, low pretreatment BIM RNA levels from *EGFR*-mutant patients were associated with decreased tumor shrinkage and a shorter PFS after treatment with EGFR TKIs.[45] The reasons for these differences in baseline BIM levels remain unclear. One recent report suggests that a genetic polymorphism in *BIM* results in alternative splicing

Table 2. Major Mechanisms of Acquired Resistance Identified in Clinical Specimens

Mechanism	Estimated Frequency (%)	References
EGFR TKI resistance		
Genetic alterations in *EGFR*		
T790M mutations	50	48-51
D761Y, T854A, and L747S mutations	< 5	42, 52, 53
EGFR amplification	8	50
Bypass signaling tracts		
MET amplification	5-22	35, 50, 51
HER2 amplification	12	54
PIK3CA mutations	5	50
BRAF mutations	1	55
CRKL amplification	9	56
HGF overexpression	1 of 2 cases	57
Phenotypic alterations		
Transformation to small-cell lung cancer	3-14	50, 51
ALK TKI resistance		
Genetic alterations in ALK		
ALK secondary mutations (eg, L1196M)	22-36	58-61
ALK gene amplification	7-18	60, 61
Bypass signaling tracts		
EGFR activation	44	60
KIT gene amplification	15	60

Abbreviations: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; ALK, anaplastic lymphoma kinase.

and altered BIM function, which may contribute to intrinsic resistance in some patients.[47]

ACQUIRED RESISTANCE

Mechanisms of acquired resistance in oncogene-driven malignancies are broadly divided into two categories. The first involves development of additional genetic alterations in the primary oncogene, which facilitates continued downstream signaling. This commonly arises through secondary mutations in the kinase target or through gene amplification of the kinase itself (Table 2).[8,62-64] Alternatively, resistance can develop independent of genetic changes in the target. This occurs through activation of downstream signaling pathways, changes in tumor histology, or alterations in drug metabolism.[8,27,62,65,66]

Secondary Mutations

Experience with imatinib resistance in CML has informed approaches to acquired resistance in other malignancies. Secondary mutations in the *ABL* kinase domain are common causes of TKI resistance in CML.[67,68] Among the more than 50 secondary mutations identified to date, the most common involves a threonine-to-isoleucine substitution at position 315 (T315I) of *ABL*, the so-called gatekeeper residue.[8] This mutation reduces imatinib binding but preserves ABL kinase activity.[67] In *EGFR*-mutant NSCLC, the earliest reports of TKI resistance identified an analogous secondary mutation in exon 20 of *EGFR*, leading to a threonine-to-methionine substitution within the gatekeeper residue at position 790 (T790M).[48,49] Secondary T790M mutations have since been found in approximately 50% of TKI-resistant, *EGFR*-mutant patients, establishing this alteration as the dominant resistance mechanism in the clinic.[50,51]

Although other gatekeeper mutations sterically impede TKI binding, T790M causes resistance predominantly through changes in adenosine triphosphate (ATP) affinity.[69] *EGFR*-mutant tumors are generally sensitive to competitive inhibitors because such mutations reduce the receptor's affinity for ATP. The addition of T790M, however, restores the ATP affinity of the kinase back to wild-type levels, re-establishing ATP as the favored substrate rather than the TKI. When coexpressed with classic *EGFR* sensitizing mutations, T790M confers resistance in vitro and in transgenic mice.[48,49,70] Furthermore, biochemical studies demonstrate that such dual mutations result in enhanced kinase activity and oncogenicity.[71,72] Despite these findings, preclinical and retrospective clinical studies suggest that T790M may actually confer a growth disadvantage relative to TKI-sensitive parental cells.[73,74]

T790M mutations have also been rarely detected within treatment-naive specimens.[31-33] In most patients, however, it is unclear whether these mutations arise de novo during therapy or whether EGFR-directed therapies select for preexisting clones. In addition to T790M, three additional secondary *EGFR* mutations have been associated with TKI resistance: D761Y, T854A, and L747S (Fig 1).[42,52,53] The structural basis for how these mutations confer resistance is unknown. Collectively, non-T790M secondary mutations are relatively uncommon and, in vitro, confer less pronounced resistance.

Secondary mutations in the *ALK* kinase domain have been identified in approximately 30% of *ALK*-positive patients with crizotinib

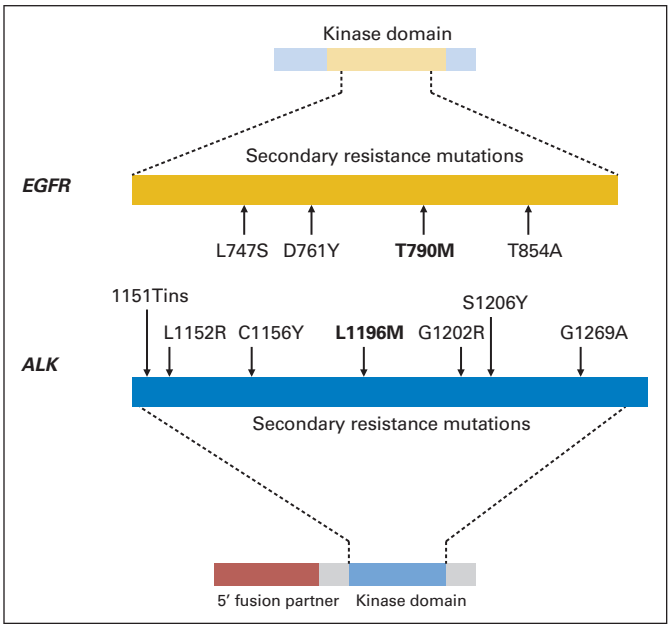

Fig 1. Comparison of the number and distribution of secondary resistance mutations in the tyrosine kinase domains of the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*). In *EGFR*-positive patients with acquired tyrosine kinase inhibitor (TKI) resistance, four different second-site mutations in *EGFR* have been identified in clinical specimens. The gatekeeper mutation T790M (bold) is the most common, present in approximately 50% of patients at the time of resistance. The remaining *EGFR* secondary mutations are present at low frequencies. In contrast, seven different secondary mutations have been identified in *ALK*-positive patients at the time of TKI resistance, including the L1196M gatekeeper mutation (bold). Despite this wider distribution of secondary mutations within the *ALK* tyrosine kinase domain, such mutations are found in only approximately 30% of patients.

resistance.[58-61,75] The earliest description involved an *ALK*-positive patient who developed disease progression after receiving crizotinib for 5 months.[58] Analysis of pleural fluid from this patient revealed two nonoverlapping mutations, L1196M and C1156Y, within the *ALK* kinase domain. Each independently conferred crizotinib resistance in vitro. The L1196M substitution is notable because it involves the *ALK* gatekeeper residue, analogous to T790M in *EGFR*. The L1196M mutation, which replaces a leucine moiety with a bulkier methionine residue, likely causes resistance by steric interference with crizotinib binding.

Since the initial case report of crizotinib resistance, additional second-site *ALK* mutations have been identified in patient-derived NSCLC specimens (1151Tins, L1152R, G1202R, S1206Y, and G1269A).[59-61] Mutations in a majority of these residues were also found in accelerated in vitro mutagenesis screening.[76] A separate *ALK* secondary mutation, F1174L, has also been reported in a crizotinib-resistant inflammatory myofibroblastic tumor.[77] Interestingly, the F1174 residue is among the most commonly mutated sites in neuroblastoma.[78]

ALK secondary mutations in NSCLC are distributed throughout the kinase domain, including the solvent front (G1202R, S1206Y), gatekeeper residue (L1196M), ATP-binding pocket (G1269A), and *N*-terminal to the αC-helix (1151Tins, L1152R, and C1156Y).[58-61,75] In vitro, *ALK* secondary mutations confer differential sensitivities to crizotinib and second-generation ALK TKIs.[60] For example, the *ALK* S1206Y mutation confers lower degrees of crizotinib resistance compared with G1202R, L1196M, and 1151Tins mutations. It remains unclear, however, whether variations in *ALK* secondary mutations translate into different clinical responses to next-generation ALK inhibitors.

It is noteworthy that *ALK*-positive patients, like those with CML, develop multiple secondary mutations at the time of TKI resistance. This is in contrast to *EGFR*-mutant patients, in whom T790M is essentially the sole secondary mutation observed clinically. Although in vitro mutagenesis experiments identified several additional *EGFR* resistance mutations, T790M was the only mutation consistently identified in all screens.[79] One potential explanation for this finding is that the *EGFR*-mutant kinase, which already possesses one alteration, may be unable to accommodate diverse drug-resistant, secondary mutations without compromising kinase function.[61] Still another consideration is that gefitinib and erlotinib bind to the active conformation of EGFR, whereas crizotinib and imatinib bind to the inactive conformations of ALK and BCR-ABL, respectively.[27] This may limit the spectrum of secondary *EGFR* mutations to those that influence drug binding in the ATP pocket.

Target Gene Amplification

Target gene amplification is another cause of acquired resistance that was first identified in CML.[50,61,67,69,80] Gene amplification may shift the intracellular balance between kinase and TKI in favor of the kinase. Gene amplification may also augment the effects of secondary resistance mutations if both are present simultaneously. For example, in a report of 37 *EGFR*-mutant patients with resistance to TKIs, *EGFR* amplification was identified in three patients (8%).[50] Interestingly, all three patients had simultaneous T790M mutations. Consistent with preclinical models, two of these patients seemed to have selective amplification of the T790M-containing allele.[50,81]

ALK fusion gene amplification has also been identified as a cause of crizotinib resistance.[60,61,80] This was initially suggested by cell line models in which amplification of wild-type *EML4-ALK* was sufficient to confer crizotinib resistance.[80] Subsequent studies have confirmed *ALK* fusion gene amplification in resistant clinical specimens.[60,61] In one report, high-level amplification was identified in one (7%) of 15 specimens,[60] whereas a separate study showed *ALK* copy number gain in two (18%) of 11 patients.[61] One of these patients also had a secondary *ALK* G1269A mutation.

Bypass Signaling

TKI resistance can also develop through reactivation of downstream signaling pathways via bypass tracts (Fig 2). One well-described example in *EGFR*-mutant NSCLC is through *MET* amplification.[35,82] Initially identified in 22% of EGFR TKI-resistant specimens, *MET* amplification confers resistance through ERBB3-mediated activation of downstream PI3K/AKT signaling, effectively bypassing the inhibited EGFR.[35] Activation of MET through its ligand, hepatocyte growth factor, may also promote resistance.[57,83] In more recent studies of EGFR TKI resistance, *MET* amplification has been identified in only 5% of specimens, perhaps reflecting differences in testing methodology and thresholds compared with those of earlier reports.[50,51] Interestingly, *MET*-amplified subclones have been identified at low frequencies in untreated specimens.[34] In a majority of these cases, the dominant mechanism of resistance at the time of disease progression was *MET* amplification, suggesting that these preexisting cells emerged as dominant clones as a result of selective pressure.

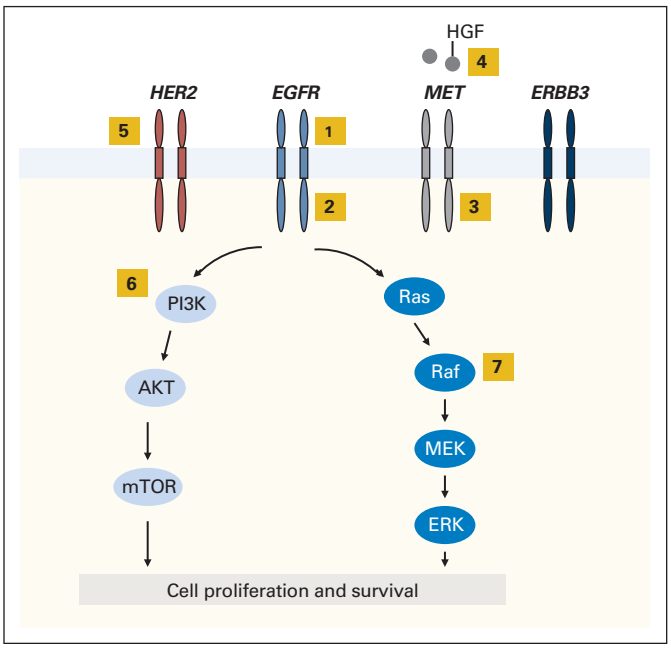

Fig 2. Mechanisms of acquired resistance to epidermal growth factor receptor (*EGFR*) tyrosine kinase inhibitors identified in *EGFR*-positive patient specimens. Activation of EGFR results in downstream signaling through the PI3K/AKT and RAS/RAF/MEK/ERK pathways, leading to cell proliferation and survival. The highlighted yellow boxes designate points along these pathways and others that have been associated with resistance to EGFR TKIs: (1) *EGFR* gene amplification, (2) secondary mutations in *EGFR* (eg, T790M), (3) *MET* amplification, (4) hepatocyte growth factor (HGF) –mediated MET activation, (5) *HER2* amplification, (6) *PIK3CA* mutations, and (7) *BRAF* mutations.

Several additional bypass tracts have been implicated in EGFR TKI resistance.[50,54-56] Recently, *HER2* amplification was identified in three (12%) of 26 *EGFR*-mutant patients with acquired resistance to TKIs.[54] When amplified, *HER2* is believed to function in parallel to the inhibited EGFR to reactivate common downstream signaling pathways. Recently, genetic alterations in effectors downstream from *EGFR* have also been identified as potential mediators of resistance.[50,55,84] For example, *PIK3CA* mutations have been identified in 5% of *EGFR*-mutant patients with acquired resistance.[50] Preclinical studies suggest that these mutations confer resistance by activating downstream AKT.[84] Recent studies have also focused on RAS/MAPK signaling as a source of EGFR TKI resistance because *KRAS* mutations have long been associated with primary resistance to EGFR inhibitors.[85] Despite their role in primary resistance, no *KRAS* mutations have been detected in *EGFR*-mutant patients with acquired resistance.[49,50,55] However, point mutations in *BRAF*, another member of the MAPK pathway, have been described.[55]

Bypass tracts also contribute to resistance in *ALK*-positive NSCLC. Preclinical studies identified EGFR coactivation as one potential mechanism of crizotinib resistance.[59,60,86] In these models, EGFR activation did not occur through mutation. Instead, increases in EGFR ligands EGF[86] and amphiregulin[59] were observed. Recently, a collection of crizotinib-resistant, *ALK*-positive tumor specimens were examined for evidence of EGFR activation.[60] Four (44%) of nine specimens demonstrated increased EGFR phosphorylation compared with precrizotinib samples, suggesting that EGFR upregulation may serve as a clinically relevant bypass track. A separate study also described one *ALK*-positive patient treated with crizotinib who was later found to have an *EGFR* mutation and a negative ALK FISH test in a repeat biopsy specimen.[61] In this latter report, *KRAS* mutations were also identified in two *ALK*-positive patients after disease progression occurred during treatment with crizotinib, although one patient harbored this mutation pretreatment. Lastly, *KIT* gene amplification has been identified in two (15%) of 13 crizotinib-resistant specimens, suggesting that this signaling pathway may also be co-opted to mediate resistance.[60]

Phenotypic Alterations

Several groups have reported changes in tumor histology on development of resistance to EGFR TKIs. The most dramatic examples include transformation from NSCLC to small-cell lung cancer (SCLC) in a subset of patients.[87,88] In one study of 37 *EGFR*-mutant patients with acquired resistance, repeat biopsies identified SCLC in five patients (14%), all of whom had adenocarcinoma at baseline.[50] Interestingly, the original *EGFR* mutations were present in all SCLC specimens. Samples were negative for T790M and *MET* amplification, although one patient developed a new *PIK3CA* mutation. In a separate cohort of 106 *EGFR*-mutant patients, SCLC or high-grade neuroendocrine carcinoma were found in three TKI-resistant patients.[51] The mechanistic basis for these observations remains unclear. Similar histologic changes have not been identified in *ALK*-positive patients.[60]

Another histologic change observed in EGFR TKI-resistant specimens is an epithelial to mesenchymal transition (EMT).[50,89,90] EMT is characterized by loss of epithelial markers (eg, E-cadherin) and gain of mesenchymal features, including surface expression of vimentin.[91] EMT is associated with enhanced motility, invasiveness, and in vitro EGFR TKI resistance.[92-94] Clinically, EMT has been recognized in a subset of EGFR TKI-resistant specimens.[50] The biology underlying this change and its impact on resistance remains unknown.

Other Mechanisms of Resistance

A subset of *EGFR*-mutant and *ALK*-positive patients have unknown mechanisms of TKI resistance. In preclinical models, EGFR TKI resistance has also been associated with insulin growth factor receptor signaling,[95-97] nuclear factor κB activation,[98] and loss of *PTEN*.[99] Among *ALK*-positive patients, loss of the *ALK* fusion oncogene has also been raised as a potential mechanism of resistance.[61] Additionally, resistance may be influenced by pharmacokinetic considerations. Gefitinib, erlotinib, and crizotinib are oral medications, which may be affected by absorption, patient compliance, drug–drug interactions, and metabolism. In CML, for example, plasma trough concentrations of imatinib have been correlated with response to therapy.[100-102] Similarly, patient adherence rates to imatinib have been identified as independent predictors of response.[103]

Polyclonal Resistance

Occasionally, multiple different resistance mechanisms are found within the same biopsy specimen.[50,51,60,61,82] Moreover, different resistance mechanisms may be found in separate tumor deposits within the same patient. For example, Yu et al[104] recently identified an *EGFR*-mutant patient with T790M in one resistant sample and *HER2* amplification in a separate specimen, underscoring the potential for clonal divergence across metastatic sites. Patients may have additional tumor heterogeneity in the form of TKI-sensitive cells admixed with resistant cells. Furthermore, the overall composition of such tumor populations may evolve with changes in therapy. For instance, *EGFR*-mutant and *ALK*-positive patients can experience a disease "flare" on TKI discontinuation, presumably because of accelerated growth of TKI-sensitive clones once selective pressure from the drug is removed.[105,106] The dynamic nature of resistance underscores the value of repeat biopsies at each new phase of treatment to advance our understanding of resistance and guide clinical decision making. Nevertheless, care must be taken to balance biopsy-related risks and ensure adequate informed consent.[107,108] This also emphasizes the need to develop noninvasive tools for monitoring resistance, such as mutational analysis of plasma DNA or circulating tumor cells.[109,110]

TREATMENT APPROACHES

Knowledge of the mechanisms underlying TKI resistance may inform new treatment strategies. We therefore conclude with an overview of treatment approaches for patients with TKI resistance. A more comprehensive discussion is beyond the scope of this review.

TKI Continuation Beyond Progression

In routine practice, oncologists typically discontinue a given therapy at the time of disease progression. It remains unclear, however, whether similar approaches should apply to TKIs in *EGFR*-mutant and *ALK*-positive patients, because resistance may be heterogeneous and TKI discontinuation may precipitate a disease flare.[105,106] In cases of isolated progression (eg, CNS), local therapies followed by continuation of the relevant targeted therapy may be a viable approach in select patients.[111-114] In *EGFR*-mutant patients for whom a switch to cytotoxic chemotherapy is ultimately

Table 3. Ongoing Trials of Next-Generation ALK Inhibitors or ALK Inhibitor Combinations

Compound(s)	Company	NCT Identifier	Description	Reference
AP26113	Ariad	01449461	Phase I/II study of AP26113 in crizotinib-naïve and crizotinib-resistant, *ALK*-positive patients.	132
ASP3026	Astellas	01401504	Phase I trial of ASP3026 in patients with solid tumors.	133
CH5424802	Chugai	01588028	Phase I/II study of CH5424802 in crizotinib-naïve and crizotinib-resistant, *ALK*-positive patients.	135
LDK378	Novartis	01283516	Phase I trial of LDK378 in crizotinib-naïve and crizotinib-resistant, *ALK*-positive patients.	131
		01685138	Phase II trial of LDK378 in crizotinib-naïve, *ALK*-positive patients.	
		01685060	Phase II trial of LDK378 in *ALK*-positive patients previously treated with crizotinib and chemotherapy.	
X-396	Xcovery	01625234	Phase I study of X-396 in patients with solid tumors, including *ALK*-positive patients treated with crizotinib or other second-generation ALK TKIs.	NA
Crizotinib + STA-9090	Pfizer/Synta	01579994	Phase I/II trial of combination crizotinib and STA-9090 (HSP90 inhibitor) in crizotinib-naïve, *ALK*-positive patients.	NA
Crizotinib + AT13387	Pfizer/Astex	01712217	Phase I/II trial of AT13387 (HSP90 inhibitor) alone or in combination with crizotinib in *ALK*-positive patients.	NA

Abbreviations: ALK, anaplastic lymphoma kinase; HSP90, Heat Shock Protein 90; NA, not applicable; NCT, National Clinical Trial.

deemed necessary, however, it remains unclear whether EGFR TKIs should be continued with standard chemotherapy. Several prospective clinical trials evaluating this question are currently ongoing (NCT01544179, NCT01310036).

Alternative Dosing

Another strategy is to use alternative doses or schedules of TKIs. In CML and GIST, for example, imatinib dose escalation has been effective in some patients experiencing disease progression at standard imatinib doses.[24,115,116] In *EGFR*-mutant NSCLC, different dosing strategies may also be relevant because the FDA-approved dose of erlotinib was determined in unselected patients. Recent evolutionary mathematical modeling studies have proposed that alternative EGFR TKI doses and schedules may produce comparable results while delaying development of resistance.[73,117]

Next-Generation TKIs

Initial strategies to combat acquired resistance have centered on using next-generation TKIs. This approach has met with success in other oncogene-driven malignancies. In CML, four next-generation TKIs (dasatinib, nilotinib, bosutinib, and ponatinib) have shown activity in imatinib-resistant disease.[118-123] One agent, ponatinib, is of particular note because it seems to overcome the gatekeeper T315I mutation.[123] Unfortunately, similar strategies have been less successful in *EGFR*-mutant NSCLC. Second-generation EGFR TKIs, such as neratinib, dacomitinib, and afatinib, differ from gefitinib and erlotinib in that they form irreversible covalent bonds with EGFR.[124] These agents also possess activity against other *ERBB* family members (eg, *HER2*). In preclinical models, irreversible EGFR TKIs demonstrated promising activity against T790M.[125,126] Unfortunately, clinical trials of these agents in patients with acquired resistance have been largely disappointing, likely as a result of dose limitations from toxicity caused by inhibiting wild-type EGFR.[127,128]

Recently, third-generation EGFR inhibitors, such as WZ4002 and CO-1686, have been developed. In preclinical studies, these compounds are active against cell lines and murine models harboring T790M mutations.[129,130] Moreover, WZ4002 and CO-1686 both seem to spare wild-type EGFR in vitro and in vivo. It is therefore hoped that these mutant-selective inhibitors will be able to overcome T790M-mediated resistance while producing less toxicity in the clinic.

Next-generation TKIs are also being investigated in *ALK*-positive patients. Indeed, five agents are currently in clinical testing[131-135] (Table 3). In preliminary reporting, two of these compounds (LDK378 and AP26113) were associated with high ORRs in phase I trials of *ALK*-positive patients with crizotinib resistance.[131,132] Both agents also demonstrated activity against brain metastases. This raises the possibility that next-generation ALK inhibitors may control disease in the CNS, which is among the most common sites of relapse among patients undergoing treatment with crizotinib. The mechanisms of crizotinib resistance for responders versus nonresponders to second-generation ALK TKIs have not been reported. Nevertheless, these encouraging early results suggest that use of more potent and/or structurally distinct ALK TKIs may be a promising strategy.

Combinatorial Strategies

The diversity of resistance mechanisms in NSCLC provides a rationale for combinatorial approaches. Such strategies commonly aim to inhibit the primary oncogene in addition to compensatory signaling pathways. One such approach in *EGFR*-mutant NSCLC has been to use dual MET and EGFR TKIs in those patients with *MET* amplification.[35,136] Similar combination strategies may have a role in *ALK*-positive NSCLC. As detailed earlier, *KIT* gene amplification and EGFR activation are possible mediators of crizotinib resistance. Given the availability of KIT and EGFR inhibitors already in clinical practice, KIT and EGFR may be effective targets for combination therapy. Indeed, crizotinib in combination with KIT inhibitors[60] or EGFR inhibitors[59,60,86] demonstrated activity in cell lines with upregulation of each respective kinase.

Combination strategies may also be considered when resistance arises through secondary mutations in the primary oncogene. In transgenic mouse models harboring *EGFR* T790M mutations, concurrent administration of the irreversible EGFR TKI afatinib and the EGFR monoclonal antibody cetuximab resulted in dramatic tumor shrinkage.[137] In a phase I/II trial of this combination, responses were observed in 40% of patients with EGFR TKI resistance.[138] Common

adverse events were rash and diarrhea. The mechanisms underlying disease activity from this combination remain unclear.

Additional combinations are currently being investigated. Given the association between BIM levels and apoptotic response to TKIs, proposed strategies include combinations of EGFR TKIs and modulators of apoptosis.[42-45] Still another approach to combat resistance in NSCLC is to target the molecular chaperone heat shock protein 90 (HSP90) because certain oncogenic kinases rely on this protein for proper folding. HSP90 inhibitors have demonstrated efficacy in *EGFR*-mutant cell lines and murine models harboring secondary mutations, including T790M.[139] Clinical studies using HSP90 inhibitors in patients with EGFR TKI resistance are ongoing, but responses have been reported.[140] Preclinical and early clinical findings suggest that HSP90 inhibitors may also have activity in *ALK*-positive patients.[60,80,140-143] HSP90 inhibitors may therefore be attractive options for use alone or in combination with TKIs in the management of resistance.

In summary, although kinase-directed therapies have reshaped treatment approaches in oncogene-driven NSCLC, these therapies have been universally limited by the development of resistance. It is therefore vital to develop new paradigms for understanding the mechanisms driving TKI resistance. *EGFR*-mutant and *ALK*-rearranged lung cancers offer instructive conceptual frameworks. Both highlight common principles of resistance, such as the development of secondary mutations in the target kinase, target gene amplification, and activation of bypass tracts. However, experiences in both malignancies also demonstrate the complexity, heterogeneity, and dynamic nature of resistance, suggesting that resistance will need to be approached on a truly "personalized" basis. Although future directions include development of noninvasive genotyping tools, the dynamic nature of resistance highlights the current importance of serial biopsies at the time of disease progression. Such reassessments of the changing molecular profiles of tumors may influence the development of novel therapeutic strategies and inform rational trial design.

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