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New Drugs

Novel therapeutic strategies for patients with NSCLC that do not respond to treatment with EGFR inhibitors

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ABSTRACT

Introduction: Treatment with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) yields tumour responses in non-small cell lung cancer (NSCLC) patients harbouring activating EGFR mutations. However, even in long-lasting responses, resistance to EGFR TKIs invariably occurs.
Areas covered: This review examines resistance mechanisms to EGFR TKI treatment, which mainly arise from secondary EGFR mutations. Other resistance-inducing processes include mesenchymal–epithelial transition factor (MET) amplification, epithelial–mesenchymal transformation, phenotypic change from NSCLC to small-cell lung carcinoma, and modifications in parallel signalling pathways. Current therapeutic strategies to overcome these EGFR TKI resistance mechanisms focus on the inhibition or blocking of multiple members of the ErbB family. Several molecules which target multiple ErbB receptors are being investigated in NSCLC and other indications including afatinib, an ErbB Family Blocker, as well as dacomitinib and lapatinib. Novel, non-quinazoline, EGFR inhibitors, that also target EGFR activating and resistance (T790M) mutations, are currently under clinical development. Other therapeutic strategies include inhibition of parallel and downstream pathways, using agents which target heat shock protein (HSP)90 or

Abbreviations: AEG-1, astrocyte elevated gene-1; ALK, anaplastic lymphoma kinase; ATP, adenosine triphosphate; BARD1, BRCA1-associated protein 1; BIM, B-cell lymphoma 2 interacting mediator of cell death; BRAF, v-raf murine sarcoma viral oncogene homolog B1; BRCA1, breast cancer 1, early onset; CNS, central nervous system; CRKL, crk-like protein; DCR, disease control rate; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transformation; EphB4, ephrin type-B receptor 4; ER, oestrogen receptor; ERK, extracellular-signal-regulated kinases; HER, human epidermal growth factor receptor; HGF, hepatocyte growth factor; HSP90, heat shock protein 90; IGF-1R, insulin-like growth factor 1 receptor; KRAS, Kirsten rat sarcoma viral oncogene homologue; MBP-QP, mutation-biased PCR quenching probe; MEK, mitogen-activated protein kinase; MET, mesenchymal–epithelial transition factor; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NSCLC, non-small-cell lung cancer; ORR, overall response rate; OS, overall survival; PARP, poly (ADP-ribose) polymerase; PCR, polymerase chain reaction; PFS, progression free survival; PI3K, phosphoinositide-3-kinase; PIK3CA, phosphoinositide-3-kinase, catalytic, alpha polypeptide; PTEN, phosphatase and tensin homologue; RECIST, Response Evaluation Criteria in Solid Tumours; RR, response rate; SCLC, small-cell lung cancer; TGF, transforming growth factor; TKI, tyrosine kinase inhibitors; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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poly (ADP-ribose) polymerase in addition to mammalian target of rapamycin (mTOR), monoclonal antibodies against the insulin-like growth factor-1 receptor, and fulvestrant-mediated oestrogen receptor regulation.

Conclusion: Improved understanding of mechanisms underlying resistance to EGFR TKIs emphasises the importance of a genotype-guided approach to therapy. Elucidation of resistance mechanisms is indeed crucial to target innovative therapeutic approaches and to improve the efficacy of anticancer regimes in NSCLC.

Introduction

Non-small cell lung cancer (NSCLC) is the most common form of lung cancer, accounting for approximately 85% of cases. Disease staging is essential in defining clinical management, and surgical resection remains the best option in early stage disease. However, 70% of patients have locally advanced or metastatic disease at diagnosis. To date first-line platinum-based chemotherapy has represented the standard treatment for these patients, achieving about 30% as response rates (RR) and an approximate 12-months median overall survival (OS). More recently, the molecular biology of lung cancer has been shown to play an important part in its pathology. Genotyping for key mutations has become clinically relevant, guiding the success of new therapies in NSCLC compared with traditional chemotherapy [1].

Development of EGFR TKIs

One particular area of research interest has focused on the ErbB Family, whose four members – epidermal growth factor receptor (EGFR/ErbB1), human epidermal growth factor receptor 2 (HER2/ErbB2), ErbB3 and ErbB4 – are central for the regulation of downstream signalling pathways important for tumour cell proliferation, survival, migration and metastasis (Fig. 1). Targeting EGFR has become an essential strategy in the treatment of NSCLC [2].

Two reversible EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, are currently commercially available for the treatment of NSCLC. These agents were first investigated in unselected NSCLC patients with encouraging results [3–6] but subsequent trials proved disappointing [7–9]. However, more recently clinical trials have shown clinical benefit of both gefitinib and erlotinib in a specific population of NSCLC patients with tumours bearing *EGFR* activating mutations [10–17]. The two most common EGFR mutations, accounting for >85% of all EGFR alterations, are in-frame deletions in exon 19 (del19) or point mutations in exon 21 (L858R) [18] while exon 18 mutations are less frequent (approximately 4%) [19,20]. The del19 and L858R mutations are present in ~10% of Caucasian patients and 30–50% of Asian patients with NSCLC [21] and are sensitive to treatment with reversible EGFR TKIs [22].

Resistance to EGFR TKIs

While most (>75%) NSCLC patients with somatic EGFR mutations show initial responses to treatment with the reversible TKIs

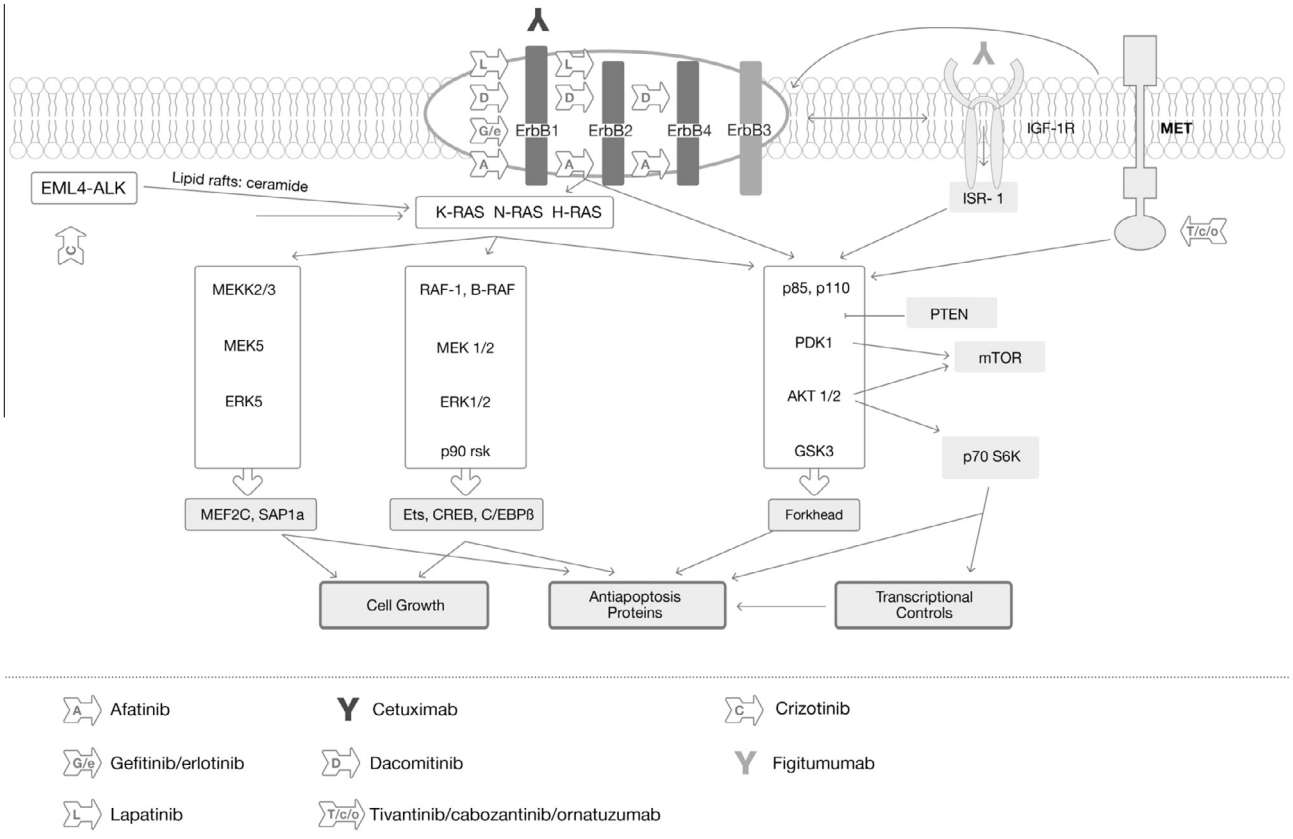

Fig. 1. Receptors and signalling pathways involved in the development of NSCLC.

[21] they invariably develop or 'acquire' resistance to these agents [21,23]. There are also a number of patients who do not respond to initial treatment with reversible EGFR TKIs and these patients are deemed to have primary or *de novo* resistance.

Primary resistance

There are several mechanisms of primary resistance (Table 1) some of which are common to acquired resistance but are present prior to EGFR TKI treatment.

De novo T790M EGFR mutation

The primary mechanism of *de novo* resistance is thought to be the T790M *EGFR* mutation which is present before EGFR TKI treatment (31.5–35% of tumours) [24,25] and may contribute to primary resistance. Specifically, the presence of the compound mutant *EGFR* is postulated to contribute to the Darwinian selection of pre-existing drug-resistant clones [26,27]. Thus, the fraction of the T790M allele would increase during EGFR TKI therapy to a threshold, which allows us to consider resistance 'acquired'. As a consequence the T790M detection rate changes from 31.5–35% in tumours pre-EGFR TKI therapy [24,25] to 83.3% post-EGFR TKI treatment [27]. These detection rates are obtained by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI–TOF MS), which is a highly sensitive method for detection of EGFR mutations. The detection rate of T790M is 2.8% in TKI-naive patients by direct sequencing. This low prevalence of *de novo* T790M detected by direct sequencing may be a result of the low copy number of T790M present in patients' tumour specimens. For this reason the evaluation of studies assessing the clinical role of T790M should take into account the type of detection method utilised.

However, the existence of the T790M mutation does not necessarily indicate a poorer outcome compared with patients without the T790M mutation. Oxnard and Sordella found that the combined *EGFR* mutations (del19 or L858R plus T790M), identified at the time of clinical progression on erlotinib, were associated with longer survival [28]. Likewise, Fujita et al. observed that patients who were strongly positive for T790M had significantly longer time to treatment failure on reversible EGFR TKIs than patients without T790M or those with modest positivity for the mutation (frequency of positive signals obtained from colony hybridization) [24]. Finally, T790M mutations have recently been associated with better progression-free survival (PFS) in EGFR-mutant NSCLC patients receiving chemotherapy [29]. These results suggest that the presence of the T790M mutation in a tumour with activating EGFR mutations does not necessarily preclude treatment with an EGFR TKI. Secondly, T790M has a prognostic and predictive value for EGFR TKI treatment outcomes; therefore, routine assessment of the T790M mutation in the diagnostic biopsy is warranted. Moreover, a better understanding of the role of T790M is crucial for the development of effective treatments to overcome this problem.

Brca1

The expression of *Breast Cancer Type 1 susceptibility protein* (BRCA1) has also been evaluated as a predictive marker of outcome in NSCLC patients treated with an EGFR TKI [25]. For NSCLC patients bearing a T790M mutation pre-TKI exposure, low BRCA1

Table 1
Resistance factors (mutations) to EGFR TKIs.

Type of mutation	Outcome
Primary resistance mechanisms*	
Basal T790M mutations	*De novo* reversible EGR TKI resistance. T790M mutation associated with a decreased progression-free survival in patients with NSCLC who received TKI treatment [25]
BRCA1 expression	Low levels of BRCA1 could improve the current negative perspectives and offer a longer progression-free survival with erlotinib [25]
NF-κB signalling	NF-κB hyperactivation predicts worse response and survival in erlotinib-treated NSCLC patients harbouring *EGFR*-mutant tumours [30]
BIM expression and polymorphism	A pro-apoptotic member of the B-cell CLL/lymphoma 2 (BCL2) which has an important role in resistance to EGFR TKIs [31ó34]
Acquired resistance mechanisms*	
Acquired T790M mutations	Resistance to reversible EGFR TKI treatment (affects over 50% of the cases of acquired resistance) [24, 35ó37]
MET amplification	MET, a receptor tyrosine kinase, stimulates the ErbB3-dependent activation of PI3K/Akt signalling, generating resistance to EGFR TKIs [39]
HGF amplification	Has been identified as a resistance factor in NSCLC patients [41ó44]
AXL upregulation	Expression of AXL and its ligand, GAS6, is increased and contributes to resistance in TKI-resistant EGFR-mutant NSCLC [45]
Epithelial-mesenchymal transformation	Common process in cancer pathogenesis and has been observed in NSCLC tumours with acquired resistance to reversible EGFR TKIs [46]
Conversion from NSCLC to SCLC	This transition seems to be specific to cells with resistance to EGFR TKI [46,49]
CRKL amplification	Induces resistance to gefitinib by activating extracellular signal-regulated kinase signalling [50,51]

* Some mechanisms may be common to both primary and acquired resistance. BIM, B-cell lymphoma 2 interacting mediator of cell death; BRCA1, breast cancer 1, early onset; CRKL, crk-like protein; EGFR, epidermal growth factor receptor; HGF, hepatocyte growth factor; MET, mesenchymal–epithelial transition factor; NSCLC, non small cell lung carcinoma; PIK3, phosphoinositide-3-kinase; TKI, tyrosine kinase inhibitor.

levels may improve the impact of T790M and promote a longer progression-free survival (PFS) with erlotinib. This suggests that establishing the presence of the T790M mutation and BRCA1 levels could aid appropriate treatment selection for such patients [25]. Furthermore, the coexpression of BRCA1 and the associated oncogene, astrocyte elevated gene-1, could represent a marker for prognosis in patients with wild-type *EGFR* and response to erlotinib treatment in patients with *EGFR* mutations [30].

NF-κB signalling and IκB

A recent study performed a pooled shRNA screen to identify genes that, when silenced, enhanced sensitivity to the EGFR TKI erlotinib [31]. Results revealed a potentially important role for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signalling in regulating EGFR oncogene dependence in *EGFR*-mutant NSCLC. Genetic or pharmacological inhibition of NF-κB significantly enhanced responses to erlotinib in *in vitro* and *in vivo* models of *EGFR*-mutant NSCLC [31]. Furthermore, clinical studies using *EGFR*-mutant NSCLC specimens from erlotinib-treated patients showed that NF-κB hyperactivation, as marked by low tumour levels of IκB, predicted worse response and survival than for patients treated with chemotherapy. Additional prospective studies validating NF-κB signalling as a predictive biomarker of EGFR TKI response and as a therapeutic target in *EGFR*-mutant NSCLC patients are underway.

BIM expression and polymorphisms

BIM, also known as BCL2-like 11, is a proapoptotic protein that is overexpressed in different malignancies. BIM upregulation is required for induction of apoptosis by EGFR TKIs in EGFR-mutant NSCLC and low BIM mRNA levels are thought to be a marker of primary resistance in these tumours [32]. Indeed BIM mRNA expression has recently been shown to be a biomarker of survival in EGFR-mutant NSCLC [33]. A study by Ng et al. has assessed the presence or absence of the *BIM* deletion polymorphism in 141 subjects with NSCLC from Singapore and Japan who were known to have activating mutations in EGFR and who received TKI therapy. The authors concluded that a common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to EGFR TKIs in cancer [34]. Recent studies have showed that HDAC inhibition can epigenetically restore BIM function *in vitro* and apoptotic sensitivity to EGFR-TKI, in cases of EGFR mutant NSCLC where resistance to EGFR-TKI is associated with a common BIM polymorphism [35].

Acquired resistance

In 2010, Jackman et al. proposed criteria to define and classify acquired resistance to EGFR TKIs in NSCLC patients with somatic *EGFR* gene mutations (Table 2) [22]. Many mechanisms of acquired drug resistance to reversible EGFR TKIs have been postulated (Table 1).

EGFR resistance mutations

In approximately 50–83% of EGFR mutation-positive NSCLC patients, resistance to a reversible EGFR TKIs is attributed to development or acquirement of a second *EGFR* mutation, the T790M mutation [24,36–38]. Several other mutations, including D761Y, L747S and T854A, have also been implicated in acquired resistance in patients who have previously responded to EGFR TKIs [2,39]. The reports of these mutations are rare and mainly attributed to single patient case reports in the literature but they do demonstrate the role of secondary mutations in acquired resistance.

MET and HGF amplification

Another significant group of cancers (approximately 20%) acquire resistance to reversible EGFR TKIs following the amplification of the mesenchymal–epithelial transition factor (MET) receptor tyrosine kinase (RTK), which in combination with ErbB3 activates different signalling pathways from EGFR [40]. MET, an RTK and its ligand, hepatocyte growth factor (HGF), regulate multiple cellular processes that stimulate cell proliferation, invasion, and angiogenesis. Alterations in MET (mutation, amplification or translocation) can produce tumourigenic effects. MET amplification stimulates the ErbB3-dependent activation of phosphoinositide-3-kinase (PI3K)/Akt signalling, which by-passes the effects of EGFR TKIs [40]. MET amplification is detected in 7% of EGFR TKI-naïve NSCLC patients who undergo surgical resection and approximately 20% of patients with acquired EGFR TKI resistance [40,41]. Evidence suggests that EGFR inhibition induces HGF-mediated clonal selection of pre-treatment MET amplification [42]. HGF is, therefore, another important resistance factor in NSCLC patients [43]. It is tempting to speculate that HGF production by stroma may also partly explain the discordant emergence of clinical resistance in some tissues such as liver, bone and brain, while pulmonary disease continues to respond to erlotinib treatment [42]. Researchers have discovered that HGF induces gefitinib resistance by restoring the PI3K/Akt pathway through Gab1, but not via EGFR or ErbB3 [42,43]. A recent study showed that the transient blockade of the PI3K/Akt pathway by PI-103 and gefitinib could overcome HGF-mediated resistance to EGFR TKIs by inducing apoptosis in *EGFR*-mutant lung cancer [44]. These observations suggest that targeting MET and HGF may counteract TKI resistance in EGFR-mutant lung cancer. However HGF overexpression detected by immunohistochemistry showed a favourable prognostic value [45].

AXL upregulation

AXL is a member of the TAM (Tyro3-AXL-Mer) family of RTKs, assuming broad functions in tumour cell growth, proliferation, migration, adhesion and chemosensitivity. An important and previously underappreciated role for AXL signalling in acquired EGFR TKI resistance in *EGFR*-mutant NSCLC was recently revealed [46]. In TKI-resistant *EGFR*-mutant NSCLC, increased expression of AXL and its ligand, GAS6 was observed and was required for resistance; genetic or pharmacological AXL inhibition restored sensitivity to

Table 2
Proposed criteria for acquired resistance to EGFR TKIs in NSCLC (adapted from Jackman et al., 2010) [22].

All patients should have the following	
1.	Received previous treatment with single-agent EGFR TKI (e.g. gefitinib or erlotinib)
2.	Have either or both of the following: a. An EGFR mutation-positive tumour, for which the EGFR mutation is known to be associated with drug sensitivity e.g. G719X, del19, L858R, L861Q b. An objective clinical benefit to EGFR TKI treatment
3.	Have shown systemic progression of disease (RECIST or WHO) within the last 30 days while on continuous treatment with gefitinib or erlotinib
4.	Received no intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy

EGFR = epidermal growth factor receptor; TKIs = tyrosine kinase inhibitors; NSCLC = non-small cell lung cancer; RECIST = Response Evaluation Criteria in Solid Tumours; WHO = World Health Organization.

erlotinib. Interestingly, elevated AXL and GAS6 co-existed with T790M in a minority of tumours, suggesting that these mechanisms may cooperate to promote resistance. In contrast, upregulation of AXL and MET was not shown, indicating that AXL or MET kinase activation alone is sufficient to function as a kinase-mediated bypass mechanism of EGFR TKI resistance. Prospective validation of AXL and GAS6 as biomarkers of EGFR TKI resistance and evaluation of promising therapeutic targets in *EGFR*-mutant NSCLC are ongoing.

Epithelial–mesenchymal transformation

Epithelial–mesenchymal transformation (EMT) is a common process in cancer pathogenesis and has been observed in NSCLC tumours with acquired resistance to reversible EGFR TKIs [47]. EMT includes processes underlying an increased potential for cancer cells to migrate to other tissues, such as the loss of epithelial cell characteristics and the development of new mesenchymal markers.

EMT has been associated with EGFR TKI resistance because cell behaviour can vary depending on their transformation stage. For example, cells containing wild-type *EGFR* that retained certain epithelial characteristics, such as E-cadherin expression, were more sensitive to erlotinib, whereas those that expressed mesenchymal markers such as vimentin and/or fibronectin were erlotinib-resistant [48]. A recent study suggested that MED12 loss induces an EMT-like phenotype associated with resistance to chemotherapy in colon cancer and to gefitinib in lung cancer [49]. As MED12 is partly cytoplasmic (where it negatively regulates transforming growth factor [TGF]-βR2 through physical interaction), TGF-βR inhibition restores drug responsiveness in MED12(KD) cells, highlighting a strategy to treat drug-resistant tumours with MED12 loss [49]. Interestingly, a recent study, also reported a relationship between EMT and erlotinib acquired resistance in an erlotinib sensitive lung cancer cell-line harbouring an EGFR deletion mutation. The authors found that cells acquired mesenchymal phenotype and exhibited down-regulation of E-cadherin expression, while the Histone deacetylase inhibitor, MS-275, restored E-cadherin expression and partial sensitivity to erlotinib [50]. Expression studies of these markers may be a very important source of diagnostic information.

Conversion of NSCLC to SCLC

Sequist et al. first described certain phenotypic changes in tumours with acquired resistance to TKIs. These changes affected a significant number of patients (14% of those studied) presenting with NSCLC whose biopsies – taken at the time of TKI resistance – revealed a small-cell lung cancer (SCLC) phenotype [47]. This transformation may be indicative of a pluripotent population of *EGFR*-mutant cancer cells or cancer stem cells as the source of resistance. The transition from NSCLC to SCLC appears specific to EGFR TKI-resistant cells. While the original *EGFR* mutation was maintained in all patients, none developed *EGFR* T790M or MET amplification. These patients received etoposide-based chemotherapy and showed a response similar to 'classical' SCLC. More recently, however a study by Yu et al. has shown that in EGFR mutation-positive NSCLC patients with acquired resistance to reversible EGFR TKIs, T790M, MET amplification and SCLC transformations have also been simultaneously observed [51]. Therefore, rebiopsy in lung cancer patients with acquired resistance to EGFR inhibitors can provide information about tumour cell phenotype, which can be relevant to choose an appropriate therapeutic strategy.

CRKL amplification

Multiple NSCLC cell lines and 3% of lung cancer specimens exhibit high-level amplification at cytoband 22q11.21 containing the crk-like protein (*CRKL*) gene, which contributes to cell proliferation and survival [52]. CRKL over-expression promotes anchorage-independent growth and tumourigenicity via SOS1-RAS-RAF-ERK and SRC-C3G-RAP1 pathway activation. Description of CRKL overexpression in an EGFR mutant cells induces resistance to gefitinib. The appearance of CRKL amplification in lung adenocarcinoma post-treatment with EGFR-inhibitors, suggests CRKL as an additional mediator of acquired resistance to epidermal growth factor receptor (EGFR)-inhibitors and credential it as a potential therapeutic target for a subset of NSCLC [52]. Hence, amplification and over-expression of CRKL contribute to oncogenic phenotypes in lung cancer, with relevance for therapy [53].

Other receptor mutations and signalling pathways involved in EGFR TKI resistance

Several receptor mutations as well as other signalling pathways and receptors have been shown to be modified in EGFR TKIs resistant tumours. These include increased expression of vascular endothelial growth factor (VEGF) which has been shown post-TKI therapy in patients with different malignancies, including squamous cell carcinoma and GEO (well differentiated colon cancer cell line). The insulin-like growth factor-1 receptor (IGF-1R) has been described as activating many of the same signalling pathways as EGFR [54]. EGFR TKI treatment increased IGF-1R expression, leading to the activation of PI3K/Akt signalling and increased resistance to EGFR TKIs [55]. In about 5% of NSCLC patients harbouring EGFR mutations, who developed resistance to EGFR TKIs, phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) mutation has been identified [47]. v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutations, which affect the RAS pathway, have also been observed in 1% of tumours with acquired resistance [56]. *HER2* amplication has been detected in NSCLC inducing an unfavourable prognostic value. It is mutually exclusive with T790M mutation in EGFR and has been found in 12% of lung cancers which developed resistance to EGFR TKIs [57]. *MAPK1* can also be amplified in lung cancer patients with acquired resistance to EGFR TKIs and has been identified in 5% of these patients and is mutually exclusive with both EGFR T790M mutation and MET amplification [58]. More recently preclinical work has shown that resistance to the EGFR TKI erlotinib is associated with reduced expression of neurofibromin [59]. Neurofibromin is a RAS GTPase encoded by the *NF1* gene. Erlotinib failed to fully inhibit RAS-ERK signalling when neurofibromin levels were reduced but treatment of neurofibromin-deficient lung cancers with a mitogen-activated protein kinase (MEK) inhibitor restored sensitivity to erlotinib. Furthermore, low levels of NF1 expression have been observed in EGFR TKI resistant lung adenocarcinoma patients [59]. Other potential mechanisms include upregulation of JAK2, an upstream STAT signalling pathway, and phosphatase and tensin homologue (PTEN) loss [2,60].

Monitoring resistance to EGFR TKI

Large-scale EGFR mutation screening interventions based on tumour tissue samples have proven their utility in NSCLC and can play an important role in clinical treatment decisions [61]. However, obtaining tumour tissue is challenging; for this reason, more sensitive and/or non-invasive techniques requiring a small amount of tissue or involving nucleic acid genotyping in blood samples have been tested for implementation in clinical practice.

New polymerase chain reaction techniques

Aside from *BRCA1*, other genes potentially involved in erlotinib resistance have been explored using NanoString® [62]. This tool

utilises digital technology based on a direct multiplexed measurement of targeted gene expression, allowing the expression analysis of 48 genes in a single reaction. Using NanoString®, both BRCA1-associated protein 1 (BARD1) and astrocyte elevated gene-1 (AEG-1) were found to predict PFS in erlotinib-treated *EGFR*-mutant NSCLC [62]. Hence, wider adoption of NanoString® may have important implications for optimal NSCLC management.

Peripheral blood samples for monitoring EGFR mutations

In NSCLC patients for whom obtaining tissue samples is challenging non-invasive tissue sampling methods could be useful. Blood samples drawn from NSCLC patients may serve as a source of tumoural DNA for the detection of EGFR mutations. The equivalence of EGFR mutations in serum samples and matched tumour tissue from patients with advanced disease was shown using two detection methods, mutant-enriched polymerase chain reaction (PCR)-based DNA sequencing and non-enriched sequencing [63]. A reported concordance rate of 93.1% for mutant-enriched sequencing supports its recommendation for routine practice.

Results obtained with a new non-invasive method for determining the presence of T790M using DNA extracted from plasma samples – the mutation-biased PCR quenching probe (MBP-QP) [64] – were comparable with other non-invasive DNA-based mutation detection systems. However, the MBP-QP method confers major advantages in terms of simplicity and sensitivity for detecting T790M in plasma samples, suggesting real utility in standard care.

Strategies for overcoming resistance to EGFR TKIs

New drugs affecting the ErbB signalling pathways

Recently, attention has focused on the simultaneous blocking of multiple members of the ErbB Family, achieving prolonged inhibition of EGFR signalling and reducing the development of resistance. Several new agents that block or inhibit the ErbB signalling pathways are currently under development or have been recently approved (afatinib in NSCLC) (Table 3 and Fig. 1).

Afatinib

Afatinib (BIBW 2992; Boehringer Ingelheim) is an orally bioavailable ErbB Family Blocker that irreversibly blocks signalling from EGFR (ErbB1), HER2 (ErbB2) and ErbB4 [65,66] and also blocks the transphosphorylation of ErbB3; thus blocking all relevant ErbB Family dimers [65].

In vitro and *in vivo*, afatinib has shown similar potency against the EGFR L858R mutation versus gefitinib and against the *HER2* YVMA mutant versus the EGFR/HER2 TKI, lapatinib [65,66]. Moreover, afatinib showed significantly greater activity against the TKI-resistant *EGFR* double mutant, L858R/T790M, than gefitinib. Furthermore, afatinib was more effective than erlotinib, gefitinib, and lapatinib in inhibiting human NSCLC cell lines harbouring wild-type *EGFR* or the L858R/T790M double mutant. Similarly, in xenograft models of NSCLC, afatinib has suppressed tumour growth to a greater extent than gefitinib or lapatinib and was also effective in xenografts resistant to EGFR TKIs [65]. These preclinical data suggested the potential for afatinib to offer clinical benefit to patients with ErbB Family-driven tumours. Overall afatinib has a low potential for drug–drug interactions [67]. Afatinib is not metabolised but enzyme-catalysed metabolic reactions and is not an inhibitor or an inducer of the CYP enzymes [67]. *In vitro* studies have shown that afatinib interacts with the drug transport systems involving p-glycoprotein (P-gp) and Phase I studies support the adjustment of afatinib dose as tolerated in patients requiring co-administration of P-gp inhibitors/inducers [67,68]. Afatinib is dosed once daily and should preferably not be taken with food; Phase I studies have shown that when consumed with a high fat meal exposure to afatinib is reduced [68,69].

Afatinib is being investigated in NSCLC in the LUX-Lung clinical trial programme as a first-line treatment in patients with EGFR-activating mutations (LUX-Lung 2, 3, 6 and 7) and as a second- or third-line treatment in patients previously treated with EGFR TKIs (LUX-Lung 1, 4 and 5) [70]. Afatinib is also being assessed in LUX-Lung 8, a Phase III, randomized trial comparing afatinib with

Table 3
Drugs targeting EGFR signalling pathways in NSCLC.

Agent	Detail
Afatinib	ErbB Family Blocker that irreversibly blocks signalling from EGFR (ErbB1), HER2 (ErbB2) and ErbB4 tyrosine kinases and transphosphorylation of ErbB3. It is active against *EGFR* mutations targeted by erlotinib and gefitinib but also against those insensitive to these therapies, that is the wild-type and the mutant forms of *EGFR* and *HER2*.
Cetuximab	Chimeric monoclonal antibody inhibitor of EGFR was associated with CRs in mice with tumours harbouring the T790M mutation or the L858R mutation. Under study in combination with afatinib.
Dacomitinib	Agent with activity against wild-type EGFR, HER2, and ErbB4 and effective against NSCLC cell lines with deletion of *EGFR* exon 19 and L858R mutation and L858R/T790M mutation.
Lapatinib	Reversible EGFR/HER2 TKI. It has limited activity in patients with advanced NSCLC.
AZD9291	Irreversible TKI targeting both EGFR activating and resistance (T790M) mutations. Limited inhibition in wild-type EGFR.
CO-1686	Irreversible TKI targeting with both EGFR activating and resistance (T790M) mutations. Limited activity in wild-type EGFR.
XL647	An oral small-molecule inhibitor of multiple receptor tyrosine kinases, including EGFR, VEGFR2, HER2 and EphB4.
Pelitinib, AV-412/MP-412 and BMS-599626	These are new EGFR tyrosine kinase inhibitors which are under development.

CR, complete response; EGFR, epidermal growth factor receptor; EphB4, ephrin type-B receptor 4; HER2, human epidermal growth factor receptor 2; NSCLC, non-small-cell lung carcinoma; TKI, tyrosine kinase inhibitor; VEGFR2, vascular endothelial growth factor receptor 2.

erlotinib in patients with advanced squamous cell NSCLC, who have progressed after platinum-based chemotherapy. The Phase IIb/III LUX-Lung 1 trial evaluated afatinib versus placebo in patients with NSCLC who had experienced prior treatment failure on reversible EGFR TKIs, gefitinib and/or erlotinib, and one or two lines of chemotherapy [71]. The median PFS was significantly longer ($P < 0.0001$) and response was higher with afatinib (PRs in 29 patients versus one patient) when compared with placebo. Although this did not translate into an OS benefit. The trial did not meet its primary endpoint since not survival benefit was observed with afatinib in the overall population (afatinib 10.8 months versus 12.0 months in the placebo). Interpretation of the lack of benefit in OS during the trial may be complicated by cancer treatments administered post-progression and the fact that afatinib was not the final treatment patients received. Indeed 79% and 68% of patients in the placebo and afatinib arms, respectively, received additional treatment. Afatinib treatment in LUX-Lung 1 was also associated with significant improvement in NSCLC-related symptoms and quality of life (QoL; $P \leqslant 0.05$) [72]. In the first-line setting, the clinical activity of afatinib was assessed in the proof-of-concept, Phase II LUX-Lung 2 study, which enrolled 129 patients with *EGFR*-mutation-positive NSCLC (>80% with Del19 or L858R mutations) [73]; 61 patients received afatinib first-line and 68 patients received afatinib as second-line therapy. Regardless of starting dose (50 mg ($n = 99$) or 40 mg ($n = 30$)), afatinib demonstrated notable anti-tumour activity in the selected patient population, with two complete responses (CRs) and 77 PRs; thus representing an overall response rate (ORR) of 61% (independent review). There was no significant difference in achieving an objective response between those who received afatinib treatment first-line (40 [66%] of 61) versus second-line (39 [57%] of 68; odds ratio [OR]: 0·71, 95% confidence interval [CI]: 0·35–1·44) or between those who received afatinib 40 mg as first dose (18 [60%] of 30) versus 50 mg as first dose (61 [62%] of 99; OR 1.07, 95% CI: 0.46–2.47). The subsequent LUX-Lung 3 trial was the largest randomised, global prospective Phase III study of afatinib versus cisplatin/pemetrexed as first-line treatment for patients with advanced adenocarcinoma of the lung harbouring EGFR-activating mutations [74]. The median PFS for afatinib treatment was significantly longer than for cisplatin/pemetrexed in the overall study population and in patients with common *EGFR* mutations, del19 and L858R. Recent results from LUX-Lung 6, the companion Phase III study to LUX-Lung 3, reinforce the superiority of afatinib over chemotherapy (gemcitabine/cisplatin) alone in the *EGFR* mutation-positive NSCLC patient population [75]. The safety profile of afatinib has demonstrated consistency across studies; the most frequent adverse events, diarrhoea and rash, were manageable [76,77]. On the basis of these key studies, afatinib monotherapy has gained approval for the treatment of EGFR TKI-naïve patients with locally advanced or metastatic NSCLC and activating *EGFR* mutations. Interestingly, recently a data analysis from LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6 have shown that afatinib is active in lung tumours harbouring uncommon EGFR mutations, such as G719X, L861Q and S768I [78]. The rate and duration of response was comparable with that previously observed in patients with common mutations in these trials. While the response rate was low in tumours with *de novo* T790M mutations and insertions in exon 20 durable tumour control was achieved in some patients. In LUX-Lung 3 [78].

Afatinib has also been studied in combination with cetuximab, a chimeric mAb against EGFR. Afatinib plus cetuximab was associated with CRs in mice with tumours harbouring the T790M mutation or the L858R mutation [79,80]. Preliminary results from a Phase I trial of *EGFR*-mutant NSCLC patients with progressive disease following treatment with erlotinib or gefitinib (NCT01090011) showed disease control in all patients ($n = 22$) treated with afatinib 40 mg plus cetuximab 500 mg/m^2 and confirmed PR in 8 patients (36%) including 4 patients with T790M mutation [81]. These findings are in contrast to negative results from a Phase I/II trial of cetuximab and erlotinib in patients with lung adenocarcinoma and acquired resistance to erlotinib [82].

Dacomitinib

Dacomitinib (PF-00299804; Pfizer) is active against wild-type EGFR, HER2, and ErbB4. Pre-clinical *in vitro* and *in vivo* studies showed activity for dacomitinib against *EGFR*-sensitising mutations, the *EGFR* T790M resistance mutation, and wild-type and mutant *HER2* [83]. Dacomitinib is metabolised by CYP2D6. Administration of drugs that are highly dependent on CYP2D6 metabolism may require dose adjustment or substitution with an alternative medication. Recent Phase I pharmacokinetic data suggest that dacomitinib exposure may be slightly affected by moderate hepatic impairment [84]. Since many patients with advanced cancer have liver metastases leading to impaired liver function, dose adjustment may be required.

Dacomitinib was clinically active in a two-arm Phase II trial in patients with advanced NSCLC who had failed 1 or 2 prior chemotherapy regimens and prior erlotinib treatment [85]. Other studies provided evidence of additional clinical activity in advanced lung carcinoma [86], versus erlotinib as second- or third-line therapy in patients with advanced NSCLC [87], and in Kirsten rat sarcoma viral oncogene homologue (*KRAS*) wild-type patients refractory to at least one chemotherapy regimen and erlotinib [88]. Interim results from an ongoing Phase II open label trial (NCT00818441) of dacomitinib showed that 74% of patients with advanced lung adenocarcinoma and *EGFR*-activating mutations experienced PR and remained progression-free at 1 year, and that the median PFS was 17 months [89]. Diarrhoea and skin and nail changes were common side effects.

Based on PFS benefits with dacomitinib versus erlotinib in a previous Phase II study of pre-treated patients [90], a randomised, double-blind, Phase III trial (ARCHER 1009) was initiated. The study was designed to compare dacomitinib with erlotinib in locally advanced or metastatic NSCLC after at least one prior chemotherapy regimen in two co-primary populations – all patients and patients with KRAS wild-type NSCLC [91]. Initial results of the study have recently been released and have shown that the study did not meet its primary objective and no significant improvement in PFS was observed in dacomitinib-treated patients versus those who received erlotinib [92]. A second study, BR.26, which is a double-blind placebo controlled randomized Phase III study assessing dacomitinib in patients with incurable stage IIIB/IV NSCLC after failure of standard therapy for advanced or metastatic disease has also recently release of initial findings. The study did not meet its objective of prolonging OS versus placebo [92]. A third study, ARCHER 1050, is ongoing and will assess dacomitinib versus gefitinib in treatment-naïve patients with EGFR-mutant advanced NSCLC. The results are expected in 2015 [92].

Other agents in development

Several new agents which target the ErbB Family and their signalling pathways are in development. Most recently these include AZD9291 and CO-1686. AZD9291 is an irreversible selective tyrosine kinase inhibitor that targets both EGFR activating and resistance (T790M) mutations. Preclinical work has demonstrated that AZD9291 potently inhibits EGFR phosphorylation across a number of *in vitro* cell lines harbouring EGFR-mutations including T790M [93]. While *in vivo* AZD9291 treatment has been associated with profound growth regression across multiple EGFR mutation-positive (PC9; 250% growth inhibition) and EGFR mutation and T790M positive (H1975; 132% growth inhibition) tumour models [93]. Tumour growth inhibition was associated with

profound inhibition of EGFR activity and key downstream signaling pathways and chronic long-term treatment of these xenograft tumours with AZD9291 led to a complete and sustained macroscopic response [93]. Preliminary data from a Phase I dose-escalation study in patients with advanced NSCLC who have progressed following EGFR TKI treatment ($n = 18$ plus T790M expansion cohorts [$n = 9$]) are encouraging with reports of about 50% Response Evaluation Criteria in Solid Tumours (RECIST) responses, at the starting dose level of 20 mg once daily ($n = 2$ confirmed partial responses in T790M mutation-positive patients) with good tolerability [93,94].

CO-1686 is an irreversible TKI that also targets EGFR activating and resistance (T790M) mutations. Preclinical work has demonstrated that oral administration of CO-1686 as single agent induces tumour regression in EGFR-mutated NSCLC tumour xenograft and transgenic models [95]. No inhibition of wild-type EGFR is observed. Interestingly in NSCLC cells with acquired resistance to CO-1686 *in vitro*, there was no evidence of additional mutations or amplification of the EGFR gene, but resistant cells exhibited signs of epithelial–mesenchymal transition and demonstrated increased sensitivity to AKT inhibitors. Initial reports from a Phase I dose-finding study are encouraging with CO-1686 demonstrating good tolerability and efficacy against proven T790M positive EGFR mutant NSCLC, with reports of about 67% responses, and a strong suggestion of a dose–response relationship [96].

XL647 is an oral small-molecule inhibitor of multiple RTKs, including EGFR, VEGFR2, HER2 and ephrin type-B receptor 4 (EphB4). XL647, administered in an intermittent or daily-dosing schedule, exhibited antitumour activity with an objective response rate (ORR) of 3% in TKI-resistant patients selected for *EGFR*-activating mutations [97]. The EGFR-driven component of NSCLC combined with resistance likely precludes the prolonged use of reversible or weak irreversible inhibitors in NSCLC. Neratinib, an EGFR and HER2 TKI, failed to produce a response in advanced NSCLC patients bearing the TKI-resistant T790M mutation. Even maximally tolerated doses of neratinib may be insufficient to prevent potential development of *EGFR* T790M. To overcome secondary acquired resistance to current quinazoline-derived EGFR TKIs, novel non-quinazoline EGFR inhibitors have been developed to specifically target T790M. Such agents have shown a powerful selective inhibition *in vitro* against *EGFR* T790M [98]. The EGFR TKI, icotinib hydrochloride (BPI-2009H), is being tested in preclinical studies, and has shown a significant effect [99,100]. Other new EGFR-targeted agents under development include the irreversible TKIs, pelitinib (EKB-569; EGFR, HER2, and ErbB4) and AV-412/MP-412 (EGFR and HER2), and BMS-599626, a reversible TKI (EGFR/HER2) [101,102].

Agents acting on alternative signalling pathways

Agents acting on other signalling pathways that complement the EGFR pathway are also being evaluated in clinical trials of advanced NSCLC (Table 4).

MET inhibitors

MET inhibitors are able to overcome EGFR TKI resistance. Even though specific studies of MET inhibitors for EGFR resistant have not been conducted yet, some evidence has emerged concerning the concomitant inhibition of MET and EGFR pathway. However the positive, encouraging results, reported in the phase II trials [103,104], were not confirmed in the subsequent, phase III studies. The phase III, MET Lung study (NCT01456325), of MET-inhibitor Onartuzumab (MetMAb) plus Erlotinib versus placebo plus Erlotinib, in previously treated, MET-positive, advanced NSCLC, was stopped, following an interim analysis that suggested a lack of clinically meaningful efficacy. A second phase III study exploring onartuzumab plus erolotinib in patients with MET/EGFR-positive advanced NSCLC (NCT02031744) is currently ongoing. Two randomized, phase III trials, MARQUEE and ATTENTION trials, comparing tivantinib plus erlotinib versus placebo plus erlotinib in non-squamous NSCLC, failed to meet their primary endpoint of OS. However, the secondary endpoint of PFS was significantly extended in favour of tivantinib combinations [105].

Anti-IGF-1R antibodies

There is a close relationship between the signalling of IGF-1R and EGFR, which supposes activation of the IGF-1R pathway when

Table 4
Drugs inhibiting alternative signalling pathways.

Agent	Detail
Anti-IGFR antibodies	
Figitumumab	Has shown activity in a phase II trial with previously untreated patients and those with advanced NSCLC who received paclitaxel/carboplatin with or without figitumumab
R1507	Binds to the extracellular domain of IGF-1R with high selectivity and inhibits receptor activation and function. It has been previously tested in some pre-clinical models, demonstrating anticancer activity in some cancers, including NSCLC
Heat Shock Protein inhibitors	Have been shown to be suppressors of the EGFR-mediated signalling in erlotinib-sensitive and erlotinib-resistant cell lines.
mTOR inhibitors	Everolimus, produced objective responses in 7.1% of NSCLC patients who had previously failed chemotherapy and in 2.3% of patients who had failed chemotherapy and an EGFR TKI
PARP inhibitors	Olaparib, combined with gefitinib is being compared with gefitinib monotherapy in patients with NSCLC bearing EGFR mutations by the Spanish Lung Cancer Group
Oestrogen receptor antagonists	The combination of fulvestrant with gefitinib resulted in greater inhibition of growth in cell cultures and xenograft models of NSCLC

ALK, anaplastic lymphoma kinase; ATP, adenosine triphosphate; EGFR, epidermal growth factor receptor; HSP90, heat shock protein 90; IGF-1R, insulin-like growth factor 1 receptor; MET, mesenchymal–epithelial transition factor; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; TKI, tyrosine kinase inhibitor.

EGFR is inhibited and the consequent increase in tumour cellular proliferation.

Figitumumab (CP-751,871; Pfizer) is a mAb targeted against IGF-1R [106]. In a Phase II trial of treatment-naïve or advanced NSCLC patients who received paclitaxel/carboplatin with or without figitumumab, RR was 54% versus 42% in the chemotherapy alone arm [107]. The addition of figitumumab to chemotherapy also improved PFS, compared with chemotherapy alone. However, patient enrollment in a Phase III clinical trial testing figitumumab plus paclitaxel/carboplatin was halted due to lack of efficacy.

Another mAb against IGF-1R is R1507. A Phase II trial randomly assigned patients with advanced-stage NSCLC who progressed following one or two prior regimens to receive erlotinib (150 mg orally once a day) in combination with either placebo, R1507 9 mg/kg weekly, or R1507 16 mg/kg intravenously once every 3 weeks [108]. The combination of R1507 with erlotinib did not confer a survival advantage over erlotinib alone in an unselected group of patients with advanced NSCLC [108].

Heat shock protein – HSP90 inhibitors

The heat shock protein (HSP)90 chaperone mediates conformational changes in the ErbB Family, MET, and various downstream kinases, including Akt. Therefore, inhibiting the action of HSP90 is a potentially viable treatment strategy given that *EGFR* mutations associated with resistance to EGFR TKIs do not compromise the ability of HSP90 to regulate ErbB Family members [109]. Moreover, HSP90 inhibitors have been described as suppressors of EGFR-mediated signalling in erlotinib-sensitive and erlotinib-resistant cell lines [109]. More recently preclinical work with the HSP90 agent, CH5164840, has demonstrated potent antitumour activity and is highly effective in combination with erlotinib against NSCLC tumours with EGFR overexpression and mutations [110]. These findings have been translated into the clinical setting where a Phase II study has recently reported interim data from 16 NSCLC EGFR mutation-positive patients who had progressed on EGFR-TKIs who were treated with the HSP90 inhibitor, AUY922, in combination with erlotinib. The ORR was 13% (2/16; both patients were T790M positive. Four other patients have stable disease for at least 8 weeks [111]. Another phase II study was conducted in patients with previously treated, advanced NSCLC, stratified by molecular status. Interestingly, clinical activity of AUY922 was mainly observed in patients with ALK-positive and EGFR-mutant NSCLC, reporting a 18% of RR, and an estimated 34% of median PFS rate in the latter subgroup of patients [112].

mTOR inhibitors

Another pathway of interest in NSCLC is the Akt/mTOR, which is activated by mutant HER2. In a preclinical HER2-driven transgenic murine lung cancer model, synergistic anti-tumour activity was observed with the combination of a HER family inhibitor and an mTOR inhibitor compared with either drug alone [113]. The mTOR inhibitor, everolimus was evaluated in a Phase II trial of patients with advanced NSCLC after one or two previous chemotherapy regimens or chemotherapy plus an EGFR TKI [114]. Everolimus achieved objective responses in 7.1% of patients who had previously failed chemotherapy, and in 2.3% of patients who had failed chemotherapy and an EGFR-TKI. A Phase I/II trial evaluated everolimus plus erlotinib versus erlotinib alone in patients with advanced NSCLC who progressed after more than two previous lines of chemotherapy, showing an improved disease control rate (DCR) [115].

PARP inhibitors

Poly adenosine diphosphate (ADP)-ribose polymerases (PARPs) are a family of nuclear enzymes that play a critical role in cellular processing of DNA damage through the base excision repair pathway. PARP inhibition can be directly cytotoxic to tumour cells, thus agents targeting PARP could be suitable for enhancing radio/chemotherapy and overcoming drug resistance [116]. A large, multicentre, prospective study, conducted by the Spanish Lung Cancer Group, will evaluate the clinical efficacy and safety profile of olaparib, a potent, orally active, PARP inhibitor, plus gefitinib versus gefitinib monotherapy in patients with NSCLC bearing *EGFR* mutations (NCT01513174).

Controlling oestrogens

The oestrogen receptor (ER) beta has been detected in NSCLC cells [117]. In fact, ERs are expressed independently of gender and histology [118] and oestrogens stimulate growth of NSCLC cells and tumour xenografts [118,119]. EGFR and ER pathways share signalling molecules; thus, activation of EGFR results in activation of ERs. Studies in cell cultures and xenographic models have shown that the combination of the ER antagonist, fulvestrant and gefitinib resulted in an additional inhibition of growth [118,120]. In an analysis of 317 NSCLC tumours it has been shown that oestrogen receptor α and β expression distinguishes a subset of NSCLC that has defined clinicopathologic and genetic features. Indeed data from this study demonstrated a positive correlation between oestrogen receptor α expression and EGFR mutations in lung adenocarcinoma [121]. A recent study has suggested that lung cancer in female never-smokers is frequently associated with an EGFR mutation and ERα expression, with a correlation between both markers. These findings suggest the possibility of treating this population by targeting both hormonal factors and genetic abnormalities [122]. Several Phase II trials are evaluating the hypothesis that the combination of fulvestrant and an EGFR inhibitor, such as erlotinib [118,119] or gefitinib [117] will provide improved efficacy over the EGFR inhibitor alone in NSCLC patients.

HDAC inhibitors

In vitro studies have demonstrated that gefitinib resistant cell lines were simultaneously treated with the histone deacetylase (HDAC) inhibitor vorinostat and gefitinib a synergistic effect was observed in four out of five of the cell lines tested [123]. These data suggest that HDAC inhibitors overcome EGFR-TKI resistance. It is postulated that this is achieved by the upregulation of E-cadherin expression which facilitates synergy between the HDAC and EGFR-TKI. There may also be a role of ErbB3 in this process [123]. Another study has recently shown that the HDAC inhibitor Vorinostat increased expression of the pro-apoptotic BH3 domain-containing the isoform of BIM, epigenetically restoring BIM function and EGFR-TKI sensitivity, in EGFR-mutant NSCLC, where resistance to TKI is associated with BIM polymorphism [124]. However, the effectiveness of this treatment strategy in overcoming EGFR-TKI acquired resistance, remains to be proven in clinical setting. In a phase I/II trial, the combination of Erlotinib and Vorinostat has shown no magniful activity in NSCLC patients with EGFR mutations, after Erlotinib progression [125]. The results of another randomized Phase II study have shown that patients with advanced NSCLC, who have progressed on erlotinib, who also have elevated E-cadherin, have a better outcome when treated with erlotinib plus entinostat (OS = 9.4 months), another HDAC inhibitor, versus erlotinib alone (OS = 5.4 months) [126].

Novel treatment paradigms

The move towards targeted therapy also requires changes to existing treatment approaches.

Accelerated progression after EGFR TKI discontinuation in patients with acquired resistance

An accelerated progression of disease in patients with *EGFR*-mutant lung cancer and acquired resistance to erlotinib or gefitinib after discontinuation of reversible EGFR TKI therapy has been observed. The first description of this phenomenon was published by Riely et al. in a prospective assessment of discontinuation and reinitiation of erlotinib or gefitinib in patients with acquired resistance to these agents [127]. The study recommended implementation of a randomised trial to assess the value of continuing erlotinib or gefitinib after development of acquired resistance [127]. More recently, Chaft et al. evaluated disease progression in patients with *EGFR*-mutant lung cancer with acquired resistance who needed to discontinue EGFR TKI [128]. In 40 of 61 patients (95% CI, 14–35), accelerated progression was observed and was associated with shorter time to progression on initial TKI ($P = 0.002$) and the presence of pleural ($P = 0.03$) or central nervous system (CNS) disease ($P = 0.01$). No association with the presence of T790M at the time of acquired resistance was found. These results suggest that washout periods must be minimised in these patients [128].

Continued treatment post-progression with EGFR TKI

EGFR TKI treatment should normally stop when disease progression is confirmed, even though treatment discontinuation is usually followed by an acute exacerbation of the disease. In certain conditions, continuous administration of EGFR TKI following disease progression appears to be a valid treatment option [129,130] as it can be suggested that tumour cell populations remain sensitive to EGFR TKIs after progression. Therefore, post-progression EGFR TKI treatment combined with chemotherapy is plausible, and early indications suggest that the choice of chemotherapy will depend on avoiding intolerable adverse events due to the amplified toxicity of the combination. Two randomized, phase III trials, PREFER and IMPRESS (NCT01544179), are currently investigating the effectiveness of continuing EGFR TKI, Erlotinib or Gefitinib, after progression on first-line TKI treatment, combined with platinum based chemotherapy versus chemotherapy alone of note, new agents which target the ErbB Family are also being investigated in combination with chemotherapy beyond progression. The second part of the LUX-Lung 5, phase III, randomized study, aims to investigate the use of afatinib with chemotherapy in patients who progress on afatinib alone. In this study patients have previously been treated with erlotinib/gefitinib and therefore highly selected for the presence of EGFR mutations [131]. Further investigation will determine the effectiveness of such combination therapy, but there is evidence to suggest that the tumour genotype and phenotype may evolve dynamically under the selective pressure of TKI therapies considering that genetic mechanisms of resistance are lost in the absence of the continued selective pressure of the EGFR inhibitor treatment. Hence, in order to know how best to prevent or overcome resistance to treatment, there is a need for continuing assessment of the genotypic and histological evolution of the cancer over the course of therapy in each patient.

Re-treatment with EGFR TKIs after having completed treatment with EGFR TKIs

Conventional chemotherapy is usually considered the only option for patients who progress following an initial response to EGFR TKIs. However, it has been observed that some NSCLC cell lines are able to regain sensitivity to TKIs [37], which suggests the action of a non-mutational and reversible EGFR TKI drug resistance mechanism. In a retrospective study of patients with stage IV NSCLC who progressed after previously achieving long term disease control on EGFR TKI treatment, subsequent treatment with standard chemotherapy and, at renewed progression, retreatment with erlotinib (alone or in combination with cetuximab) was considered a viable option [132]. Several evidences support the re-treatment with the same [133–137], or different [138–143] EGFR-TKI in patients who showed SD during 1st TKI treatment, while lower activity is reported for those patients who had PD to 1st TKI [144]. The highly heterogeneous nature of NSCLC tumours with respect to the mutations causing both the initial tumour and treatment response, which can also change in response to treatment, can create an apparent paradox whereby failure of a treatment at one stage of the disease does not mean that the same treatment might not be beneficial at a later stage. Intratumoural heterogeneity is therefore a subject of interest, and its importance in the development of biomarker strategies and drug development is increasingly recognised.

Approaches based on different growth rate of resistant cells lines to EGFR TKIs

Most advanced NSCLCs with activating *EGFR* mutations respond initially to EGFR TKIs. Chmielecki et al. theorised that the current dosing schedules of gefitinib and erlotinib, created to target wild-type *EGFR*, were not optimised for resistant EGFR [145]. In order to investigate this further, isogenic TKI-sensitive and TKI-resistant pairs of cell lines were developed to replicate the behaviour of human tumours. Observations that the drug-resistant *EGFR*-mutant cells exhibited a slower growth were used to create evolutionary mathematical cancer models, which were able to predict alternative therapeutic strategies to prolong the clinical benefit of TKIs against *EGFR*-mutant NSCLCs, by delaying the development of resistance [145]. These models predicted that high-dose pulses with low-dose continuous therapy were likely to be the most effective in preventing the development of resistance [146].

Combining radiotherapy with EGFR TKIs in patients with brain metastases

Brain metastases are a common and devastating consequence of disease progression in patients with NSCLC. The administration of radiotherapy to control brain metastases has proven survival benefit [147]. Several studies using growth factor inhibitors to modify tumour proliferation and/or radiosensitivity, report the potential to improve tumour control [148]. Combining first-line erlotinib/gefitinib with early multi-target radiotherapy is very effective in selected patients who respond to TKI, when the status of *EGFR* mutations is unknown before treatment [149]. In another study, the presence of *EGFR* mutations and the administration of EGFR TKI during radiotherapy independently conferred radiosensitivity in brain metastases of lung adenocarcinoma, with the best response rate achieved in the subgroup of patients who received TKI during radiotherapy [147]. Furthermore, experimental models show that gefitinib could inhibit cellular proliferation and enhance tumour response to radiation [147]. To summarise, continuous administration of EGFR TKI during and following radiotherapy for progressive disease in brain metastases appears to be a potential treatment option [129] but further work is needed to fully assess the use of this approach.

Use of local ablative therapy for oligoprogressive disease with continued TKI treatment

Disease progression is often observed at limited sites in patients with EGFR mutation-positive NSCLC. It is now postulated that there is a role for local ablative therapy (LAT) in patients with

CNS and/or limited systemic disease progression. In many cases disease progression is due to the treatment not penetrating the CNS. Consequently, it is unlikely that a patient will have developed systemic resistance to a drug and may be deriving significant ongoing benefit from its use [150]. This idea is based on reports in the literature of the benefit of radiation therapy on isolated CNS progression in patients with EGFR mutant NSCLC being treated with EGFR TKIs who also received continued systemic administration of the TKI in the absence of systemic progression [129]. A recent study has extended the use of LAT to those NSCLC patients with limited systemic disease progression or 'oligoprogressive disease' [150]. The authors of this study hypothesised that treatment of systemic progression before resistant clones can be spread will allow disease control to be achieved until the resistant clones can multiply and become detectable. In addition, there is also the theory that other, non-progressing sites, will benefit from targeted therapy due to continued suppression of sensitive clones that have not yet developed acquired resistance [150]. In patients with metastatic anaplastic lymphoma kinase (ALK)+ NSCLC treated with crizotinib ($n = 38$) and EGFR-MT NSCLC treated with erlotinib ($n = 27$) a subset ($n = 25/51$) with either non-leptomeningeal CNS and/or $\leqslant 4$ sites of extra-CNS progression (oligoprogressive disease) suitable for LAT received either radiation ($n = 24$) or surgery ($n = 1$) to these sites and continued on the same TKI. After LAT 19/25 patients progressed again, with median PFS of 6.2 months [150].

Combining chemotherapy with TKIs

Several randomized trials have shown no significant improvement of survival by combining EGFR-TKIs and chemotherapy in a population unselected for EGFR [15,7,151,8,152,153].

However, one of the such trials, the CALGB30406, reported evidence to suggest that EGFR mutations identify patients most likely to benefit [15]. More recently, the FASTACT2 trial demonstrated that intercalated chemotherapy and erlotinib is a viable first-line option for patients with non-small-cell lung cancer with EGFR mutation-positive disease or selected patients with unknown EGFR mutation status [154]. Survival outcomes were significantly prolonged with intercalated combination, leading a median PFS of 16.8 months and a median OS of 31.4 months, only in patients with an activating EGFR gene mutation [154]. These results are similar to those reported in phase 3 trials of single-agent EGFR TKIs in east Asian [155] and European populations [17,156] Therefore the current debate is: will the intercalated strategy ultimately delay or prevent the onset of acquired resistance? Which are the mechanism of how a TKI intercalated with chemotherapy might delay the onset of acquired resistance? The best schedule (timing, sequence, ...) of TKI and chemotherapy is still unknown. Further randomised studies are needed, to compare the new intercalated approach versus TKI alone followed by chemotherapy treatment, which remains the standard of care in this subgroup of patients.

Conclusions

The treatment of NSCLC with reversible EGFR TKIs is limited by the development of acquired resistance. The aetiology of resistance to EGFR TKIs is most often caused by mutations in the *EGFR* gene – most commonly T790M – but also attributed to MET or HGF, EMT cell transformations, phenotypic change from NSCLC to SCLC and signalling pathway changes. Modifications in parallel signalling pathways, such as the amplification of the *CRKL* oncogene have also been considered as inducers of resistance effects in NSCLC. It is clear that the blockade of each of these alterations could have therapeutic potential. The search for pathways to overcome the resistance to EGFR TKIs has been a focus of research in recent years with several potentially useful agents currently under development. The most clinically advanced agent in development is the ErbB Family Blocker, afatinib, which has shown encouraging results in several trials, and has received approval as monotherapy for the treatment of EGFR TKI-naïve patients with locally advanced or metastatic NSCLC and activating *EGFR* mutations. Promising results emerged from early phase I studies, investigating the safety and activity of a new class of non-quinazoline, EGFR inhibitors, that also target EGFR activating and resistance (T790M) mutations. Another possibility is the use of inhibitors of parallel signalling pathways, such as MET, HSP90, mTOR, PARP inhibitors or anti-IGF-1R mAbs. The regulation of the oestrogen receptor with fulvestrant is also a therapeutic option, in combination with EGFR TKIs.

As drug resistance appears to be pleomorphic, changes to standard treatment approaches, including different schedules and combinations, may also be an effective strategy in circumventing resistance. However, appropriate pharmacological evaluation should always be accompanied by a deeper understanding of the genetic alterations of tumour cells and of tumour heterogeneity, emphasising the need for continuous monitoring of the tumour genotype by both rebiopsy or liquid biopsy in order to lead decision-making. The evaluation of resistance development should also be carried out, because the standard definition of disease progression according to RECIST criteria has not always been related to clinical worsening during EGFR TKI treatment.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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