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Occupy EGFR

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1-2 Sentence Summary

Erlotinib and gefitinib inhibit growth of non-small cell lung cancer tumors that harbor activating EGFR mutations, but are ineffective against EGFR variants found in glioblastoma. New studies by Barkovich et al. (1) and Vivanco et al. (2) show that these drugs only occupy the active sites of glioblastoma-derived EGFR mutants to a limited extent, and fail to inhibit the activated receptor. Other EGFR inhibitors that target distinct receptor conformations are more effective in glioblastoma. These studies reveal distinct drug selectivities for different EGFR mutations, and show that analysis of binding site occupancy should be considered as a biomarker for inhibitor efficacy in targeting EGFR.

The epidermal growth factor receptor (EGFR) is a primary target of more than five FDA-approved targeted oncology agents, some of which are small molecule tyrosine kinase inhibitors (TKIs) and others are therapeutic antibodies. Effective clinical application of these agents presents several major challenges. The first is the identification of patients whose tumors are in fact dependent on EGFR. This has been illustrated best in non-small cell lung cancer (NSCLC). Broad initial clinical trials of EGFR-targeted TKIs in NSCLC gave disappointing results, but highlighted a subset of patients with activating EGFR mutations that responded very well to treatment. The second major challenge is acquired resistance, a current area of intense activity. A third challenge is highlighted by two papers in this issue of *Cancer Discovery*, which show clearly that not all EGFR-activating mutations are equal and suggest strategies to identify the differences and exploit them in the clinic.

Clinical trials in NSCLC with the EGFR-targeted TKIs erlotinib and gefitinib led to the discovery that the tumors of responding patients all harbored mutations in the EGFR intracellular tyrosine kinase domain (3). Patients with these mutations frequently showed dramatic responses (4). These studies appeared to identify a subgroup of NSCLC cases in which the EGFR is clearly the oncogenic driver—raising the hope that all tumors with EGFR mutations might be responsive to these TKIs, and motivating efforts to identify EGFR mutations in many other cancers. Meanwhile, clinical experience with glioblastoma (GBM) has told a different, and rather puzzling, story. GBM was the first human tumor type in which amplification and mutation of EGFR was reported (5), frequently expressing so-called ‘variant III’ EGFR (EGFRvIII) that has a 267aa deletion from its extracellular region (6) that activates the receptor. It seemed reasonable to expect that GBM patients might be primary beneficiaries of EGFR-targeted agents—but this has unfortunately not been the case. GBM patients expressing EGFRvIII have not responded well to gefitinib or erlotinib for reasons that remain unclear—although it is also true that the mechanism of EGFRvIII activation remains obscure (7).

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The study by Mellinghoff and colleagues in this issue (2) sheds important new light on the resistance of EGFRvIII (and other EGFR mutations in GBM) to erlotinib and gefitinib. This group previously identified several extracellularly-mutated EGFR variants in GBM (8) and, given the poor responses seen to erlotinib and gefitinib, asked whether they actually represent oncogenic driver mutations. They show quite clearly in their new report that GBM-derived cells expressing extracellularly-mutated forms of EGFR are addicted to the receptor: EGFR depletion by RNAi was sufficient to induce cell death. Despite the EGFR addiction of these cells, initial efforts to inhibit the receptor and to induce cell death with erlotinib or CI-1033 (a covalent/irreversible EGFR inhibitor) were not successful—in stark contrast to the effectiveness of these inhibitors in EGFR-addicted NSCLC cell-lines and in NSCLC patients. By investigating the effects of several different EGFR inhibitors, Vivanco et al. (2) made the remarkable discovery that efficacy depends on the ‘type’ of the EGFR-targeted TKI that is used (Figure 1). Erlotinib and CI-1033 are so-called ‘type I’ TKIs that bind the active conformation of the kinase domain (9), and are ineffective in GBM. By contrast, Vivanco et al. (2) found that ‘type II’ inhibitors—which selectively bind the inactive conformation of the receptor’s kinase domain—were effective inhibitors of EGFR variants found in GBM (including EGFRvIII) and efficiently induced cell death in EGFR-mutated GBM cells. Specifically, the type II inhibitors used were lapatinib and HKI-272, which are reversible and covalent/irreversible EGFR/ErbB2 dual-specific TKIs respectively that selectively bind the inactive conformation of EGFR’s kinase domain (10, 11).

Just as type I inhibitors such as erlotinib are more effective in killing EGFR-addicted NSCLC cells than GBM cells, so is the reverse also true. Whereas the type II inhibitor lapatinib induced significant death in EGFR-addicted GBM cells, it was substantially less effective against NSCLC-derived EGFR variants, consistent with recent *in vitro* studies showing that EGFR harboring NSCLC-derived mutations is resistant to lapatinib (12)—or indeed cetuximab. EGFR mutations found in NSCLC are almost exclusively intracellular, within the tyrosine kinase domain of the receptor (3). The GBM mutations (including that in EGFRvIII) are exclusively extracellular (2, 8). The fact that the different classes of activating EGFR mutations yield receptor variants that are responsive to quite distinct types of EGFR-targeted TKIs implies that they activate the receptor in different ways. Basal phosphorylation of EGFR promoted by all extracellular mutations tested by Vivanco et al. (2) is more potently inhibited by lapatinib than by erlotinib, whereas the opposite is true for NSCLC-derived intracellular kinase domain mutations. By contrast, regardless of which mutations it harbors, the EGF-activated receptor is most effectively inhibited by erlotinib.

This dichotomy in inhibitor sensitivity of oncogenic EGFR variants has important clinical implications, and other activating (and resistance) mutations may show an even wider range of specificities. Vivanco et al. (2) were able to show that the sensitivity of GBM-derived EGFR variants to type II inhibitors correlates with the abilities of the inhibitors to displace ATP from the kinase domain’s binding site. In the accompanying paper, Barkovich et al. (1) describe an elegant approach for monitoring inhibitor occupancy of the kinase domain using a fluorescent affinity probe for the EGFR ATP-binding site. When added to cells expressing EGFR, this probe interacts specifically with the receptor and becomes covalently linked to a cysteine in the ATP-binding site. It associates only with the ‘empty’ ATP-binding site, and therefore competes with both endogenous ATP and any other ATP-competitive inhibitor that is present in the cell. Accordingly, the probe can be used to monitor the extent to which EGFR active sites are ‘left unoccupied’ following treatment with a particular ATP-competitive inhibitor such as erlotinib or gefitinib. Using this approach, Barkovich et al. (1) showed—in an isogenic background—that erlotinib occupies NSCLC-derived EGFR variants to a significantly greater extent than it does EGFRvIII, consistent with the findings of Vivanco et al. (2) outlined above. Utilizing this fluorescent probe for dynamic studies also allowed Barkovich et al. (1) to provide mechanistic insight, establishing that the rate of

dissociation of erlotinib and gefitinib (both type I inhibitors) from the kinase domain's active site is much more rapid for EGFRvIII than for NSCLC-derived EGFR variants (which are more effectively inhibited). The data of Vivanco et al. (2) suggest that similar studies with lapatinib would find the converse. Monitoring such kinetic differences for a series of reversible inhibitors would offer a valuable window into their likely effects on newly discovered oncogenic alleles – for EGFR and other receptor tyrosine kinases (RTKs).

Both Vivanco et al. (2) and Barkovich et al. (1) make the observation that near complete inhibition of EGFR is required to promote cell death or cell cycle arrest – for NSCLC or GBM. To achieve this in a GBM cell line with lapatinib, drug concentrations in the range of 2 μ M were required (2) – in excess of levels that were achieved in most patients in a 44-patient clinical trial that Vivanco et al. report (2). Nonetheless, studies with GBM tumor sphere cell lines and xenografts clearly demonstrated that lapatinib can be effective against EGFR-driven tumors if concentrations in this range can be reached (2), arguing for the development of new type II EGFR-specific TKIs with different pharmacokinetics and/or higher affinity for the EGFR kinase domain. As a tool in developing such agents (whether type I or type II), Barkovich et al. (1) propose the use of kinase site occupancy as a biomarker for assessing EGFR inhibitor efficacy against different activated EGFR alleles. As echoed by studies with several TKIs, they find that the antiproliferative effects of erlotinib (and its effects on downstream signaling) correlate poorly with levels of EGFR autophosphorylation. Correlation with kinase site occupancy by erlotinib was much stronger – supporting its analysis as a biomarker.

Taken together, these two reports illuminate new avenues for extending the relative success of EGFR-targeted TKIs in NSCLC to other cancers – potentially reversing the disappointment experienced to date in GBM. Tools to monitor kinase site occupancy by new inhibitors for EGFR (and other RTKs) hold significant promise as a novel biomarker. Both sets of findings also underline the need to understand the different ways in which oncogenic EGFR alleles are activated in order to target them effectively. For EGFR mutations found in NSCLC, structural studies explain quite satisfyingly how they activate the receptor by destabilizing the 'autoinhibited' conformation of the kinase and allowing it to adopt a constitutively active structure (13). The predicted destabilization of the inactive conformation explains why type II EGFR inhibitors (such as lapatinib) will not be very effective in NSCLC. It is much less clear, though, how the extracellular EGFR mutations found in GBM patients (8) can cause EGFR to be most effectively inhibited by TKIs that target the inactive conformation. These extracellular mutations—which clearly elevate basal autophosphorylation – may promote weak dimerization of the receptor in which the kinase domain cycles between the active and inactive conformations. It has been shown that lapatinib dissociates from the EGFR kinase domain over 30 times more slowly than erlotinib or gefitinib (11). Since lapatinib selectively binds the inactive kinase conformation, its slow dissociation could 'trap' the kinase in this inactive state – thus inhibiting the mutated receptor. Dissociation of erlotinib and gefitinib from the kinase domain appears to be accelerated in EGFRvIII compared with the wild-type receptor in the studies of Barkovich et al. (1), perhaps indicating that the basal activity of this variant arises from a unique, partly active, conformation induced specifically by the mutation. Invoking such partly active states appears to be the only way to rationalize reduced sensitivity to inhibitors that are known to bind to the active conformation of the kinase domain. The nature of mutation-specific activated states in these and other oncogenic EGFR alleles should be investigated using the approaches described in this issue by Barkovich et al. (1) and Vivanco et al. (2), underlining the need to understand molecular mechanism in parallel with the identification of new oncogenic driver mutations.

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Figure 1.

Cartoon representation of the active and inactive conformations of the EGFR kinase domain. In the inactive state (right), the key α C helix is displaced, breaking an important salt bridge between a glutamate in α C (red sticks) and a lysine in the ATP-binding site (blue sticks). In the active conformation (left), α C is moved in towards the center of the N-lobe, so that this salt bridge forms to stabilize ATP binding. Type I inhibitors such as gefitinib and erlotinib bind to the active conformation (left), whereas type II inhibitors (such as lapatinib) bind only to the inactive conformation. As reported by Vivanco et al. (2), only type I inhibitors are effective in NSCLC, whereas only type II inhibitors effectively inhibit EGFR variants found in glioblastoma