

IN THE SPOTLIGHT

Occupy EGFR

Jin H. Park and Mark A. Lemmon

Summary: Erlotinib and gefitinib inhibit the growth of non-small cell lung cancer tumors that harbor activating epidermal growth factor receptor (EGFR) mutations but are ineffective against EGFR variants found in glioblastoma. New studies by Barkovich and colleagues and Vivanco and colleagues 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 the treatment of glioblastoma. These studies reveal distinct drug selectivities for different EGFR mutations and show that an analysis of binding-site occupancy should be considered as a biomarker for inhibitor efficacy in targeting EGFR. *Cancer Discov*; 2(5); 398–400. ©2012 AACR.

Commentary on Barkovich et al., p. 450 (1) and Vivanco et al., p. 458 (2).

Epidermal growth factor receptor (EGFR) is a primary target of more than 5 targeted oncology agents approved by the U.S. Food and Drug Administration. Some of these are small-molecule tyrosine kinase inhibitors (TKI) and others are therapeutic antibodies. The 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 provided disappointing results but highlighted a subset of patients with activating EGFR mutations who responded very well to treatment. The second major challenge is acquired resistance, a current area of intense activity. A third challenge is highlighted by 2 articles (1, 2) in this issue of *Cancer Discovery* in which the authors 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.

Authors' Affiliation: Department of Biochemistry and Biophysics and Graduate Group in Biochemistry and Molecular Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Corresponding Author: Mark A. Lemmon, Department of Biochemistry and Biophysics, University of Pennsylvania Perelman School of Medicine, 809C Stellar-Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19194-6059. Phone 215-898-3072; E-mail: mlemmon@mail.med.upenn.edu

doi: 10.1158/2159-8290.CD-12-0144

©2012 American Association for Cancer Research.

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); it frequently expresses so-called variant III EGFR (EGFRvIII), which has a 267aa deletion from its extracellular region (6) that activates the receptor. It seemed reasonable to expect that patients with GBM might be primary beneficiaries of EGFR-targeted agents, but this has unfortunately not been the case. Patients with GBM 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).

The study by Vivanco and colleagues (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 observed with erlotinib and gefitinib, now ask 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 RNA interference was sufficient to induce cell death. Despite the addiction of these cells to EGFR, initial efforts to inhibit the receptor and to induce cell death with erlotinib or CI-1033 (a covalent/irreversible EGFR inhibitor) were not successful, a finding in stark contrast to the effectiveness of these inhibitors in EGFR-addicted NSCLC cell-lines and in patients with NSCLC.

By investigating the effects of several different EGFR inhibitors, Vivanco and colleagues (2) made the remarkable discovery that efficacy depends on the “type” of the EGFR-targeted TKI that is used (Fig. 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 patients with GBM. By contrast, Vivanco and colleagues (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

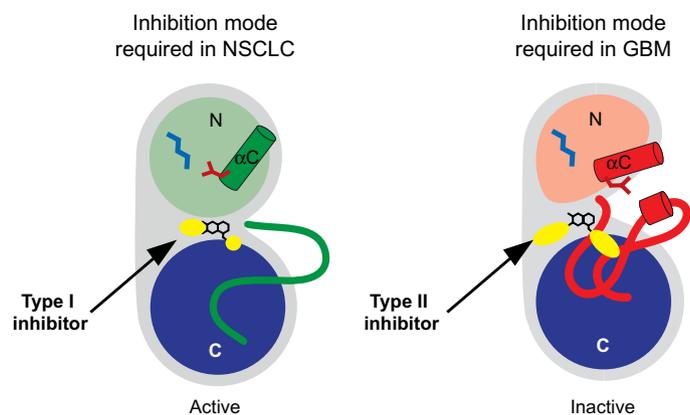


Figure 1. Illustration of the active and inactive conformations of the EGFR kinase domain. In the inactive state (right), the key α C helix is displaced, breaking an important salt bridge between a glutamate in α C (red sticks) and a lysine in the ATP-binding site (blue sticks). In the active conformation (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 (e.g., lapatinib) bind only to the inactive conformation. As reported by Vivanco and colleagues (2), only type I inhibitors are effective in NSCLC (left), whereas only type II inhibitors effectively inhibit EGFR variants found in GBM (right).

(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, the reverse also is 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, a finding consistent with recent *in vitro* studies in which investigators demonstrated 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 and colleagues (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 and colleagues (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 article, Barkovich and colleagues (1) describe an elegant approach for monitoring the inhibitor occupancy of the kinase domain in cells by 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" after treatment with a particular ATP-competitive inhibitor such as erlotinib or gefitinib. Using this approach, Barkovich and colleagues (1) showed—in an isogenic background—that erlotinib occupies NSCLC-derived EGFR variants to a significantly greater extent than it does EGFRvIII, which is consistent with the findings of Vivanco and colleagues (2) outlined above. The use of this fluorescent probe for dynamic studies also allowed Barkovich and colleagues (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 and colleagues (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.

Both Vivanco and colleagues (2) and Barkovich and colleagues (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), which is in excess of levels that were achieved in most patients in a 44-patient clinical trial that Vivanco and colleagues report (2). Nonetheless, studies with GBM tumor sphere cell lines and xenografts clearly showed that lapatinib can be effective against EGFR-driven tumors if concentrations in this range can be reached (2), which argues for the development of new type II EGFR-specific TKIs with different pharmacokinetics and/or greater affinity for the EGFR kinase domain. As a tool in developing such agents (whether type I or type II), Barkovich and colleagues (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 2 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 receptor tyrosine kinases) hold significant promise as novel biomarkers. Both sets of findings also underline the need to understand the different ways in which oncogenic EGFR alleles are activated if they are to be targeted 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). On the other hand, 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, however, how the extracellular EGFR mutations found in patients with GBM (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 more than 30 times more slowly than erlotinib or gefitinib (11). Because 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 and colleagues (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 by use of the approaches described in this issue by Barkovich and colleagues (1) and Vivanco and colleagues (2), underlining the need to understand molecular mechanism in parallel with the identification of new oncogenic driver mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M.A. Lemmon

Writing, review, and/or revision of the manuscript: J.H. Park, M.A. Lemmon

Administrative, technical, or material support: J.H. Park

Received April 2, 2012; accepted April 2, 2012; published online May 10, 2012.

REFERENCES

- Barkovich KJ, Hariono S, Garske AL, Zhang J, Blair JA, Fan Q-W, et al. Kinetics of inhibitor cycling underlie therapeutic disparities between EGFR-driven lung and brain cancers. *Cancer Discov* 2012; 2:450–7.
- Vivanco I, Robins HI, Rohle D, Campos C, Grommes C, Ngienphu PL, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. *Cancer Discov* 2012;2:458–71.
- Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007; 7:169–181.
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–39.
- Liebermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature* 1985;313:144–7.
- Sugawa N, Ekstrand AJ, James CD, Collins VP. Identical splicing of aberrant epidermal growth factor receptor transcripts from amplified rearranged genes in human glioblastomas. *Proc Natl Acad Sci U S A* 1990;87:8602–6.
- Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010;141:1117–34.
- Lee JC, Vivanco I, Beroukheim R, Huang JH, Feng WL, DeBiasi RM, et al. Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. *PLoS Med* 2006;3:e485.
- Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. *J Biol Chem* 2002;277:46265–72.
- Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. *Proc Natl Acad Sci U S A* 2008;105:2070–5.
- Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH, et al. A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells. *Cancer Res* 2004;64:6652–9.
- Wang Z, Longo PA, Tarrant MK, Kim K, Head S, Leahy DJ, et al. Mechanistic insights into the activation of oncogenic forms of EGF receptor. *Nat Struct Mol Biol* 2011;18:1388–93.
- Jura N, Zhang X, Endres NF, Seeliger MA, Schindler T, Kuriyan J. Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. *Mol Cell* 2011;42:9–22.

CANCER DISCOVERY

Occupy EGFR

Jin H. Park and Mark A. Lemmon

Cancer Discovery 2012;2:398-400.

Updated version Access the most recent version of this article at:
<http://cancerdiscovery.aacrjournals.org/content/2/5/398>

Cited articles This article cites 13 articles, 4 of which you can access for free at:
<http://cancerdiscovery.aacrjournals.org/content/2/5/398.full#ref-list-1>

Citing articles This article has been cited by 2 HighWire-hosted articles. Access the articles at:
<http://cancerdiscovery.aacrjournals.org/content/2/5/398.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerdiscovery.aacrjournals.org/content/2/5/398>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.