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Rational, biologically based treatment of *EGFR*-mutant non-small-cell lung cancer

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Abstract

Epidermal growth factor receptor (*EGFR*)-mutant non-small-cell lung cancer (NSCLC) was first recognized in 2004 as a distinct, clinically relevant molecular subset of lung cancer. The disease has been the subject of intensive research at both the basic scientific and clinical levels, becoming a paradigm for how to understand and treat oncogene-driven carcinomas. Although patients with *EGFR*-mutant tumours have increased sensitivity to tyrosine kinase inhibitors (TKIs), primary and acquired resistance to these agents remains a major clinical problem. This Review summarizes recent developments aimed at treating and ultimately curing the disease.

Cancers of the lung, the leading cause of cancer-related death in the United States, accounted for 30% of all male cancer deaths and 26% of all female cancer deaths in 2009 (REF. 1). The overall 5-year survival rate of patients with metastatic disease remains less than 15%[2]. However, emerging data suggest that considerable progress has been made in the treatment of subsets of patients with lung cancer (FIG. 1). Lessons from these patients can hopefully serve as a model for how to make advances against the entire disease.

Historically, lung cancer was considered as one entity arising from the lung. In the mid-1970s, investigators showed that different histological subtypes had differential sensitivities to chemotherapeutic agents[3]. The trend towards subdividing lung cancer into ever more meaningful clinically relevant subsets has continued, with the appreciation that there are major histological differences among the main lung cancer subtypes, including small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), which is comprised of adenocarcinoma, squamous cell carcinoma and large-cell carcinoma. Today, further subcategorization is fuelled by the realization that tumours can be defined by various molecular criteria. One of the most promising treatment strategies exploits the discovery that distinct subsets of cancers harbour specific driver mutations in genes that encode signalling proteins that are crucial for cellular proliferation and survival. Targeting the activity of these mutant proteins can lead to cell death and therapeutic benefit. This finding serves as the basis for the concept of oncogene addiction[4], implying that tumours have Achilles heels that can be targeted with specific agents.

This Review focuses on one particular molecularly defined subset of NSCLC that harbours activating mutations in the epidermal growth factor receptor (*EGFR*) gene. EGFR belongs to

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a family of receptor tyrosine kinases (TKs) that includes EGFR, ERBB2 (also known as HER2), ERBB3 (also known as HER3) and ERBB4 (also known as HER4). Structurally, each receptor is composed of an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain. All family members have intrinsic TK activity, except ERBB3. The receptors exist as inactive monomers. On binding to ligands, such as EGF and transforming growth factor-α, the receptors undergo conformational changes that facilitate homodimerization or heterodimerization. Growth factor-induced receptor dimerization of EGFR is followed by intermolecular autophosphorylation of key tyrosine residues in the activation loop of catalytic TK domains through the transfer of γ-phosphates from bound adenosine triphosphate (ATP). Subsequently, appropriate adaptor or signalling molecules with SRC homology 2 and protein tyrosine-binding domains bind to carboxy-terminal phosphotyrosines and recruit proteins involved in downstream signalling events that control multiple cellular processes, including proliferation and survival[5]. Selective blockade of EGFR and ERBB2 has been shown to be an effective therapeutic approach against multiple epithelial cancers.

EGFR-mutant tumours were first discovered in 2004 and currently represent the best-studied example of oncogene addiction in lung cancer. *EGFR*-mutant tumours most often display adenocarcinoma histology and are associated with a better prognosis than *EGFR* wild-type tumours[6]. After more than 30 years, during which the overall survival (OS) of patients with metastatic lung cancer remained at no more than 1 year, recent data have shown that patients with metastatic *EGFR*-mutant tumours treated with first-generation EGFR TK inhibitors (TKIs) can have a median survival of more than 2 years (FIG. 1). Unfortunately, primary resistance to TKIs is still observed and acquired resistance limits the prolonged effectiveness of currently available TKIs. Here, we discuss recent strategies aimed at targeting mutant EGFR with an emphasis on approaches to overcome resistance to TKIs.

First-generation anti-EGFR therapy

The rationale for targeting EGFR in cancer has been extensively reviewed[5]. Notably, the first-generation anti-EGFR therapies developed in the 1990s were all directed against the wild-type receptor, which was shown to be overexpressed in many epithelial cancer types. Therapeutic agents include the small molecule TKIs gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; Genentech/OSI Pharmaceuticals) and the EGFR-specific antibody cetuximab (Erbitux; ImClone/ Merck/Bristol–Myers Squibb).

During the development of the first EGFR TKI, gefitinib, investigators worldwide noted that strikingly ten of 100 patients with previously heavily treated NSCLC had objective radiographic responses[7–10]. In confirmatory Phase II studies[11,12] (TABLE 1), the major clinical characteristics of responding patients were found to be adenocarcinoma histology, East Asian ethnicity, a history of never smoking cigarettes and female gender[12,13]. In 2004, *EGFR* kinase domain mutations were discovered, found to be associated with the clinical characteristics of responding patients and linked to an increased sensitivity of lung tumours to gefitinib and the related compound, erlotinib[14–16]. Scepticism surrounding this link was fuelled by multiple inconclusive correlative studies of large clinical trials. In most of these studies, the percentage of tumours that could be evaluated for mutations and the proportion of mutant tumours among entire cohorts was extremely low (FIG. 2). The reasons for such poor tumour accrual include the retrospective nature of these studies and the fact that, in the absence of specific tissue requirements, most of the patients diagnosed with advanced and/or metastatic NSCLC had insufficient tissue for molecular analysis (FIG. 2).

In the past 5 years, however, at least nine prospective single-arm studies for patients with advanced NSCLC and activating EGFR mutations have validated the benefit of EGFR TKIs

in *EGFR*-mutant lung cancer (reviewed in REF. 17). Trials were performed in East Asia, the United States and Europe, with either gefitinib or erlotinib. Radiographic response rates (RRs) ranged from 55% to 91%, and progression-free survival (PFS) and time to progression (TTP) from 7.7 months to 13.3 months. For comparison, RRs in unselected patients with NSCLC who were treated with gefitinib and erlotinib were 8.0% to 8.9%, with a median TTP of 2.2 months to 3.0 months in two large studies[18,19] (TABLE 1).

In 2009, two landmark randomized prospective Phase III studies (the Iressa Pan-Asia Study (IPASS) and WJTOG3405) showed that an EGFR TKI is superior to chemotherapy as an initial treatment for *EGFR*-mutant lung cancer[20,21] (TABLE 1). The IPASS enrolled East Asian individuals who had never smoked (never smokers) or former light smokers with lung adenocarcinoma. The PFS of patients with *EGFR*-mutant tumours was significantly longer among those who received gefitinib than among those who received carboplatin–paclitaxel (hazard ratio (HR) for progression or death, 0.48; 95% confidence interval (CI), 0.36–0.64; $p < 0.001$), whereas the PFS of patients with wild-type *EGFR* tumours was significantly longer among those who received chemotherapy (HR for progression or death with gefitinib, 2.85; 95% CI, 2.05–3.98). In the WJTOG3405 study, which enrolled Japanese patients with lung tumours harbouring *EGFR* mutations, the gefitinib group also had a significantly longer median PFS of 9.2 months (95% CI, 8.0–13.9) compared with 6.3 months (95% CI, 5.8–7.8; HR, 0.489; 95% CI, 0.336–0.710; log-rank $p < 0.0001$) in the cisplatin plus docetaxel group. Erlotinib has similarly been shown to be highly effective in patients with *EGFR*-mutant tumours[22,23]. A summary of major trials with anti-EGFR therapies is listed in TABLE 1.

More recently, another randomized prospective Phase III study (NEJ002) in patients with untreated *EGFR*-mutant tumours confirmed the benefit of first-line EGFR TKI (gefitinib) versus chemotherapy and further hinted that the order of treatment is important[24]. Unlike previous studies, 95% of the patients whose disease progressed on first-line carboplatin–paclitaxel crossed over to gefitinib therapy. Strikingly, the median OS in the gefitinib group was 7 months longer than that in the chemotherapy group (30.5 months versus 23.6 months). Moreover, the rate of response to gefitinib was slightly worse in the second-line setting than in the first-line setting (58.5% versus 73.7%). To determine whether EGFR TKIs are truly more effective in the first-line versus the second-line setting further studies are warranted.

A small proportion (1–20%, depending on the trial) of patients with no detectable *EGFR*-activating mutations show a radiographic response when treated with EGFR TKIs[20,25,26]. This observation can be partly explained by the fact that all molecular diagnostic tests for *EGFR* mutations have an inherent limit of detection[27]. However, it is possible that other genetic alterations may activate the EGFR signalling pathway in the absence of intrinsic gene mutations. For example, disease in patients with mucoepidermoid carcinomas (MECs) of the salivary and bronchial glands with wild-type EGFR has responded to gefitinib[28,29], and MEC cell lines are sensitive to EGFR TKIs *in vitro*[30]. As MECs harbour a recurrent mucoepidermoid carcinoma translocated 1 (*MECT1*)–mastermind-like 2 (*MAML2*) fusion[31] that induces expression of the EGFR ligand amphiregulin[30], one possibility is that gefitinib sensitivity is mediated by the action of the aberrant fusion protein.

Other predictive beneficial biomarkers have been proposed for EGFR TKIs, notably EGFR expression measured by immunohistochemistry (IHC) and *EGFR* copy number assessed by fluorescent *in situ* hybridization (FISH)[32–37]. Although EGFR IHC has not been found to be informative, increased *EGFR* copy number (that is, high polysomy and gene amplification) was shown to be associated with OS benefit in retrospective studies[32–34,36]. However, prospective studies have not validated *EGFR* FISH as a useful biomarker.

Whether erlotinib and gefitinib can be considered equally efficacious in the first-line setting relative to chemotherapy is currently unknown. Although no direct comparative effectiveness trials exist that have compared gefitinib with erlotinib in patients with *EGFR*-mutant tumours, the data suggest that there are no major differences between them. The two drugs are dosed differently (that is, erlotinib is administered at its maximum-tolerated dose whereas gefitinib is not); however, both EGFR inhibitors have similar, strongly correlated inhibitory patterns in *EGFR*-mutated cells *in vitro*[38,39]. In patients, the major mechanisms of primary and acquired resistance (see below) are the same for both drugs[40,41], indicating that they have the same target. Finally, similar response, PFS and survival rates have been observed for erlotinib and gefitinib[21,22,42].

In contrast to the link between *EGFR* mutations and EGFR TKIs, the role of *EGFR* mutations in predicting sensitivity to EGFR-specific antibodies is not clear. Cetuximab is a human–murine chimeric IgG1 monoclonal antibody that binds to the extracellular domain of EGFR and blocks EGFR signalling[43]. The antibody has been US Food and Drug Administration (FDA) approved for the treatment of colorectal and head and neck cancers[44,45] but its role in NSCLC remains to be established. A single-arm study in unselected patients with previously treated disease showed a RR of only 4.5%[46] and, despite cetuximab showing a promising additive effect with chemotherapy[47], two Phase III studies (FLEX and BMS099) in chemotherapy-naive patients showed conflicting results regarding OS[48,49] (TABLE 1). No links between *EGFR* mutations and sensitivity to cetuximab have been found, although only a limited number of patients has been studied[50,51]. As cetuximab interferes with EGFR ligand binding and subsequent receptor dimerization, *EGFR* mutations that confer ligand independence may abrogate the efficacy of this agent[52]. Interestingly, in mouse models of lung cancer driven by EGFR-L858R (exon 21), cetuximab can induce dramatic tumour regressions[53,54] but the drug is not effective as a single agent against an exon 19 deletion[53] or T790M mutant[54] (see below). The reasons for this discrepancy are unknown and might be related to different structural or conformational properties of the different mutants.

Biology of EGFR mutations

In lung cancer, activating mutations in *EGFR* occur in exons encoding the kinase domain (exons 18 to 21; summarized in FIG. 3). *EGFR* mutations are usually heterozygous, with the mutant allele also showing gene amplification[55,56]. Multiple genomic studies have shown that *EGFR*-mutant NSCLCs represent distinct disease phenotypes that have unique expression, mutation and copy number signatures[57–59]. For example, *EGFR*-mutant NSCLCs rarely harbour serine/threonine kinase 11 (*STK11*; also known as *LKB1*) mutations and are associated with a concurrent loss of the negative regulatory dual specificity phosphatase 4 (*DUSP4*) and the tumour suppressor cyclin-dependent kinase inhibitor 2A (*CDKN2A*; which encodes p16) genes[59].

The crystal structures of the L858R and G719S TKI-sensitive EGFR mutants show that these substitutions activate the kinase through disruption of autoinhibitory interactions, resulting in receptors with 50-fold more activity compared with their wild-type counterparts[60–62]. A separate crystal structure suggests the presence of an activating region in the juxtamembrane domain of EGFR[63]. To date, the crystal structure of the exon 19 deletion EGFR mutant has not been determined. Biochemical data further show enhanced kinase activity and transformation capabilities of *EGFR* in the presence of L858R or the exon 19 deletion[64,65]. In contrast to wild-type EGFR, the presence of a TKI-sensitive mutation results in preferential binding of gefitinib or erlotinib versus ATP.

As *EGFR*-mutant NSCLC cells are dependent on this aberrant kinase signalling for survival, inhibition of this pathway with the TKIs erlotinib and gefitinib results in cell death that is mediated through the intrinsic apoptotic pathway. This process is dependent on BIM, a BCL-2 pro-apoptotic family member regulated by ERK signalling[66–69] (FIG. 4). The downregulation of the induced myeloid leukaemia cell differentiation 1 (MCL1) protein — an anti-apoptotic protein regulated by PI3K signalling — also seems to be important[70].

One area of contention is whether *EGFR*-mutant tumours display similar biological characteristics in both East Asian and Caucasian patients. Currently, no convincing data exist to suggest that there are major differences between these two groups of patients. At the molecular level, the sequences of exons encoding a portion of the kinase domain (exons 18 to 21) from Asians and non-Asians and the range of the major drug-sensitive mutations are grossly similar (for example, see REF. [71] and FIG. 3). In preclinical models, transgenic mouse lung tumours harbouring EGFR-L858R or an exon 19-deletion mutant similarly respond to TKIs regardless of whether the mutation is on a mixed genetic (B6×CBA×FVB) background[72] or on a pure (FVB) background[53], indicating that genetic background does not substantially affect drug sensitivity. Clinically, the prognostic value of the *EGFR* mutation in patients undergoing surgery is approximately the same in Japan and the United States[6,73]. The RRs and survival rates are remarkably similar across populations[21,42]. Finally, the major mechanisms of acquired resistance in patients (see below) are the same in East Asian and Caucasian populations[40,41].

Primary resistance to EGFR TKIs

Lung tumours can show *de novo* resistance (primary resistance) to TKI therapy, even in the presence of an activating mutation in EGFR. Recent work has uncovered many of the molecular mechanisms underlying this primary resistance.

TKI resistance in the presence of an EGFR mutation

Among patients with *EGFR*-mutant tumours, a 75% RR is observed, indicating that approximately 25% of cases do not respond to a TKI (compared with 90% of unselected patients with NSCLC). Some of this can be explained by the fact that patients may experience tumour shrinkage, but the reduction in tumour size is not sufficient to fulfil response evaluation criteria in solid tumours (RECIST)[74]. According to these criteria, the unidimensional measurement of a tumour must shrink by 30% or more to be counted as a partial response. Tumour shrinkage of 20–25% may be beneficial to patients, but would only be considered as ‘stable disease’ by these guidelines.

Drug-resistant EGFR mutations

Some *EGFR* mutations, although they occur in exons 18 to 21, are associated with primary resistance to EGFR TKIs (FIG. 3). For example, small insertions or duplications in exon 20 (such as D770_N771, ins NPG, ins SVQ, insG and N771T; see REF. [75] for a complete list of insertions) are observed in ~5% of NSCLCs. *In vitro* studies have shown that such mutations are less sensitive to EGFR TKIs than the exon 19 deletion and L858R mutants[76]. Consistent with these data, most patients with tumours harbouring exon 20 insertions show progressive disease while taking EGFR TKIs[75]. Similarly, some patients present with a *de novo* resistance T790M mutation, which is encoded by exon 20 (REFS 77–79). As this mutation is more commonly found in patients with acquired resistance, the data are discussed in more detail below.

Primary TKI resistance may also be mediated by other rarer mutations in EGFR that occur together with drug-sensitive mutations (FIG. 3). For example, the drug-sensitive G719C

mutation[14] can co-occur *de novo* with an E709A mutation[80]. *In vitro*, the double-mutant receptor has been shown to be less sensitive to EGFR drugs than the G719C mutant alone[81].

Other genomic alterations that co-occur with EGFR mutations

Another reason why tumours with drug-sensitive *EGFR* mutations may not respond to treatment with EGFR inhibitors is the presence of other genetic lesions that affect signalling downstream of EGFR. For example, mutations in *PIK3CA,* the p110α catalytic subunit of PI3K, are found in approximately 2% of NSCLCs and can co-occur with EGFR mutations[82]. The addition of a constitutively active PI3K mutant (E545K) has been shown to confer gefitinib resistance, at least *in vitro*[83]. Similarly, loss of PTEN expression in *EGFR*-mutant cells correlates with decreased sensitivity to EGFR TKIs[84]. PTEN loss in NSCLC cells, although rare (<5%), uncouples EGFR from negative-feedback mechanisms, resulting in decreased degradation mediated by CBL, an E3 ubiquitin protein ligase that targets molecules for proteasomal destruction[84,85].

Crosstalk between EGFR and insulin-like growth factor receptor 1 (IGF1R) has also been implicated as a potential mechanism of disease persistence in *EGFR*-mutant cell line models[86,87] (FIG. 4). For example, some *EGFR*-mutant cells undergo only G1 cell cycle phase arrest in the presence of erlotinib, but undergo apoptosis when co-treated with an IGF1R-specific antibody[87]. In another study, *EGFR*-mutant NSCLC cells persisting after treatment with gefitinib gave rise to populations of cells of mixed sensitivity[86]. After further investigation, these persistent cells showed a distinct chromatin state that was mediated through IGF1R signalling and the histone demethylase, lysine-specific demethylase 5A (KDM5A)[86].

Resistance in EGFR-wild-type tumours

The IPASS clinical trial demonstrated that most tumours without detectable *EGFR* kinase domain mutations are insensitive to gefitinib[20]. Tumours wild-type for *EGFR* often harbour somatic mutations in other genes encoding signalling molecules. Thus, primary drug insensitivity is linked to the absence of drug-sensitizing mutations in *EGFR* and is more likely to be a result of mutations in other genes. Activating mutations occurring at codons 12 and 13 in the GTPase domain of *KRAS* are observed in 15–25% of NSCLCs and occur almost only in *EGFR*-wild-type tumours. *KRAS* mutations are found more frequently in tumours from former or current smokers compared with never smokers, and in tumours from Caucasians compared with East Asians, for reasons which are poorly understood. The initial observation that *KRAS*-mutant lung tumours are resistant to EGFR TKIs[88] has been well validated[89]. However, although *KRAS* mutations are used routinely as a negative predictor of benefit from EGFR-specific antibody therapy in colorectal carcinoma (FIG. 2), *KRAS* mutation testing has not been widely adopted in lung cancer.

Approximately 2–3% of NSCLCs harbour mutations in *BRAF*, which encodes a signalling molecule downstream of EGFR[90–92]. Similar to *KRAS* mutations, *BRAF* mutations are also mutually exclusive with changes in *EGFR*. The most common change in BRAF, V600E, is found in a large subset of melanomas, colon and thyroid cancer[91], and confers sensitivity to specific small-molecule V600E inhibitors[93] as well as MEK inhibitors[94]. NSCLC cell lines harbouring BRAF V600E are also sensitive to the MEK inhibitor PD0325901 but are resistant to EGFR inhibition[95]. A Phase II MEK inhibitor trial with PD0325901 showed little efficacy in advanced NSCLC; however, patients were not preselected by mutation status[96].

Another 5% of tumours harbour translocations in anaplastic lymphoma kinase (*ALK*)[97,98]. So far, most of these oncogenic rearrangements involve the echinoderm microtubule-

associated protein-like 4 (*EML4*) as the 5′ partner of *ALK*. Multiple different EML4–ALK variants have been identified, but all involve the tyrosine kinase portion of ALK and have variable lengths of EML4 (REF. 99). Similar to *KRAS*- and *BRAF*-mutant tumours, most of ALK fusion-positive tumours lack other 'driver' mutations. Clinically, *ALK* fusion-positive tumours are insensitive to EGFR TKIs[100].

Primary resistance to EGFR TKIs may also be mediated by non-mutation-based mechanisms. One example involves increased expression of hepatocyte growth factor (HGF), the ligand for the MET receptor tyrosine kinase[101]. HGF binding increases MET-mediated activation of the PI3K–AKT pathway, decreasing the ability of an EGFR TKI to effectively inhibit this signalling cascade. In contrast to the role of MET in acquired resistance (see below), primary resistance owing to increased HGF activation of MET is channelled through GAB1, not ERBB3 (REFS 101,102).

Acquired resistance to EGFR TKIs

Until recently, the clinical definition of acquired resistance (secondary resistance) to EGFR TKIs in lung cancer was not uniform. To minimize reporting of false- positive and false-negative activity in clinical trials and to facilitate the identification of agents that truly overcome acquired resistance to gefitinib and erlotinib, the following clinical and molecular criteria were recently proposed to more precisely define acquired resistance to EGFR TKIs[103]: previous treatment with a single-agent EGFR TKI (for example, gefitinib or erlotinib); a tumour that harbours an *EGFR* mutation known to be associated with drug sensitivity and/or objective clinical benefit from treatment with an EGFR TKI; systemic progression of disease (by RECIST or radiological criteria put forth by the World Health Organization) while on continuous treatment with gefitinib or erlotinib within the past 30 days; and no intervening systemic therapy between the cessation of gefitinib or erlotinib and the initiation of new therapy. The relatively simple definition proposed should lead to a more uniform approach to investigating the problem of acquired resistance to EGFR.

Second-site EGFR mutations

Chronic myelogenous leukaemia (CML) cells harbouring *ABL* translocations and gastrointestinal stromal tumour (GIST) cells harbouring activating *KIT* mutations are highly sensitive to the ABL and KIT inhibitor, imatinib (Gleevec; Novartis). When tumours relapse, a common mechanism of resistance is the emergence of second-site mutations in *ABL* and *KIT*[104,105]. A major secondary mutation involves a threo-nine gatekeeper residue in these proteins; the change to a bulkier isoleucine residue alters drug binding in both ABL (T315I) and KIT (T670I) (FIG. 5). Analogously, patients with *EGFR*-mutant tumours who develop acquired resistance to EGFR TKIs often develop a second-site mutation in the threonine gatekeeper residue at position 790, T790M. This mutation occurs exclusively in *cis* with the primary activating mutations in *EGFR*. Unlike CML and GIST, in which the gatekeeper mutation is found in 20–25% of patients with acquired resistance to imatinib, the T790M mutation in EGFR is found in 50% of *EGFR*-mutant tumours with acquired resistance to erlotinib or gefitinib[106,107] (FIG. 5). One possible explanation for the discrepancy in the frequency of gatekeeper mutations is that imatinib binds to ABL and KIT in their inactive conformations, whereas gefitinib and erlotinib bind to EGFRs in their active conformations[56] (TABLE 2). Therefore, any mutations that disrupt the inactive conformation of ABL or KIT can lead to imatinib resistance, whereas only mutations that interfere with drug binding in the EGFR ATP pocket may confer resistance to gefitinib and erlotinib.

The T790M mutation is almost never found in progressive brain or central nervous system lesions. This observation may be due to a lack of selective pressure, as the concentration of EGFR TKI that reaches the brain is 100-fold less than that found in blood[108,109].

Rarely, T790M mutations can be found in the germ line of patients (0.54% of never smokers with lung cancer). This variant seems to be associated with increased genetic susceptibility to lung cancer, which usually occurs after the age of 50 (REFS 110–112). Tumours in these patients often contain an additional activating mutation in *EGFR*, suggesting that additional genetic events (such as other changes in *EGFR*) are required for tumorigenesis.

At least two molecular mechanisms explain how T790M confers drug resistance. First, substitution of a bulky methionine for threonine at position 790 leads to altered drug binding in the ATP pocket of EGFR. Second, introduction of the T790M mutation increases the ATP affinity of the EGFR-L858R mutant by more than an order of magnitude, in effect restoring ATP affinity back to the level of wild-type EGFR. This restoration closes the therapeutic window that is opened by the diminished ATP affinity of the oncogenic mutants, which are normally more easily inhibited relative to wild-type EGFR[61,113].

Biochemical studies investigating the properties of T790M have shown synergistic kinase activity and transformation potential when the mutation is present in the context of an *EGFR*-sensitizing mutation[64,65]. However, despite this enhanced oncogenicity of T790M-harbouring EGFR, patients with this form of acquired resistance can display slow rates of disease progression[114]. Following the discontinuation of TKI therapy, disease flares have also been reported[115], suggesting that a proportion of cells in a resistant tumour cell population remain sensitive to EGFR inhibition (discussed below). Although erlotinib and gefitinib have limited activity against tumours with T790M-harbouring EGFR[67], multiple re-responses to EGFR TKIs following a short hiatus without targeted therapy have been reported[115–119]. The biology underlying this phenomenon has yet to be elucidated.

A recent study suggests that T790M-harbouring resistant clones may also be found at a very low frequency in untreated *EGFR*-mutant lung cancers. Using highly sensitive mutation detection techniques, EGFR T790M mutations were detected at an allele frequency of one in 500 in pretreatment tumour samples from patients with metastatic NSCLC[77]. Whether such mutations pre-exist in early-stage tumours has not yet been reported. One caveat, however, is that the polymerase chain reaction-based kit (DxS Ltd) used to detect the T790M mutations in this study has been associated with a high false-positive rate, leading the manufacturer to delete that mutation from its range. Thus, further studies are needed to confirm these findings.

Three other second-site mutations in *EGFR* have been associated with acquired resistance, including L747S (exon 19)[120], D761Y (exon 19)[56] and T854A (exon 21 in the activation loop)[121] (FIGS 3,5). Like T790, T854 is a drug contact residue and mutation to the smaller hydrophobic alanine residue may increase the size of the selectivity pocket, negatively impacting erlotinib binding[122]. L747 occurs at the start of the loop between the β3 strand and the α-C helix and is thought to shift the equilibrium towards the active conformation of the receptor[123]. D761 occurs in the a-C helix and a salt bridge formed by D761 may be disrupted by mutation to tyrosine, affecting the catalytic cleft of the receptor[124]. Consistent with the clinical data, D761Y, T790M and T854A were all identified in a comprehensive resistance mutation screen with erlotinib *in vitro*[122]. Among the resistance mutations, T790M confers the highest degree of drug resistance.

MET amplification

Amplification of the *MET* oncogene is observed in up to 20% of *EGFR*-mutant NSCLCs after TKI failure, independently of the T790M mutation[40,41]. Cells with *MET* amplification seem to undergo a kinase switch and rely on MET signalling through the ERBB3 pathway to maintain activation of AKT through increased phosphorylation in the presence of EGFR TKIs (FIG. 4). In addition to its role in *de novo* resistance (discussed above), the MET ligand HGF can play a part in acquired resistance to TKIs[101]. In one study, tumour cells with *MET* amplification were detected at a low frequency using high-throughput FISH in four patients with untreated *EGFR*-mutant tumours who all developed acquired resistance to gefitinib or erlotinib through *MET* amplification[102]. By contrast, pre-existing amplification was found only rarely in tumours from patients (one of eight) who did not develop resistance by *MET* amplification. Collectively, these data suggest that TKI therapy may select for pre-existing cells with *MET* amplification.

Other mechanisms of acquired resistance

Tumours from approximately 40% of patients with acquired resistance do not harbour a second-site mutation or *MET* amplification. Thus, multiple investigations to identify resistance mechanisms are ongoing. One promising approach involves the study of acquired resistance in mouse models of EGFR TKI-sensitive lung tumours[125]. Prolonged exposure of mice harbouring EGFR-L858R-driven and exon 19 deletion-driven lung tumours led to the development of resistant tumours that harboured the secondary T790M change and/or *Met* amplification. Further analysis of these models may reveal novel mechanisms faster than by studying humans.

The epithelial to mesenchymal transition (EMT) has been associated with resistance to EGFR TKIs *in vitro*[126]. Recently, investigators confirmed that EMT can also be found in patient tumours [127]. The shift in signalling networks resulting from EMT may alleviate dependence on EGFR signalling[128]. Increased IGF1R signalling has also been associated with acquired resistance, albeit only in an *in vitro* model using a cell line that expressed high levels of wild-type EGFR[129].

Another unexplained observation is that some patients originally diagnosed with *EGFR*-mutant lung adenocarcinoma who develop acquired resistance display SCLC at the time of relapse. Three such cases have been reported[130–132]. In patients in whom the mechanisms of resistance have been examined, none displayed the T790M mutation or *MET* amplification, but tumours have been found to harbour *EGFR* drug-sensitizing mutations. This remains an area of active investigation.

Overcoming resistance to TKIs

Primary resistance

As stated above, primary resistance falls into four main categories: TKI resistance in the presence of a drug-sensitizing *EGFR* mutation; drug-resistant *EGFR* mutations; genomic alterations that co-occur with *EGFR* mutations; and *EGFR*-wild-type tumours. Different strategies are needed to overcome resistance for each category (FIGS 4,6).

As the first category of resistance may be related to semantics (whether tumour shrinkage meets established radiographic criteria), this is not an active area of investigation. However, as the first-generation EGFR TKIs were originally developed against wild-type EGFR and EGFR mutations were only discovered after the initial development of these drugs, there is a rationale to pursue trials to determine the optimum upfront treatments for patients with tumours that harbour EGFR mutations (FIG. 6). One potential strategy involves taking

advantage of the requirement for BIM and enhancing TKI-induced apoptosis by adding a BCL-2 inhibitor[66] (FIG. 4). Such an approach, which seems promising *in vitro*, could lead to more profound responses and delayed TTP. For drug-resistant *EGFR* mutations, such as exon 20 insertions and duplications, other EGFR TKIs may be more effective. For example, the second-generation EGFR TKI PF-00299804 compound (Pfizer; discussed further below) has been shown to induce a partial response in a least one patient with an EGFR exon 20 insertion[133]. For genomic alterations that co-occur with EGFR mutations, drug combinations could be pursued (FIGS 4,6). For example, as IGF1R signalling can mediate disease persistence through the PI3K–AKT pathway[87], addition of a GF1R-specific antibody (reviewed in REF. 134) or a PI3K or AKT inhibitor to TKI treatment could be beneficial. Finally, for *EGFR*-wild-type tumours, multiple approaches are being taken, based on the presence of other driver mutations (in genes such as *ALK*, *KRAS* and *BRAF*) that are found in these tumours. This topic has been reviewed elsewhere (for example, REF. 135).

Acquired resistance

Acquired resistance remains a major clinical problem in *EGFR*-mutant lung cancer, usually occurring within a year of starting treatment. Based on the molecular mechanisms discussed above and additional studies discussed below, multiple clinical trials have been initiated and/or are being planned.

Second-generation EGFR TKIs

Second-generation EGFR inhibitors were touted to overcome T790M-mediated resistance[136,137]. These agents were shown, at least in pre-clinical models, to be more potent against the second-site mutation than gefitinib or erlotinib. However, their clinical efficacy remains to be established (TABLE 2).

Irreversible EGFR inhibitors make a covalent bond with C797 of the EGFR (TABLE 2). The first drug tested, HKI-272 (neratinib; Wyeth), showed promising preclinical results[138] but no responses were reported in a Phase I trial involving 14 patients with NSCLC with EGFR-positive tumours (measured by IHC) and six patients who had previously progressed on erlotinib achieved stable disease[139]. The Phase II trial showed an overall RR of 3% but no responses were observed in patients who had tumours that harboured T790M[140]. Interestingly, three of the four patients with a G719X mutation had a partial response and the remaining patient had stable disease. This drug is no longer being developed for the treatment of lung cancer.

Multiple other second-generation irreversible EGFR inhibitors have also entered the clinic. BIBW2992 (afatinib; Boehringer Ingelheim) has potent activity against EGFR and ERBB2 and can overcome T790M-mediated resistance *in vitro* and *in vivo*[121,141] (TABLE 2). Multiple Phase II and Phase III trials are currently underway in patients with *EGFR*-mutant and TKI-naive NSCLC and in patients who have progressed on previous TKI treatment. PF-00299804 also binds irreversibly with activity against all ERBB family members (TABLE 2). It has also shown efficacy against H1975 (EGFR L858R and T790M) cells and xenograft models[142,143]. Results from early clinical trials are pending.

Continuous exposure of *EGFR*-mutant NSCLC cell lines to gefitinib or erlotinib has derived clinically relevant mechanisms of acquired resistance (for example, cells with T790M or *MET* amplification)[41,83,144], validating this approach as an *in vitro* tool to anticipate resistance mechanisms. Using a similar strategy, HKI-272 and BIBW2992 also select for T790M-harbouring clones[54,145]. *In vitro*, gefitinib-resistant cells already harbouring T790M further amplify the T790M allele on exposure to PF-0299804 (REF. 146). The limited efficacy of second-generation irreversible EGFR TKIs has been corroborated by

chemogenomic profiling of a panel of irreversible compounds[113]. The growth inhibitory potential of these agents was limited by decreased target binding in the presence of the T790M mutation. Collectively, these data suggest that these three irreversible inhibitors are more potent than erlotinib against T790M but that their clinical efficacy will be limited by pharmacokinetic issues — that is, can the levels of drug achieved in patients be high enough to inhibit T790M without toxicity?

Third-generation EGFR TKIs

Recently, a new compound, WZ4002 (Gatekeeper Pharmaceuticals), was discovered through screening different core chemical scaffolds for their ability to fit in the ATP-binding pocket of EGFR specifically in the presence of T790M[147]. Instead of the quinazoline core used in all reversible and irreversible inhibitors to date (TABLE 2), WZ4002 is built on an anilinopyrimidine core that fits the gatekeeper mutation while binding irreversibly to C797. In contrast to existing second-generation EGFR TKIs, this agent selectively targets T790M-harbouring receptors (TABLE 2) and induces greater growth inhibitory effects *in vitro* and *in vivo* against double-mutant EGFRs than those harbouring only drug-sensitizing mutations or wild-type EGFR. Therefore, the data suggest that T790M-harbouring receptors will be inhibited effectively at doses that will not affect wild-type EGFR and cause toxicity.

Drug combinations

Based on the mechanisms of primary and acquired resistance in *EGFR*-mutant NSCLC, several rational combinations have been tested in preclinical models (FIGS 4,6). To simultaneously target signalling from EGFR and its downstream target AKT, irreversible EGFR inhibitors have been paired with mTOR inhibitors. Combination therapy with BIBW2992 and rapamycin resulted in greater tumour shrinkage than either agent alone in transgenic mice with T790M-containing lung tumours[141,148]. Whether this strategy is efficacious in patients with acquired resistance remains to be established. Dual inhibition of EGFR with BIBW2992 and cetuximab also seems a promising strategy, because only this combination of agents effectively targets EGFR T790M[54] (FIG. 4). A clinical trial with these agents is currently underway.

As MET signalling can also contribute to TKI resistance, multiple MET inhibitors are being investigated for their potential activity in tumours that harbour these resistance mechanisms (FIG. 4). Antibodies targeting HGF (AMG102), MET (MetMAb) and small molecular inhibitors of MET (reviewed in REF. 149) are currently in development (FIGS 4,6). Notably, many of these trials may demonstrate limited efficacy. For example, trials of a second-generation EGFR TKI will not address tumours with *MET* amplification, and trials with a MET inhibitor plus gefitinib or erlotinib will not address tumours harbouring T790M. Moreover, heterogeneous mechanisms of resistance can exist in different tumours in the same individual (see below).

Finally, the question of whether to continue treatment with an EGFR TKI in patients who develop acquired resistance and do not participate in clinical trials with EGFR inhibitors has not yet been answered. In standard oncology practice, progression on TKI leads to the permanent discontinuation of that therapy and the initiation of an alternative therapy, usually one involving cytotoxic agents. However, the disease flares and re-responses to drug discussed above suggest that continued EGFR TKI suppression is likely to be beneficial even after disease progression has developed.

Tumour heterogeneity and resistance

The exact percentage of resistant cells necessary to confer what appears clinically as radiographic progression within a tumour lesion is currently unknown. Moreover, whether

resistant tumours are a homogeneous mass of TKI-resistant cells or a heterogeneous mixture of sensitive and resistant cells is almost impossible to determine from routine biopsy samples. However, several lines of evidence support the hypothesis that resistant tumours are a mixture of sensitive and resistant cells. First, the pre-existence of rare cells harbouring *MET* amplification and/or the T790M mutation in untreated tumours suggest that these subpopulations may be selected for over the course of TKI therapy[77,102]. Second, the re-treatment phenomenon (discussed above) suggests that different populations of tumour cells may become dominant under different conditions[118]. Collectively, these data suggest that at every stage of treatment, patients' tumours should ideally be freshly profiled as comprehensively as possible to assign the most appropriate and rationally based therapy.

Preventing or delaying acquired resistance

An alternative long-term strategy to address the problem of acquired resistance to TKIs is to delay or prevent the acquisition of resistance (FIG. 6). One approach is to investigate the effect of different dosing strategies using existing drugs, as the optimal dosing regimens for *EGFR*-mutant tumours have not been studied. Mathematical modelling suggests that different dosing schedules may influence the time to acquired resistance without compromising efficacy[150]. A similar observation in CML has been documented in which transient inhibition of breakpoint cluster region (BCR)–ABL with dasatinib (Sprycel; Bristol–Myers Squibb) induced similar killing rates as chronic exposure[151]. Alternatively, multiple potential combination strategies have been elucidated to treat patients earlier rather than later in the course of their disease (FIG. 4).

Perspective

Over a short period of time, translational research has described a new clinically relevant molecular subset of NSCLC that is defined by *EGFR* mutations. Today, patients with metastatic disease can achieve survival rates at least double that of patients with wild-type *EGFR* tumours. Through the rational dissection of the mechanisms of drug sensitivity and resistance, promising strategies have been defined to further improve the outcomes of patients with *EGFR*-mutant lung cancer. This molecular-centric approach will hopefully serve as a paradigm for how to understand and treat other cancers for which targets and/or targeted therapies have already been or remain to be established.

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Glossary

Driver mutation	An oncogenic mutation that induces and sustains tumorigenesis
Oncogene addiction	The phenomenon in which cancer cells become dependent on or addicted to signalling from oncogenic mutants for survival
Primary resistance	The initial resistance to therapy

Gefitinib	The first quinazoline-based reversible small-molecule EGFR tyrosine kinase inhibitor
Erlotinib	An FDA-approved quinazoline-based EGFR inhibitor
Prospective single-arm study	A clinical trial in which a drug is administered in a prospective manner to a single group of patients (defined by certain characteristics) to see whether their condition improves. Single-arm studies are distinct from two-arm studies, in which a group of patients is randomly administered one of two possible treatments (for example, an experimental treatment versus standard treatment) to determine which treatment is better
Response rate	RR. The proportion of patients undergoing a documented radiographic response as determined by response evaluation criteria in solid tumours
Progression-free survival	PFS. The length of time during and after treatment in which a disease does not progress
Time to progression	TTP. Time from the beginning of treatment until treatment failure
Never smoker	An individual who has smoked <100 cigarettes in their lifetime
Former light smoker	An individual who has stopped smoking for at least 15 years previously and has a total of ≤10 pack-years of smoking
Carboplatin–paclitaxel	An example of a platinum doublet for first-line treatment of NSCLC
Hazard ratio	HR. A measure of how often an event happens in one group compared with how often it happens in another group
Confidence interval	CI. A calculated value that shows the range in which a particular treatment effect is likely to be observed
Chimeric IgG monoclonal antibody	A recombinant antibody made from two species (in the case of cetuximab, the fusion contains human and mouse sequences)
Acquired resistance	Resistance that develops after the initial response to therapy
Gatekeeper residue	A conserved residue that lies at the opening of the ATP-binding pocket in several kinases
Disease flare	Rapid tumour growth following withdrawal of therapy
Resistance mutation screen	A comprehensive cell-based screen to identify all potential mutations in a target gene that confer resistance to a given agent
Irreversible EGFR inhibitor	A small-molecule inhibitor that binds permanently in the ATP-binding pocket of EGFR through a covalent bond at C797
Chemogenomic profiling	The technique of coupling chemical compound sensitivity to genomic signatures

Quinazoline core	A scaffold built on the fusion of a benzene ring and a pyrimidine ring
Anilinopyrimidine core	A scaffold built on an anilino group and pyrimidine ring

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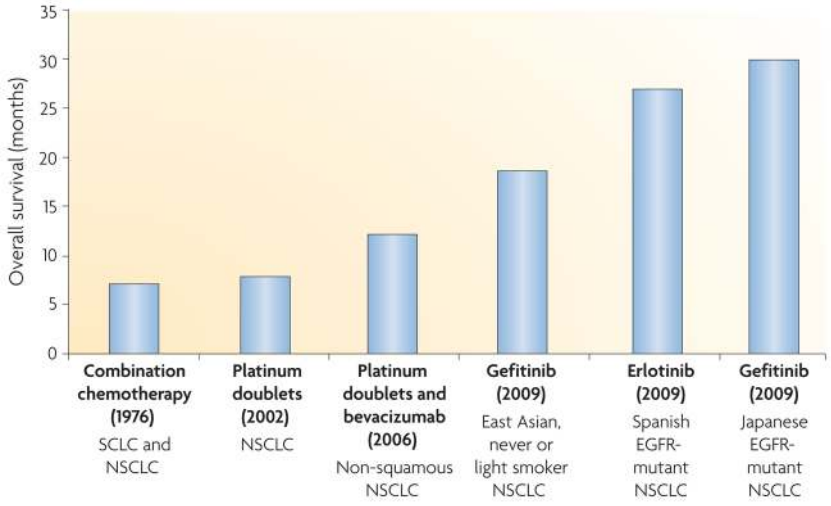

Figure 1. Progress in the treatment of metastatic lung cancer

In 1976, a chemotherapy trial studied all patients with lung cancer, regardless of whether they had small-cell lung cancer (SCLC) or non-small-cell lung cancer (NSCLC)[3]. In 2002, a landmark chemotherapy trial involving platinum doublets studied all patients with NSCLC, regardless of histological subtype (adenocarcinoma, squamous cell carcinoma and large-cell carcinoma)[152]. In 2006, bevacizumab (Avastin; Genentech/ Roche) was shown to confer an overall survival benefit when added to chemotherapy for patients with non-squamous NSCLC[153]. The smoking history of patients was not recorded. In 2009, trials in epidermal growth factor receptor (*EGFR*)-mutant lung cancer with EGFR tyrosine kinase inhibitors (TKIs) demonstrated the longest survival rates currently seen for NSCLC[20,21,47]. Notably, patients with *EGFR*-mutant lung tumours also have a better prognosis in the absence of therapy compared with those with *EGFR*-wild-type tumours[20].

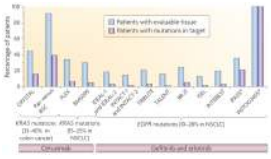

Figure 2. Tissue accrual across multiple trials

Trials in colon cancer (left side of graph), in which *KRAS* mutations are observed in 33–40% of tumour samples, were highly efficient at collecting tissue samples (45–92% patients had suitable tissue available for molecular analyses and 16–40% of patients had *KRAS* mutations). Based on the poor responses observed in patients with *KRAS*-mutant tumours, the KRAS biomarker was easily found to be a negative predictor of anti-epidermal growth factor receptor (EGFR) therapy (for example, cetuximab and panitumumab (Vectibix; Amgen) efficacy[154,155]). By contrast, in lung cancer, the role of *KRAS* mutations (FLEX and BMSO99 trials) could not be accurately determined in trials with cetuximab. The prevalence of *KRAS* mutations is 15–25%, only 30–34% of patients had tissue available for analysis and only 5–6% of patients had *KRAS* mutations[47,49]. Similarly, study of the role of *EGFR* mutations has been hampered by low tissue accrual (right section of the graph). *EGFR* mutations are found in 10–28% of patients with non-small-cell lung cancer (NSCLC), but tissue accrual in the major trials involving EGFR tyrosine kinase inhibitors (IDEAL-1, IDEAL-2, INTACT-1, INTACT-2, TRIBUTE, TALENT, BR.21, ISEL and INTEREST) was < 24% (blue bars)[20,21,34,156–160]. Of the patients with available tumour samples, the percentage that harboured an *EGFR* mutation (purple bars) was <5%, making it difficult to draw conclusions. However, in IPASS and WJTOG3405, in which these percentages were much higher, *EGFR* mutations were readily found to be a positive predictor of benefit. BSC, best supportive care; Pan, panitumumab. *Represents clinically enriched trials.

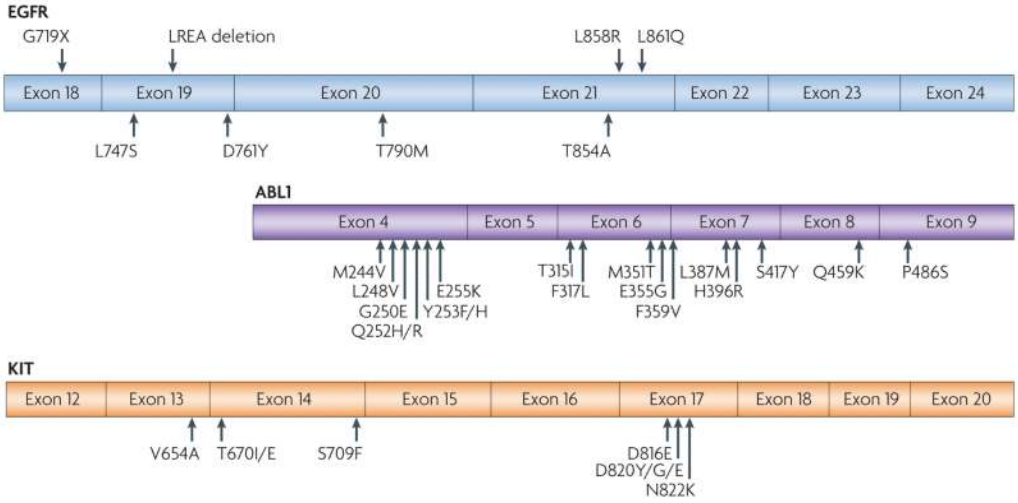

Figure 3. Comparison of TKI-sensitive and TKI-resistant mutations in cancer-derived mutant TKs

Epidermal growth factor receptor (*EGFR*)-mutant lung cancer, breakpoint cluster region (*BCR*)–*ABL*-driven chronic myelogenous leukaemia (CML) and *KIT*-mutant gastrointestinal stromal tumour (GIST) have all been treated effectively with specific tyrosine kinase inhibitors (TKIs); that is, gefitinib or erlotinib for lung cancer, imatinib for CML and imatinib for GIST. Activating drug-sensitive mutations are shown on the top of EGFR. TKI-resistant mutations are depicted on the bottom of each kinase domain schematic. The most common activating mutations in *EGFR* are a point mutation in exon 21, which substitutes an arginine for a leucine (L858R), and a small deletion in exon 19 that removes four amino acids (LREA). Together, these genomic changes account for ~90% of TKI-sensitive mutations that are observed in *EGFR*-mutant tumours. Other major drug-sensitive mutations include G719X (encoded by exon 18) and L861Q (exon 21).

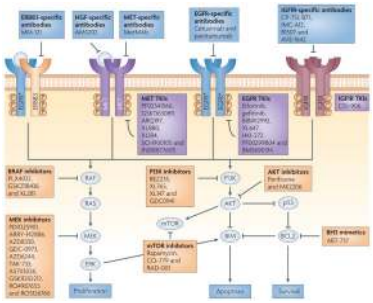

Figure 4. Multi-pathway inhibition as a strategy to treat EGFR-mutant NSCLC

Epidermal growth factor receptor (EGFR) mutants (starred) propagate signals through the PI3K–AKT and ERK pathways. Cross-activation of other membrane-bound receptor tyrosine kinases occurs under tyrosine kinase inhibitor (TKI)-sensitive states and following the development of acquired resistance (arrows). The boxes depict a sample of the targeted agents available for the treatment of the disease at various stages (see FIG. 6 for more details). IGF1R, insulin-like growth factor receptor 1; NSCLC, non-small-cell lung cancer; P, phosphorylation.

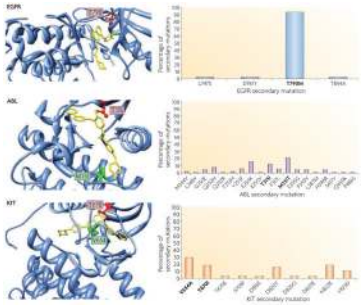

Figure 5. Comparison of second-site mutation frequency following development of acquired resistance to TKI therapy

All patients with epidermal growth factor receptor (*EGFR*)-mutant non-small-cell lung cancer (NSCLC) will inevitably develop acquired resistance following treatment with the tyrosine kinase inhibitors (TKIs) gefitinib or erlotinib. In ~50% of cases, resistance is attributed to a second-site mutation in *EGFR*. The change of the gatekeeper threonine to a methionine (T790M) accounts for ~90% of secondary mutations observed in *EGFR*[56,107,121]. By contrast, second-site resistance mutations found in ABL and *KIT* following treatment with imatinib in chronic myelogenous leukaemia (CML) and gastrointestinal stromal tumour (GIST), respectively, are found across the kinase domain (see graphs on right side of figure). Mutations affecting the analogous gatekeeper residue in ABL (T315)[161,162] and KIT (T670)[163,164] are observed in less than 20% of cases. Gatekeeper residues are shown in red in the crystal structures. For ABL and KIT, the most common secondary mutation is shown in green. EGFR is shown crystallized with gefitinib (yellow); ABL and KIT were both crystallized with imatinib (yellow). Crystal structures were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB Data Bank; see Further information; accession numbers 2ITY (EGFR)[60], 2HYY (ABL)[165] and 1T46 (KIT)[166]). The structural graphics were produced using the University of California San Francisco (USCF) Chimera package[167] (see Further information) from the Resource for Biocomputing, Visualization and Informatics at the USCF, USA.

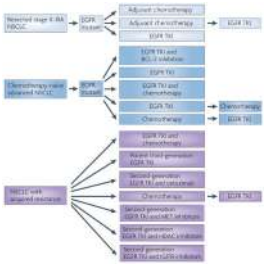

Figure 6. Potential treatment strategies to cure EGFR-mutant lung cancer

The optimal treatment strategies for patients with epidermal growth factor receptor (*EGFR*)-mutant tumours that present with early-stage disease (pale blue, top), late-stage disease (blue, middle) and acquired resistance (purple, bottom) are an active area of investigation. Patients with resectable tumours may benefit from adjuvant chemotherapy, tyrosine kinase inhibitors (TKIs) or both in varying sequence of treatment. Patients with late-stage disease may benefit from combination therapy with a TKI, which may delay or prevent the emergence of acquired resistance. For example, agents targeting the apoptotic pathway combined with TKIs enhance cell death of *EGFR*-mutant cells in preclinical models[66,68,69,123]. Alternatively, the addition of chemotherapy before, after or concurrent with TKI treatment may induce a synergistic response. Finally, in the case of acquired resistance, continuation of the TKI in combination with various other agents may be the most beneficial strategy. However, the selection of additional therapies depends heavily on the molecular composition of the tumour and the mechanism of resistance. HDAC, histone deacetylase; IGF1R, insulin-like growth factor receptor 1; NSCLC, non-small-cell lung cancer.

Table 1

select clinical trials in lung cancer involving anti-EGFR therapies

Trial	Type	Drugs	enrollment criteria	rr (%) (EGFR TKI versus other)	Median time to treatment failure (months) (EGFR TKI versus other therapy)	refs
Gefitinib						
IDEAL-1, IDEAL-2	Phase II	Gefitinib (250 mg versus 500 mg)	Unselected previously treated NSCLC	18.4–19.0 (IDEAL-1) and 9–12 (IDEAL-2)	2.7–2.8 (IDEAL-1) and 1.5–1.7 (IDEAL-2)	11, 12
ISEL	Phase III	Gefitinib versus placebo	Unselected previously treated NSCLC	8.0 versus 1.3	3.0 versus 2.6	19
INTACT-1, INTACT-2	Phase III	Chemotherapy ± gefitinib (250 mg versus 500 mg)	Unselected chemotherapy-naive NSCLC	50.3–51.2 versus 47.2 (INTACT-1) and 30 versus 28.7 (INTACT-2)	5.5–5.8 versus 6.0 (INTACT-1) and 4.6–5.3 versus 5.0 (INTACT-2)	168, 169
INTEREST	Phase III	Gefitinib versus docetaxel	Unselected previously treated NSCLC	9.1 versus 7.6	2.2 versus 2.2	157
IPASS	Phase III	Gefitinib versus chemotherapy	East Asian never or light smokers with chemotherapy-naive lung adenocarcinoma	43.0 versus 32.2* 71.2 versus 47.3‡	5.7 versus 5.8* 9.5 versus 6.3‡	20
WJTOG3405	Phase III	Gefitinib versus chemotherapy	Japanese *EGFR*-mutant chemotherapy-naive NSCLC	62.1 versus 32.2	9.2 versus 6.3	21
NEJ002	Phase III	Gefitinib versus chemotherapy	Japanese *EGFR*-mutant chemotherapy-naive NSCLC	73.7 versus 30.7	10.8 versus 5.4	24
Erlotinib						
NA	Phase II	Erlotinib	NSCLC with BAC features	22	4	13
BR.21	Phase III	Erlotinib versus placebo	Unselected previously treated NSCLC	8.9 versus <1	2.2 versus 1.8	18
TALENT	Phase III	Chemotherapy ± erlotinib	Unselected chemotherapy-naive NSCLC	31.5 versus 29.9	6.4 versus 6.0	158
TRIBUTE	Phase III	Chemotherapy ± erlotinib	Unselected chemotherapy-naive NSCLC	21.5 versus 19.3	5.1 versus 4.9	170
SLCG	Single arm	Erlotinib	Spanish *EGFR*-mutant NSCLC	70.6	14	23
Cetuximab						
FLEX	Phase III	Chemotherapy ± cetuximab	EGFR IHC-positive chemotherapy-naive NSCLC	36 versus 29	4.8 versus 4.8	171
BMS099	Phase III	Chemotherapy ± cetuximab	Unselected chemotherapy-naive NSCLC	25.7 versus 17.2	4.4 versus 4.2	49

BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; NA, not applicable; NSCLC, non-small-cell lung cancer; RR, response rate; TKI, tyrosine kinase inhibitor.

*Statistic for the entire population.

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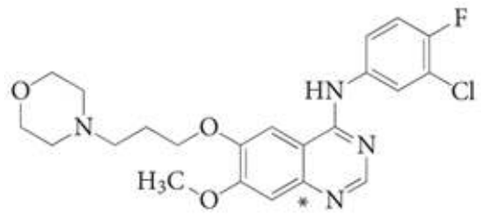
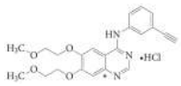
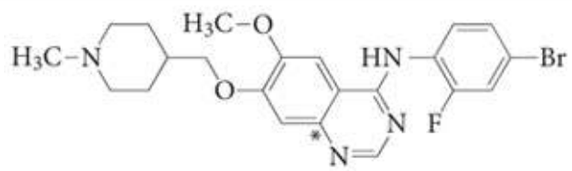
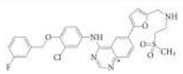
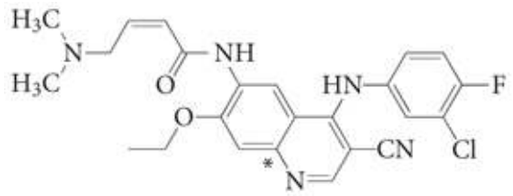
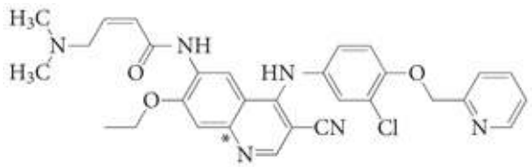
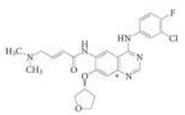
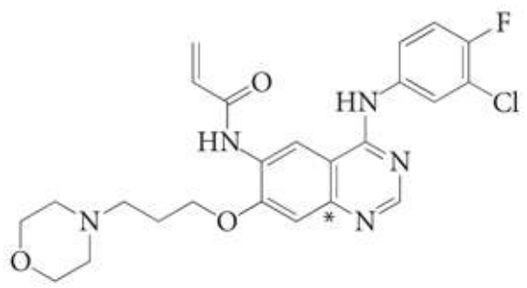
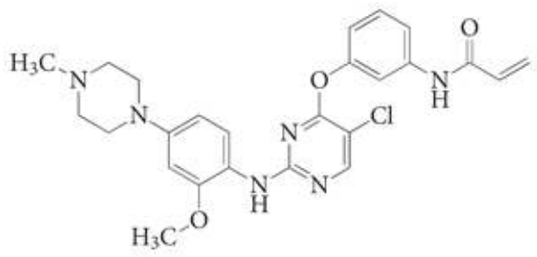
‡Statistic for the patients only with EGFR-mutant tumours.

Table 2

summary of current small-molecule EGFR inhibitors

Drug structure	Drug name	Generic name	Trade name	Company	Target	EGFR inhibition	Conformation	FDA approved	Refs
Reversible									
	ZD1839	Gefitinib	Iressa	AstraZeneca	EGFR	Mut>T790M	Active		172
	OSI-774	Erlotinib	Tarceva	OSI/Genentech	EGFR	Mut>T790M	Active	NSCLC	173
	XL647			Exelixis/Symphony Evolution	EGFR, ERBB2, VEGFR and EPHB4	Mut>T790M			174
	ZD6474	Vandetanib	Zactima	AstraZeneca	EGFR and VEGFR	Mut>T790M			175
	GW572016	Lapatinib	Tykerb	GlaxoSmithKline	EGFR and ERBB2	Unknown	Inactive	Breast	176
Irreversible (bind covalently to C797)									
	EKB-569	Pelitinib		Wyeth	EGFR	Mut>T790M			177

Drug structure	Drug name	Generic name	Trade name	Company	Target	EGFR inhibition	Conformation	FDA approved	Refs
	HKI-272	Neratinib		Wyeth	EGFR and ERBB2	Mut>T790M			178
	BIBW2992	Afatinib		Boehringer Ingelheim	EGFR and ERBB2	Mut>T790M			141
	CI-1033	Canertinib		Pfizer	pan-ERBB	Unknown			179
	PF00299804			Pfizer	pan-ERBB	Mut>T790M			142
	WZ4002			Gatekeeper Pharmaceuticals	EGFR	T790M>Mut			147

EGFR, epidermal growth factor receptor; EPHB4, ephrin type-B receptor 4; FDA, US Food and Drug Administration; Mut, mutant EGFR (exon deletion or L858R); NSCLC, non-small-cell lung cancer; VEGFR, vascular endothelial growth factor receptor; WT, wild-type EGFR.

*Quinazoline cores.