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### Authors

Tetsu, Osamu  
Hangauer, Matthew J  
Phuchareon, Janyaporn  
[et al.](#)

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## Drug Resistance to EGFR Inhibitors in Lung Cancer

Osamu Tetsu<sup>1,2,\*</sup>, Matthew J. Hangauer<sup>2</sup>, Janyaporn Phuchareon<sup>1,2</sup>, David W. Eisele<sup>1,2</sup>, and Frank McCormick<sup>2</sup>

<sup>1</sup>Department of Otolaryngology-Head and Neck Surgery, School of Medicine, University of California San Francisco, San Francisco, CA 94143 USA

<sup>2</sup>UCSF Helen Diller Family Comprehensive Cancer Center, School of Medicine, University of California, San Francisco, San Francisco, CA 94143 USA

### Abstract

**Background**—The discovery of mutations in epidermal growth factor receptor (EGFR) has dramatically changed the treatment of patients with non-small cell lung cancer (NSCLC)—the leading cause of cancer death worldwide. EGFR-targeted therapies show considerable promise, but drug resistance has become a substantial issue.

**Methods**—We reviewed the literature to provide an overview of the drug resistance to EGFR tyrosine kinase inhibitors (TKIs) in NSCLC.

**Results**—The mechanisms causing primary, acquired, and persistent drug resistance to TKIs vary. Researchers and clinicians, who have used study findings to develop more effective therapeutic approaches, have found that the sequential use of single agents presents a formidable challenge, suggesting that multi-drug combinations must be considered.

**Conclusions**—In the era of precision medicine, oncologists should promptly obtain an accurate diagnosis of drug resistance in each patient to design the most relevant combination therapy to overcome patient-specific drug resistance.

### Keywords

non-small-cell lung cancer; epidermal growth factor receptor; tyrosine kinase inhibitors; drug resistance; cancer dormancy; persister cells; monotherapy; combination therapy; precision medicine

### Introduction

Lung cancer is the leading cause of cancer death worldwide [1, 2]. It is classified into two major histologic types based on microscopic features: small cell lung cancer (SCLC; ~15%) and non-small cell lung cancer (NSCLC; ~85%). Because NSCLC represents a heterogeneous group of cancers, it is further divided into three different pathological subtypes: adenocarcinoma (40%), squamous cell carcinoma (25-30%), and large cell carcinoma (10-15%) [3]

\* Correspondence to: Osamu Tetsu, MD, PhD, 1450 3<sup>RD</sup> Street, UCSF Helen Diller Family Cancer Research Building, San Francisco, CA 94143-0128 (USA), Osamu.Tetsu@ucsf.edu, Phone: 415-514-0870, Fax: 415-502-1712.

These pathological classifications are clinically important, as treatment decisions have depended on tumor histology [4, 5]. For example, localized NSCLC in an early stage is mainly treated with surgery followed by adjuvant chemotherapy [4]. In contrast, SCLC—even in an early stage—has rarely been treated surgically, as it tends to be more aggressive and spread more rapidly [5]. Thus, SCLC is usually treated with chemo- and radiotherapy. However, histological distinctions are no longer sufficient for determining treatment plans [6].

Molecular characterization of NSCLCs has provided valuable information for diagnosis, prognosis, and treatment [6]. In fact, the discovery of mutations in epidermal growth factor receptor (EGFR) and chromosomal translocations in anaplastic lymphoma kinase (ALK) has dramatically changed the treatment of patients with lung adenocarcinoma [7]. Targeted therapies are currently approved for these abnormalities and show considerable promise [1, 2, 7]. However, drug resistance has become a substantial issue [1, 2, 8-11].

In this review article, we focus on EGFR-targeted therapy and present an overview of drug resistance in NSCLC. We also discuss therapeutic strategies designed to circumvent drug resistance to EGFR inhibitors.

## Methods

A search of the literature revealed 131 articles (through November 15, 2015) relevant to drug resistance to EGFR TKIs in NSCLC.

## Results

### Molecular Profiling of Lung Adenocarcinomas

Recent studies have demonstrated that lung adenocarcinomas have recurrent mutations in multiple oncogenes: KRAS (32%), STK11 (17%), EGFR (11%), neurofibromin 1 (NF1; 11%), BRAF (7%), MET (7%), human epidermal growth factor receptor 2 (HER2; 3%), PTEN (3%), ROS1 (2%), ALK (1%), AKT1 (1%), RET (<1%), HRAS (<1%), NRAS (<1%), MEK1 (<1%), and PIK3CA (<1%) [12-14]. With the exception of PIK3CA [14], these mutations are mutually exclusive. Therefore, genetic profiling of NSCLC allows precise molecular classification of the disease. It can also be used to predict the potential efficacy of targeted therapy for each individual with adenocarcinoma [7, 10-13]. In fact, evaluation of gene mutations in EGFR and chromosomal rearrangements of the gene encoding ALK (most commonly resulting in an EML4-ALK fusion gene) are now considered to be the standard of care in advanced-stage pulmonary adenocarcinomas [7]. Intriguingly, EGFR mutations are more prevalent in patients with adenocarcinoma from East Asia who have never smoked or whose habit was light, whereas KRAS mutations are most frequent in Caucasian patients with adenocarcinoma in North America and Europe who have been long-time or heavy smokers [15, 16].

### Oncogenic Addiction and Targeted Therapy

The genesis and progression of human cancer involves multiple genetic and epigenetic alterations [17, 18]. However, the inactivation of a single oncogene can often impair these

altered cells' survival [19]. This phenomenon—known as oncogene addiction—has provided a rationale for molecular targeted therapy. The use in lung cancer of selective tyrosine kinase inhibitors (TKIs) for EGFR or ALK represents such examples [7]. Here we focus on EGFR TKIs.

EGFR is a receptor tyrosine kinase (RTK) that exists on the cell surface [20]. It belongs to the EGFR/HER family, consisting of four members: EGFR, HER2, HER3, and HER4. EGFR becomes activated by overexpression or by ligand-dependent or -independent mechanisms. Ligand-independent activation of the receptor occurs as gain-of-function mutations with the mutant allele showing gene amplification [21, 22].

Gefitinib (Iressa) was the first agent designed to target EGFR [22, 23]. It received US Food and Drug Administration (FDA) approval for the treatment of NSCLC, yet its activity was limited to 10-20% of patients with refractory lung cancer [1, 2]. In 2002, two retrospective cohort studies unexpectedly revealed a correlation between dramatic clinical responses to the TKI and activating EGFR mutations in the catalytic kinase domain of EGFR [21, 22]. Almost similar observations were obtained from treatment with another EGFR TKI, erlotinib (Tarceva), which was approved by the FDA in November 2004 [2, 24-26]. Approximately 85% of EGFR mutations are either exon 19 deletions (45%) or the missense mutation L858R (an amino acid substitution at position 858 from leucine to arginine) in exon 21 (41%) within the kinase domain [27, 28].

### **Heterogeneous Initial Responses to EGFR TKIs in NSCLCs**

A recent randomized phase-3 clinical trial with erlotinib found tumor reduction >90% in only 5% of patients (complete to near-complete response [29]). The remainder achieved a partial response or maintained stable disease, even though they too had TKI-sensitive EGFR mutations. The heterogeneous nature of this primary response raised questions of its causation—whether attributable to resistance inherent in the tumor cells or to acute drug-tolerance, or both.

### **Primary and Acquired Drug Resistance**

Drug resistance is a major obstacle to the success of targeted therapies, including EGFR TKIs [1, 2]. Based on tumor response to the initial therapy, drug resistance is classified as either primary (also called innate or intrinsic) or acquired (adaptive or secondary) [2, 10]. Patients with primary resistance do not respond at all to treatment, while those with acquired resistance may initially respond completely or partially, only to fail to do so over time. Approximately, 10% of patients harboring TKI-sensitive EGFR mutations show primary resistance to TKIs [2, 10, 29].

### **Primary Drug Resistance to EGFR TKIs**

Primary resistance to EGFR TKIs in NSCLCs is mostly associated with wild-type EGFR [30]. It also develops from activated mutations in KRAS or BRAF, or loss of function of the apoptotic protein Bim, or some uncommon EGFR mutation [31-34]. More recently, the tumor microenvironment or activation of NF- $\kappa$ B signaling has also been shown to elicit primary resistance [35-38].

**Wild-type EGFR**—Although erlotinib can prolong survival in patients with unselected NSCLC after first- or second-line chemotherapy [39], the benefit from EGFR TKIs is small in the 90% of NSCLC patients with wild-type EGFR [30, 40]. A recent clinical trial confirmed this observation. Only 3% of patients harboring wild-type EGFR had a partial response to erlotinib; in the remainder, docetaxel (Taxotere) was more effective [41].

**KRAS and BRAF Mutations**—EGFR, KRAS and BRAF mutations are mutually exclusive [14]. In approximately 30% of NSCLCs, activating KRAS mutations are observed at codons 12 or 13 and are considered to constitute a negative predictor for EGFR-targeted therapies [31]. Despite EGFR inhibition, KRAS mutations constitutively activate downstream MAPK signaling, presenting another mechanism that contributes to primary resistance.

Likewise, 7% of NSCLCs harbor mutations in BRAF. The most common change in BRAF is the V600E mutation (an amino acid substitution at position 600 from valine to glutamic acid), which confers resistance to EGFR TKIs but shows increased sensitivity to specific smallmolecule V600E inhibitors as well as to MEK inhibitors [32].

**Bim Polymorphism**—Bim is a BH3-only protein, which is essential for apoptosis and caspase induction in EGFR-mutated NSCLC cells [42-44]. Thus, reduction of Bim expression in NSCLC may cause drug resistance to TKIs. A recent report demonstrated that genetic polymorphism generates alternative splicing variants of Bim protein lacking the BH3 domain, which is sufficient to confer primary resistance to TKIs in NSCLCs harboring EGFR mutations [33].

**Various EGFR Mutations**—The most common activating EGFR mutations (85%) are either L858R or exon 19 deletions, which confer drug sensitivity to EGFR TKIs [27, 34]. However, less common EGFR mutations also exist. Among them, G719X in exon 18 (a substitution at position 719 from glycine to some other amino acid; 3%) and L861Q in exon 21 (an amino acid substitution at position 861 from leucine to glutamic acid; 2%) appear sensitive to TKIs [27, 34].

However, not all EGFR mutations are equally sensitive [27]; some cause TKI resistance. For example, small insertions or duplications in exon 20, which account for 5-10% of EGFR mutations, are associated with primary resistance, except for the rare case with EGFR exon 20 insertion A763\_Y764insFQEA [45, 46].

A recent study demonstrated that, although the vast majority of cancer cells harbor classic activating EGFR mutations, the EGFR TKI resistance conferring T790M EGFR mutation (an amino acid substitution from threonine to methionine at position 790) can be found within rare cells in primary tumors. Subsequent clonal selection of these preexisting EGFR TKI resistant cells during EGFR TKI treatment may contribute to drug resistance [47, 48].

**Tumor Microenvironment**—The latest studies have demonstrated that RTK ligands secreted through paracrine, autocrine, and endocrine mechanisms in the tumor microenvironment are also important determinants of primary therapeutic responses to

anticancer kinase inhibitors [35-37]. Indeed, hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and neuregulin 1 (NRG1) confer primary drug resistance to a large number of cancer cell lines by activating RTKs and thus stimulating either the Ras/MAPK- or PI3K/AKT pro-survival pathway or both [35]. HGF-mediated activation of the RTK MET is suspected as the most important cause of primary resistance to anticancer agents [35-37].

**Activation of NF- $\kappa$ B Signaling**—NF- $\kappa$ B signaling activation was recently identified as a mechanism of primary resistance to EGFR TKI [38]. Low expression of I $\kappa$ B, the NF- $\kappa$ B inhibitor, was a predictor of a poor clinical outcome in patients treated with erlotinib [38]. Thus, combined therapy with EGFR TKI and an inhibitor of NF- $\kappa$ B signaling can enhance primary responses in a subset of NSCLC harboring EGFR mutations.

### Acquired Drug Resistance to EGFR TKIs

Acquired resistance to EGFR TKIs develops after an average of a year of continuous treatment [1, 2]. A clinical definition has recently been proposed by Jackman et al. [49]. According to their criteria, the tumors should harbor TKI-sensitive EGFR mutations such as L858R or exon 19 deletions, should have responded either partially or completely (unless stable disease has been present for more than six months), and have demonstrated systemic progression.

Four different mechanisms of acquired drug resistance to EGFR TKIs have been reported [1, 2]: EGFR target alterations in the drug target itself (such as T790M secondary mutation [30, 45]), which can nullify the activity of gefitinib or erlotinib without changing the RTK activity; activation of alternative signaling pathways to bypass the EGFR inhibition (such as amplification of RTK MET [50, 51] or HER2 [52, 53], activation of another oncogenic driver, BRAF [54], or inactivation of the tumor suppressors PTEN [55, 56] or NF1[57]); a lineage switch through histological transformation from NSCLC to SCLC [58-60] or epithelial-mesenchymal transition (EMT) [61-63]; and intratumor heterogeneity [64].

**T790M and Other Secondary EGFR Mutations**—Acquired resistance to gefitinib and erlotinib is predominantly mediated by the development of the T790M EGFR secondary mutation, which occurs in 50-65% of patients with EGFR mutation and TKI resistance [1, 2, 8, 30, 45]. Threonine 790 is the gatekeeper residue in EGFR, lies within the ATP-binding pocket of EGFR, and influences drug effectiveness [65]. In accordance with this notion, a recent report demonstrated that the T790M mutation in EGFR confers drug resistance by increasing the affinity for ATP [66]. In addition, a further chromosomal amplification of the gene locus may enhance the inhibitory effect of T790M [67].

Gatekeeper mutations can be a common mechanism of acquired drug resistance to targeted therapies in cancer [68]. In fact, analogous mutations are reported in malignances exposed to various TKIs: imatinib-resistant T315I BCR-ABL fusion kinase in chronic myelogenous leukemia (CML) [69]; imatinib-resistant T670I KIT in gastrointestinal stromal tumor (GIST) [70]; and crizotinib-resistant L1196M ALK fusion gene in NSCLC [71].

Other rarer TKI-induced EGFR mutations, constituting <10% of all secondary substitutions in EGFR, have been reported: L747S [42], D761Y [72], and T854A [73]. Although these

non-T790M mutations have been associated with acquired resistance to TKIs, their drug-resistant mechanism is not yet clear [74].

**Activation of Alternative Pathways**—Recently, a common mechanism of acquired resistance to EGFR TKIs has been reported as a result of an RTK switch, such as amplification of MET [50,51] or HER2 [52, 53] or activation of HER3 [75, 76], insulin-like growth factor 1 receptor (IGF-1R) [77, 78], or fibroblast growth factor receptor 1 (FGFR1) [79, 80].

**MET Amplification:** Amplification of the MET gene is identified in 5-20% of EGFR-mutated NSCLCs with acquired drug resistance to EGFR TKIs [50]. Amplified MET specifically makes a heterodimeric complex with HER3, one of four members of the EGFR kinase family, and activates the PI3K/AKT pathway to bypass EGFR inhibition [50]. MET amplification is not mutually exclusive with the T790M secondary mutation; indeed, the latter is seen in 50% [51]. Thus, it is possible to conjecture that MET-amplified clones are selected after exposure to EGFR TKIs and then acquire the T790M mutation. In fact, MET amplification is found in 2-4% of previously untreated EGFR-mutated NSCLCs [51]. In any case, the population harboring activating EGFR mutations and MET amplification can achieve a clinical benefit from combined therapy with EGFR and MET inhibitors, as shown in a recent phase-III clinical trial [81].

**HER2 Amplification:** HER2 is amplified in 12% of tumors with acquired resistance, whereas the gene amplification is found in only 1% of untreated lung adenocarcinoma [52, 53]. No mutations in HER2 are found in this population. Importantly, HER2 amplification and T790M mutation occur in a mutually exclusive manner. These observations clearly suggest that HER2 amplification is an alternative mechanism of drug resistance to EGFR TKIs [2]. Indeed, HER2 is a potential therapeutic target because its overexpression or knockdown can confer, respectively, resistance or sensitivity to TKIs in NSCLC cells [53].

**HER3 Activation:** HER3 is activated independent of MET amplification in NSCLCs, and may contribute acquired drug resistance to EGFR TKI [75, 76]. HER3 lacks several catalytically important residues and is thought to be an inactive pseudokinase [82]. However, it can form a heterodimeric complex with EGFR/HER2 to stimulate downstream cell signaling [83, 84] and can be activated by its ligand NRG1 through an autocrine mechanism [85].

In NSCLC cells, HER3 couples with EGFR to activate the PI3K/AKT pathway in gefitinib-sensitive NSCLC cell lines, but not gefitinib-resistant lines, suggesting that NRG1-bound HER3 may predominantly dimerize with RTKs other than EGFR to promote acquired resistance to TKIs [76]. This mechanism may provide a rationale for the combined treatment of NSCLC patients with erlotinib and patritumab, an anti-HER3 monoclonal antibody [86].

**IGF-1R Activation:** IGF-1R has also been proposed to have a role in mediating acquired drug resistance to EGFR TKIs [77, 78], and in fact was reported as a biomarker for resistance to the TKI in NSCLC [78]. Unfortunately, data are conflicting: IGF-1R expression

was significantly associated with longer survival in NSCLC patients treated with gefitinib [87]. Further studies are warranted.

**FGFR1 Activation:** FGFR1 is activated by its ligand fibroblast growth factor (FGF) 2 through an autocrine mechanism and confers acquired drug resistance in NSCLC cells [79, 80].

**BRAF Mutations:** Mutations occurring in other driver oncogenes may also affect acquired drug resistance to EGFR TKIs. BRAF, but not KRAS, mutations are found in TKI-resistant NSCLC tumors, although their frequency is very low [54].

**Loss of PTEN Expression:** PTEN is a tumor suppressor gene controlling the PI3K/AKT pathway [88], and its mutation or loss of expression has been reported as a potential mechanism of acquired drug resistance [55, 56].

**Reduced NF1 Expression:** NF1 is a negative regulator of the Ras oncogene through stimulation of Ras GTPase activity [89]. TKI-induced low levels of NF1 expression have been associated with primary and acquired resistance to EGFR TKIs in NSCLC patients [57]. Treatment of NF1-deficient lung cancers with MEK inhibitors has restored sensitivity to TKIs, suggesting that combination therapy with MEK and EGFR inhibitors may have a clinical benefit [57, 90].

**Histologic Transformation—**In a rare phenomenon of acquired drug resistance, two lineage switches have been reported: histologic transformation of EGFR-mutated NSCLC to SCLC [58-60]; and epithelial-mesenchymal transition (EMT) [61-63]. The former has been reported in 2–14% of patients with acquired resistance to EGFR TKI [58-60]. These new SCLCs continued to harbor the initial activating EGFR mutations and were highly sensitive to the chemotherapy regimens for SCLC [60].

The lineage switch from NSCLC adenocarcinoma to SCLC was recently reported to involve the loss of RB1 (retinoblastoma 1) and EGFR proteins [91]. Actually, primary SCLCs are known to have a high prevalence of inactivating mutations in RB1 and TP53 [92]. In contrast, EGFR mutations and gene amplifications are rarely found in sporadic SCLCs [92]. Thus, the loss of RB1 expression can be the most likely scenario for lineage switching [91]. The study also suggested that NSCLC and SCLC may share the same cells of origin [93]. Alveolar type II cells might have the potential [94], but further studies are required.

EMT is a cellular mechanism critical for normal development, wound healing, and cancer metastasis [95]. In the process of EMT, cells undergo a lineage switch from epithelial to mesenchymal phenotype, which causes loss of cellular polarity, resulting in high motility and increased invasion capability [95]. EMT induction can be triggered by various cell signals, including TGF-beta and Notch-1, and may confer acquired drug resistance [61, 62]. A recent study demonstrated that the mechanisms leading to EMT in TKI-treated patients may involve activation of AXL RTK [63]. AXL overexpression has been found in 20% of NSCLC samples with progressive disease after treatment with EGFR TKI. However, how



EMT promotes TKI drug resistance remains unclear, and additional investigation is warranted.

**Intratumor Heterogeneity**—Intratumor heterogeneity may also account for drug resistance [64]: e.g., activating EGFR mutations and MET amplification were shown to co-exist a minor subset of cells in previously untreated TKI-sensitive NSCLC [51]. Subsequent clonal selection of the doubly positive cells could result in acquired drug resistance by affecting additional genomic alterations such as T790M EGFR [51].

### Cancer Dormancy and Acquired Drug Resistance

The origin of resistant cells remains to be elucidated, but they must arise from surviving populations [96-98]. These cells lie temporarily dormant or quiescent as a means of circumventing the effects of the given therapy, but eventually regain proliferative capacity [99]. The dormant cells resist chemotherapy because they do not divide until the environment is favorable to resume cell proliferation [100]. In the clinical setting, cancer dormancy is observed as a “grace period” after treatment [101]: signs and symptoms of cancer have disappeared, but the patients occasionally carry surviving tumor cells in local and distant bodily regions.

**Drug-tolerant Persister Cells**—In a recent study by Sharma et al., cultured cells from various cancer tissues remained after targeted or cytotoxic chemotherapy [102]. This phenomenon can affect up to 10% of a genetically homogeneous drug-sensitive population; thus, these drug-tolerant cells are far more numerous than might be expected from resistance based on the acquisition of de novo genetic mutations. Importantly, however, it was shown to be reversible, supporting a non-mutational mechanism.

In an analogy to microbial persister cells, these non-mutational drug-resistant cells were defined as drug-tolerant persisters. In 1942 Gladys Hobby first described the phenomenon of bacterial persistence, whereby penicillin was found to kill the vast majority of cultured streptococcal cells, but 1% remained intact [103, 104]. These residual cells were characterized in 1944 by Joseph Bigger as “bacterial persisters” [105].

In lung cancers, drug-tolerant persisters emerge during treatment as a subset of the cells within primary tumors [96, 102]. Although initially quiescent, the persister cells can soon begin to propagate in the presence of EGFR TKIs, becoming drug-tolerant expanded persisters. These may be cancer stem or mesenchymal cells because of this ability to escape the effect of drug treatment by becoming quiescent [102]. Epigenetic mechanisms have been proposed to explain this distinct lineage-switching [102]. However, in our investigation of the small TKI-tolerant subset of NSCLC cells we could not detect known lung cancer stem cell markers, nor could we uncover expression changes in mesenchymal markers [97, 98]. This suggested that a different mechanism could also cause the quiescent status in NSCLC cells after EGFR inhibition.

**Drug Resistance of Dormant NSCLC Cells to EGFR TKIs**—As mentioned above, we recently studied the mechanism by which a small subset of cells remains viable after EGFR inhibition, despite cell death in the vast majority [96-98, 106, 107]. Our study

demonstrated that EGFR inhibition in lung cancer cells generates a drug-tolerant subpopulation by blocking AKT activity and thus inactivating Ets-1 function (Figure 1). The remaining cells enter a dormant, non-dividing quiescent state (G0/G1 arrest) because of the inhibited transactivation of Ets-1 target genes cyclins D1, D3, and E2. Moreover, Ets-1 inactivation inhibits transcription of dual specificity phosphatase 6 (DUSP6), a negative regulator specific for ERK1/2. As a result, ERK1/2 is activated, which combines with c-Src to renew activation of the Ras/MAPK pathway, causing increased cell survival by accelerating Bim protein turnover. These observations may explain why a small subset of quiescent persister cells can tolerate TKIs, leading to acquired drug resistance.

### Overcoming Drug Resistance to EGFR Inhibitors

Among the numerous therapeutic investigations to improve patient outcomes [2, 8], a focus has narrowed on T790M EGFR, the most common mechanism of drug resistance to gefitinib and erlotinib. Below is an overview of the past decade's battles to overcome T790M-mediated resistance.

**Retreatment with the Same TKI after a Treatment Interruption**—Treating patients with other anti-cancer agents occasionally restores sensitivity to EGFR TKIs (Figure 2A) [108]. A chemotherapy regimen that interrupts the TKI therapy for a period of time, i.e. a “drug holiday,” may effect a restoration of the TKI's efficacy [8]. Nevertheless, there is a risk that dormant cancer cells might rapidly expand in some cases when the TKI treatment is suddenly stopped [99, 100].

**T790M-specific EGFR TKIs**—In contrast to the first-generation reversible EGFR TKIs (i.e., gefitinib and erlotinib), the second-generation TKIs such as afatinib (Gilotrif) [109], dacomitinib (PF-00299804) [110], and neratinib (HKI-272) [111] bind irreversibly to EGFR and other EGFR family members, including HER2 [8]. Therefore, the agents were thought to be effective for patients with the T790M EGFR drug-resistant mutation [112, 113]. However, early-phase clinical trials found no objective response in this population [114-116].

In contrast, the third-generation TKIs (AZD9291 [117], rociletinib (CO-1686) [118], and WZ4002 [119]) inhibit both mutations of EGFR activation and resistance, while they have a modest activity with wild-type EGFR (Figure 2B) [8]. In early-phase studies, AZD9291 and rociletinib demonstrated promising response rates (~60%) in tumors with acquired T790M EGFR mutation [117, 120, 121]. Unfortunately, acquired resistance to both developed in EGFR with or without the T790M mutation, demonstrating intratumor heterogeneity [122-126].

Of the third-site EGFR mutations (L718Q, L844V and C797S [125]), C797S was found in approximately 40% of AZD9291-resistant T790M-positive tumors. L858R, T790M, and C797S EGFR mutation-positive cells showed a partial sensitivity to cetuximab, a monoclonal antibody against EGFR [125]. More recently, additional mechanisms of drug resistance to AZD9291 have been reported: loss of the T790M mutation and EGFR gene amplification; and SCLC histologic transformation [122]. These findings suggest that

stepwise monotherapies represent a formidable challenge in drug-resistant cells and that simultaneous multi-drug combination therapies should be considered [127].

**Targeting Drug-tolerant Persister Cells**—The drug-tolerant persister cells could be a therapeutic target, as reduction of this subset may prevent emergent resistance to EGFR inhibitors (Figure 2C) [97, 98]. Drug-tolerant cells were previously shown to be sensitive to histone deacetylase (HDAC) inhibitor [102]. However, the combined therapy of erlotinib and entinostat, an HDAC inhibitor, did not improve clinical outcome [128].

Recently, we proposed combined EGFR and MEK inhibition therapy [96-98, 106, 107]. Our study demonstrated that addition of a MEK inhibitor enhances programmed cell death by rewiring apoptotic signaling after EGFR inhibition in persister cells. Therefore, to decrease the probability of emergent resistance to EGFR TKIs in NSCLCs, combined TKI and MEK inhibitor treatment should be considered. A recent report from another laboratory has also proposed this novel therapy, which is thought to be effective not only in TKI-sensitive activating EGFR mutations, but also in acquired resistance with the T790M second-site EGFR mutation [129]. Interestingly, a separate report showed that ERK1/2 reactivation accompanied DUSP6 reduction after L858R/T790M EGFR-selective inhibition in gefitinib-resistant cells [130]. Thus, a clear rationale for combined treatment exists, regardless of whether drug resistance is persistent or acquired. A randomized double-blind trial is necessary before this novel therapy can be integrated into the management of EGFR-mutated NSCLCs in the clinical setting.

## Conclusions

Over the past decade, EGFR-targeted therapies have dramatically changed the treatment of patients with lung adenocarcinoma. However, drug resistance has become a substantial issue. Recent studies have identified the mechanisms of primary, acquired, and persistent drug resistance to TKIs (Table 1), and researchers and clinicians have used these findings to develop therapeutic approaches (Table 2). However, the stepwise use of single agents presents a formidable challenge. This suggests that researchers and clinicians should consider multi-drug combinations to overcome drug resistance. In this era of precision medicine, oncologists must promptly obtain an accurate diagnosis of drug resistance during the individual clinical course to design the most relevant combination to overcome the patient-specific drug resistance in this population [131].

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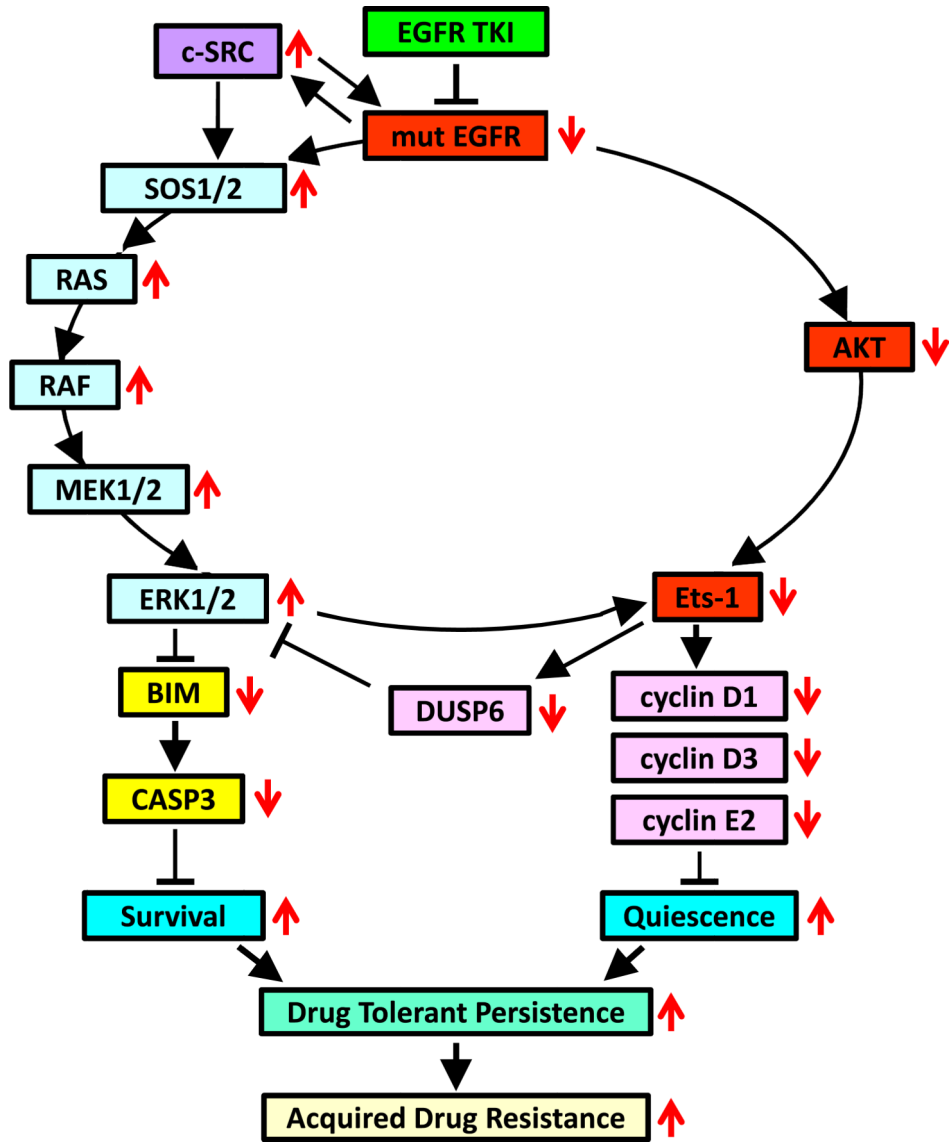
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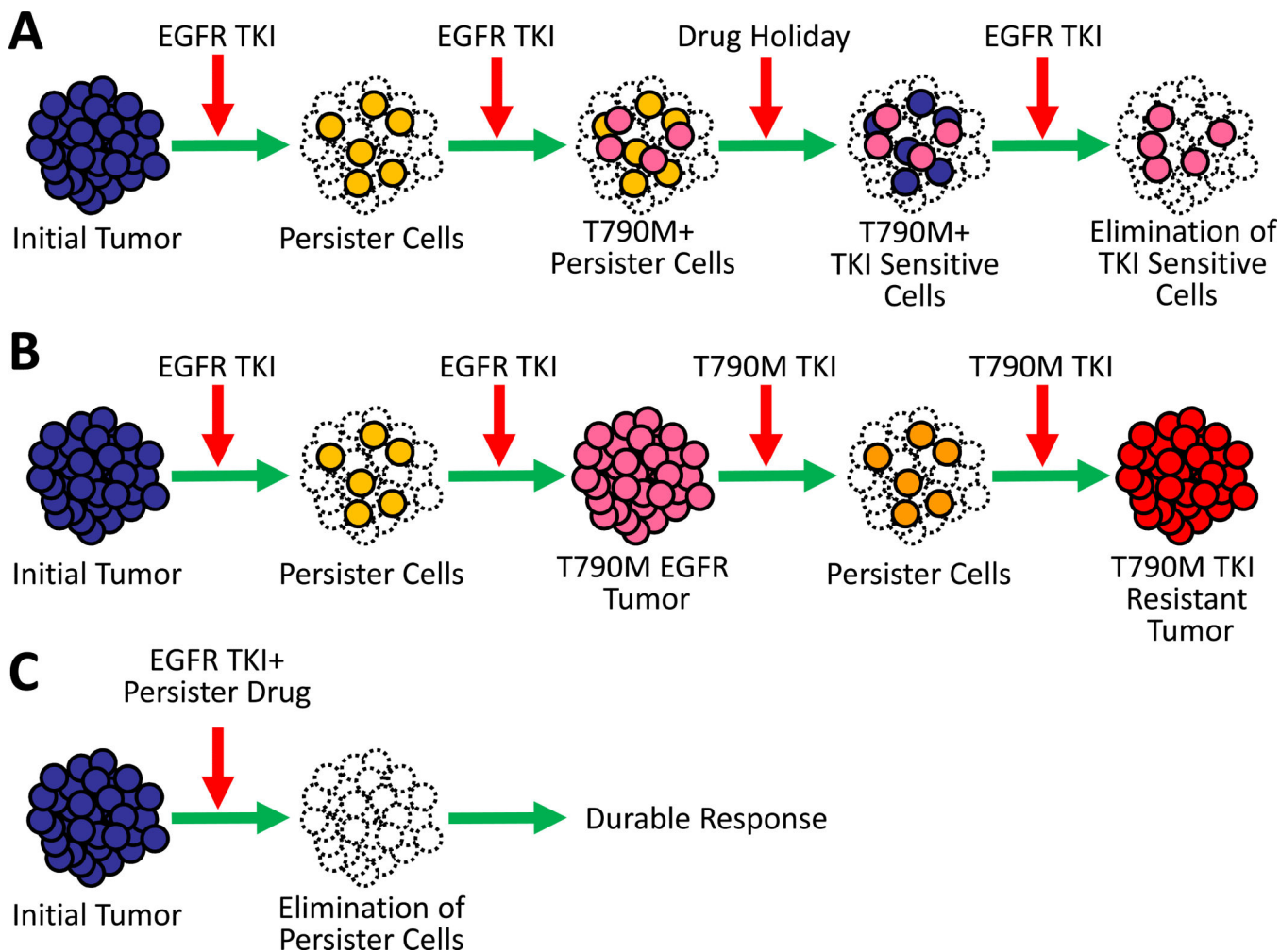
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**Figure 1.** Schematic of the molecular mechanism of EGFR inhibition and drug resistance in EGFR-mutated NSCLC cells. Increases and decreases in activity/expression of signaling molecules or biological outcomes resulting from EGFR inhibition are indicated by red arrows.

**Figure 2.**

Different strategies to overcome drug resistance to EGFR inhibitors.

(A) Retreatment with the same TKI after a treatment interruption. Resistant tumors are often composed of mixed populations of sensitive and resistant cancer cells. A chemotherapy regimen that interrupts the TKI therapy for a period of time, i.e. a “drug holiday,” may effect a restoration of the TKI’s efficacy.

(B) Administration of T790M-specific EGFR TKIs. The third-generation TKIs inhibit both mutations of EGFR activation and resistance. Early-phase studies demonstrated promising response rates in tumors with acquired T790M EGFR mutation. Unfortunately, acquired resistance developed in EGFR with or without the T790M mutation.

(C) Targeting drug-tolerant persister cells. The drug-tolerant persister cells could be a therapeutic target, as reduction of this subset may prevent emergent resistance to EGFR inhibitors. We and others recently proposed combined EGFR and MEK inhibition therapy.

**Table 1**

## Mechanisms of Drug Resistance to EGFR TKIs

Primary Resistance	Acquired Resistance	Persistent Resistance
Wild-type EGFR [30, 41]	<i>Target alterations</i>	AKT inhibition [96]
KRAS mutation [31]	T790M EGFR [65]	Epigenetics [102]
BRAF mutation [32]	L747S [42], D761Y [72], or T854A [73] EGFR	
Bim polymorphism [33]	<i>Activation of alternative pathways</i>	
EGFR exon 20 insertions or duplications [45, 46]	MET amplification [50, 81]	
T790M EGFR [47, 48]	HER2 amplification [52, 53]	
Tumor microenvironment [35-37]	HER3 activation [75, 76]	
NF- $\kappa$ B signaling activation [38]	IGF-1R activation [77, 78]	
	FGF1 activation [79, 80]	
	BRAF mutation [54]	
	PTEN loss [55, 56]	
	NF1 reduction [57]	
	<i>Histologic Transformation</i>	
	SCLC histologic transformation [58-60]	
	EMT histologic transformation [61-63]	
	<i>Intratumor heterogeneity</i> [64]	

**Table 2**

## Targeted Therapy: EGFR TKIs

1 <sup>st</sup> -generation	2 <sup>nd</sup> -generation	3 <sup>rd</sup> -generation
Gefitinib [23, 24]	Afatinib [109]	AZD9291 [117]
Erlotinib [24-26]	Dacomitinib [110]	Rociletinib [118]
	Neratinib [111]	WZ4002 [119]

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