compounds target the stem cell population effectively. This is important because these cells are thought to be the source of the residual disease that remains even after long-term drug treatment.

If it lives up to its promise, DCC-2036 will play a role in the CML and AML stories. Regardless of its final contribution to the clinical management of these diseases, switch control drugs are certainly a very elegant solution to the BCR-ABL^{T315I} problem.

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Resisting Targeted Therapy: Fifty Ways to Leave Your EGFR

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Despite the promise of the new generation of molecularly targeted drugs, intrinsic and acquired resistance is proving to be as problematic as with cytotoxic drugs. Two recent papers have identified novel ways by which non-small cell lung cancers can exhibit resistance to EGFR inhibitors and suggest new therapeutic workarounds.

Paul Simon famously sang about the fifty ways to leave your lover. Two recent studies by Sequist et al. (2011) and Bivona et al. (2011) have revealed several additional new ways by which non-small cell lung cancers (NSCLC) can escape the clutches of small molecule inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase. Whether there will turn out to be fifty, or even more mechanisms through which resistance can be mediated—either de novo or acquired during treatment—remains to be seen. What is clear is that there are a growing number of ingenious molecular means by which cancers can circumvent inhibitors of EGFR and other oncogenic kinases. Although representing therapeutic challenges to the clinician, these mechanisms also suggest rational new therapeutic opportunities to improve clinical outcomes. Furthermore, by using kinase inhibitors as chemical probes (Workman and Collins, 2010) to interrogate human cancers, we are gaining considerable fundamental as well as translational insights into the diverse mechanisms of human oncogenesis.

The extraordinary success of imatinib in prolonging the lives of patients with chronic myeloid leukemia (CML) through the inhibition of the pathogenic tyrosine kinase activity of BCR-ABL has had a major scientific impact in validating the concept of single kinase addiction in the clinic (Druker et al., 2006). Furthermore, the principle of treating such oncogene addiction (Weinstein, 2002) with inhibitors of the respective major driver kinases has proved to be applicable to many other types of cancer. On the other hand, the tumor regression and prolonged survival obtained are commonly not as sustained as in CML (Sawyers, 2009).

NSCLC is a leading cause of death worldwide (www.who.int/cancer/en/, http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900), and cytotoxic chemotherapy has limited effectiveness. Approximately 10% of NSCLCs in western populations harbor somatic mutations in exons encoding the tyrosine kinase domain of EGFR, and these occur with an increased frequency in adenocarcinomas arising in nonsmokers, females, and individuals of Asian ethnicity. These mutations cause activated signaling

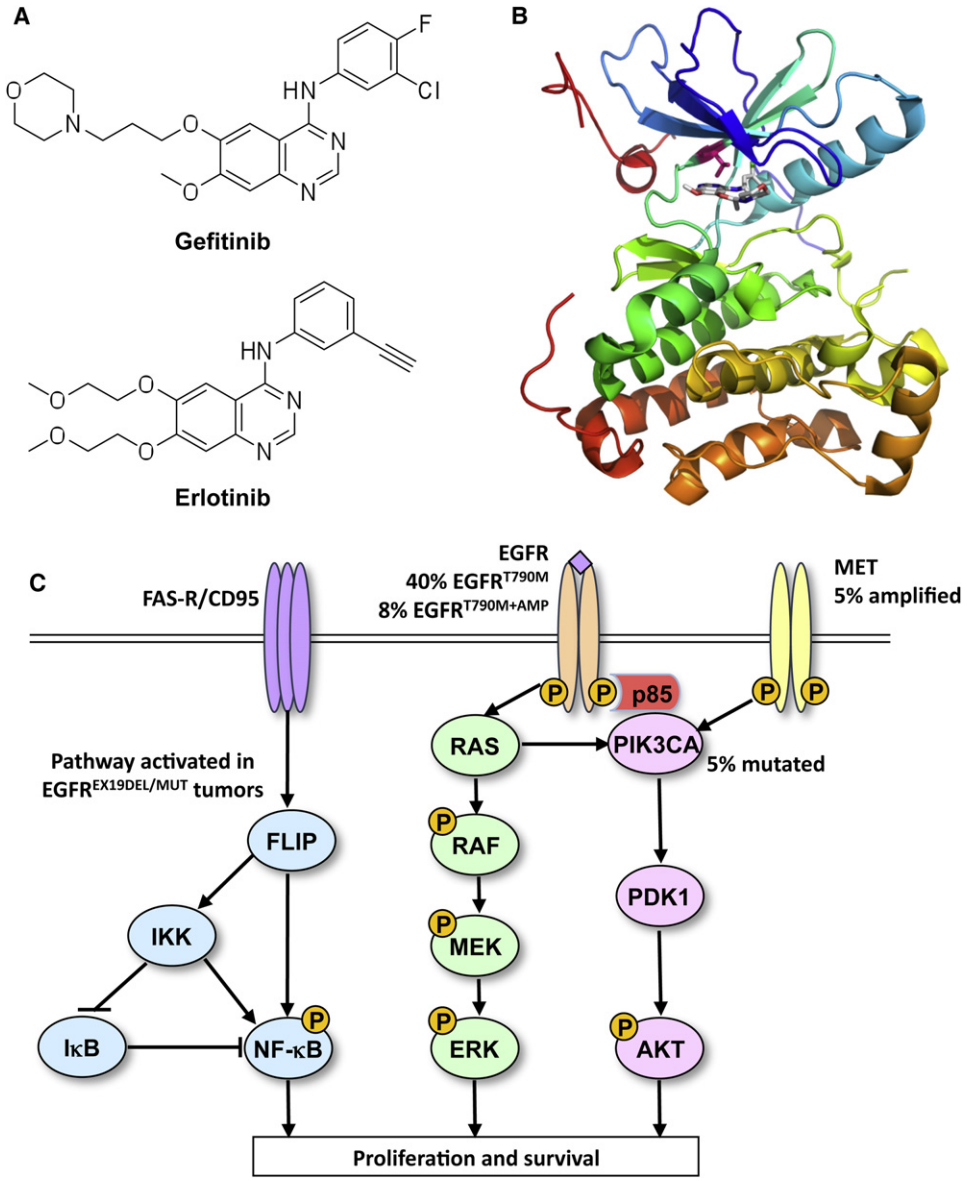

Figure 1. Ways to leave your EGFR inhibitor: Biochemical pathways leading to resistance to small molecule EGFR drugs such as gefitinib and erlotinib

(A) Structures of two approved EGFR TKIs, gefitinib and erlotinib, used in the treatment of NSCLC.

(B) Ribbon diagram of wild-type human EGFR (PDB code 2ITY), illustrating binding of gefitinib to the active site of the kinase. The magenta ball-stick (located just above the gefitinib molecule in the active site) indicates the gatekeeper residue (threonine[790]) that is commonly mutated to methionine (T790M), resulting in reduced inhibitor binding and drug resistance.

(C) Simplified pathway diagram of EGFR signaling through RAS/MEK/ERK and PI3K/PDK1/AKT indicating the points of mutation/amplification in EGFR TKI resistance as reported by Sequist and colleagues. The resistance mechanisms include the *EGFR* T790M gatekeeper mutation, amplification of *EGFR* T790M, *MET* amplification, and *PI3KCA* mutation (note that additional epithelial to mesenchymal transition changes and transformation from the NSCLC to the SCLC phenotype also lead to resistance but are not covered by this illustration). The illustration also shows the FAS/NF-κB signaling arm downstream of the FAS death receptor that was shown to be important in TKI resistance by Bivona and colleagues.

downstream of the receptor and commonly result in dramatic responses to the selective reversible tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib (Figure 1A), thus defining a subset of NSCLC characterized by addiction to the activated EGFR pathway (Haber et al., 2005). Despite around 70% of EGFR mutant NSCLC patients experiencing significant and durable tumor regressions with EGFR TKIs, 30% fail to respond, and the vast majority of responders develop acquired resistance (Mok et al., 2009; Rosell et al., 2009; Jackman et al., 2010). Roughly half the mutant EGFR NSCLC cases with acquired resistance to TKIs have a secondary "gatekeeper" mutation in EGFR (T790M) (Figure 1B) that reduces drug binding to the kinase target, while a further 15%–20% have amplifications in the MET receptor gene, providing a bypass signaling pathway through kinase switching or via receptor heterodimerization (see references in Sequist et al., 2011).

In the first of the two new papers, Sequist et al. (2011) obtained tumor biopsies before and after treatment from 37 EGFR mutant NSCLC patients who were initially responsive to TKIs. Tumor DNA was profiled to detect mutations in 13 key cancer genes. The now familiar gatekeeper mutation in *EGFR* (49% of cases) and amplification of *MET* (5%) were confirmed (Figure 1C). Important new findings were that clinical resistance was also associated with amplification of the *EGFR* T790M resistant allele (8%) and mutation of *PIK3CA* that encodes the p110α catalytic subunit of PI3 kinase (5%), the latter being consistent with laboratory studies in which introduction of *PIK3CA* into NSCLC cells induced TKI resistance. Activated PI3 kinase likely operates independently downstream of EGFR (Figure 1C).

Interestingly, Sequist et al. (2011) observed several additional (5%) cases in which TKI resistance developed by cells undergoing a transition from the epithelial to the more aggressive mesenchymal phenotype—also seen in laboratory studies and detected here by routine pathology and biomarker expression. Surprisingly, in 14% of patients, TKI resistance was associated with transformation from NSCLC into the phenotype of small cell lung cancers (SCLC), which then duly responded in four out of five cases to standard treatments for that disease.

Sequist et al. (2011) also obtained multiple longitudinal samples in three TKI-resistant NSCLC patients. In two patients, responses were seen after reintroducing TKI treatment following a drug-free interval after resistance developed, and these responses were concurrent with a loss of detectability of the T790M resistance mutation. In the third patient, TKI resistance was associated with emergence of the SCLC phenotype (together with a *PIK3CA* mutation), leading to successful implementation of SCLC-based chemo-radiation therapy; subsequent reappearance of adenocarcinoma lacking both neuroendocrine markers and the *PIK3CA* mutation led to successful readministration of TKI therapy, after which TKI-resistant disease with SCLC phenotype and *PIK3CA* mutation once again reemerged. Though anecdotal, these results support other findings (discussed by Sequist et al., 2011), indicating that resistance mechanisms are lost without the continued selective pressure of TKI therapy.

In the other new paper, Bivona et al. (2011) took a different approach. They screened 2000 "cancer relevant genes" using short hairpin RNAs to identify genes that, when silenced, would specifically sensitize the NSCLC cell line H1650, which is resistant to TKI treatment despite having a mutation associated with sensitivity to TKIs and lacking known clinical resistance mechanisms. Of the 36 screen hits, 18 genes, including *FAS/CD95*, were involved directly or indirectly with NF-κB signaling. Since FAS death receptor and NF-κB signaling are involved in cell survival and can promote tumor growth, Bivona et al. (2011) focused on these. Following thorough validation of hits in additional models, they showed that knockdown of the RELA subunit of NF-κB and of the functionally related c-FLIP or RIPK also increased sensitivity to EGFR TKI therapy but not to cytotoxic agents in the TKI-resistant cells. Further biochemical data supported the view that persistent NF-κB signaling was implicated in resistance to TKI-induced apoptosis, and the results were extended to tumor xenograft models, in which silencing of *FAS* and *RELA* increased the response of H1650 cancers in vivo to EGFR TKI treatment.

Since IκB kinase (IKKβ or IKBKB) decreases IκB stability leading to NF-κB activation, NF-κB pathway blockade can be achieved by inhibition of IKKβ. Consistent with this, Bivona et al. (2011) showed that sensitization to TKI treatment in resistant NSCLC models could be obtained using a small molecule IKKβ inhibitor in vitro and with IKKβ knockdown in tumor xenografts.

Demonstrating the clinical relevance of the findings, Bivona et al. (2011) went on to show that low expression of the NF-κB inhibitory protein IκB, indicative of a "high NF-κB activation state," was predictive of a poorer clinical outcome in a cohort of erlotinib-treated EGFR mutant NSCLC patients lacking evidence of the T790M gatekeeper mutation. In another cohort, IκB expression did not predict outcome from surgery and chemotherapy, indicating specificity. Thus, the results suggest that combining an NF-κB pathway inhibitor with EGFR inhibitors may be therapeutically advantageous.

The results obtained in these two important studies are consistent with an emerging overall model in which resistance can develop at the genetic level, owing to Darwinian clonal selection—in which the drug treatment represents an additional selective pressure—as well as at the biochemical level, involving feedback loops in the pathway or network and reversible epigenetic mechanisms.

Overall, the new results stress the importance of understanding the multiple molecular mechanisms of de novo and acquired resistance to TKIs in NSCLC and of modifying the therapy accordingly, including use of single drugs or more likely combinatorial treatments that are designed to overcome resistance. Therapeutic opportunities for EGFR-addicted NSCLC that are indicated by the two recent studies include the use of existing or novel EGFR TKIs, together with inhibitors of MET, the PI3 kinase pathway, and IKKβ plus SCLC cytotoxic agents. If confirmed in larger prospective studies, the results would reinforce the ongoing selection of treatment options informed by repeated tumor profiling in order to reverse or avoid drug resistance. Clearly, the development of less invasive multiplex profiling methods, such as analysis of circulating tumor cells and plasma DNA sequencing, and perhaps in the future proteomic and metabolomic analysis, would help with this, especially if there turn out to be fifty ways to escape from EGFR therapy.

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