

Published in final edited form as:

Nat Med. 2013 November ; 19(11): 1389–1400. doi:10.1038/nm.3388.

The quest to overcome resistance to EGFR-targeted therapies in cancer

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Abstract

All patients with metastatic lung, colorectal, pancreatic or head and neck cancers who initially benefit from epidermal growth factor receptor (EGFR)-targeted therapies eventually develop resistance. An increasing understanding of the number and complexity of resistance mechanisms highlights the Herculean challenge of killing tumors that are resistant to EGFR inhibitors. Our growing knowledge of resistance pathways provides an opportunity to develop new mechanism-based inhibitors and combination therapies to prevent or overcome therapeutic resistance in tumors. We present a comprehensive review of resistance pathways to EGFR-targeted therapies in lung, colorectal and head and neck cancers and discuss therapeutic strategies that are designed to circumvent resistance.

Oncologists are confronted with a formidable challenge in overcoming cancers with innate or acquired resistance to targeted therapies. This dilemma is especially acute in cancers that are dependent on EGFR activation: the initial enthusiasm over substantial clinical responses to EGFR tyrosine kinase inhibitors (TKIs) and monoclonal antibodies has now been tempered by the identification of an ever-increasing number of *de novo* and acquired resistance mechanisms.

EGFR addiction and signaling in cancer

EGFR (also known as *ERBB1* or *HER1*) belongs to the ERBB family of cell-surface receptor tyrosine kinases that also includes *HER2* (also known as *NEU* or *ERBB2* (ref. 1)). EGF binding to EGFR triggers homodimerization or heterodimerization of this receptor with other ERBB members, namely HER2, receptor phosphorylation and activation of downstream effectors such as RAS–RAF–MEK–ERK–MAPK and PI3K–AKT–mTOR,

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COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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leading to cell proliferation² (Fig. 1). Other EGFR ligands include transforming growth factor- α (TGF- α), amphiregulin, epigen, betacellulin, heparin-binding EGF and epiregulin³. Wild-type EGFR signaling contributes to tumor cell proliferation, evasion of apoptosis, angiogenesis and metastasis².

The crucial importance of EGFR to tumor cell survival in lung adenocarcinoma highlights the concept of ‘oncogene addiction’ as defined by Weinstein in 2002 whereby a cancer cell becomes dependent on a specific oncogenic signaling pathway⁴. Drugs that inhibit mutant EGFR such as erlotinib turn off this key pathway and lead to tumor cell death through the BCL-2 family member BIM (also called BCL2L11). Since EGF was first identified by Stanley Cohen in 1962, considerable advances have been made in the understanding of EGF-mediated signaling and the therapeutic application of this knowledge (Fig. 2).

Whether there is addiction to EGFR signaling in cancers of the head and neck, colon and pancreas is less clear than in lung cancer: EGFR-targeted therapies are either combined with chemotherapy to be effective or are much less effective as single-agent therapies when compared to the initial response rates to EGFR TKIs in lung adenocarcinoma (Supplementary Table 1).

Therapeutic targeting of EGFR signaling

Therapies targeting EGFR signaling are part of the arsenal of agents that are used to treat lung, colorectal, pancreatic and head and neck cancers (Supplementary Table 1). Specific drugs used include erlotinib and gefitinib, which reversibly inhibit the EGFR tyrosine kinase domain by competitively binding with ATP, and the monoclonal antibodies (mAbs) cetuximab (a chimeric mouse-human IgG1 antibody) and panitumumab (a fully humanized IgG2 antibody). Cetuximab and panitumumab block ligand binding to the extracellular domain of EGFR, promote receptor internalization and mediate antibody- and complement-mediated cytotoxicity². Antibody- or complement-mediated killing may be more effective with cetuximab as compared to panitumumab, as the IgG1 subclass is more effective than IgG2 at activating complement and the Fc receptor on immune effector cells⁵.

EGFR-activating mutations cluster in the catalytic kinase domain, are detected almost exclusively in adenocarcinomas of the lung, display up to a 50-fold increase in kinase activity by abrogating autoinhibition⁶ and are capable of oncogenic transformation of fibroblast and lung epithelial cells⁷. Activating mutations are heterozygous, and the mutant *EGFR* allele is frequently amplified. Although over 100 different mutations in the *EGFR* kinase domain have been identified in adenocarcinomas of the lung, the majority of patients harbor one of seven mutations⁸, the clinical properties of which are summarized in Table 1. The common *EGFR*-activating mutations, exon 19 deletions and L858R, which account for 85% of all *EGFR* mutations, predict sensitivity to the EGFR TKIs (gefitinib, erlotinib and afatinib) in preclinical models and in patients with lung cancer. With the exception of rare cases of familial lung adenocarcinoma^{9,10}, most *EGFR* mutations in lung adenocarcinoma are somatic.

The superiority of first-line gefitinib and erlotinib over conventional cytotoxic chemotherapy, both in terms of response rates and progression-free survival in patients with

EGFR-mutant adenocarcinoma, has been well established in clinical trials in Western and Asian populations^{11–14}. Similarities in overall survival between EGFR TKIs as compared to chemotherapy occur because patients who progress on either treatment cross over to the other; that is, a patient who progresses on an EGFR TKI will receive chemotherapy and vice versa. Trials of cetuximab in combination with chemotherapy in lung cancer have been more disappointing. Although the response rate with the addition of cetuximab is higher than that for chemotherapy alone, there was no statistically significant difference in progression-free survival and only an ~1 month improvement in overall survival in combination with cisplatin and vinorelbine^{15,16}. Cetuximab in combination with chemotherapy or radiation in head and neck cancers has produced more encouraging results in terms of overall survival^{17,18}. Cetuximab and panitumumab are also approved for use in colorectal cancer in combination with chemotherapy or, when other options are exhausted, as single agents^{19–24}. Erlotinib produces an ~2 week increase in overall survival in pancreatic cancer when given in combination with gemcitabine as compared to gemcitabine monotherapy²⁵, which is interesting given that EGFR signaling has been implicated in *KRAS*-mediated development of pancreatic cancer in preclinical mouse models^{26,27}.

Mechanisms of resistance to EGFR-targeted therapies

Despite the demonstrated benefits of EGFR-targeting agents, not all patients with cancer respond to treatment, and any gain in the median progression-free survival with these therapies compared to chemotherapy is, rather disappointingly, still less than 1 year. Intrinsic, *de novo* or primary resistance is defined as the failure to respond to small-molecule or antibody inhibitors. Primary resistance is distinct from failure to respond due to insufficient drug exposure. This failure can occur when EGFR TKIs are coadministered with drugs such as fenofibrate, which induce CYP3A4 (thereby increasing erlotinib metabolism), or proton pump inhibitors and H2-receptor antagonists, which decrease pH-dependent drug solubility^{28,29}. Acquired resistance occurs in patients who initially benefit from EGFR-targeted therapies. A clinical definition of acquired resistance to EGFR TKIs has been proposed and may also be expanded to also include EGFR-targeting mAbs: acquired resistance is systemic progression (by Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) criteria) after a complete or partial response or >6 months of stable disease after treatment with a targeted therapy³⁰.

Resistance mechanisms to EGFR small-molecule inhibitors or antibodies that have been validated in patients may be grouped into four categories (Fig. 3, Table 2 and Supplementary Tables 1 and 2): mutation of EGFR to a drug-resistant state, for example, through the T790M or S492R mutations, which abrogate the activity of gefitinib or erlotinib and cetuximab, respectively, but do not diminish the kinase activity of the receptor; ‘oncogenic shift,’ or activation of a bypass signaling pathway (such as *MET* amplification, *HER2* upregulation or *KRAS* activation)³¹; impairment of a pathway that is essential for EGFR TKI-mediated apoptosis, such as germline intronic deletions that remove the BH3 domain of *BIM*³²; and histologic transformation to small cell lung cancer or an epithelial-mesenchymal transition³³. All four resistance mechanisms have been observed to occur in patients with lung adenocarcinoma with resistance to EGFR TKIs, and some mechanisms, such as T790M, occur in both acquired and innate resistance³⁴.

Secondary *EGFR* mutations

The most common mechanism of resistance to TKIs in *EGFR*-mutant lung cancer is the T790M ‘gatekeeper’ mutation, which is found in approximately 60% of patients with acquired resistance³³. Secondary kinase mutations are a common mechanism of acquired resistance across other cancers that demonstrate oncogene addiction, and these mutations represent a form of oncogenic drift. Examples include ABL T315I in chronic myeloid leukemia (CML)³⁵, KIT T670I in gastrointestinal stromal tumors³⁶ and ALK L1196M in adenocarcinoma³⁷. The *EGFR* T790M and ALK F1174L³⁸ mutations are mechanistically similar in that they increase the kinase affinity for ATP by approximately fivefold, which decreases sensitivity to ATP-competitive reversible inhibitors such as erlotinib or crizotinib³⁹. Germline T790M mutations have been reported in families with inherited lung cancer¹⁰. The T790M mutation lowers the growth kinetics of tumor cells⁴⁰ and may be present before treatment with *EGFR* TKIs^{9,41}. In colorectal cancer, an acquired mutation in the extracellular domain of *EGFR* (S492R) abrogates cetuximab binding, leading to clinical resistance⁴². Tumor cells with this mutation remain susceptible to panitumumab, and a patient with cetuximab resistance and an *EGFR* S492R mutation responded to panitumumab⁴². This difference in susceptibility between the two antibodies is likely due to differences in their interactions with *EGFR*, as the amino acid involved is located at the interface between antibody–receptor binding and affects the binding of cetuximab but not panitumumab.

In contrast to resistance to cytotoxic chemotherapy, which may be mediated by altered cellular import or efflux of drugs⁴³, resistance to *EGFR*-targeted therapies may occur through multiple, interacting pathways (Fig. 1). Signaling pathways that stimulate cell growth may be thought of as a network in which loss of one node diverts pro-survival or proliferation stimuli through other nodes. Numerous pathways are clinically validated to trigger resistance to *EGFR* TKIs and monoclonal antibodies, including amplification of *MET* or *HER2* (refs. 44–46), loss of *PTEN*^{47,48} and activation of *KRAS*, *PIK3CA* and *BRAF*^{49–52}. These pathways lead to persistent activation of downstream signaling despite *EGFR* inhibition and hence block the apoptosis or decreased cellular proliferation that is normally mediated by *EGFR* inhibition. The activation of these pathways may be complementary and interchangeable across different cancers: for example, *BRAF* activation triggers resistance to *EGFR* TKIs in *EGFR*-mutant lung cancer⁵¹, and *EGFR* mediates resistance to vemurafenib in colon cancers with mutant *BRAF* V600E^{53,54}.

How, when or why a tumor selects one pathway over another to overcome resistance remains unknown. For example, in lung cancer, *EGFR*-activating mutations are mutually exclusive to activating mutations in *KRAS*, whereas in colorectal cancer, *KRAS* activation is a mechanism of innate and acquired resistance to *EGFR*-targeting mAbs. Common culprits such as *HER2* and *MET* have been implicated in resistance across lung, head and neck and colorectal cancers (Table 2). The bias of familiarity may also bend researchers’ search for resistance toward these and other well-studied pathways.

That the *EGFR* T790M mutation is by far the most common mechanism of resistance to *EGFR* TKIs in lung cancer highlights the ‘addiction’ of *EGFR*-mutant lung cancer: a simple

amino acid substitution resurrects the ability of the tumor to proliferate when challenged with an EGFR TKI. In contrast, in colorectal cancer, resistance to EGFR-targeted mAbs is mediated primarily through other signaling networks such as *KRAS*, *BRAF*, *PIK3CA* and *HER2* (Table 2 and Supplementary Tables 1 and 2). This distinction between EGFR functioning as a driver oncogene in a subset of lung cancers compared to its role as one of the many pathways that contribute to tumor growth in colorectal and other cancers is likely to be clinically relevant for three reasons. First, the durability and rate of response for EGFR-targeted monotherapy is much higher in lung cancers that are ‘addicted’ to EGFR signaling (response rate of 64–83% and progression-free survival of 9.7–13.1 months for erlotinib in *EGFR*-mutant lung cancer compared to a response rate of 12.8% and progression-free survival of 3.7 months for cetuximab monotherapy in colorectal cancer). Indeed, erlotinib behaves similarly to cetuximab in colorectal cancer in terms of response rate (9%) and progression-free survival (2.2 months) as a second-line treatment for patients with lung cancer unselected for *EGFR* mutation status (Supplementary Table 1). Second, the combination of erlotinib with chemotherapy in *EGFR*-mutant lung cancer does not improve the response rate, progression-free survival or overall survival compared to chemotherapy alone⁵⁵, whereas the combination of cetuximab or panitumumab with chemotherapy in colorectal cancer results in a marked improvement in response rate and progression-free survival compared with chemotherapy alone (Supplementary Table 1). Third, it is much easier to pharmacologically overcome resistance due to a change in a driver oncogene (for example, EGFR T790M) than resistance due to activation of an accessory pathway. This is because overcoming accessory pathway activation requires combinations of targeted agents (with all the attendant toxicities, discussed below) and the activated pathway may be pharmacologically intractable (for example, *KRAS*).

Resistance through histologic transformation

The epithelial-to-mesenchymal transition (EMT), as manifest by loss of E-cadherin expression and increased expression of fibronectin and vimentin, was reported as an EGFR resistance mechanism in lung adenocarcinoma cell lines in 2005 (ref. 56) and in patients treated with EGFR TKIs independent of a T790M mutation in 2010 (ref. 57). This process may be mediated by the activation of AXL kinase as a potential resistance mechanism that is associated with an increase in the EMT, along with AKT activation, and the small-molecule AXL inhibitors MP-470 and XL-880 restore sensitivity to erlotinib⁵⁸. Other pathways that have been reported to be involved in the histologic transformation of EGFR TKI-resistant tumors include Notch-1 and TGF- β ^{59,60}. Transformation of EGFR TKI-resistant adenocarcinoma to small cell lung cancer was reported in 2010 (ref. 61), and patients whose tumors have undergone such a change may benefit from treatment with etoposide and cisplatin, which is a standard chemotherapy regimen for small cell lung cancer. Although histologic transformation accounts for resistance in a minority of patients (~3% in some case series)³³, this unique and rare phenomenon underscores the role that EGF signaling has in development, as was first observed by Stanley Cohen in 1962 (ref. 62).

Development of criteria to assess the clinical relevance of resistance mechanisms

An ever-increasing list of alternative signaling pathways and mechanisms has been reported to mediate resistance to EGFR TKIs in lung cancer cell-based models (Supplementary

Table 3). These pathways and mechanisms include PTEN downregulation or loss^{63,64}, *CRKL* amplification⁶⁵, increased vascular endothelial growth factor (VEGF) production⁶⁶, activation of HER2, HER3 or fibroblast growth factor receptor (FGFR)^{67–71}, JAK signaling⁷², acquisition of stem cell–like properties⁷³, involvement of tumor-associated fibro-blasts⁷⁴ and EGFR ubiquitination⁷⁵. Through modulation of microRNAs, *MET* expression has been proposed to upregulate genes that are involved in the EMT and downregulate the expression of genes that are involved in mediating apoptosis, such as *BIM*⁷⁶. Liposomal transfection of lung adenocarcinoma cell lines and mouse xenograft models with microRNA targeting EGFR effectively suppresses growth of EGFR TKI–sensitive and –resistant (T790M) tumors⁷⁷. *EGFR*-mutant cell lines propagated in the presence of an EGFR TKI demonstrate activation of other oncogenic pathways such as *PI3K*, *AKT* and *HER2* and *HER3*—in effect, the ‘baton’ is passed from one oncogenic pathway to another. In 2010, another layer of complexity was added to the understanding of EGFR TKI resistance with the observation that chromatin modification mediated by insulin-like growth factor 1 (IGF-1) signaling confers resistance to erlotinib⁷⁸. Cells that acquire resistance through chromatin modification are sensitive to histone deacetylase inhibitors, and treatment with IGF inhibitors prevents the development of resistance⁷⁸.

Criteria to determine the clinical relevance of a possible resistance mechanism have been proposed and include that (i) the mechanism is necessary to generate resistance (that is, knockdown or inhibition of the resistance pathway restores sensitivity to EGFR inhibition); (ii) the mechanism is sufficient to confer resistance (that is, inappropriate activation of the mechanism confers the resistance phenotype); and (iii) the resistance mechanism is clinically observed in patients who progress on therapies that inhibit EGFR⁷⁹. These criteria are helpful in distinguishing true, clinically relevant resistance mechanisms from ‘passenger’ mutations that are found in tumor evolution or mechanisms that are limited solely to *in vitro* observations⁷⁹.

The criteria described above will be helpful in determining which combination treatments will progress to clinical trials. Additional practical criteria to move a combination treatment into the clinic include that (i) the pathway shown to mediate resistance must be detected by techniques currently used in molecular pathology (that is, DNA sequencing or immunohistochemistry); (ii) a sufficient number of patients will have the resistance mechanism to allow for statistical power in a clinical trial; and (iii) some clinical information will be available on the drug that targets the resistance pathway, for example, the drug will ideally have progressed through phase 1 as a single agent, and its toxicities should be clinically manageable. These criteria are drawn from experience in combining other targeted therapies with EGFR TKIs in lung cancer, which, as discussed below, has been difficult and disappointing.

Strategies to overcome resistance

The identification of various resistance mechanisms is essential to developing a strategy to overcome resistance and prolong the efficacy of EGFR-targeted therapies. Current clinical approaches to combat resistance in lung adenocarcinoma include irreversible and mutant-selective inhibitors of EGFR, combination of cetuximab and afatinib and combination of an

EGFR inhibitor with a drug targeting a resistance pathway, such as the combination of erlotinib and a *MET* inhibitor (Figs. 1 and 3). As further research is performed on resistance to EGFR-targeted therapies in colorectal, head and neck and pancreatic cancers, we predict that even more therapeutic opportunities will arise.

Next-generation EGFR inhibitors

In the late 1990s, irreversible inhibitors of EGFR were developed to increase the potency of inhibition through covalent modification of Cys797 in the ATP binding cleft of EGFR, thereby reducing competition from millimolar concentrations of intracellular ATP^{80,81}. In 2005, the activity of irreversible EGFR inhibitors against lung adenocarcinoma cells harboring an EGFR T790M mutation was noted in preclinical models^{82,83}. This discovery was taken one step further in 2009 with the report of EGFR T790M mutant-specific inhibitors⁸⁴. Irreversible EGFR inhibitors currently in clinical development include afatinib (BIBW 2992) and dacomitinib (PF00299804), which both also inhibit the kinase activity of HER2 and HER4 (ref. 85), and CO-1686, which specifically targets EGFR T790M⁸⁶. AZD9291, another EGFR T790M-specific inhibitor, has shown promising activity in phase I trials of patients with acquired resistance through this mechanism⁸⁷. The development of the irreversible EGFR inhibitors neratinib (HKI-272) and canertinib (CI-1033) in lung cancer was discontinued because of a lack of efficacy and dose-limiting diarrhea^{81,88}. Midostaurin (PKC412), an inhibitor of protein kinase C (PKC), FLT and KIT that is currently in clinical development in acute myelogenous leukemia, was found to selectively target EGFR T790M with greater selectivity compared to wild-type EGFR than the irreversible inhibitors afatinib and neratinib⁸⁹. Likewise, AP26113, an ALK inhibitor, was also found to selectively inhibit EGFR T790M⁹⁰. These findings raise the intriguing possibility that midostaurin and AP26113 may have off-target effects that are similar to those of imatinib, a drug that was originally developed to inhibit BCR-ABL in chronic myelogenous leukemia but was later found to inhibit KIT in gastrointestinal stromal tumors⁹¹.

Clinical trials of patients with *EGFR*-mutant adenocarcinoma have demonstrated the efficacy of afatinib and dacomitinib in first-line treatment (Table 2 and Supplementary Table 4), and significant benefits in terms of response rate and progression-free survival have been seen for afatinib as compared to cytotoxic chemotherapy⁹². The results of second-line treatment with these inhibitors in patients with *EGFR*-mutant lung cancer who progress on an EGFR TKI are more disappointing, with afatinib showing an 8.2% response rate⁹³. This result may be due to the fact that physiologic doses of current-generation irreversible EGFR TKIs do not fully inhibit EGFR T790M and in fact select for cells with amplification of this mutation^{94,95}. Dose escalation of current irreversible EGFR inhibitors in the clinic is limited by on-target inhibition of wild-type EGFR, which leads to EGFR-mediated toxicity (skin rash). Whether mutant-selective EGFR inhibitors such as CO-1686 and AZD9291 are clinically effective in patients with EGFR T790M mutant lung cancers remains unknown, and we are still awaiting the results of ongoing clinical trials. An alternative strategy has been dual targeting of EGFR by combining afatinib and cetuximab. This approach has been found to be effective in a mouse model of EGFR T790M⁹⁶, as well as in patients with *EGFR*-mutant lung cancer who developed acquired resistance to erlotinib⁹⁷ (Supplementary

Table 4). Interestingly, both patients with EGFR T790M and those without this mutation appeared to benefit from this therapeutic approach. The mechanistic basis for a benefit of afatinib and cetuximab in patients with *EGFR*-mutant or erlotinib-resistant lung adenocarcinoma that do not harbor a T790M mutation is not currently known. This benefit requires an irreversible EGFR inhibitor, as the combination of erlotinib and cetuximab in patients with acquired resistance to erlotinib failed to show any benefit in patients with lung cancer or in *KRAS*-mutant colorectal cancer^{98,99}. Afatinib and dacomitinib have demonstrated efficacy in patients with metastatic or refractory head and neck cancer, with afatinib showing noninferiority compared to cetuximab in a phase 2 trial^{100,101}.

Combination strategies

Preclinical studies identifying mechanisms of resistance to EGFR TKIs have been translated into several completed or ongoing clinical trials, including combinations of drugs that target MET or hepatocyte growth factor (HGF)¹⁰², dasatinib¹⁰³, everolimus¹⁰⁴, bortezomib¹⁰⁵, bevacizumab¹⁰⁶, sunitinib¹⁰⁷ and cetuximab^{97,98}. So far, however, none except the combination of afatinib and cetuximab in lung cancer⁹⁸ and cetuximab and erlotinib in metastatic chemotherapy-refractory colorectal cancer⁹⁹ has been very effective clinically. There are multiple potential reasons for these observations. Many clinical trials are undertaken without prospectively evaluating or targeting the specific subpopulation of drug-resistant patients when, on the basis of preclinical data, the addition of the new agent may be clinically effective. For example, *MET* amplification has been detected as an acquired resistance mechanism in ~10–20% of patients with lung cancer who progress on an EGFR TKI, yet clinical trials combining a MET inhibitor with erlotinib did not prospectively select for this population of patients¹⁰⁸. A common clinical development strategy is to add a new agent to erlotinib in order to overcome erlotinib resistance. However, this strategy, although perhaps easier from a regulatory standpoint because erlotinib has been approved by the US Food and Drug Administration, does not consider that EGFR T790M is the most common mechanism of erlotinib resistance (detected in ~50–60% of patients), and it is unlikely that any erlotinib combination will overcome this specific drug resistance mechanism.

Another limitation of clinical trials involving combination therapies is the overlapping toxicities of agents that make up the combination. The majority of kinase inhibitors are administered daily, and the dose taken forward for clinical development is based on toxicity and not target inhibition. This approach predicts a high likelihood that any combination approach will be more toxic, and therefore the tolerable doses of each agent in the combination may be suboptimal. In a phase 1/2 trial of erlotinib and XL-184, which inhibits MET, vascular endothelial growth factor receptor 2 (VEGFR2) and RET, the recommended phase 2 doses of the combination were 50 mg of erlotinib and 125 mg of XL-184, both of which are below the registered single-agent doses of each¹⁰⁹. Significant dose-limiting toxicities were encountered at higher doses of each drug when given in combination¹⁰⁹. Given the lack of efficacy of the combinations of targeted therapies tested so far and the dose-limiting toxicities encountered, the ideal partner to an EGFR TKI may be a drug that acts by an entirely different mechanism. For example, promising candidates that have been proposed include antibodies to the T cell inhibitory receptor programmed death-1 (PD1) or its ligand (PD-L1), given the efficacy of this therapy in patients with cancer who have

progressed on multiple lines of treatment^{110,111}. However, whether the expression of PD1 or PD-L1 is upregulated in EGFR inhibitor-resistant cancers remains to be determined.

One potential solution to overcome multiple mechanisms of resistance is to target downstream pathways that mediate the balance between survival and apoptosis. ROR1 is a pseudokinase that is regulated by the homeodomain transcription factor NKX2-1, is essential for lung development¹¹² and is thought to regulate the balance between survival and apoptosis. Knockdown of ROR1 is sufficient to inhibit the growth of lung cancer cell lines with multiple mechanisms of acquired resistance, including EGFR T790M, *MET* amplification and HGF overexpression¹¹³. PUMA is a BH3 BCL-2 effector of apoptosis that is induced along with BIM after EGFR inhibition and is essential for apoptosis induced by EGFR TKIs¹¹⁴. Inhibition of PI3K-AKT signaling leads to PUMA expression through nuclear translocation of the FOXO transcription factors¹¹⁴. A therapeutic strategy that directly activates the machinery that is necessary for apoptosis may circumvent multiple mechanisms of acquired resistance to EGFR-targeted therapies and may therefore be a broader approach than aiming to inhibit one resistance mechanism at a time. Key to the success of such an approach is ensuring specificity in targeting cancer cells while avoiding toxicity in the rapidly dividing cells found in the bone marrow and epithelium.

Ongoing research and challenges

Origins of resistance

Whether the resistance mechanisms to EGFR-targeted therapy highlighted in this article (Table 2 and Supplementary Tables 2 and 3) evolve under selective pressure from drug exposure or already exist before treatment is a matter of ongoing investigation. What is clear is that tumors that rely on EGFR signaling may draw on an arsenal of resistance mechanisms to overcome targeted therapy. This capability manifests in the clinic through response rates and times to progression that are disappointing compared to those seen with TKIs in the treatment of CML.

An analogy has been made between drug resistance in HIV and targeted therapies in cancer: both diseases are subject to high mutational rates, may benefit from combination treatment and may be difficult to eradicate entirely^{115,116}. Re-biopsy studies in patients with *EGFR*-mutant, erlotinib-resistant lung adenocarcinoma at progression suggest that the number of resistance mutations may be limited to a handful of suspects, including the T790M mutation (63% of patients), small cell lung cancer transformation (3%), amplification of *MET* (5%) or *HER2* (13%) or overlapping mechanisms (4%)³³. However, such reports may underestimate the number of resistance mechanisms, as resistance pathways that have been reported more recently, such as nuclear factor- κ B (NF- κ B), were not examined. Upfront use of combination therapies with different mechanisms, as is done in HIV treatment, may circumvent the development of resistance. Unlike HIV, which has a defined set of foreign protein drug targets—reverse transcriptase, protease, integrase and gp41—cancer may appropriate numerous normal signaling pathways, and inhibiting these pathways may not be tolerated, as this inhibition affects the normal physiologic functions of these pathways. In this Review, in which we evaluated ~5,000 abstracts published on EGFR in cancer, we counted at least 20 different nodes in various signaling networks that have been reported to

mediate resistance to EGFR-targeting strategies (Table 2). Given that each pathway is comprised of multiple protein members, each of which may undergo mutation, the number of potential resistance pathways is staggering.

Tumor heterogeneity and resistance

Because of genetic heterogeneity, tumors may exhibit different mechanisms of resistance at different sites within a patient. For example, the degree of *MET* amplification with or without a T790M mutation varied among metastatic sites sampled at autopsy from patients who died of TKI-resistant *EGFR*-mutant lung adenocarcinoma¹¹⁷, and *MET* amplification and the T790M mutation have been observed in the same tumor¹¹⁸. Discordance in *EGFR* mutation status within a tumor or at a metastatic site has been observed and was proposed to explain the mixed responses to EGFR TKI treatment^{119–121}. As more sensitive sequencing methods are used, however, heterogeneity in *EGFR* mutations is noted to occur much less frequently than when less sensitive methods are used^{121,122}. *KRAS* mutations are associated with decreased responsiveness to EGFR TKIs or monoclonal antibodies in patients unselected for *EGFR* mutational status¹²³. Discrepancy between *KRAS* mutational status between the primary tumor and a metastatic site has been noted in a patient with *EGFR*-mutant lung cancer¹²⁴. Tumor heterogeneity may also be of therapeutic benefit after resistance occurs, as clinical responses to the reintroduction of EGFR TKIs have been noted¹²⁵. The T790M mutation confers a growth disadvantage, and after removal of selective pressure from an EGFR TKI, further tumor growth may be driven by clones lacking T790M, leaving the tumor vulnerable to retreatment by an EGFR TKI⁴⁰.

The lack of complete understanding of genetic heterogeneity in patients with cancer can limit the ability to develop and/or interpret the efficacy of therapies and select patients for clinical trials that are biomarker driven. Because only one site of drug resistance is typically biopsied and the mechanism of resistance is determined from that biopsy, we may often assume that all of the non-biopsied sites of disease harbor the same mechanism of resistance. It is crucial to bear in mind that sequence data from a tumor biopsy represents the genetic makeup of that isolated piece of tissue. More data from clinical trials and sequential biopsies from multiple sites are needed to distinguish whether a drug fails because of lack of efficacy against one particular resistance mechanism or because of resistance heterogeneity.

Monitoring the evolution of drug resistance

How can a strategy that pairs a resistance mechanism with a therapy directed at overcoming resistance be applied clinically? One can imagine application of an adaptive clinical trial design to combat resistance to EGFR TKIs¹²⁶: patients with *EGFR*-mutant lung cancer who progress on primary TKI therapy undergo either re-biopsy or the detection of circulating tumor DNA or tumor cells to analyze mechanisms of resistance. These patients may then be adaptively randomized to receive additional therapy that specifically targets their resistance mechanism (for example, treatment with a MET or I κ B kinase inhibitor). The success of this approach depends on the ability to rapidly detect resistance mechanisms using small amounts of DNA. The EGFR T790M mutation has been successfully detected in serum¹²⁷ and circulating tumor cells¹²⁸, and sequencing of *EGFR*, *TP53*, *BRAF* and *RAS* in circulating tumor DNA has been reported^{129,130}. Proteomic attempts to determine serum

biomarkers that predict susceptibility and resistance to EGFR TKIs have shown some promise in lung and head and neck cancers^{131,132}. In the next decade, patients who progress on an EGFR-targeted therapy may undergo circulating tumor or tumor cell DNA analysis and be paired with a therapy that is tailored to overcome resistance. Coupled with advances in sequencing technologies, including targeted next-generation sequencing and whole-exome sequencing, these technologies may be able to detect drug resistance mechanisms noninvasively before clinical resistance or clinical consequences develop.

Preventing compared to treating drug resistance

Another method to delay the development of resistance involves alteration of EGFR TKI dose and schedule on the basis of the observation that drug-sensitive cells grow more rapidly than those with an acquired EGFR T790M mutation⁴⁰. This phenomenon has also been reported in a study of patient-derived mouse xenografts of BRAF V600E melanoma, which demonstrated that discontinuous dosing strategies may prolong the duration of vemurafenib response as a result of drug dependency in resistant cells¹³³. Tumor flares have been reported in patients who discontinue an EGFR TKI because of disease progression or inability to tolerate treatment^{104,134,135}, as well as in patients with progression on an EGFR TKI who have responded after a treatment ‘holiday’^{136,137}. In both cases, disease progression on EGFR TKI treatment is likely due to slow growth of a resistant clone (such as T790M) followed by fast growth of sensitive clones once the selective pressure of the EGFR TKI is removed. Mathematical and evolutionary modeling support the idea that high-dose pulses of EGFR TKI therapy along with low-dose maintenance may prevent the emergence of resistance^{40,138}. In a case report, a patient who experienced progression on erlotinib and afatinib demonstrated a remarkable response to weekly high-dose (1,500 mg) erlotinib¹³⁹. A relationship between drug plasma concentration and imatinib efficacy is observed in the treatment of CML: patients who achieve higher imatinib plasma concentrations have an increased response rate that is more rapid and durable compared to patients with lower plasma concentrations^{140,141}. Meta-analyses show that the presence of a rash after EGFR TKI treatment, which indicates inhibition of cutaneous EGFR signaling, correlates with improved progression-free and overall survival^{142,143}.

Preventing the emergence of drug resistance is an alternative strategy to developing a treatment approach for each of the potential resistance mechanisms. If a strategy could be developed that prevented not just one but a broad array of drug resistance mechanisms, it could have substantial clinical impact. However, it is not currently clear which combinations of agents need to be combined with EGFR TKIs or monoclonal antibodies to achieve this effect. In addition, any such combination needs to be tolerable, as the anticipation and hope would be for a longer duration of treatment than what is currently achievable.

Combining targeted therapies by understanding differences in effectiveness across various cancers

Oncologists hope to cure cancer; if that is not possible, they hope to turn it into a chronic disease, and if that is not possible, they aim to provide a longer progression-free survival. This spectrum is seen in targeted agents that cure certain cancers (retinoic acid in combination with chemotherapy in acute promyelocytic leukemia), turn other cancers into

chronic diseases (imatinib in CML) and increase progression-free survival in others (imatinib in gastrointestinal stromal tumors, crizotinib, erlotinib and gefitinib in lung cancer and vemurafenib in melanoma) (Fig. 4). It is not clear whether differences in the biology of the tumors (that is, liquid as compared to solid) or the activating pathways underlie the variations in effectiveness of different targeted agents in different cancers. One potential explanation lies in the high mutation rates described as ‘genomic chaos’ that are observed in solid tumors¹⁴⁴. Other explanations may include a higher degree of clonality and greater drug exposure in hematologic as compared to solid tumors. The situation we are currently in with creating combinations of targeted therapies may parallel the early days of cytotoxic chemotherapy before the advent of combination treatments, such as 5-fluorouracil, oxaliplatin and leucovorin (FOLFOX), cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), bleomycin, etoposide and cisplatin (BEP) and doxorubicin, cyclophosphamide and paclitaxel (AC→T), that are now well known to patients and oncologists. Determination of optimal therapeutic sequences and combinations will likely take dozens of clinical trials involving thousands of patients. One can hope that knowledge of resistance mechanisms will accelerate clinical translation by guiding trial design.

Conclusions

Oncogene addiction was described by Weinstein as being the “Achilles heel of cancer”⁴. A decade later, therapies targeted to some oncogenes grant a reprieve to patients lucky enough to have select driver mutations, and remissions last years in some cases (Fig. 4). For patients with *EGFR*-mutant lung cancer, the outlook, although better than in patients without such a mutation, is guarded, with tumor responses lasting months if they occur at all. Cancer may be more like the mythical, multiheaded Hydra battled by Hercules because of its ability to resist targeted therapies through evolution and tumor heterogeneity.

One lesson for cancer researchers and clinicians is the importance of perseverance, creativity and collaboration. Whether we defeat tumor resistance using combinations of drugs, immunotherapy, new dosing strategies or an as-yet-undiscovered approach, our success cannot come soon enough for our patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study is supported by US National Institutes of Health grants RO1CA114465 (P.A.J.), R01CA135257 (P.A.J.) and P01CA154303 (P.A.J.) and by an American Society of Clinical Oncology Young Investigator Award (C.R.C.).

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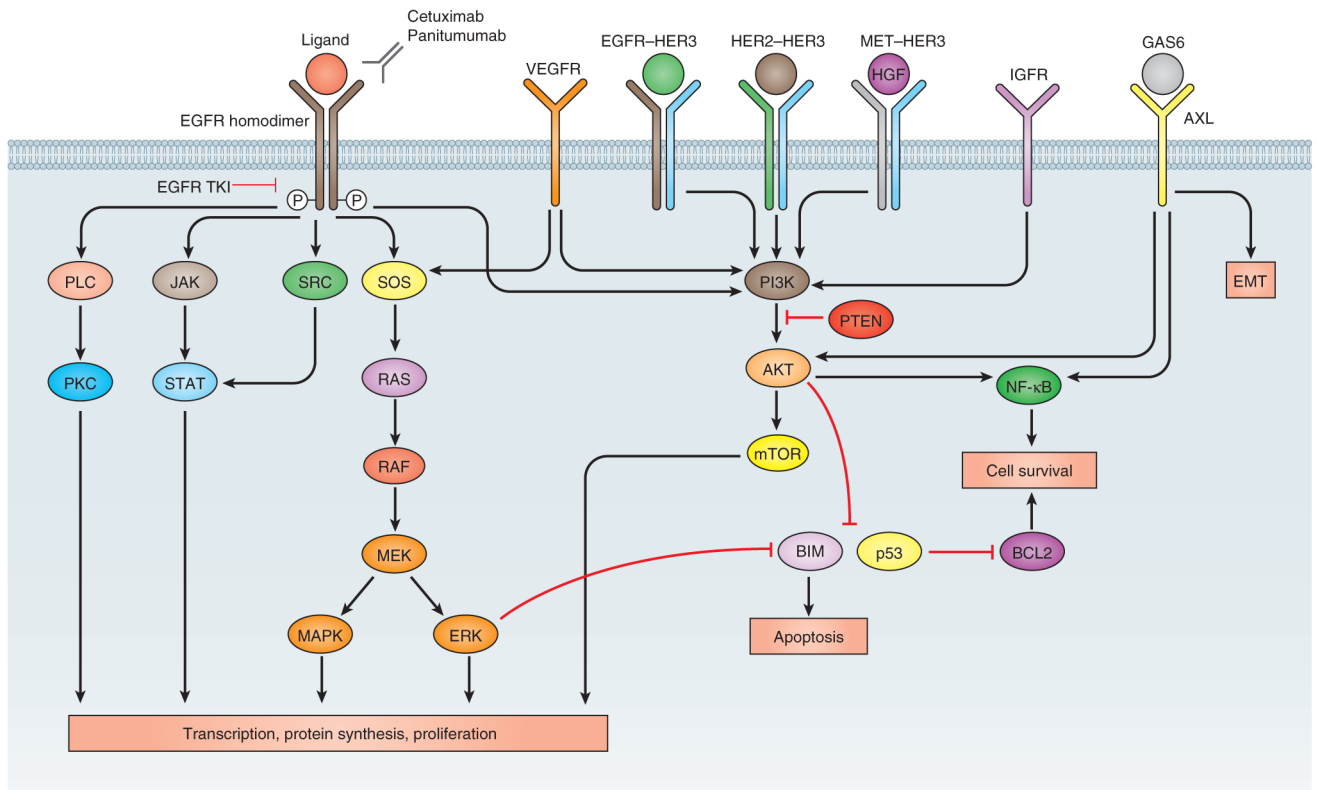


Figure 1.

EGFR signaling pathways. Activation of EGFR leads to downstream signaling pathways that ultimately drive tumor proliferation or impair apoptosis. These pathways mediate resistance through crosstalk or inappropriate activation but also provide targets for drugs to overcome resistance. IGFR, insulin-like growth factor receptor; PLC, phospholipase C; GAS6, growth arrest-specific 6.

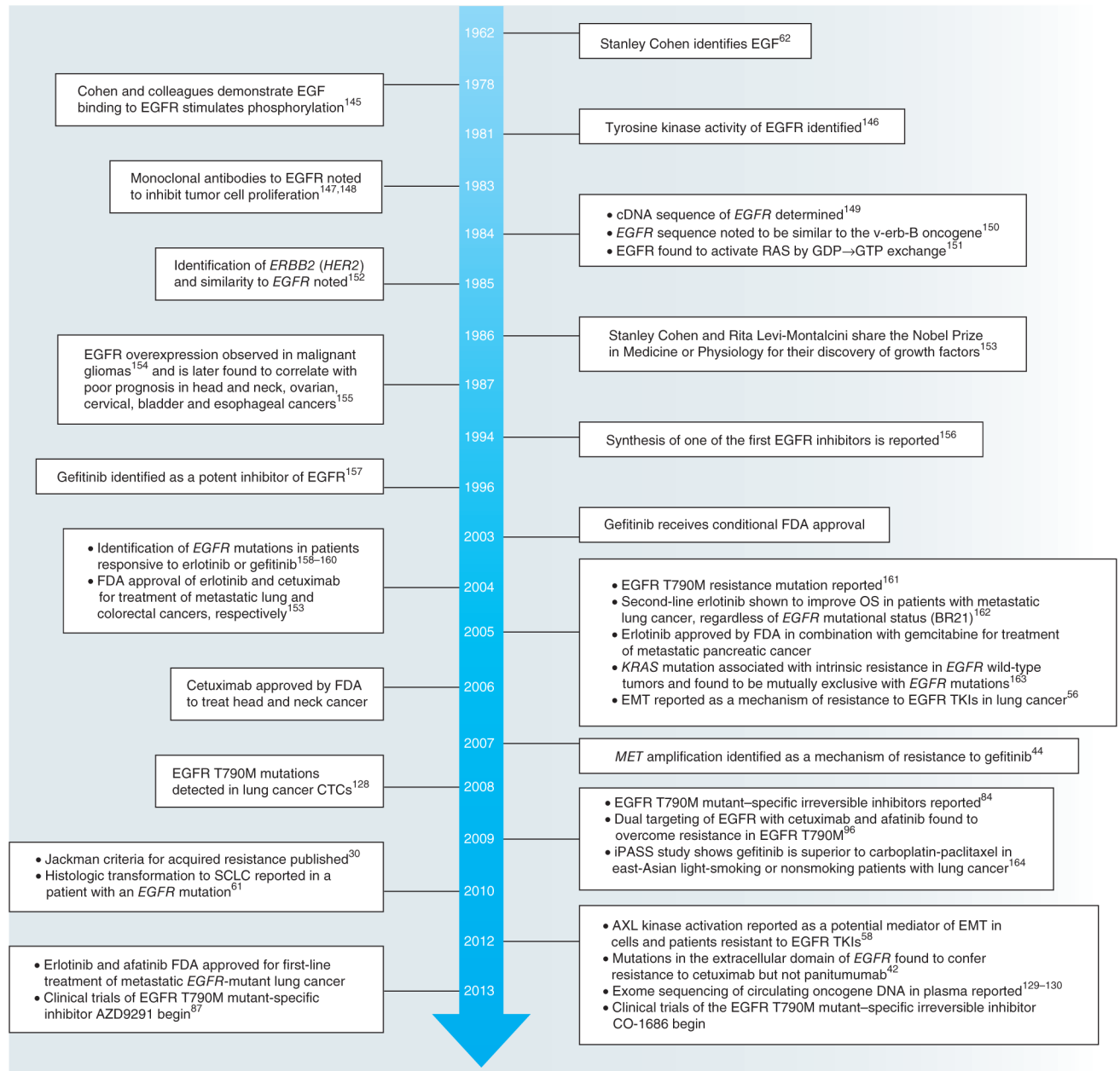


Figure 2.

Timeline of key discoveries in the EGFR field. The timeline charts important findings from basic and clinical research into EGFR and its role in cancer^{30,42,44,56,58,61,62,84,87,96,129,130,145–164} (adapted from ref. 153). FDA, US Food and Drug Administration; OS, overall survival; CTCs, circulating tumor cells; SCLC, small cell lung cancer; iPASS, Iressa Pan-Asia Study.

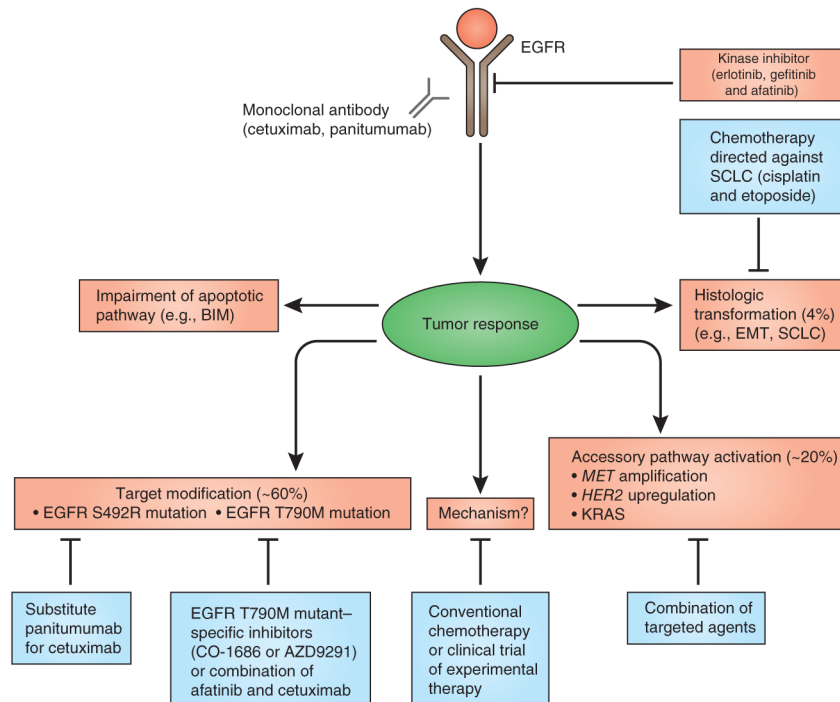


Figure 3. Clinically validated resistance mechanisms to EGFR inhibitors. Treatment with EGFR-targeted therapy results in tumor responses, which are blunted by the selection or evolution of clones with resistant EGFR (T790M or S492R), oncogenic shift (activation, upregulation or amplification of a bypass pathway), inhibition of apoptosis or histologic transformation (figure adapted from ref. 79).

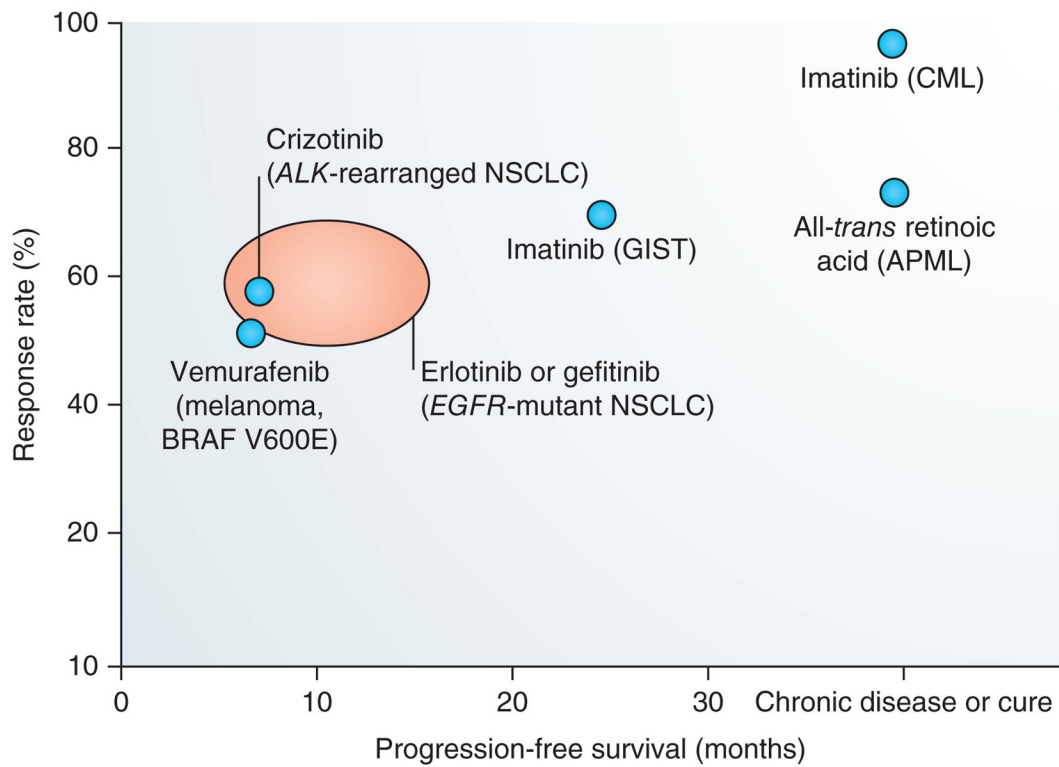


Figure 4.

Efficacy of targeted therapies in cancer treatment. Response rates and impact on progression-free survival vary widely among targeted therapies. The orange oval represents the reported response rates and progression-free survival for EGFR TKIs in *EGFR*-mutant lung adenocarcinoma (adapted from Supplementary Table 1). Imatinib targets BCR-ABL, has a ~95% response rate and has turned CML into a chronic disease for many patients¹⁶⁵. *All-trans* retinoic acid targets the PML-RAR fusion protein and may cure acute promyelocytic leukemia (APML) when given in combination with chemotherapy¹⁶⁶. For solid tumors such as melanoma and lung cancer, targeted therapies have a lower response rate and less of an impact on progression-free survival¹⁶⁷⁻¹⁶⁹. GIST, gastrointestinal stromal tumors.

Table 1

EGFR-activating and resistance mutations in adenocarcinoma of the lung

Mutation	Frequency in <i>EGFR</i> -mutant lung adenocarcinoma (%)	Clinical properties					
		Response rate to <i>EGFR</i> TKIs		Median PFS		Median OS	
		%	Reference	Months	Reference	Months	Reference
Exon 19 deletions	45	82.8	13	11.5	13	30.8	171
		84.8	170	9.0	14	34	174
		63	171	11	12	17.7	172
		64	172	14.6	171	33.1	173
		70	173	12	174		
				9.3	172		
				9.8	173		
L858R (exon 21)	40	67.3	13	10.8	13	14.8	171
		60.9	170	9.6	14	8	174
		50 ^a	171	8.4 ^b	12	20.5 ^c	172
		62	172	9.7	171		
Exon 20 insertions	2-9			5	174		
				6.9	172		
G719X	3	~50	178	8.1	179	16.4	179
L861X	2	60	179	6		15.2	
Exon 19 insertions	1						
T790M	0.5-3 (in some case series)						

The variable response to *EGFR* TKIs is thought to be related to the effect of varying insertion length on the drug-binding pocket¹⁷⁵. Median OS of 16 months¹⁷⁶ in one series and 4 years in another¹⁷⁷.

^a $P = 0.39$ compared to exon 19 deletions in this series.

^b $P = 0.075$ compared to exon 19 deletions in this series.

^c $P = 0.65$ compared to exon 19 deletions in this series.

PFS, progression-free survival; OS, overall survival.

Table 2

Overview of EGFR resistance mechanisms

	Lung (EGFR TKIs)	Colon (EGFR mAbs)	Head and neck (cetuximab)
Primary resistance	<i>EGFR</i> exon 20 insertion ^{7,a} <i>BIM</i> deletion ^{32,a} <i>EGFR</i> T790M ^{41,179}	<i>KRAS</i> ^{47,48,a} <i>PIK3CA</i> exon 20 (refs. 47-48) ^a <i>BRAF</i> mutation ^{47,48,a} <i>PTEN</i> deletion ^{47,48,a}	
Acquired resistance			
EGFR modification	T790M ^{82,a}	S492R ^{42,a}	<i>EGFRvIII</i> ¹⁸¹
Alternative pathway activation	<i>BRAF</i> ^{51,a} <i>CRKL</i> ⁶⁵ <i>DAPK</i> ¹⁸² <i>FGF</i> ⁶⁹⁻⁷¹ <i>HER2</i> (ref. 45) ^a <i>HER3</i> (ref. 68) <i>IGF</i> ^{183,184} <i>JAK2</i> (ref. 72) <i>MED12</i> (ref. 185) <i>MET</i> ^{44,a} <i>NF-κB</i> ^{186,a} <i>PTEN</i> loss ^{63,64} <i>PUMA</i> ¹¹⁴ <i>ROR1</i> (ref. 113) <i>VEGF</i> ¹⁸⁷	<i>HER2</i> (ref. 46) ^a <i>IGF</i> ^{189,a} <i>KRAS</i> ^{49,50,a} <i>MET</i> ¹⁹⁰	<i>Aurora</i> ¹⁹¹ <i>HER2</i> <i>HER3</i> <i>MET</i> ¹⁹²
Histologic transformation	Acquisition of stem cell properties ⁷³ EMT (AXL, Notch-1 or TGF-β activation) ^{57-60,185,a} Small cell lung cancer transformation ^{188,a}		EMT ^{193,194}

^aMechanisms have also been identified in patient tumors.