

REVIEW

Systemic treatment in EGFR-ALK NSCLC patients: second line therapy and beyond

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ABSTRACT

Lung cancer is the most frequently diagnosed cancer and a leading cause of cancer mortality worldwide, with adenocarcinoma being the most common histological subtype. Deeper understanding of the pathobiology of non-small cell lung cancer (NSCLC) has led to the development of small molecules that target genetic mutations known to play critical roles in progression to metastatic disease and to influence response to targeted therapies. The principle goal of precision medicine is to define those patient populations most likely to respond to targeted therapies. However, the cancer genome landscape is composed of relatively few “mountains” [representing the most commonly mutated genes like KRAS, epidermal growth factor (EGFR), and anaplastic lymphoma kinase (ALK)] and a vast number of “hills” (representing low frequency but potentially actionable mutations). Low-frequency lesions that affect a druggable gene product allow a relatively small population of cancer patients for targeted therapy to be selected.

KEYWORDS

Lung cancer; epidermal growth factor (*EGFR*); anaplastic lymphoma kinase fusions (*ALK* fusions); tyrosine kinase inhibitors (TKIs); TKI resistance

Introduction

In recent years, diagnosis and treatment of patients with advanced lung cancer have undergone transformational changes. The current paradigm for prescribing novel targeted therapies is based on selecting patients according to the presence of specific oncogenic abnormalities in the tumor. The efficacy of therapy targeted at a specific oncogene is convincing evidence of “oncogene addiction”, or the concept that some cancers rely on or are “addicted to” a specific gene for their survival and proliferation¹.

The first such abnormalities discovered in lung cancer were epidermal growth factor (*EGFR*) kinase domain mutations; tumors with these mutations were found to be sensitive to EGFR

tyrosine kinase inhibitors (TKIs)². The echinoderm microtubule-associated protein-like 4- anaplastic lymphoma kinase (*EML4-ALK*) fusion has emerged as the second most important driver oncogene in lung cancer and the first targetable fusion oncogene to be identified in 4%-6% of lung adenocarcinomas^{3,4}. Crizotinib, an oral small-molecule inhibitor of ALK and c-MET receptor kinases, is now approved for treatment of *ALK* positive advanced non-small cell lung cancer (NSCLC), based on the results of two pivotal studies⁵⁻⁷.

Among *EGFR* or *ALK* mutated NSCLCs, the percentage of complete response is negligible and therefore novel, effective, safe treatments need to be tested and developed. To this end, repurposing an existing drug for treatment of NSCLC is also a worthy goal.

EGFR NSCLC patients: second line therapy and beyond

Our group was the first to evaluate the feasibility of large-scale screening for *EGFR* mutations in patients with advanced

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NSCLC and analyze the association between the mutations and the outcome of erlotinib treatment⁸. Since the introduction of erlotinib and gefitinib, patients with metastatic *EGFR* positive lung cancer can be offered a therapeutic alternative that has proven its superiority over standard platinum-based chemotherapy^{2,9}. In the EURTAC study, in which erlotinib was compared with platinum-doublet chemotherapy as first-line treatment for patients with *EGFR*-mutant NSCLC, erlotinib demonstrated a significant improvement in the overall response rate (ORR) and median progression free survival (PFS)². On the basis of this study, in May 2013 the U.S Food and Drug Administration (FDA) approved erlotinib for use in patients with lung cancers harboring *EGFR* exon 19 deletions and *EGFR* L858R substitutions².

Whereas reversible *EGFR* TKIs compete with ATP in the kinase domain of *EGFR*, 2nd generation *EGFR* TKIs, also compete for ATP binding but then covalently bind at the edge of the ATP binding cleft on Cys773 of *EGFR* via the Michael mechanism (addition of nucleophile to an α , β unsaturated carbonyl)¹⁰. Currently there are two lead 2nd generation *EGFR* TKI candidates, afatinib and dacomitinib, that are active against *EGFR* mutations with acquired resistance to erlotinib or gefitinib¹¹. Afatinib exhibits superior anticancer activity in lung cancer patients harboring gefitinib/erlotinib-resistant mutant *EGFR* (including T790M, exon 20 insertion, and T790M/L858R double mutation)¹². Based on the results of the LUX-Lung 3, the FDA has approved afatinib as a new first-line treatment for patients with metastatic *EGFR*-mutated NSCLC¹³. In the pooled analysis from LUX-Lung 3 and 6, presented this year at ASCO, afatinib prolonged survival of lung cancer patients whose tumors have common *EGFR* mutations by a median of three months compared with standard chemotherapy and significantly reduced the risk of death by 19%. The most pronounced reduction in risk of death was 41% in patients whose tumors had the most common exon 19 deletion *EGFR* mutation; for patients with the L8585R mutation there was no impact on overall survival¹⁴.

Despite the recent paradigm shift in the treatment of NSCLC patients, with a move toward biomarker-directed therapy, preclinical data has seldom been incorporated into clinical practice, since the existence of multiple resistance mechanisms complicates the selection of optimal biomarkers. Among patients progressing to first generation *EGFR* TKIs, 50% have tumors with a secondary T790M mutation¹⁵. The emergence of the T790M *EGFR* gatekeeper mutation and up-regulation of downstream signaling by *MET* amplification have been described as the two main mechanisms responsible for acquired resistance¹⁶. However a phase III trial enrolling only patients with *MET*-positive tumors was stopped in early March 2014 due

to futility; there was no evidence to suggest a positive effect of addition of onartuzumab to erlotinib¹⁷.

Other mechanisms include *EGFR* amplifications, *PI3KCA* mutations or a transition from epithelial to mesenchymal differentiation. For a small percentage of resistant tumors, histological transformation occurs to small cell lung cancer (SCLC)¹⁶. We recently reported possible causes of resistance to *EGFR* TKIs in *EGFR*-mutant NSCLC patients: the high co-existence of the pretreatment somatic T790M mutation, with a clear impact on PFS, and the role of *BIM* mRNA expression as an independent prognostic marker¹⁸. Pretreatment T790M can be detected in more than 60% of cases¹⁸. In fact, using a PCR-PNA assay pretreatment, T790M mutations were detected in 65.26% of patients¹⁸. These results reinforce the need for 2nd and 3rd generation *EGFR* TKIs, while taking into account existing data that suggest use of erlotinib or gefitinib beyond progression, with added chemotherapy, radiotherapy or best supportive care may improve survival¹⁹. Although afatinib and dacomitinib have been introduced to overcome acquired resistance, they showed limited efficacy in NSCLC with T790M and were more than 100-fold less potent in NSCLC cells with *EGFR* T790M mutation than in NSCLC cells with *EGFR* activating mutation²⁰.

CO-1686 is a novel covalent inhibitor that irreversibly and selectively targets both the initial activating *EGFR* mutations and the T790M secondary acquired resistance mutation²¹. To investigate its use as a single agent, CO-1686 is being evaluated in a phase I/II trial in *EGFR*-mutant NSCLC patients previously treated with first-line gefitinib or erlotinib (NCT01526928)²². In the phase I study, and based on early findings from the ongoing phase II trial, the agent yielded a 58% ORR across all dose levels in trial participants with biopsy-confirmed *EGFR* T790M mutations. Additionally, the compound did not cause the skin rash and diarrhea commonly associated with earlier generations of *EGFR* inhibitors²³. AZD9291 showed high activity in preclinical studies and was well tolerated in animal models. Xenograft studies identified a breakdown metabolite of AZD9291 called AZS104 that is about five times as potent as AZD9291 itself²⁴. In the phase I study of AZD9291 in *EGFR* mutant patients resistant to standard *EGFR* TKIs, 50% of patients experienced tumor shrinkage and the drug worked particularly well in patients with the T790M mutation²⁵.

A consistent proportion of *EGFR* mutant patients, approximately 30%, never respond to *EGFR* TKIs due to primary resistance. *BIM* mRNA expression could enrich the molecular diagnosis in patients with *EGFR* mutations, by identifying patients unlikely to respond and those with stable disease for whom treatment may not be effective due to early adaptive resistance^{18,26}. It is worth mentioning that, besides *BIM*

expression, a common BIM deletion polymorphism impairs generation of the proapoptotic isoform required for EGFR TKIs²⁷. This polymorphism confers an inherent drug-resistant phenotype that can be circumvented with the addition of the histone deacetylase (HDAC) inhibitor vorinostat to EGFR TKIs²⁸.

The two primary signaling pathways activated by *EGFR* include the mitogen-activated protein kinase (MAPK), and the phosphoinositide-3-kinase (PI3K), axes. Src tyrosine kinases and activation of the signal transducer and activator of transcription 3 (STAT3) pathways and downstream signaling have also been well documented²⁹. EGFR phosphorylation leads to recruitment of multiple effector proteins through recognition and binding of Src-homology 2 domain-containing phosphatase 2 (SHP2) to phosphotyrosine motifs on the receptor²⁹. SHP2 (encoded by *PTPN11*) is a ubiquitously expressed SH2 domain-containing protein tyrosine phosphatase (PTP). Despite its direct function in protein dephosphorylation, SHP2 plays an overall positive role in transducing signals initiated from growth factors/cytokines and extracellular matrix proteins, and initiating various downstream signaling cascades, including the PI3K and MAPK^{29,30}. By contrast, SHP2 functions as a negative regulator of the JAK/STAT pathway³¹.

In 2004, Sordella and colleagues were able to demonstrate the differential EGF-induced tyrosine phosphorylation pattern seen with wild-type (WT) and mutant EGFR receptors³². For instance, Y845 is highly phosphorylated in the L858R missense mutant, but not in the WT or deletion mutant, and hence appears to be unique in distinguishing between the two types of *EGFR* mutations³². Y845 (pY845) phosphorylation stabilizes the activation loop, maintains the enzyme in an active state, and regulates STAT3/5 activity³². Surprisingly, the *EGFR* L858R mutation leads to decreased ability to activate ERK compared to WT *EGFR* which correlates with decreased EGFR internalization, reduced phosphorylation of SHP2, hyperactivity of STAT3 and reduced sensitivity to gefitinib³³. Lazzara and colleagues found that SHP2 Y542 phosphorylation was not induced in response to EGF in the H3255 cells, which harbor the missense L858R exon 21 mutation, suggesting that SHP2 activity may be less efficiently promoted by *EGFR* L858R and the STAT3 pathway may be more active³⁴. The main problem is that STAT3 activation is not abrogated by single EGFR TKI treatment with gefitinib, erlotinib, afatinib or dacomitinib^{20,34,36}. Indeed, the second generation irreversible *EGFR* TKIs, afatinib or dacomitinib, not only do not abrogate, but may even induce STAT3 phosphorylation in gefitinib or erlotinib resistant cell lines such as H1975 or PC9-R20.

Afatinib activates interleukin-6 receptor (IL-6R)/JAK1/

STAT3 signaling via autocrine IL-6 secretion in both cells. Blockade of IL-6R/JAK1 significantly increased sensitivity to afatinib through inhibition of afatinib-induced STAT3 activation. The role of the paracrine IL-6R/JAK1/STAT3 loop between stroma and cancer cells in the development of drug resistance is crucial²⁰. Yao *et al.* uncovered the existence of a subpopulation of cells intrinsically resistant to erlotinib which display features suggestive of epithelial-to-mesenchymal transition (EMT) in NSCLC-derived cell lines and early-stage tumors before erlotinib treatment³⁷. Activation of TGF-beta-mediated signaling was sufficient to induce these phenotypes. An increased TGF-beta-dependent IL-6 secretion released previously addicted lung tumor cells from their EGFR dependency. Therefore, both tumor cell-autonomous mechanisms and/or activation of the tumor microenvironment could contribute to primary and acquired erlotinib resistance, and as such, treatments based on EGFR inhibition may not be sufficient for the effective treatment of lung-cancer patients harboring mutant *EGFR*³⁷. Combination of EGFR TKIs with drugs that target the IL-6R/JAK1/STAT3 pathway can be a rational synthetic lethal approach to overcome this mechanism of resistance (Figure 1).

Furthermore, tumor cells exposed to reversible or irreversible EGFR TKIs display early resistance dependent on MET-independent activation of BCL-2/BCL-XL survival signaling²⁶. According to the study of Fan and colleagues, such cells displayed a quiescence-like state that was readily reversed after withdrawal of targeted inhibitors. BCL-2 induction and p-STAT3 (Y705) activation were found within the residual tumor cells surviving the initial antitumor response to targeted therapies²⁶.

Although MAPK signaling is more commonly associated with receptor tyrosine kinases, it has been demonstrated that TGFBR1 also has tyrosine kinase activity. TGFBR1 directly phosphorylates tyrosine on ShcA, allowing for interaction with Grb2 and Sos. This leads to Ras activation, downstream ERK phosphorylation and BIM downregulation³⁸. Furthermore, TGF-b can induce expression of the transcription factor Snail, which leads to repression of E-cadherin transcription. SMAD-independent activation of Snail is a result of RAS/ERK activation. Although the mechanism remains incomplete and deserves further study, in the tumor setting of aberrant TGF-b signaling, TGF-b actively represses E-cadherin and promotes EMT³⁸.

More interesting is the fact that ADAM metallopeptidase domain 17 (ADAM17) or tumor necrosis factor alpha converting enzyme (TACE) targeting of TGFBR1 has been linked to ERK activation in nonprostate cells, resulting in shedding of the TGFBR1 ectodomain³⁸. There is evidence that active vitamin D (1,25(OH)₂D) therapy effectively inhibits ADAM17 and suppresses TGF/EGFR-pathway³⁹. Finally, Yuan

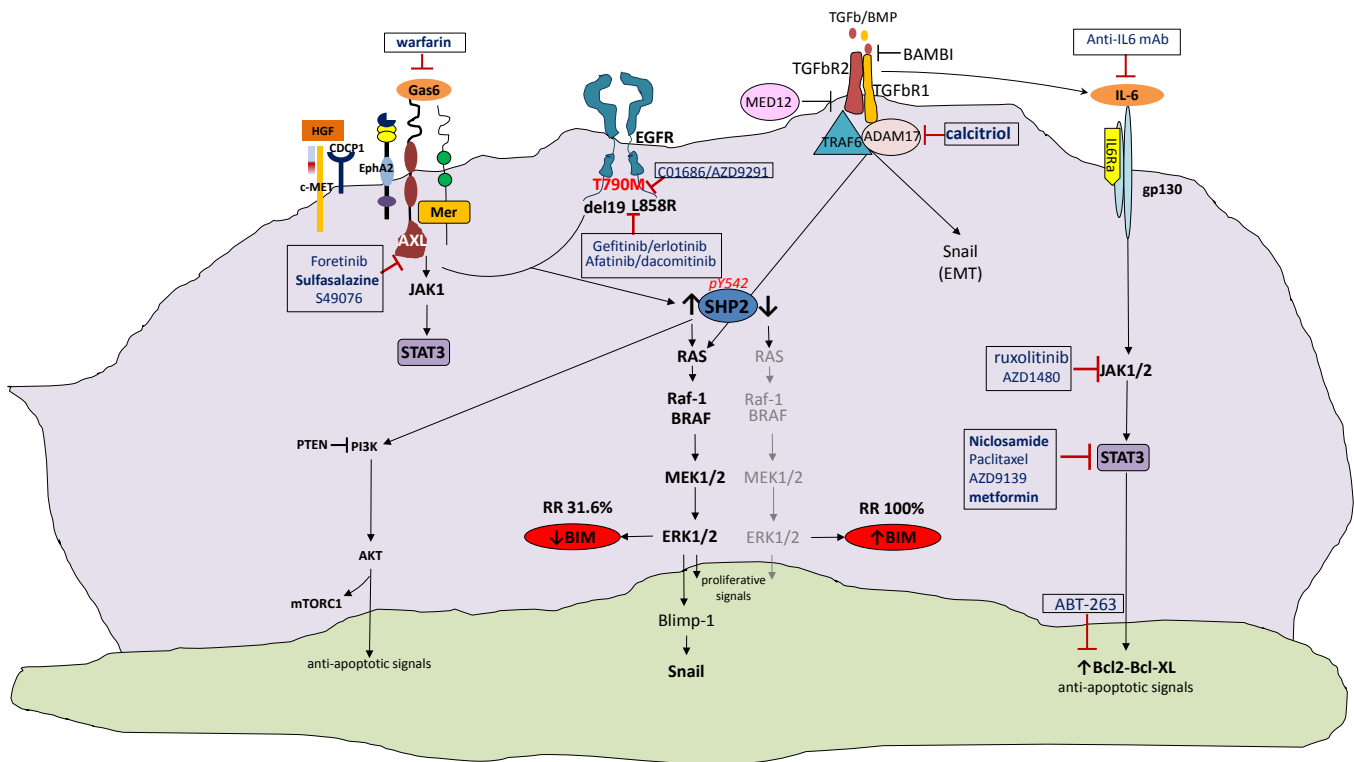


Figure 1 Crosstalk signaling pathways as potential mechanisms of resistance to reversible or irreversible EGFR TKIs. EGFR, epidermal growth factor; TKIs, tyrosine kinase inhibitors.

et al. recently demonstrated that a novel long noncoding RNA activated by TGF- β (lncRNA-ATB) amplifies the prometastatic effect of TGF- β via two independent mechanisms: firstly by inhibiting miR-200s, releasing activity of ZEB1 and ZEB2 and promoting EMT, and secondly by stabilizing *IL11*-mRNA and increasing autocrine IL-11-STAT3 signaling^{40,41}.

BIM expression in treatment naïve cancers predicts responsiveness to EGFR TKIs, but almost 2/3 of patients have low *BIM* mRNA levels at baseline¹⁸. SHP2, which is downstream of both EGFR and several other tyrosine kinase receptors, is required for sustained activation of ERK and *BIM* downregulation^{42,43}. Upon activation of MET by its ligand, hepatocyte growth factor (HGF) which is provided by stromal cells, EGFR signaling is dramatically altered⁴⁴. Indeed, HGF enables EGFR to interact with proteins which are known to be markers of a highly metastatic phenotype like the CUB domain-containing protein-1 (CDCP1), Ephrin Type-A Receptor 2 (EphA2), but mainly AXL and Mer; these interactions cannot be affected by EGFR TKI treatment. Interestingly, STAT3 signaling can be hyperactive due to upstream pathways including not only IL-6 and JAK but also AXL, providing further opportunities for combination therapies (**Figure 1**).

ALK NSCLC patients: second line therapy and beyond

The novel *ALK* fusion is formed by a rearrangement occurring on the short arm of chromosome 2 and involves the N-terminal portion of the EML4 protein and the intracellular signaling portion of the ALK tyrosine kinase receptor⁴⁵. *EML4-ALK* generates a transforming tyrosine kinase with as many as nine different variants identified⁴⁶. ALK-dependent mitogenic signaling is largely mediated via the RAS/MAP kinase pathway as well as ALK-driven PI3K activation. The JAK/STAT3 pathway also provides essential survival signals and modulates cellular metabolism regulating the mitochondrial oxidation chain. STAT3 is activated by ALK, either directly or through JAK⁴⁷. Crizotinib, an ATP-competitive aminopyridine inhibits tyrosine phosphorylation of ALK with an IC₅₀ of 20-40 nM and response in 57% of patients with ALK-rearrangement positive lung cancer⁵. However, most patients develop resistance to crizotinib, typically within one to two years. Studies of ALK-rearranged lung cancers with acquired resistance to crizotinib have identified *ALK* fusion gene amplification and secondary ALK TK domain mutations (L1196M and G1269A) in about one third of cases⁴⁸.

The success of a targeted drug is critically dependent on a specific and sensitive screening assay to detect the molecular drug target. The gold standard for detection of predictive *ALK* rearrangements is currently break-apart fluorescence in situ hybridization (FISH) as it is able to detect all known *ALK*-rearrangements and was clinically validated in crizotinib clinical trials⁷. However, the *ALK* FISH assay is fraught with technical challenges, including FISH signal instability and scoring difficulties. Although *ALK* FISH is clinically validated, the assay can be technically challenging and other diagnostic modalities are being explored, including immunohistochemistry (IHC) and reverse transcriptase–polymerase chain reaction (RT-PCR). Very interestingly, high frequency of *ALK* rearrangements in thyroid cancer from atomic bomb survivors in Japan was detected by a highly sensitive RT-PCR assay from archival paraffin blocks but was not confirmed by FISH or other methods⁴⁹. Targeted resequencing has recently been proved to be a promising method for *ALK* gene fusion detection in NSCLC, with results correlating significantly with those from FISH, RT-PCR, and IHC⁵⁰.

As with the majority of targeted agents, the tremendous excitement and enthusiasm sparked by crizotinib is tempered by the reality that a fraction of the target tumors are refractory from time of treatment initiation and most patients will eventually relapse and develop resistance after initial response. *ALK* kinase mutations and *ALK* fusion copy number gain (CNG) have been termed *ALK*-dominant mechanisms of resistance since tumors harboring these mechanisms presumably preserve *ALK* signaling despite the presence of crizotinib and are still dependent on that pathway for survival⁵¹. Data from several second-generation *ALK* inhibitors [ceritinib (LDK378), AP26113, (alectinib)] demonstrate response rates of 55% to 60% in crizotinib-resistant *ALK* positive NSCLC patients, with observed or predicted disease control rates of approximately 90%⁵¹. Ceritinib (LDK378) is an oral, small-molecule, ATP-competitive, *ALK* TKI52. Ceritinib achieved 56% response rate in patients previously treated with crizotinib with various resistance mutations in *ALK*; also in patients without detectable mutations⁵². On April 29th 2014, the FDA granted accelerated approval to ceritinib for treatment of patients with *ALK* positive, metastatic NSCLC with disease progression on or intolerant to crizotinib. The approval of ceritinib was based on the results of a multicenter, single-arm, open-label clinical trial enrolling 163 patients with metastatic *ALK*-positive NSCLC who had progressed on or were intolerant to crizotinib. This study is part of the ongoing clinical trial program in this patient population. Zykadia achieved an ORR of 54.6% and a median duration of response of 7.4 months based on investigator assessment⁵³. However, while ceritinib is able to effectively suppress many

crizotinib-resistant mutations, the G1202R and F1174V/C mutants are resistant to ceritinib⁴⁸.

One of the questions surrounding second-generation *ALK* inhibitors is exactly how they will fit into the treatment landscape of *ALK* positive NSCLC and whether they stand a chance of outshining crizotinib at the frontline. Furthermore, encouraging early clinical results in NSCLC have demonstrated that ganetespib, a novel triazolone inhibitor of HSP90, may offer a potential strategy to target *ALK* inhibition by inducing substantial antitumor responses and overcoming acquired resistance in patients with *ALK* positive lung cancer. When combined with ganetespib, crizotinib displayed superior antitumor efficacy compared with monotherapy in H3122 NSCLC xenografts⁵⁴. Recently, Richards *et al.* found that sensitivity of *EML4-ALK* variants to HSP90 inhibitors differs depending on whether the breakpoint of the fusion protein interrupts the globular TAPE (tandem atypical propeller in EMLs) domain. They determined the molecular structure of a conserved, tubulin-binding region of *EML1* that reveals an unexpected protein fold⁵⁵. This region is disrupted in almost 70% of *EML4-ALK* fusions found in patients, causing them to be sensitive to drugs that target HSP90, a cellular factor that stabilizes misfolded protein. These findings suggest that the truncation of a globular domain at the translocation breakpoint may prove generally predictive of HSP90 inhibitor sensitivity in cancers driven by fusion oncogenes⁵⁵.

Other “*ALK*-independent” mechanisms, such as the activation of compensatory signaling pathways, may also confer resistance to targeted *ALK* agents. Bypass signaling, by *v*-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) or *EGFR*, has also been described as a mechanism of resistance to crizotinib in *ALK* positive NSCLC⁵¹. EGF-induced activation of *EGFR*, *HER2*, and *HER3*, as well as a reduced level of *ALK* activation, was associated with sustained downstream signaling in the presence of *ALK* inhibitors, indicative of a shift in survival dependency from the *ALK* signaling pathway to *HER* family pathways in *ALK*-TKI-resistant cells⁵⁶. The combination of an *ALK* inhibitor and an *EGFR* inhibitor induced apoptosis, further supporting the notion that the *EGFR* signaling pathway contributes to survival in cells resistant to *ALK* inhibitors⁵⁶. *ALK* rearrangements and *EGFR* mutations could coexist in a small subgroup of NSCLC. Advanced pulmonary adenocarcinomas with such co-alterations could have diverse responses to *EGFR*-TKIs and crizotinib⁵⁷. Relative phospho-*ALK* and phospho-*EGFR* levels could predict the efficacy of *EGFR*-TKI and crizotinib. Yang *et al.* recently described co-altered *EGFR* and *ALK* in a large cohort of NSCLC and found that 3.9% of *EGFR* mutant and 18.6% of *ALK* rearranged tumors have co-

alterations⁵⁷. Tumors harboring co-altered *EGFR* and *ALK* could have diverse responses to first-line EGFR-TKIs, which were associated with phospho-EGFR levels⁵⁷. Phospho-ALK levels correlated with efficacy of subsequent crizotinib treatment. In clinical practice, we should pay attention to the specific biological behavior and corresponding management of NSCLC with dual altered EGFR and ALK genes⁵⁷.

ALK and its ligand pleiotrophin are required for self-renewal and tumorigenicity of glioblastoma stem cells (GSCs)⁵⁸. The pleiotrophin-ALK axis is activated by SOX2 and may be a promising target for therapy in ALK signaling-dependent tumors⁵⁸. Finally, Takezawa *et al.* demonstrated that expression of BIM and survivin are independently regulated by ERK and STAT3 signaling pathways, respectively, and are implicated in ALK-TKI-induced apoptosis in NSCLC cells positive for EML4-ALK⁵⁹. A selective inhibitor of ALK kinase activity (more potent than crizotinib), NVP-TAE68, inhibits STAT3 phosphorylation and downregulates survivin in H2228 cells, but fails to inhibit ERK phosphorylation and upregulate BIM. In a recent study, inhibition of both STAT3 and ERK pathways with the combination of TAE684 and a MEK inhibitor, AZD6244, was associated with a marked increase in the number of apoptotic cells⁶⁰ (Figure 2).

Conclusion

TKIs are effective anti-cancer therapies but resistance to these agents eventually develops. Several models of resistance to TKIs have been studied, including resistance to EGFR inhibitors or ALK inhibitors. A decade after the discovery of *EGFR* mutations, treatment is still incomplete and growing evidence indicates that targeting STAT3 or upstream or downstream components could cause induce synthetic lethal effects and achieve better outcomes. Repurposing drugs in combination with gefitinib, erlotinib, afatinib and dacomitinib is being investigated in cell cultures, subcutaneous & orthotopic xenograft models and clinical tumor samples. Clearly, the use of oncogene-addicted, highly drug-sensitive cell line models to identify escape mechanisms should be a strategy for identifying and overcoming resistance. However, it is of paramount importance that whenever possible, a biopsy is taken at time of cancer recurrence in patients who become resistant to targeted therapies so that these mechanisms can be confirmed or refuted. By identifying how a patient's cancer becomes refractory to targeted therapies, we will be well positioned to design rational treatment strategies to re-induce remissions. Only with expanding knowledge of these escape mechanisms, availability of drugs targeting escape pathways,

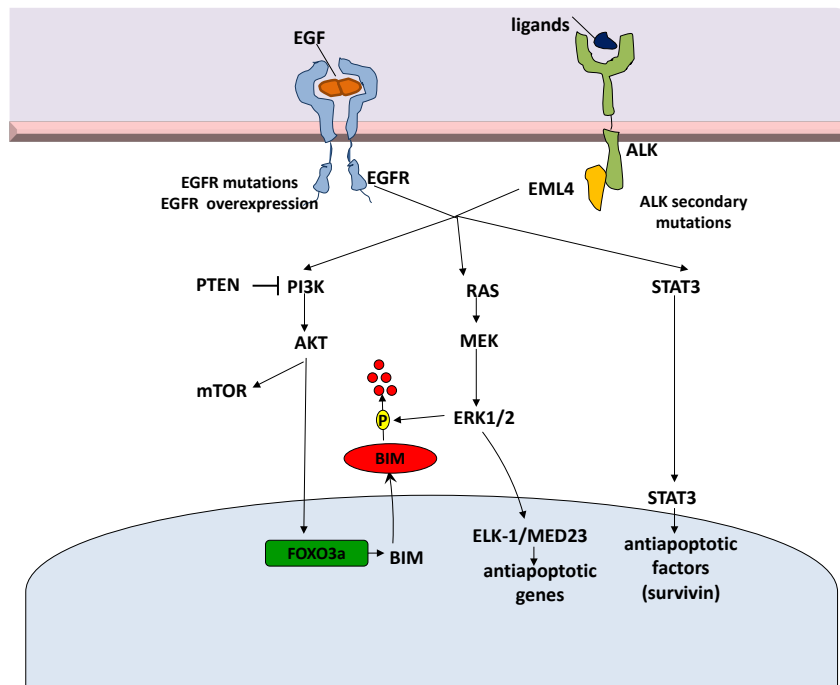


Figure 2 EML4-ALK signaling pathways and potential mechanisms of resistance to targeted therapies. EML4-ALK, echinoderm microtubule-associated protein-like 4- anaplastic lymphoma kinase.

and examples of such molecular data informing treatment decisions resulting in good clinical outcome, can re-biopsy and molecular profiling of recurrent drug-resistant tumors be widely incorporated into clinical practice.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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