A major goal in drug discovery over the past several decades has been the development of selective and potent kinase inhibitors. The development of EGFR receptor (EGFR) tyrosine kinase inhibitors (TKI) represents an excellent example. In 1994, chemists at Parke-Davis synthesized quinazoline 45, one of the first small molecules to show selectivity against the kinase activity of EGFR (1). Quinazoline-based drugs, such as gefitinib and erlotinib, were eventually approved by the U.S. Food and Drug Administration (FDA) in the 2000s as first-generation EGFR TKIs (2). The drugs were originally developed as reversible ATP-competitive agents against wild-type EGFR. However, in 2004, investigators identified gefitinib and erlotinib as most effective against forms of EGFR that are mutated within the kinase domain in certain lung cancers (3, 4). One hallmark of these mutant-selective TKIs is the capacity to outcompete ATP for binding in the nucleotide-binding pocket (5).

For all clinically approved kinase inhibitors thus far, a window of efficacy exists, after which patients’ tumors progress. For gefitinib and erlotinib in patients with metastatic EGFR-mutant lung cancer, this progression-free survival lasts about 1 year. Multiple mechanisms of acquired resistance to EGFR TKIs have been defined through the study of tissue obtained after progression. The most frequent mechanism in patients harboring a primary drug-sensitizing mutation [an acrylamide group predicted to covalently bond with Cys797 in EGFR, were also quinazoline based and, in fact, were still developed against wild-type EGFR. They were more potent than gefitinib/erlotinib against all forms of EGFR, including lung cancer–associated mutants, but still had differential selectivity for exon 19 deletions/L858R versus T790M. Therefore, in retrospect, it is not surprising that these drugs still select for T790M-mediated resistance in vitro and in patients (6).

More recently, efforts to circumvent resistance brought about by the T790M substitution in the hinge region of the nucleotide-binding pocket have led to the development of EGFR TKIs with a pyrimidine backbone, such as WZ-4002. WZ-4002 arose from a rational drug screen of common kinase inhibitor core scaffolds against EGFR T790M (7). Investigators intentionally selected for compounds containing an acrylamide group predicted to covalently bond with Cys797 to select for irreversible inhibitors (7). WZ-4002 is selective toward T790M-containing EGFRs in vitro and in animal models. However, its efficacy against T790M-driven tumors in patients is currently unknown (7). A phase I clinical trial of a similar compound, CO-1686, is under way.

In this issue of Cancer Discovery, Lee and colleagues (8) report the identification of a potential new class of EGFR T790M inhibitors. A variety of kinase inhibitors were first profiled for growth inhibitory activity against a panel of 705 human cancer cell lines derived from various solid tumor types with known mutated oncogenic drivers. Less than 4% of the tested cell lines exhibited strong sensitivity to the protein kinase C (PKC) inhibitor, Go6976, an indolocarbazole alkaloid natural product derived from staurosporine. Unexpectedly, 2 EGFR-mutant lines displayed strong growth inhibition by Go6976, which was independent of PKC inhibition. The compound displayed selectivity against EGFR-addicted cell lines harboring the T790M gatekeeper mutation (8). To ensure these results were due to direct inhibition of EGFR T790M, the authors conducted several orthogonal dose–response studies, including cell-based autophosphorylation, viability, and in vitro kinase assays.
Table 1. Selectivity of kinase inhibitors based on molecular target and disease

| Drug                  | Original target(s) | Original indication | Additional target(s) | Additional indication | Selectivity score, S (3 μM) | 0
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<tr>
<td>Imatinib (STI571)</td>
<td>BCR-ABL</td>
<td>CML</td>
<td>KIT, PDGFR</td>
<td>GIST</td>
<td>0.6655</td>
</tr>
<tr>
<td>Dasatinib (BM5-354825)</td>
<td>BCR-ABL</td>
<td>CML</td>
<td>SRC, KIT</td>
<td>In trials</td>
<td>0.2828</td>
</tr>
<tr>
<td>Crizotinib (PF-02341066)</td>
<td>MET</td>
<td>In trials</td>
<td>ALK fusion, ROS1</td>
<td>ALK-positive lung cancer</td>
<td>Not reported</td>
</tr>
<tr>
<td>AP26113</td>
<td>ALK fusions</td>
<td>In trials</td>
<td>EGFR-T790M</td>
<td>In trials</td>
<td>Not reported</td>
</tr>
<tr>
<td>Midostaurin (PKC412)</td>
<td>PKC, FLT3, KIT</td>
<td>In trials</td>
<td>EGFR-T790M</td>
<td>T790M-positive lung cancer</td>
<td>0.4655</td>
</tr>
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Abbreviations: CML, chronic myelogenous leukemia; GIST, gastrointestinal stromal tumor; PDGFR, platelet-derived growth factor receptor.

Lee and colleagues (8) also investigated 2 other staurosporine-derived PKC inhibitors, CEP-701 and PKC412. Both agents are currently undergoing clinical development, albeit against a different target (FLT3, frequently activated in acute myeloid leukemia). Interestingly, Lee and colleagues (8) show that PKC412 is T790M selective, without much effect on either wild-type or mutant EGFRs, including L858R or an exon 19 deletion. Compared with other EGFR TKIs, PKC412 is not as potent as the T790M-specific drug WZ-4002 at inhibiting autophosphorylation of recombinant T790M or L858R/T790M EGFR kinases, whereas BIBW-2992 seems to have slightly reduced efficacy (8). Consistent with these data, treatment of T790M-driven tumors with PKC412 in vivo preclinical models (xenografts and genetically engineered mice) leads to growth inhibition, but only transiently. Collectively, these studies provide new lead scaffolds for optimization against resistant EGFR mutants in lung cancer. Further structure–activity relationship studies, coupled with in silico or crystallographic analyses, could provide an avenue for inhibitor optimization in the hope of developing more selective and potent T790M-specific drug inhibitors.

Large-scale high-throughput studies have elucidated selectivity profiles of a variety of clinically approved and research-based TKIs. Such studies have illustrated just how difficult it is to develop kinase-selective ATP-competitive inhibitors for the more than 500 kinases, which share moderate-to-high conservation in their nucleotide-binding pockets (9). However, target promiscuity can also be advantageous, as some inhibitors may have desirable off-target potency, which can be exploited for therapeutic benefit (ref. 10; Table 1). For example, although imatinib was developed to target BCR–ABL in chronic myelogenous leukemia, its activity against KIT and PDGFRs has led to its use against mutated forms of these kinases in gastrointestinal stromal tumors. Similarly, crizotinib was originally developed as a MET inhibitor, but its off-target activity against ALK led to its FDA approval as an ALK inhibitor in ALK-fusion–positive lung cancer. Crizotinib may also be effective against ROS1 fusions in lung cancer. Dasatinib, approved for use as an ABL TKI, is being tested in other diseases for its capacity to inhibit SRC. In yet another example, AP26113, an ALK TKI in early clinical trials, was recently reported to inhibit EGFR T790M as well.

To examine the issue of kinase inhibitor target discrimination in more detail, investigators have assigned values for the selectivity of a panel of inhibitors using a “selectivity score” (10). In this system, quantitative comparisons between inhibitors and their interaction patterns have yielded a numerical ranking (10). For instance, staurosporine has the highest selectivity score of 0.87, derived from the fact that it binds 253 kinases with a $K_d < 3 \mu M$, whereas the HER2 TKI, lapatinib, has the lowest score at 0.01, because it binds only 3 kinases with a $K_d > 3 \mu M$ (10). Currently, the “optimum” selectivity score for a clinically effective agent is unknown. A highly selective inhibitor may have a very narrow indication and either fewer or more side effects, depending upon the target. A less selective inhibitor may gain broader use across a variety of targets, but with potential trade-offs involving potency, toxicity, and drug resistance. Future studies should help delineate the “sweet spot” of kinase inhibition.

In summary, Lee and colleagues (8) have shown the use of broad-based cell line screens to identify potential off-label uses for available inhibitors. Their studies also point out that reexamining the chemical backbones of small-molecule inhibitors may provide a new avenue for reducing the off-target effects of irreversible inhibitors to wild-type proteins. It is hoped that such studies will lead to novel treatments for patients with resistance to the current generation of EGFR TKIs.

**Disclosure of Potential Conflicts of Interest**

W. Pao has commercial research grants from AstraZeneca, Clovis, Symphogen, and Enzon; has ownership interest (including patents) in Molecular MD; and is a consultant/advisory board member of Bristol-Myers Squibb, Clovis, and Symphony Evolution.

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