and thus identifying regulators, such as Myc, that mediate sensitivity is critical to its success. Indeed, sensitizing cancer cells to TRAIL using combination therapy has been successfully tried before, for example, using DNA-damaging agents. But the elegant approach of targeting the resistance pathway (upregulation of Mcl-1) using the new targeted therapy Sorafenib is an appealing advance and opens avenues for combining TRAIL with small molecules or peptides that act directly on apoptotic sensitizers and effectors (such as stabilized BH3-peptides targeting Mcl-1 and/or Bak) or other drugs that destabilize Mcl-1. However, a word of caution—sensitizing TRAIL-resistant cancer cells may also sensitize normal cells to this "death" ligand. Thus, one needs to choose the "TRAIL" wisely...

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Drugging the Bad "AKT-TOR" to Overcome TKI-Resistant Lung Cancer

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EGFR kinase inhibitors constitute an important class of lung cancer treatments. While they produce dramatic responses in a subset of patients—primarily those with activating EGFR mutations—remissions are typically limited to several months due to acquired drug resistance, frequently associated with the secondary T790M mutation in EGFR. In this issue of *Cancer Cell*, Li et al. report that an irreversible EGFR kinase inhibitor, HKI-272, had limited activity in a mouse lung cancer model driven by an EGFR mutant harboring T790M and an activating mutation. However, combining HKI-272 with rapamycin promoted rapid tumor regression, suggesting a therapeutic strategy to overcome drug resistance.

Lung cancer remains the leading cause of cancer deaths worldwide. While the disease is largely refractory to conventional chemotherapy, the recent emergence of selective tyrosine kinase inhibitors (TKIs) that elicit dramatic clinical responses in a subset of treated patients represents a highly promising therapeutic development. Specifically, about 10%–20% of chemotherapy-refractory non-small-cell lung cancers (NSCLCs), which constitute the vast majority of lung cancer cases, exhibit clinical responses to gefitinib (Iressa) and erlotinib (Tarceva), small-molecule inhibitors of the epidermal growth factor receptor (EGFR) kinase (Sequist et al., 2007). Moreover, such responses have been well correlated with the presence of somatic activating EGFR kinase domain mutations in tumors and have been linked to an overall survival benefit (Sequist et al., 2007).

However, the excitement around this therapeutic advancement has been somewhat tempered by the fact that clinical responses to EGFR TKIs are of limited duration—typically, 6–9 months, due to acquired drug resistance. Paralleling the clinical experience with the BCR-ABL kinase inhibi-

tor imatinib in chronic myelogenous leukemia (CML), it is now clear that about half of all NSCLC cases in which clinical response has been followed by disease progression are associated with the acquisition of a secondary mutation, T790M, within the EGFR kinase domain, which prevents drug binding while preserving catalytic activity (Kobayashi et al., 2005; Pao et al., 2005). Interestingly, EGFR T790M alleles have been detected at lower frequency in untreated NSCLCs, suggesting that they may confer oncogenic activity to EGFR in addition to their role in acquired drug resistance (Soh et al., 2007). Thus, there has been great interest in developing "next generation" EGFR inhibitors that can potentially overcome the TKI-resistant T790M allele. Such efforts have been further motivated by the recent clinical success of dasatanib, an ABL kinase inhibitor that can induce remissions in a subset of CML patients with acquired resistance to imatinib by overcoming some of the secondary resistance mutations seen in that setting (Shah et al., 2004).

Preclinical cell culture studies have demonstrated that irreversible EGFR inhibitors constitute a class of drugs that can potentially provide a solution to the T790M TKI resistance problem (Kwak et al., 2005). Gefitinib and erlotinib bind EGFR reversibly, whereas several other investigational drugs can bind covalently to the EGFR catalytic pocket via a cysteine residue within the kinase domain. One of these drugs, HKI-272, an irreversible dual inhibitor of both EGFR and HER2, has been demonstrated to suppress EGFR signaling in the context of an EGFR double-mutation mutant that harbors both an activating "primary" mutation and the T790M substitution (Kwak et al., 2005). Moreover, HKI-272 can promote cell killing more effectively than gefitinib/erlotinib in cultured NSCLC lines harboring EGFR double-mutation mutants. However, signaling by the EGFR T790M mutant is not completely suppressed by HKI-272, and it remains to be demonstrated that these irreversible inhibitors can provide clinical benefit in NSCLC patients that have progressed following an initial response to gefitinib or erlotinib. Indeed, early clinical studies of HKI-272 have thus far failed to demonstrate objective responses in this setting (Wong et al., 2006). Hence, further preclinical development may be required before these irreversible inhibitors can be used optimally in lung cancer patients.

Mouse modeling of EGFR-driven NSCLC provides a potentially powerful system in which to examine the efficacy of such novel agents, as well as to test drug combinations that may be clinically useful. Indeed, transgenic mice expressing activated EGFR mutants have recently been reported, and these mice develop lung adenocarcinomas that exhibit histologic features characteristic of human NSCLCs, and which rapidly regress following treatment with erlotinib or HKI-272. In this issue of *Cancer Cell*, Li and colleagues (Li et al., 2007) have utilized this system to explore the utility of HKI-272 in the context of lung cancer driven by transgenic expression of an EGFR double-mutation mutant that harbors the recurrent L858R activating mutation in *cis* with a T790M substitution. They designate this mutant as the "TL" allele. Mice were generated in which the EGFR TL allele could be expressed inducibly (via doxycycline administration) and specifically in type II alveolar epithelial cells. As expected, doxycycline-treated mice developed bronchioalveolar carcinoma that could be readily visualized by MRI. Interestingly, two types of lesions were noted—lung peripheral adenocarcinomas and bronchial papillary adenocarcinomas, and both types of tumors regressed within 12 weeks of doxycycline withdrawal, indicating a requirement for mutant EGFR in maintaining the malignant phenotype.

Treatment of tumor-bearing mice with erlotinib failed to produce tumor regression, as would be expected in the context of a T790M mutation. Among HKI-272-treated mice, there was some reduction in peripheral adenocarcinomas and partial tumor regression, whereas no significant effect was seen in bronchial tumors. The mechanistic basis for this difference in HKI-272 efficacy between these two tumor types is unclear, as is the relevance of that differential tissue activity to the therapeutic potential of HKI-272 in human lung cancer. However, these unexpected findings warrant further investigation. Overall, the effect of HKI-272 treatment in this model was far less than that seen in previous studies of erlotinib-treated mice harboring EGFR L858R-driven lung tumors, raising questions regarding the therapeutic value of HKI-272 as a single agent.

Li and colleagues then demonstrated that a combination of HKI-272 and the mTOR inhibitor rapamycin caused significant regression of both peripheral and bronchial tumors in TL mice. The rationale for testing this combination was based on previous studies suggesting an important survival function for AKT-mTOR signaling downstream of EGFR (Adjei, 2006). Indeed, several previous reports indicated a synergistic inhibition of cultured lung cancer and glioma cells using EGFR TKIs together with mTOR inhibitors (Adjei, 2006; Goudar et al., 2005). The ability of the HKI-272/rapamycin combination to promote more effective suppression of EGFR signaling to S6 and AKT kinases was demonstrated both in cultured NSCLC cell lines harboring double mutant EGFR alleles as well as in lung tumors in TL mice. The investigators suggest that HKI-272 may not sufficiently overcome the biochemical drug resistance conferred by T790M and that further suppression of an essential AKT-mTOR signal downstream of EGFR is required to achieve a therapeutic response. Although the unique effect of combined treatment on both bronchial and alveolar tumors is evidence of synergy at the biologic level, the claim that this drug combination leads to synergistic inhibition of downstream signaling is not compelling and would need to be substantiated by further quantitative analysis. In fact, it is not clear that a synergistic effect of the drug combination would necessarily be

expected. The fact that doxycycline withdrawal is sufficient to promote tumor regression in this model suggests that complete suppression of EGFR signaling should lead to a therapeutic response. Thus, if EGFR signaling to mTOR and AKT is direct and linear, then one would expect, at best, only an additive effect of combining drugs that target EGFR and mTOR. Importantly, as the investigators suggest, a more effective EGFR kinase inhibitor that can overcome resistance conferred by T790M may be sufficient as a single agent in this setting.

These findings highlight the potential shortcomings of HKI-272 (and possibly other irreversible EGFR inhibitors) as effective lung cancer drugs, and they reveal a potential therapeutic role for an HKI-272/rapamycin combination. The study also demonstrates the potential complementarity of mouse and cell culture models for the testing of such agents preclinically. Li and colleagues obtained essentially identical results using cultured human NSCLC lines and transgenic mice, revealing both the limited efficacy of HKI-272 as a single agent and the potential value of the combination with rapamycin. Unexpectedly, the mouse model studies further revealed that HKI-272 was efficacious against alveolar but not bronchial tumors, and HKI-272 resistance in bronchial tumors was overcome by the addition of rapamycin. Of course, the ultimate test of efficacy of the drug combination identified here will come in the form of a clinical trial—and so, for now, we'll just have to wait.

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