Epidermal growth factor receptor mutations in lung cancer

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Abstract | The development and clinical application of inhibitors that target the epidermal growth factor receptor (EGFR) provide important insights for new lung cancer therapies, as well as for the broader field of targeted cancer therapies. We review the results of genetic, biochemical and clinical studies focused on somatic mutations of EGFR that are associated with the phenomenon of oncogene addiction, describing 'oncogenic shock' as a mechanistic explanation for the apoptosis that follows the acute treatment of susceptible cells with kinase inhibitors. Understanding the genetic heterogeneity of epithelial tumours and devising strategies to circumvent their rapid acquisition of resistance to targeted kinase inhibitors are essential to the successful use of targeted therapies in common epithelial cancers.

Neural crest
A pluripotent, ectodermally derived ridge-like cluster of cells found on either side of the neural tube in vertebrate embryos.

Cytotoxic chemotherapy
Chemicals or drugs that kill proliferating cells, especially cancer cells. Their side effects are typically related to the inhibition of normal cell proliferation, with a narrow window of selectivity for cancer cells.

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Lung cancer is the leading cause of cancer death, accounting for one third of all deaths from cancer worldwide. Like most cancers, lung cancer is a conglomeration of diseases of diverse aetiology, broadly divided into small-cell lung cancer (SCLC, comprising 20% of lung cancers), and non-small-cell lung cancer (NSCLC, comprising 80% of lung cancers). SCLC is a tumour of neural crest origin and initially responds well to chemotherapy, but commonly recurs with resistant disease. NSCLC is thought to originate in lung epithelial cells, and comprises diverse histological subtypes including adenocarcinoma, bronchioloalveolar, squamous, anaplastic and large-cell carcinomas[1]. Most patients with advanced NSCLC present with metastatic disease and, if left untreated, have a median survival after diagnosis of 4–5 months and a 1-year survival of less than 10% (REF. 2). Combination cytotoxic chemotherapy, the treatment of choice in these cases, results in a modest increase in survival at the cost of significant toxicity to the patient[3]. The advent of molecular-targeted therapeutics has therefore generated much optimism, given the perception that the limits of chemotherapy in NSCLC have been reached and that further advances in the treatment of NSCLC will have to involve radically different approaches (reviewed in REF. 4). Against this backdrop, the approval of small-molecule inhibitors of the epidermal growth factor receptor (EGFR) kinase for the treatment of NSCLC in 2003 was heralded with much fanfare, although the limitations of their efficacy have become readily apparent (reviewed in REF. 5).

The deregulation of EGFR in NSCLC

The receptor tyrosine kinase (RTK) super-family of cell-surface receptors serve as mediators of cell signalling by extra-cellular growth factors[6]. Members of the ErbB family of RTKs, such as EGFR (also known as ERBB1 or HER1), ERBB2 (also known as HER2), ERBB3 (also known as HER3) and ERBB4 (also known as HER4) have received much attention, given their strong association with malignant proliferation (reviewed in REF. 7). Increased levels of *EGFR* gene expression are observed in cancers of the head and neck, ovary, cervix, bladder, oesophagus, stomach, brain, breast, endometrium, colon and lung, and frequently seem to confer an adverse prognosis (reviewed in REFS 6,8). Extending previous observations of almost two decades ago[9,10], recent retrospective analyses have reported EGFR overexpression in 62% of NSCLC cases, and its expression is correlated with a poor prognosis[8,11,12]. In some cases, genomic analyses documented the amplification of chromosomal region 7p12, where the *EGFR* gene is located[13]. In addition to EGFR overexpression, its cognate ligands, epidermal growth factor (EGF) and transforming growth factor-α (TGFα) are also frequently expressed in NSCLCs, and can establish autocrine loops that lead to receptor hyperactivity[14,15]. The disruption of these autocrine loops is the primary rationale for antibody-based EGFR-targeted therapeutics[16].

Various strategies involving small-molecule inhibitors have also been developed to target EGFR and/or its family members, and these are in various stages of clinical testing (reviewed in REF. 17). Gefitinib

At a glance

- Advanced non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in the world.
- Epidermal growth factor receptor (EGFR) is expressed in 50% of NSCLCs, and its expression is correlated with poor prognosis. These two factors make EGFR and its family members prime candidates for the development of targeted therapeutics.
- Two EGFR-targeting small-molecule inhibitors, gefitinib (Iressa: AstraZeneca, approved in May 2003) and erlotinib (Tarceva: OSI-Genentech, approved in November 2004) received fast-track approval from the US Food and Drug Administration as treatment for patients with advanced NSCLC who had failed to respond to conventional chemotherapy.
- Early clinical data showed that 10% of patients with NSCLC responded to gefitinib or erlotinib. Although infrequent, the speed and magnitude of clinical responses were unique, as was the fact that they were seen in specific subsets of cases (non-smokers, women, East Asians and patients with adenocarcinomas with bronchioloalveolar histology).
- Molecular analysis showed that in most instances, responders harboured specific mutations in the gene that encodes EGFR. Exon 19 mutations characterized by in-frame deletions of amino-acids 747–750 account for 45% of mutations, exon 21 mutations resulting in L858R substitutions account for 40–45% of mutations, and the remaining 10% of mutations involve exon 18 and 20.
- EGFR kinase domain mutations hyperactivate the kinase and confer a dependence on the mutated kinase for the survival of the NSCLC tumour cells.
- The treatment of sensitive cells with targeted therapeutics such as gefitinib and erlotinib seems to trigger a form of 'oncogenic shock', which is postulated to result from the differential decay of downstream signals leading to a temporary predominance of apoptotic signals.
- Acquired resistance to gefitinib and erlotinib might involve the recurrent mutation T790M which affects the gatekeeper residue in the catalytic domain of the kinase that weakens the interaction of the inhibitor with its target. Resistance can be overcome *in vitro* by irreversible inhibitors of EGFR

(Iressa; AstraZeneca) and erlotinib (Tarceva; OSI Pharmaceuticals, Genentech), two small-molecule drugs that specifically target the tyrosine kinase activity of EGFR (EGFR-tyrosine kinase inhibitors (EGFR-TKIs)), received fast-track approval from the US Food and Drug Administration (FDA) in 2003 and 2004, respectively, for patients with advanced NSCLC who had failed to respond to conventional chemotherapy[5]. Both drugs are reversible inhibitors of the EGFR kinase, designed to act as competitive inhibitors of ATP-binding at the active site of the EGFR kinase[18,19]. The observation that sensitivity to gefitinib and erlotinib correlated very strongly with a newly discovered class of somatic activating mutations in the EGFR kinase domain[20–22] explained the unique subset of drug-responsive cases, notably those arising in non-smokers and more frequently in women, individuals of Asian ethnic background and those with adenocarcinoma and bronchioloalveolar histology (for a review of the recent clinical literature see Sequist *et al.*[23]). In addition to providing a genetic marker for a highly EGFR-TKI-responsive subset of NSCLCs, this correlation has also highlighted the crucial importance of mutationally activated kinases as anticancer drug targets (reviewed in REF. 24) (FIG. 1).

In unselected NSCLC samples, *EGFR* mutations are present in ~10% of cases in North America and Western Europe, but ~30–50% of cases in individuals of East Asian descent, and are associated with most (over 50%) adenocarcinomas with bronchioloalveolar features that arise in non-smokers[25–34]. *EGFR* kinase domain mutations target four exons (18–21), which encode part of the tyrosine kinase domain (the entire kinase domain is encoded by exons 18–24) and are clustered around the ATP-binding pocket of the enzyme[25,35–39]. Consistent with their purported role in the aetiology of NSCLC, recent studies have shown that exon 19 deletions that involve the LREA motif, L858R, G719S and ins 770(NPG)-mutated EGFR proteins are oncogenic in both cell culture and transgenic mouse studies[40–42]. These mutations also increase the kinase activity of EGFR, leading to the hyperactivation of downstream pro-survival pathways, and consequently confer oncogenic properties on EGFR[43–45].

Kinase domain mutations in EGFR are generally referred to as activating mutations, as they seem to result in the increased kinase activity of the receptor. However, this does not imply that these mutated EGFRs are necessarily constitutively or fully active, as their degree of ligand independence might be a function of experimental context[40,43,44,46,47]. These partially activated mutant EGFRs can be rendered fully ligand-independent, and therefore constitutively active, by second site substitutions in EGFR, such as the T790M mutation in exon 20 (REF. 46). *In vitro* biochemical studies using purified recombinant wild-type and mutant (L858R and ΔE746–A750) EGFR cytoplasmic domains have shown that mutants have increased *kcat* values and an increased *Km* for ATP[48,49]. Moreover, as has been observed in cell-based studies, the mutants show an increased sensitivity to inhibition by erlotinib (reduced *Ki*) in these *in vitro* kinase assays. The reduced ATP affinity seen with mutant kinases most probably accounts for their increased sensitivity to the selective EGFR-TKIs, which compete with ATP for binding to the catalytic site. Another study, in which the phage-display method was used to examine the interaction of a large panel of kinases with selective inhibitors, concluded that EGFR mutations, including ΔE746-A750, do not themselves affect the affinity for gefitinib and erlotinib[50].

EGFR-targeted therapy of NSCLC

For unknown reasons, EGFR kinase domain mutations seem to be restricted to a subset of NSCLC, although very rare mutations have also been reported in SCLC, cholangiocarcinoma, ovarian, colorectal, head and neck, oesophageal and pancreatic cancers[51–56]. This Review discusses the genetic and biochemical determinants of erlotinib and gefitinib sensitivity in NSCLC. In light of the rapid acquisition of resistance to these EGFR-TKIs, we discuss the mechanisms by which resistance might occur and the possibilities for alternative therapeutics.

Genetic determinants of sensitivity to gefitinib and erlotinib. Early NSCLC clinical trials with gefitinib and erlotinib were modestly encouraging, with partial responses observed in approximately 10% of treated patients with NSCLC[57–60]. Most responses were seen in East Asians, females or non-smoking patients with NSCLC. These patients had a high frequency of adenocarcinoma with bronchioloalveolar features, and many showed a dramatic and lasting response to second- or third-line

Molecular-targeted therapeutics
Chemicals or drugs that target known proteins that are important in cancer cell proliferation or survival at the same time as being dispensable to normal cells. Although side effects are typically less severe than with cytotoxic agents, the effective inhibition of the target protein might not translate into generally effective therapies, hence the importance of reliable biomarkers.

Autocrine loop
A mode of cell signalling in which soluble ligands released by cells stimulate receptors on their own cell surfaces.

Reversible inhibitors
Inhibitors that bind non-covalently with biological molecules and interfere with their activity.

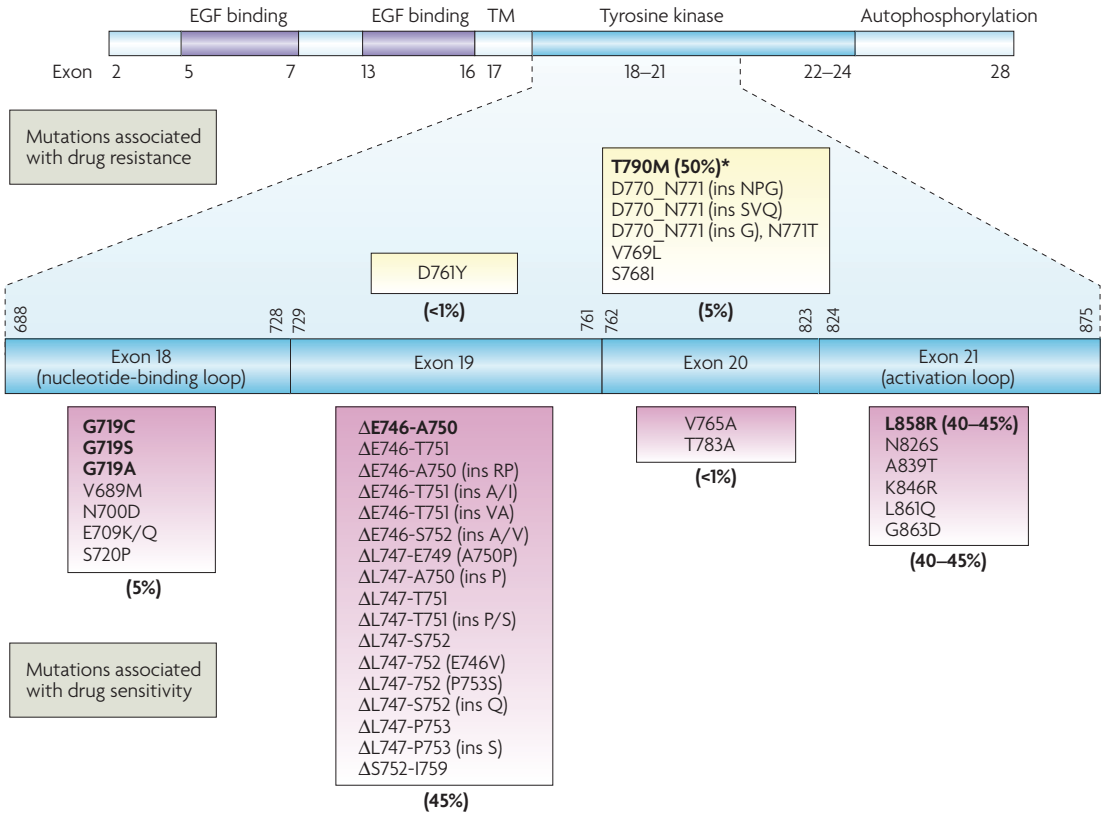


Figure 1 | **Gefitinib- and erlotinib-sensitizing mutations of EGFR in NSCLC.** A cartoon representation of epidermal growth factor receptor (EGFR) showing the distribution of exons in the extracellular domain (EGF binding), transmembrane domain (TM) and intracellular domain (comprising the tyrosine kinase and autophosphorylation regions). The cysteine-rich regions in the extracellular domain (EGF binding; purple shaded region) and the tyrosine kinase region in the intracellular domain (cyan shaded region) are also represented. Exons 18–21 in the tyrosine kinase region where the relevant mutations are located are expanded (represented by the cyan bar), and a detailed list of EGFR mutations in these exons that are associated with sensitivity (magenta boxes) or resistance (yellow boxes) to gefitinib or erlotinib is shown. The most prevalent of EGFR kinase domain mutations, accounting for 45% of EGFR mutations in non-small-cell lung cancer (NSCLC), are in-frame deletions of exon 19, nested around the LREA string of amino-acids located between residues 747–750 of the EGFR polypeptide[175]. Another recurrent mutation is the L858R substitution in exon 21, within the activation loop of EGFR, which comprises approximately 40–45% of EGFR mutations. Nucleotide substitutions in exon 18 (for example, G719C or G719S) account for another 5% of EGFR mutations, as do in-frame insertions in exon 20. The most noteworthy, clinically relevant mutation in exon 20 is T790M, which is detected in 50% of the cases (denoted by *) as a second site mutation associated with acquired gefitinib and erlotinib resistance[25,35–39]. Recently, D761Y, a T790M-like secondary mutation in exon 19 of EGFR (at the border of exon 19 and exon 20), was also reported to be associated with resistance to gefitinib and erlotinib in NSCLC cells that contain the L858R-EGFR mutation[71,176]. Although the inclusion of most of these sensitizing mutations are based on their occurrence in drug responders, increased biochemical and cellular activity of these mutations has been documented in some cases. The main mutations in each class are shown in bold type. Data compiled from[20–22,28,30,31,33,71,177].

Unselected patients
A cohort of patients identified on the basis of tissue diagnosis but not correlated with biomarkers (that is, sequencing of the *EGFR* gene was not used as a selection criterion).

Ligand independence
The activation of a receptor in the absence of interaction with its cognate ligand.

K*cat*
The overall catalytic rate of an enzyme (that is, the number of substrate molecules converted to product by each catalytic site per unit of time.

K*m*
The Michaelis–Menten constant. K*m* is a measure of the affinity of a substrate for an enzyme, and is the substrate concentration at half the maximal velocity of an enzyme.

K*i*
The dissociation constant for the binding of an inhibitor to an enzyme.

Phage-display method
A method in which proteins or peptides are displayed on the surface of filamentous bacteriophages, which can then be used to study the interaction of the peptide with other proteins or chemicals.

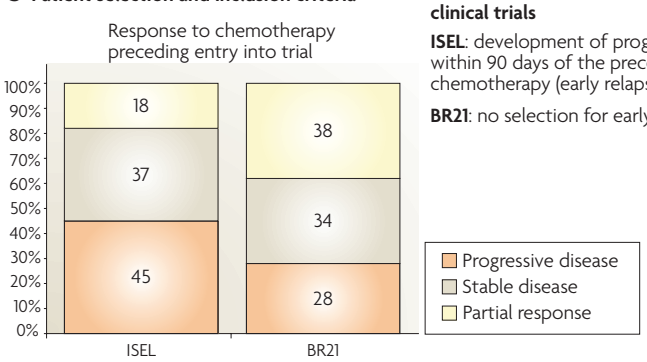
gefitinib or erlotinib monotherapy. The sequencing of the *EGFR* gene in tumour samples from these responders showed somatic gain-of-function mutations[20–22] (FIG. 1). Overall, the incidence of EGFR mutations in NSCLC among clinical responders to gefitinib or erlotinib is 77%, compared with 7% in NSCLC cases that are refractory to gefitinib or erlotinib[20–22,28,30,33,61–73]. Additional studies have shown some differences in the clinical outcomes that are associated with different mutations[27,30,74,75]. For example, NSCLCs that harbour exon 19 deletion mutations seem to respond better to gefitinib and erlotinib than tumours with point mutations in exon 21, such as L858R[30,74,75]. So far, insertion mutations in exon 20 have never been found to confer gefitinib or erlotinib sensitivity *in vitro*, nor have they ever been reported to occur in responsive cases, despite the fact that, at least in some instances (for example, ins 770 (NPG)), they seem to activate EGFR to a similar degree as sensitizing mutations in exons 19 or 21 (REF. 40).

Although EGFR mutations were present in most cases of NSCLC that were identified by virtue of their dramatic clinical response to TKIs, controversy has surrounded the predictive value of EGFR mutations in unselected patients[31,32,61,69]. Approximately 10–20% of patients who do show a partial response to gefitinib do not have identifiable EGFR mutations, indicating that EGFR mutations are not the sole determinants of TKI response[20,22,28,30,31,33,61–64,68–70,72,73,76].

a Drug dosing

Drug (study)	MTD (mg day^{-1})	Trial dose (mg day^{-1})
Gefitinib (ISEL)	600	250
Erlotinib (BR21)	150	150

b Patient selection and inclusion criteria



Criteria for inclusion in ISEL and BR21 clinical trials

ISEL: development of progressive disease within 90 days of the preceding round of chemotherapy (early relapse)

BR21: no selection for early relapse

Figure 2 | **Why gefitinib failed in the United States. a** | The maximum tolerated dose (MTD) for gefitinib and erlotinib, and the dose of the two drugs used in the ISEL and BR21 trials. Although erlotinib was used at its MTD in the BR21 trial, gefitinib was used at the sub-MTD level of 250 mg a day. **b** | The composition of the patients and their response to chemotherapy at the time of recruitment for the ISEL and BR21 trials. The histogram represents patients with progressive disease (orange), stable disease (green) and partial response (yellow). Note that the patient pool recruited to the ISEL trial had a significantly larger percentage of individuals with progressive disease as compared to patients recruited to the BR21 trial (45% versus 28%), and conversely had a lower percentage of patients that had a partial response to chemotherapy (18% versus 38%). Also shown are the inclusion criteria for patient enrollment in both of the trials. In the ISEL trial, only patients who had progressive disease within 90 days of cessation of chemotherapy were included, but no similar time-limited exclusion criterion was implemented for the inclusion of patients in the BR21 trial[83]. The differences in dosage used and the patient selection criteria might have contributed to the differences in outcomes between the two trials.

Other molecular abnormalities, including the amplification of wild-type EGFR or alterations in other ErbB family members have been detected, although it is unclear whether they account for most gefitinib-responsive cases that lack EGFR mutations[31,61,69,77–79]. In particular, the amplification of EGFR has been difficult to interpret by itself, because gene copy number alterations that affect both mutant and wild-type EGFR alleles have not been distinguished in most studies. In addition, inter-study variability stemming from the different techniques used to measure copy number, including quantitative PCR (qPCR), which provides a 'global' copy number assessment, and fluorescence *in situ* hybridization (FISH), which evaluates copy number at the single cell level, have yielded divergent results, possibly owing to the use of different threshold measurements and the distinction between specific amplification of the EGFR locus versus more general alterations in gene copy numbers linked to aneuploidy. Significantly, EGFR kinase mutations seem to be highly correlated with clinical characteristics that are predictors of TKI-responsive disease, whereas *EGFR* gene amplification, as measured by qPCR, seems to be more common in smoking-associated cancers, and does not show the same predilection towards distinct ethnic background and tumour histology[61].

Most retrospective studies to date have reported that 50–80% of EGFR-mutant NSCLCs respond to gefitinib or erlotinib, and more recent studies from Asia, where EGFR mutant NSCLC is 2–3 times more prevalent, have reported responses in more than 75% of NSCLC cases with mutant EGFR[67,80,81]. Most significantly, although improvement in overall survival for the small fraction of EGFR-mutant cases treated with gefitinib or erlotinib has not reached statistical significance in US and European studies, this has been readily apparent in Asian studies with larger fractions of mutant cases[28,31,33,62,69,73]. Taken together, the data suggest that a subset of cases, marked primarily by *EGFR* mutations and in some cases *EGFR* amplification, show dramatic responses to TKIs. These responses might occasionally be durable (that is, >3 years), but in most cases they only last for ~6–12 months before resistant disease recurs. Given the low frequency of EGFR-mutant NSCLC, a modest (2–3 months) improvement in overall survival has been observed in US and European retrospective trials, driven primarily by the stabilization of disease rather than tumour shrinkage, which is not tightly linked to the presence of *EGFR* mutations[31]. In these studies, increased *EGFR* gene copy number and high levels of aneuploidy, as measured by FISH, seemed to be more predictive of disease stabilization after treatment with TKIs[59,82]. The effect on overall survival in genotypically uncharacterized cases was observed with erlotinib (BR21 trial), but not gefitinib (ISEL trial), contributing to the withdrawal of gefitinib from the US and European market and the approval of erlotinib as third-line therapy in NSCLC irrespective of tumour genotype[59,82]. A closer examination reveals differences in the dose of the two agents, together with differences in the composition of the patient population that might account for the observed differences in outcome between gefitinib and erlotinib[83] (FIG. 2). Nonetheless, gefitinib (which is still in use in Asia) and erlotinib are comparable in virtually all laboratory analyses, and the appropriate clinical role of EGFR mutation analysis in the treatment of NSCLC remains an evolving question, awaiting prospective studies with adequate tumour analysis.

Biochemical determinants of sensitivity to gefitinib and erlotinib. Unpublished results from our laboratory suggest that sensitivity to EGFR-TKIs is not simply recapitulated by expressing the mutant constructs in transfected cells, pointing to the importance of cellular context in conferring dependency on the EGFR pathway. Furthermore, caution should be exercised in interpreting *in vitro* data using NSCLC cell lines as surrogates for clinical responses (FIG. 3). However, *in vitro* studies with NSCLC cell lines have highlighted the fact that gefitinib- and erlotinib-sensitizing mutations invariably hyperactivate the EGFR signalling pathway and promote EGFR-mediated anti-apoptotic and pro-survival signals through the Ras–Raf–MEK (mitogen-activated and extracellular-signal regulated kinase kinase)–ERK1 and ERK2 (extracellular-signal-regulated kinase 1 and 2), PI3K–Akt (phosphatidylinositol-3 kinase–Akt) and STAT3 and STAT5 (signal

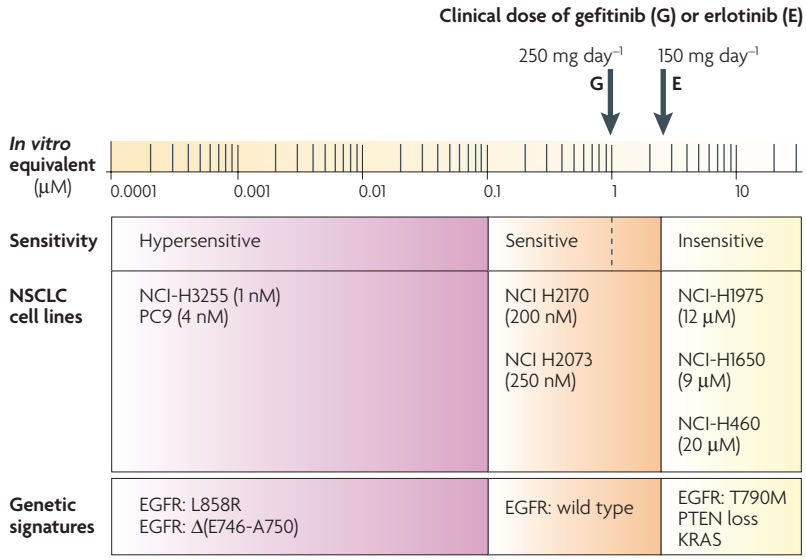


Figure 3 | **NSCLC cell lines: *in vitro* surrogates of *in vivo* drug sensitivity.** Understanding the biochemical basis of sensitivity to gefitinib (G) and erlotinib (E) has been aided by the generation and use of human tumour-derived non-small-cell lung cancer (NSCLC) cell lines that show varying degrees of sensitivity to these inhibitors, ranging from hypersensitive (IC_{50} in the low nM; graded magenta box), to sensitive (IC_{50} in the high nM; graded orange box) to extremely insensitive (IC_{50} in the high μM; graded yellow box). Representative examples of NSCLC cell lines from each category, including their distinguishing genetic features, are also shown. The hypersensitive cell lines NCI-H3255 and PC9 harbour the EGFR tyrosine kinase domain mutations L858R and ΔE746-A750, respectively. Insensitive cell lines such as NCI-H1975 and NCI-H1650, although harbouring the same kinase domain mutations (L858R and ΔE746-A750), have additional changes such as T790M (NCI-H1975), phosphatase and tensin homologue (*PTEN*) loss (NCI-H1650) or *KRAS* mutations in NCI-H460 cells. Although these cell lines have been used extensively, conclusions derived from such *in vitro* systems should be interpreted with caution in view of the off-target effects seen with these inhibitors[50], especially at supra-physiological concentrations, in excess of 1 and 2.5μM for gefitinib and erlotinib, respectively. The *in vitro* concentrations used in tissue culture roughly correlate to the plasma concentrations of these drugs in patients treated with the standard doses of these agents (250 mg a day of gefitinib and 150 mg a day of erlotinib), and have been used by researchers as a useful threshold to distinguish sensitive from insensitive and/or resistant cell lines[90,112,178–180].

transducer and activator of transcription proteins 3 and 5) pathways such that cancer cells might become dependent on a functional EGFR for their survival[43,84–86]. Interestingly, these are the same pathways that are activated after ligand engagement and are inhibited by gefitinib, including the ERK pathway involved in cell proliferation and the pro-survival Akt pathway[87–89]. The obvious implication is that shutting off EGFR with specific kinase inhibitors, antibodies or RNA interference would extinguish these proliferative and survival signals on which the tumour cell is dependent, therefore resulting in tumour cell death. Normal cells (or non-EGFR-dependent tumour cells that do not respond to gefitinib or erlotinib) remain unaffected, as their pro-survival signals are either driven by other genes or can be compensated for by other RTKs in the event of EGFR inhibition. This is consistent with the observation that gefitinib and erlotinib response in sensitive cells results in the downregulation of ERK, Akt and STAT3 and STAT5, whereas a similar downregulation is not evident in insensitive or resistant cells[43,87–90].

Oncogenic shock
A mechanism to explain oncogene addiction, in which the acute inactivation of an oncoprotein is associated with differential attenuation rates of pro-survival and pro-apoptotic signals emanating from the oncoprotein, such that apoptotic signals become predominant and kill the cancer cell.

Differential signal decay
A signalling imbalance created by the rapid decay of pro-survival signals and persistence of the relatively long-lived pro-apoptotic signals after acute oncogene inactivation.

Although these pro-survival signalling pathways are probably controlled by many RTK outputs in normal cells, their dependency on mutated and/or activated EGFR in some NSCLC tumours and cell lines bears the hallmark of oncogene addiction (BOX 1).

The molecular mechanisms that underpin oncogene addiction remain to be elucidated. As commonly understood, alterations of the signal-transduction pathways in cancer cells are thought to underlie drug hypersensitivity[91]. Based on modelling studies *in vitro*, we have recently proposed that unbalanced pro-apoptotic and pro-survival signals lead to a phenomenon that we refer to as oncogenic shock, and might account for the observed apoptotic outcome following the acute inactivation of a crucial oncogene in an addicted cancer cell[92] (FIG. 4). According to this model, an addicting oncogene gives rise to both pro-apoptotic and pro-survival signal outputs. While the oncogene is active, the pro-survival signals pre-dominate and keep the pro-apoptotic signals in check, enabling the survival and proliferation of the cancer cell. After acute oncogene inactivation, the relatively short-lived pro-survival signals decay first, whereas the longer-lasting pro-apoptotic outputs are maintained during a crucial window of time. Therefore, differential signal decay leading to a signal imbalance and a temporary predominance in pro-apoptotic outputs sets in motion the apoptotic cascade and commits the cell irrevocably to apoptosis, even if the signalling imbalance is subsequently redressed. In support of the oncogenic shock model, the apoptotic response to oncogene inactivation in oncogene-addicted cells is abrogated if the disruption of oncogene-derived signals is extended over a period of time, rather than being acute, or if pro-survival signals are transiently applied during the crucial window of time following acute withdrawal[92]. Therefore, the cell is not hard-wired to depend on a given oncogene, but rather it requires time to adapt to the loss of such a signal, and is highly susceptible to apoptosis during that window of time. The implications of this model for clinical practice, if confirmed, are considerable, as it would argue against the co-administration of TKIs with chemotherapy drugs that, by virtue of their own effects on DNA-damage checkpoints, might attenuate the acute effect of growth factor signal withdrawal. For RTKs like EGFR, it is also possible that the acute effect of EGFR-TKIs in abrogating kinase activity might be qualitatively different from that of anti-receptor antibodies, which might enable a more gradual signal attenuation, therefore explaining the differential effect of these two classes of agents on EGFR-mutant NSCLC[86].

Implicit in the oncogenic shock model is the paradoxical requirement that activated oncogenes generate pro-survival and pro-apoptotic signals simultaneously[93]. Such a coupling of antagonistic signals is well documented for *Ras*[94,95], *Src*[96,97], *BCR-ABL*[98], *EGFR*[43,99], *MYC*[100,101] and even viral oncogenes such as adenoviral *E1A*[102]. Taken together in the context of NSCLC, mutated EGFR might represent the genetic lesion to which the tumour is addicted, and the acute withdrawal of these signals by EGFR-TKIs might trigger oncogenic shock and tumour cell apoptosis.

Box 1 | **Oncogene addiction**

The term oncogene addiction was first coined in 2000 by Bernard Weinstein[91,168,169] to describe the phenomenon by which a tumour cell, despite many other genetic alterations, can become completely dependent on a single oncogenic pathway for its proliferation and/or survival. Implicit in this dependency is the fact that the tumour cell should be exquisitely sensitive to the targeted inhibition of the addicting oncogene. Beyond gefitinib- and erlotinib-responsive NSCLC, oncogene addiction is thought to explain responses of chronic myeloid leukaemia, gastrointestinal stromal tumours and chronic myelomonocytic leukaemia to imatinib, which targets the BCR-ABL, c-Kit, and platelet derived growth factor receptor-β (PDGFRβ) kinases[170]. Transgenic mouse tumour models have shown a similar addiction phenomenon, although they are somewhat biased in that the inducible expression of an oncogene is used to trigger the genesis of a tumour that is then shown to be dependent on the continued expression of the transgene for its survival (for example, HRAS in melanoma, KRAS in lung carcinoma and MYC in lymphoma and leukaemia[171–173] (reviewed in REF. 174)). An interesting parallel is the observation that, in some cases, the continued expression of the transgene does lead to the emergence of cells that have sustained additional somatic genetic lesions and have consequently acquired independence from the triggering oncogene[173]. A similar mechanism might occur in human cancers that show resistance to TKIs, despite the presence of the mutated kinase.

Resistance to EGFR-targeted therapy

Not all EGFR kinase mutations are associated with hypersensitivity to gefitinib and erlotinib. An overarching conclusion that has emerged from studies of primary insensitivity to EGFR-TKIs is that most cells that express EGFR will show effective attenuation of EGFR activity, but only in EGFR-addicted cancers will this be accompanied by tumour shrinkage. Tumours that fail to respond to gefitinib or erlotinib despite the presence of an EGFR mutation might have sustained additional genetic lesions that relieve this addiction, a mechanism that could also mediate acquired resistance in previously sensitive tumours.

Primary resistance. Recent studies suggest that insertion mutations in exon 20 of the *EGFR* gene might render the receptor about 100-fold less sensitive to EGFR-TKIs compared with other sensitizing EGFR kinase mutations[40]. It is unclear whether these mutations differ from classical activating mutations in their downstream signals (therefore attenuating oncogene addiction), or whether they do not share the differential binding affinity to ATP and the inhibitors. However, these mutations are relatively rare, and in most cases of NSCLC that fail to respond to EGFR-TKIs it is likely that genetic lesions other than EGFR are driving tumorigenesis. In most instances the T790M mutation is associated with acquired resistance. However, it has also been linked to primary resistance, occurring together with a sensitizing mutation in four unresponsive cases of NSCLC[36,39].

About 15–30% of NSCLCs harbour activating mutations in codons 12 and 13 of the *KRAS* gene[103,104]. By and large, *KRAS* and *EGFR* mutations seem to be mutually exclusive in NSCLC, and define distinct subsets of tumours, with *EGFR* mutations being characteristic of tumours that arise in non-smokers [25], whereas *KRAS* mutations are more common in smoking-associated cancers[105,106]. Mutant EGFR and KRAS might also have overlapping and/or redundant signalling roles in NSCLC aetiology[25,27,29,107], which might explain the conspicuous absence of *KRAS* mutations in EGFR-TKI-responsive tumours[108]. Mutations in *KRAS* have been proposed as a mechanism of primary resistance to gefitinib and erlotinib[108], although *KRAS* mutations are almost always found in NSCLCs with wild-type *EGFR*. Therefore, it is difficult to unequivocally determine whether insensitivity is due to the presence of mutated KRAS or the absence of mutated EGFR.

Tumour cells that are sensitive to gefitinib and erlotinib are characterized by a rapid decrease in Akt activity in response to drug treatment[87,88], and failure to downregulate Akt is a hallmark of insensitivity to the drugs[43,90]. The activation of Akt is indirectly regulated by the tumour suppressor phosphatase and tensin homologue (PTEN), which is frequently lost in human cancers[109]. Although genetic alterations in PTEN are found in <10% of cases of NSCLC[110], the absence of PTEN expression is evident in as many as 70%, and might be mediated by epigenetic mechanisms such as PTEN promoter methylation[111]. In some cell types, restoring PTEN expression is asso-ciated with increased sensitivity to gefitinib or erlotinib, suggesting that it might modulate sensitivity *in vivo*[89,112]. Insulin-like growth factor receptor 1 (IGFR1), ERBB3 or activated ERBB2 expression have also been proposed to have a role in mediating resistance to gefinitib[113–115]. However, recent studies of a large cohort of gefitinib-treated NSCLC cases failed to uncover a correlation between PTEN or IGFR1 status and response to gefitinib treatment, and have questioned the role of these proteins in mediating primary insensitivity to gefitinib[116].

Acquired resistance. Despite dramatic responses in EGFR-mutant cases of NSCLC treated with gefitinib or erlotinib, the development of drug resistance within 6–12 months of the initiation of therapy greatly limits the ability of these drugs to significantly prolong patient survival. A deeper understanding of the molecular and cellular basis of this phenomenon is crucial to the future development of alternative therapies to overcome this resistance.

A single secondary mutation in *EGFR* exon 20, T790M, is present in a subset of *EGFR*-mutant tumours that recur after an initial response to gefitinib or erlotinib[35–37]. Using allele-specific PCR, the T790M mutation is detectable in approximately 50% of patients with NSCLC who relapse after an initial response to TKIs, although in some cases the mutation seems to be underrepresented in the tumour cell genome relative to the total number of *EGFR* alleles[37,117]. This suggests that T790M might either be present in only a subset of resistant cancer cells, or might be present only in a minority of copies of the *EGFR* gene in each tumour cell[39,118]. Some studies have also shown that T790M mutations are present before the patient is exposed to the drug[25,38,56,119,120], thereby suggesting that this mutation might confer some selective advantage to tumour outgrowth and might be further selected after the exposure of the tumour to TKIs[39]. The T790M mutation in EGFR is structurally analogous to the mutated gatekeeper residue T315I in BCR-ABL,

Gatekeeper residue
Amino acids with small side chains found at the catalytic site of enzymes that, when mutated to amino acids with bulkier side chains, can sterically impede the binding of a drug at the active site of the enzyme at the same time as retaining substrate binding.

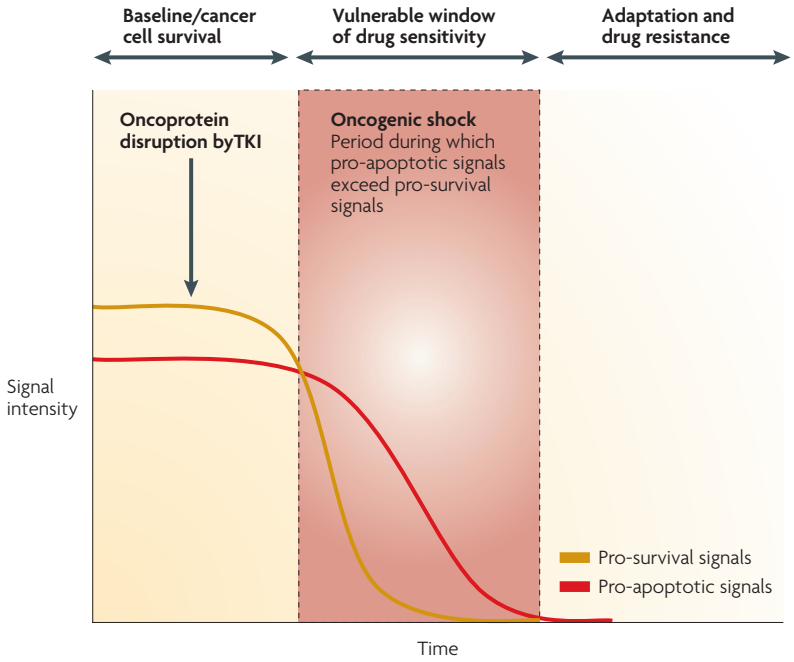


Figure 4 | **The role of differential signal attenuation in inducing oncogenic shock.** The oncogenic shock model proposes that pro-survival (orange curve) and pro-apoptotic (red curve) signals emanating from an active oncoprotein in a tumour cell are normally balanced so that the survival output predominates and results in the survival of the cancer cell. After the acute disruption of oncogene function by targeted kinase inhibitors (TKIs), pro-survival signals dissipate very rapidly, whereas pro-apoptotic signals are relatively longer lived. During this vulnerable window of drug sensitivity, the longer-lived pro-apoptotic signals gain the upper hand and cause the cells to irrevocably undergo apoptosis. One possible mechanism by which tumour cells acquire resistance to a therapeutic target is that they are able to adapt to and overcome oncogenic shock.

T670I in c-KIT and T674I in platelet-derived growth factor receptor-α (PDGFRα) that weaken the interaction of inhibitors with the kinase and that have previously been shown to confer resistance to targeted agents such as imatinib and other ATP-mimicking kinase inhibitors[121–123]. Besides T790M, the only other study of acquired resistance in clinical samples suggests that an A disintegrin and metalloproteinase 17 (ADAM17)-mediated heregulin-dependent autocrine loop activates both ERBB2 and ERBB3 signalling pathways in NSCLC and mediates resistance to EGFR-TKIs[124].

The phenomenon of acquired resistance to gefitinib has been modelled *in vitro* using highly sensitive NSCLC cell lines with EGFR mutations[125]. Mechanisms of acquired drug resistance have been defined *in vitro*, including the acquisition of (or selection for) the T790M mutation[118] and altered EGFR trafficking[37]. Other possible mechanisms that confer resistance include amplification of the mutant EGFR or the hyperactivation of downstream signalling components that circumvent EGFR inhibition, causing the increased expression of signal-attenuating molecules or cellular changes that alter the bioavailability of the drug[126]. Some studies have raised the possibility that the multi-drug resistance protein ATP-binding cassette G2 (ABCG2) might actively pump gefitinib from cells and therefore confer resistance to the drug[127,128], although others have suggested that gefitinib itself inactivates the multi-drug transporters ABCG2 and the ABC transporter P-glycoprotein[129–133]. These alternative mechanisms of gefitinib and erlotinib resistance still await validation *in vivo* — an issue confounded by the limited amounts of clinical specimens from recurrent tumours and the absence of defined genetic lesions that can be detected in tissue sections.

Irreversible inhibitors
Inhibitors that bind covalently with biological molecules and interfere with their activity.

Alternative EGFR-targeted therapeutics

The development of resistance to EGFR-TKIs calls for alternative strategies that still target EGFR signalling but circumvent the insensitivity to kinase inhibitors. Mutations such as T790M might have far-reaching implications in the context of various receptor and non-receptor tyrosine kinases, and represent a general problem that needs to be overcome in TK-targeted therapy[123]. Therefore, one of the main challenges in the treatment of NSCLC is to design inhibitors that can overcome the steric interference to drug binding conferred by the T790M mutation. Irreversible inhibitors seem to show some promise in this regard (TABLE 1). In most cases, irreversible inhibitors form a covalent bond with crucial cysteine residues — Cys797 within EGFR or Cys805 within ERBB2 — in the active site of the respective enzymes[134,135]. Given the fact that only EGFR and ERBB2 (as opposed to ERBB4) have cysteines at these corresponding positions, irreversible ErbB inhibitors show very high specificity for EGFR and ERBB2. Previous studies from our laboratory have shown that the irreversible dual EGFR and ERBB2 inhibitors, HKI-272 (REF. 136) and HKI-357 (REF. 37), as well as the irreversible EGFR inhibitor EKB-569 (REF. 137) were all able to overcome gefitinib resistance owing to T790M *in cis* with an L858R mutation in EGFR[37,138].

Interestingly, resistance to irreversible dual inhibitors is not achieved as rapidly as resistance to gefitinib and erlotinib in the laboratory[37]. Similarly, other studies have shown that the irreversible EGFR inhibitor CL-387,785 (REF. 139), and the irreversible pan-ErbB inhibitor CI-1033 (also known as canertinib)[140] can overcome resistance to L858R-mutated EGFR harbouring the T790M resistance-conferring mutation, whereas the reversible EGFR and ERBB2 inhibitor GW-572016 (also known as lapatinib) was ineffective in this regard[35,141]. CL-387,785 is also able to overcome gefitinib and erlotinib resistance mediated by in-frame insertions in exon 20 of *EGFR*[40]. A small subset of NSCLCs harbour mutations in ERBB2 (but not EGFR), and tumour cells that harbour the G776 insVG/C in ERBB2, although insensitive to erlotinib, are sensitive to the EGFR and ERBB2 dual irreversible inhibitor, HKI-272 (REF. 142). Similarly, a small subset of NSCLCs that express the EGFR mutant variant III (EGFRvIII) are also insensitive to gefitinib and erlotinib but show sensitivity to HKI-272 (REF. 143). HKI-272 is currently being evaluated in multi-center clinical trials in NSCLC patients. Therefore, several independent lines of evidence underscore the use of irreversible erbB inhibitors, especially for situations in which reversible inhibitors of EGFR lose efficacy.

Table 1 | **Targeted therapeutics currently approved or being evaluated for the treatment of NSCLC**

Class	Therapeutic	Target	Company	Stage of development (tumour type)
EGFR TKI (single reversible)	Gefitinib (Iressa; ZD-1839)	EGFR	AstraZeneca	Approved (NSCLC)
	Erlotinib (Tarceva; OSI-774)	EGFR	OSI, Genentech and Roche	Approved (NSCLC)
EGFR TKI (single irreversible)	EKB-569	EGFR	Wyeth	Phase II * (colorectal)
	CL-387,785	EGFR	Wyeth	Preclinical *
ErbB family TKI (multiple reversible)	Lapatinib (GW572016; Tykerb)	EGFR, ERBB2	GlaxoSmithKline	Phase III (breast)
ErbB family TKI (multiple irreversible)	Canertinib (CI-1033; PD183805)	EGFR, ERBB2, ERBB4	Pfizer	Phase II * (NSCLC, breast)
	HKI-272	EGFR, ERBB2	Wyeth	Phase I/II * (NSCLC, breast)
	BIBW 2992	EGFR, ERBB2	Boehringer Ingelheim	Phase I/II (breast, prostate, ovarian)
	HKI-357	EGFR, ERBB2	Wyeth	Preclinical *
RTK family TKI (multiple reversible)	ZD-6474	EGFR, ERBB2, FLT1, KDR	AstraZeneca	Phase III * (NSCLC, thyroid)
	AEE 788	EGFR, ERBB2, KDR	Novartis	Phase I/II (glioblastoma)
	XL647	EGFR, ERBB2, KDR, EPHB4	Exelexis	Phase II (NSCLC)
ErbB family heterodimerization	BMS-599626	EGFR, ERBB2	Bristol-Myers Squibb	Phase I (metastatic solid tumours)
HSP90	IPI-504	Mutant EGFR	Infinity Pharmaceuticals	Phase I/II * (multiple myeloma, GIST)
	17-AAG	Mutant EGFR	Kosan	Phase I/II * (solid tumours)

*Ability to overcome resistance to gefitinib or erlotinib. EGFR, epidermal growth factor receptor; FLT1, fms-like tyrosine kinase 1; GIST, gastrointestinal stromal tumour; KDR, kinase domain region; NSCLC, non-small-cell lung cancer; TKI, tyrosine kinase inhibitor; RTKI, receptor TKI.

Although the effectiveness of the irreversible inhibitor HKI-272 against EGFR is assumed to be responsible for its ability to suppress the proliferation of cells with acquired resistance to gefitinib and erlotinib, its ability to target ERBB2 is also of potential interest, given the role of other ErbB family members in EGFR signalling. ErbB family members undergo ligand-induced homo- and hetero-dimerization as a prelude to signal transduction after receptor activation, with each receptor dimer showing distinct ligand specificity (reviewed in REF. 7). The complex functional interactions among members of the ErbB family, combined with the ability to target several family members, might open new avenues for overcoming resistance to EGFR inhibitors.

Another strategy to overcome acquired resistance resulting from the T790M mutation is based on the observation that various EGFR mutants, including the double mutant L858R/T790M, associate with the molecular chaperone heat shock protein 90 (HSP90) (REF. 144). This interaction can be very specifically disrupted by the benzoquinone ansamysin, geldanamycin, which results in the degradation of gefitinib- and erlotinib-resistant mutant EGFR and leads to the apoptosis of EGFR-dependent tumour cells that harbour the mutated receptors[144,145]. The selective HSP90 inhibitor 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) and its derivative IPI-504 (REF. 146) are currently in clinical trials in patients with advanced solid tumours, and might be useful therapeutic alternatives for gefitinib- and erlotinib-resistant NSCLC. By overcoming the gatekeeper-residue mutations, such a strategy, if successful, might be of value for the treatment of a broad range of mutant receptor-driven cancers.

Rational combinations: is there a rationale?

At present, gefitinib and erlotinib, either alone or in combination with other regimens, are being evaluated in 157 clinical trials for various cancers (see the NCI clinical trials website). Despite some caveats[147], EGFR-targeted therapy has not been shown to have any beneficial effects in combination with standard chemotherapeutic regimens, which begs the question of whether or not there is a rationale for the combination of therapeutics in the treatment of NSCLC.

Insights into EGFR-dependent signalling might provide a first clue to the rational design of combination therapies. Research over the past 40 years has uncovered some of the crucial players in the EGFR signal-transduction pathway, which can be roughly divided into two categories: the pro-survival arm of the pathway comprising the PI3K–mTOR (mammalian target of rapamycin)–Akt cascade, and the proliferative arm consisting of the Ras–Raf–MEK–ERK pathway[148] (FIG. 5). This simplistic framework would

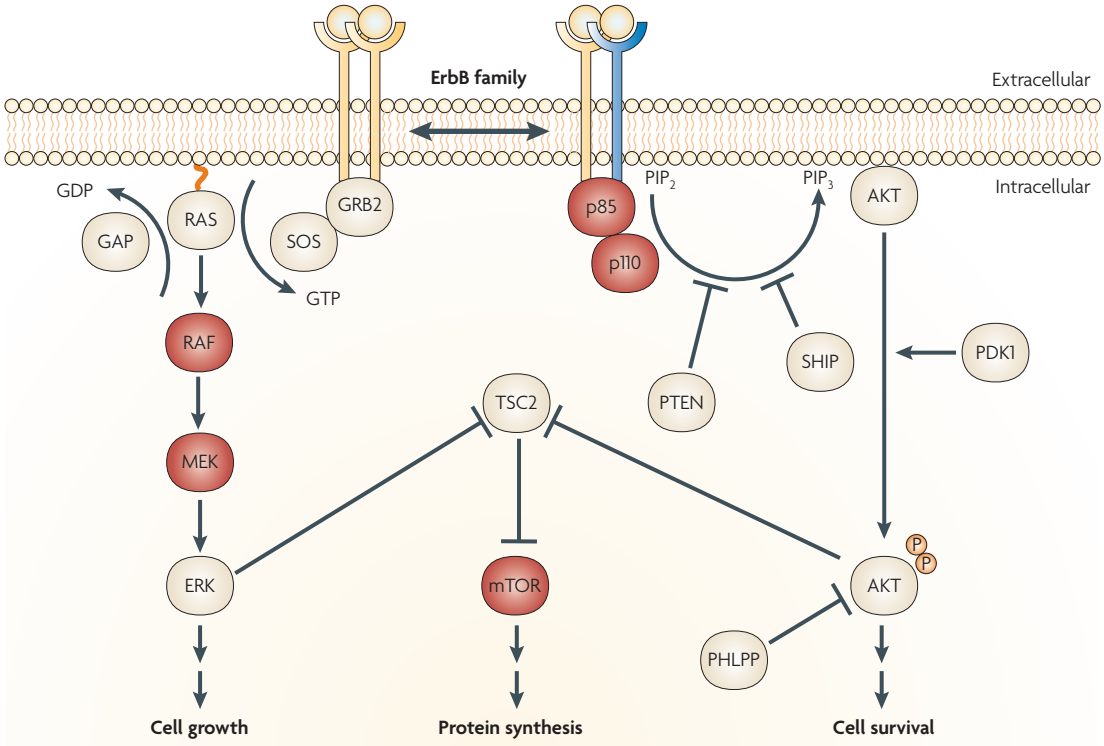


Figure 5 | **Cell-survival pathways downstream of activated erbB receptor tyrosine kinases.** Two important cell-survival pathways that operate downstream of activated ErbB transmembrane receptor tyrosine kinases (represented by pairs of yellow, and yellow and blue receptors to represent homo- and hetero-dimers, respectively), along with some of the key constituent signalling molecules are shown. The Ras–Raf–MEK–ERK pathway is shown on the left, and the phosphatidylinositol 3-kinase (PI3K)–Akt pathway is shown on the right. Key points along the pathway where targeted inhibition seems to exert a blockade are indicated by red circles, showing the relevant proteins they target (specific examples cited in TABLE 2). ERK, extracellular signal-regulated kinase; GRB2, growth factor receptor-bound protein 2; mTOR, mammalian target of rapamycin; SOS, son of sevenless.

suggest that inhibitors that target different key components of this network (in combination with EGFR-TKIs) might provide greater therapeutic efficacy, particularly in a setting where EGFR-TKI monotherapy follows a consistent pattern of diminishing returns and eventually becomes ineffective. Preclinical studies in an NSCLC cell line xenograft model have suggested that a PI3K inhibitor, PX-866, sensitizes otherwise insensitive tumours to gefitinib[149] (TABLE 2). Efforts are currently underway to develop PI3K inhibitors with greater specificity[150]. The serine-threonine kinase mTOR lies downstream of PI3K, and is inhibited by rapamycin and rapamycin analogues (TABLE 2). Preclinical studies suggest that mTOR inhibitors might also have synergistic effects when combined with targeted EGFR inhibitors[151,152]. At present, combinations of gefitinib or erlotinib with sirolimus, temsirolimus or everolimus are undergoing phase I and II evaluation in patients with advanced NSCLC, recurrent malignant glioma, prostate cancer and metastatic breast cancer[153]. However, despite their dramatic effects in some preclinical studies (reviewed in REF. 154), monotherapy with mTOR inhibitors have so far yielded disappointing results in clinical trials[155]. The selective inhibition of mTOR might in fact lead to the activation of the PI3K pathway and result in feedback activation of the pro-survival mediator Akt[156,157]. Therefore, recent studies have tried to overcome this problem through the use of dual inhibitors of PI3K and mTOR, which seem effective in preclinical studies of glioma cell lines[150]. The evaluation of these dual PI3K and mTOR inhibitors, either as monotherapy or in combination with targeted ErbB family inhibitors, in clinical studies might therefore hold considerable promise.

The Ras–MAPK (mitogen-activated protein kinase) pathway is another important cell-proliferation pathway downstream of EGFR that is frequently activated in cancer. Although mutations in Ras oncogenes do not seem to coexist with *EGFR* mutations, the pathway might be important in mediating EGFR-mutant signals, and therefore the inhibition of Ras or Raf in combination with gefitinib or erlotinib might have some benefit. The MEK inhibitor PD-325901 is currently being evaluated as a single agent in phase II clinical trials in patients with advanced NSCLC. However, activation of the Ras–MAPK pathway has not been as well correlated with response to EGFR inhibitors as the PI3K–Akt pathway[158].

In addition to manipulating components of EGFR signalling pathways, complementary molecular therapeutic approaches that involve simultaneously targeting distinct pathways have potential benefit. Although most of these approaches are empirical by nature, a rationale does exist for targeting both the tumour and stromal components of

Table 2 | **Targeted therapeutics used alone or in combination with EGFR-TKIs**

Mode of action	Therapeutic	Target	Company	Stage of development (tumour type)
p110α-specific inhibition	PX-866 (combination with gefitinib)	PI3K	ProlX Pharmaceuticals	Preclinical (NSCLC)
Rapamycin analogues	Sirolimus (combination with gefitinib)	mTOR	Wyeth	Phase I/II (NSCLC, glioblastoma)
	Temsirolimus (CCI-779; combination with erlotinib)	mTOR	Wyeth	Phase I/II (glioblastoma)
	Everolimus (RAD001; combination with gefitinib or erlotinib)	FKBP12, mTOR	Novartis	Phase I/II (NSCLC, glioblastoma, breast)
	AP23573	mTOR	Ariad	Phase I/II (endometrial)
MAPK pathway	Sorafenib (BAY49-9006; alone or in combination with erlotinib)	Raf, (KDR, p38α?)	Bayer	Phase I/II (NSCLC, glioblastoma)
	PD-325901 (single agent)	MEK	Pfizer	Phase II (NSCLC)

Representative examples of different classes of targeted inhibitors that are undergoing evaluation either alone or in combination with EGFR-TKIs are indicated. KDR, kinase domain region; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated and extracellular-signal regulated kinase kinase; mTOR, mammalian target of rapamycin; NSCLC; non-small-cell lung cancer; PI3K, phosphatidylinositol 3-kinase.

a tumour. Tumour vasculature is a particularly important target for therapeutic intervention, and it has been the basis for the development of dual inhibitors of EGFR and ERBB2 and the vascular endothelial growth factor (VEGF) receptors FLT1 (fms-like tyrosine kinase 1) and KDR (kinase domain region). Consistent with this concept, the dual EGFR and VEGFR inhibitor ZD6474[159,160] has shown efficacy in tumour xenografts that are resistant to cetuximab or gefitinib[161,162]. The use of the EGFR and VEGFR dual inhibitors ZD6464, AEE788[163] and XL647 is currently being evaluated in clinical trials[163,164].

The way forward

Experience with gefitinib and erlotinib has taught us the following important lessons: first, RTKs can be very useful targets for therapeutic intervention in epithelial cancers. Second, the targeted inhibition of RTKs might only be efficacious in a small subset of patients, and mutations in RTKs might be one of the useful predictors of response. Third, acquired TKI resistance substantially limits the therapeutic efficacy of these agents. The first lesson validates the usefulness of drug-discovery programmes focused on screening for RTK inhibitors, and reinforces the ongoing efforts of organizations engaged in this endeavor. However, the second lesson might point to the need for a reorientation of our traditional approach to drug discovery. The low frequency of genetically-defined responsive patient subsets calls for the consideration of a far broader sampling of individual cancer types, so as to achieve a representation of genetic diversity at all levels of analysis, from mutation detection in RTK genes to identifying new drug targets through functional assays and screening for efficacy in preclinical experiments. For example, to detect the 10% response to gefitinib or erlotinib typically seen in NSCLC patients, a cell-based drug screen would require a minimum of 100, and ideally 1,000, different NSCLC-derived cell lines, far beyond traditional cell-based screens. In fact, given the current cell-based screening strategies that generally involve a few cell lines representative of each tumour type, it is likely that gefitinib or erlotinib would never have been picked up as a 'hit'. A similar rationale for increased sampling size can be applied to genetic analyses of tumour samples in order to detect mutations in EGFR in an unselected cohort of NSCLC patients.

Recent genetic studies underscore the affect of large-scale genomic analyses in highlighting the complexity of the 'cancer landscape', and more importantly in pinpointing the specific genetic alterations that are key to the genesis of these tumours. For example, by analysing 120 primary lung tumours, the Sanger Center cancer kinome sequencing project showed that ERBB2 mutations occur in 4% of lung tumours[165]. In a recent whole-cancer-genome sequencing project at Johns Hopkins University in the United States, the number of distinct genetic lesions in an individual tumour were fewer than might have been predicted, but few of these were found to be recurrent, even among different cases of the same type of cancer[166], pointing to potentially small subsets of genetically-defined tumours across various histologies (different needles in different haystacks). Achieving the ambitious goals of the US National Institutes of Health (NIH) Cancer Genome Atlas (TCGA), to comprehensively annotate all cancer-associated mutations, might therefore require the analysis of many individual tumours within each histological type. Complementary functional approaches, including the use of short hairpin RNA libraries to identify genes that are essential to cancer cell viability[167], would also need to be applied across a broad spectrum of different cancer cell lines, each representing a different genetic context and potential addiction to a different oncogenic pathway. In summary, as the lessons learned from EGFR inhibition and cancer therapy continue to evolve, they have already provided a powerful example of clinical therapeutic affect achieved through an understanding of molecular abnormalities in cellular signalling, at the same time as warning of the genetic complexity in cancer that will require the coupling of different therapeutic strategies to individual genetic variation.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
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Massachusetts General Hospital Cancer Center:
http://www.massgeneral.org/cancer/
NCI clinical trials website: http://www.cancer.gov/clinicaltrials
Access to this interactive links box is free online.