

## CANCER

# Structural, Biochemical, and Clinical Characterization of Epidermal Growth Factor Receptor (EGFR) Exon 20 Insertion Mutations in Lung Cancer

Hiroyuki Yasuda,<sup>1,2\*</sup> Eunyong Park,<sup>3\*</sup> Cai-Hong Yun,<sup>4\*</sup> Natasha J. Sng,<sup>1</sup> Antonio R. Lucena-Araujo,<sup>1</sup> Wee-Lee Yeo,<sup>1</sup> Mark S. Huberman,<sup>1</sup> David W. Cohen,<sup>1†</sup> Sohei Nakayama,<sup>1</sup> Kota Ishioka,<sup>2</sup> Norihiro Yamaguchi,<sup>1</sup> Megan Hanna,<sup>3,5</sup> Geoffrey R. Oxnard,<sup>3,6</sup> Christopher S. Lathan,<sup>3,6</sup> Teresa Moran,<sup>7</sup> Lecia V. Sequist,<sup>7</sup> Jamie E. Chaff,<sup>8</sup> Gregory J. Riely,<sup>8</sup> Maria E. Arcila,<sup>8</sup> Ross A. Soo,<sup>9</sup> Matthew Meyerson,<sup>3,5,6</sup> Michael J. Eck,<sup>3‡</sup> Susumu S. Kobayashi,<sup>1‡</sup> Daniel B. Costa<sup>1‡</sup>

(Published 18 December 2013; revised 26 February 2014)

Epidermal growth factor receptor (*EGFR*) gene mutations (G719X, exon 19 deletions/insertions, L858R, and L861Q) predict favorable responses to EGFR tyrosine kinase inhibitors (TKIs) in advanced non-small cell lung cancer (NSCLC). However, *EGFR* exon 20 insertion mutations (~10% of all *EGFR* mutations) are generally associated with insensitivity to available TKIs (gefitinib, erlotinib, and afatinib). The basis of this primary resistance is poorly understood. We studied a broad subset of exon 20 insertion mutations, comparing in vitro TKI sensitivity with responses to gefitinib and erlotinib in NSCLC patients, and found that most are resistant to EGFR TKIs. The crystal structure of a representative TKI-insensitive mutant (D770\_N771insNPG) reveals an unaltered adenosine triphosphate-binding pocket, and the inserted residues form a wedge at the end of the C helix that promotes the active kinase conformation. Unlike EGFR-L858R, D770\_N771insNPG activates EGFR without increasing its affinity for EGFR TKIs. Unexpectedly, we find that EGFR-A763\_Y764insFQEA is highly sensitive to EGFR TKIs in vitro, and patients whose NSCLCs harbor this mutation respond to erlotinib. Analysis of the A763\_Y764insFQEA mutant indicates that the inserted residues shift the register of the C helix in the N-terminal direction, altering the structure in the region that is also affected by the TKI-sensitive EGFR-L858R. Our studies reveal intricate differences between EGFR mutations, their biology, and their response to EGFR TKIs.

## INTRODUCTION

Non-small cell lung cancers (NSCLCs) continue to be the leading cause of cancer-related deaths (1). Epidermal growth factor receptor (*EGFR*) gene mutations were initially reported in 2004 and defined a prevalent molecularly classified subgroup of NSCLC (2–7). The most common *EGFR* mutations are in-frame deletions around the LREA motif (amino acid residues 747 to 750) of exon 19 (45% of *EGFR* mutations) and the exon 21 L858R point mutation (40% of *EGFR* mutations) (5, 6, 8). These *EGFR* mutations are oncogenic in both cell lines and mouse models (9, 10). They activate the EGFR signaling pathway in the absence of ligand, promote downstream prosurvival and anti-apoptotic signals such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), and render *EGFR*-mutated cells

dependent on constitutively active EGFR for their survival (11, 12). The inhibition of EGFR up-regulates proapoptotic molecules (such as BIM) in models driven by EGFR-delE746\_A750 or L858R, activates the intrinsic mitochondrial apoptotic pathway, and ultimately leads to cell death (13–16). Most patients whose tumors harbor exon 19 deletions or L858R *EGFR*-activating mutations have radiographic responses to monotherapy with the reversible adenosine triphosphate (ATP)-competitive EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib (17–23) and the irreversible EGFR TKI afatinib (24). Other *EGFR* mutations have also been associated with some sensitivity to gefitinib and erlotinib. These include exon 18 point mutations in position G719 (G719A, G719C, or G719S; ~3% of *EGFR* mutations), rare in-frame exon 19 insertions (25), and the exon 21 L861Q mutant (~2% of *EGFR* mutations) (26–28).

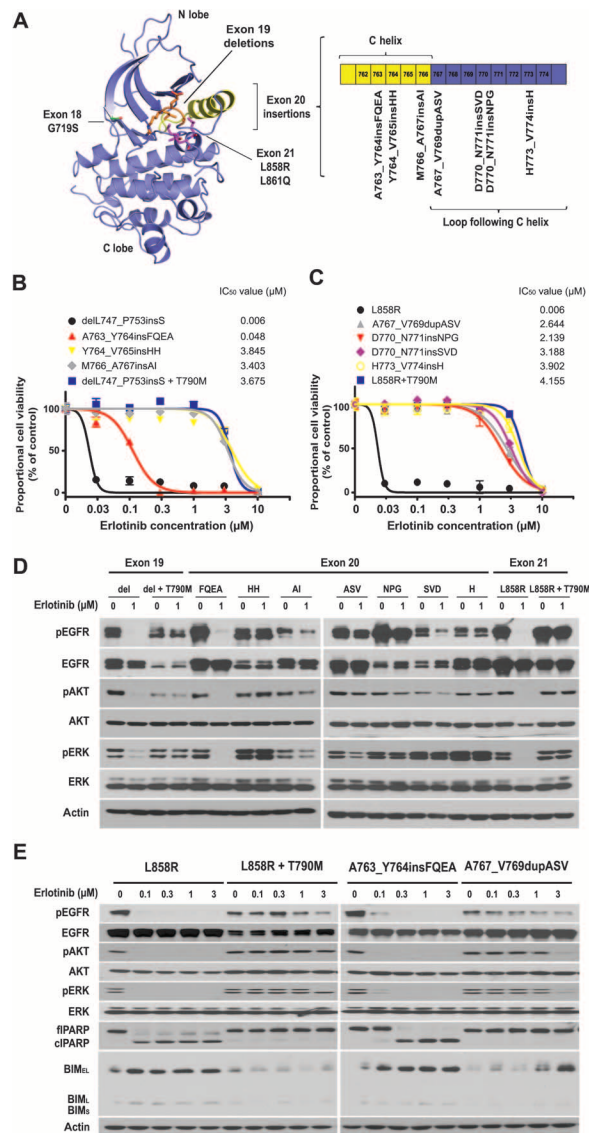
The other main group of *EGFR* mutations in NSCLC is composed of in-frame insertions within exon 20 of *EGFR* (Fig. 1A). Exon 20 insertion mutations comprise 4 to 10% of all *EGFR* mutations (27, 29–32). Most of these mutations lie near the end of the C helix within the N lobe of the kinase, after residue M766, but a small subset map to the middle of the C helix (affecting amino acids E762 to Y764) (5, 33, 34). Unlike *EGFR* exon 19 deletions and L858R-bearing tumors, most NSCLCs with *EGFR* exon 20 insertion mutations do not respond radiographically or clinically to gefitinib or erlotinib. The reported response rate (RR) is less than 5%, and most patients have short intervals of disease control (35). The precise mechanisms that determine the primary insensitivity to EGFR TKIs in the most prevalent exon 20 insertion mutations and the response of less prevalent exon 20 insertion

<sup>1</sup>Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA. <sup>2</sup>Keio University Medical School, Tokyo 160-8582, Japan. <sup>3</sup>Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA. <sup>4</sup>Peking University Health Science Center, Beijing 100191, China. <sup>5</sup>Broad Institute, Massachusetts Institute of Technology and Harvard Medical School, Boston, MA 02142, USA. <sup>6</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. <sup>7</sup>Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA. <sup>8</sup>Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College, New York, NY 10065, USA. <sup>9</sup>Cancer Science Institute of Singapore and National University Cancer Institute, National University of Singapore, Singapore 117599, Singapore.

\*These authors contributed equally to this work.

†Present address: Wexner Medical Center, The Ohio State University, Columbus, OH 43210, USA.

‡Corresponding author. E-mail: dbcosta@bidmc.harvard.edu (D.B.C.); skobayas@bidmc.harvard.edu (S.S.K.); eck@red.fci.harvard.edu (M.J.E.)



**Fig. 1. EGFR exon 20 insertion mutations and their response to EGFR TKIs.** (A) Structure of the EGFR kinase in the inactive conformation, highlighting the locations of diverse EGFR mutations [drawn from Protein Data Bank (PDB) ID 1XKK]. The schematic on the right depicts the site of EGFR exon 20 insertion mutations studied here. (B and C) Dose-dependent cell growth inhibition of Ba/F3 cells expressing EGFR-dell747\_P753insS, dell747\_P753insS + T790M, A763\_Y764insFQEA, Y764\_V765insHH, M766\_A767insAI, A767\_V769dupASV, D770\_N771insNPG, D770\_N771insSVD, H773\_V774insH, L858R, and L858R + T790M. Ba/F3 cells expressing aforementioned EGFR mutations were treated with the indicated doses of erlotinib for 72 hours. Cell survival was measured using a CellTiter Aqueous One Solution Cell Proliferation Assay. Error bars indicate SD ( $n = 3$ ). Calculated average  $IC_{50}$  values of 11 EGFR mutation types are shown ( $n = 3$ ). (D) Inhibition of EGFR signaling by erlotinib. Ba/F3 cells expressing all generated EGFR mutations were treated with 1  $\mu$ M erlotinib for 6 hours. Phosphorylation of EGFR, AKT, and ERK proteins was detected by immunoblotting. (E) Dose response of erlotinib in Ba/F3 cells expressing EGFR L858R, L858R-T790M, A763\_Y764insFQEA, and V769\_D770dupASV. The cells were treated with indicated doses of erlotinib for 24 hours. Immunoblotting was done against the indicated proteins [EGFR, AKT, and ERK, as well as full-length poly(adenosine diphosphate-ribose) polymerase (flPARP) or cleaved PARP (clPARP) and isoforms of BIM (extra long, BIM<sub>EL</sub>; long, BIM<sub>L</sub>; and short, BIM<sub>S</sub>)].

mutations to gefitinib or erlotinib remain elusive. We herein elucidate the molecular and structural mechanisms that underlie the patterns of response or resistance of EGFR exon 20 insertion mutations to EGFR TKIs.

## RESULTS

### Sensitivity of EGFR exon 20 insertion mutations to EGFR TKIs in vitro

To better understand the patterns of resistance or response to EGFR TKIs of exon 20 insertion mutations, we studied representative EGFR exon 20 mutations using in vitro systems. We selected two mutations that lie within the C helix [A763\_Y764insFQEA (structurally identical to D761\_E762insEAFQ) and Y764\_V765insHH] and five mutations that lie at the end of the helix or within the loop following it [M766\_A767insAI, A767\_V769dupASV (identical to V769\_D770insASV), D770\_N771insNPG, D770\_N771insSVD (identical to S768\_D770dupSVD), and H773\_V774insH (identical to P772\_H773insH)] (Fig. 1A and table S1). In aggregate, these mutations represent more than half of reported EGFR exon 20 insertion mutations (31, 32, 35). In addition, we used gefitinib/erlotinib-sensitive [L858R and exon 19 deletion mutations (dell747\_S752 and dell747\_P753insS)] and gefitinib/erlotinib-resistant (L858R + T790M and exon 19 deletion mutations + T790M) mutations as assay controls (36, 37).

We created Ba/F3 cells stably expressing the aforementioned EGFR mutations. All were able to proliferate in the absence of interleukin-3 (fig. S1), indicating transforming ability of these mutations. We next measured proliferation in the presence of erlotinib and showed that only cells with EGFR-dell747\_P753insS, L858R, and the atypical A763\_Y764insFQEA—among all EGFR exon 20 insertions—were inhibited by erlotinib concentrations less than 0.1  $\mu$ M (Fig. 1, B and C, and table S2). All other EGFR exon 20 insertions and T790M-bearing mutations had 50% inhibitory concentrations ( $IC_{50}$ s) that exceeded 2  $\mu$ M erlotinib (Fig. 1, B and C, and table S2). Nearly identical results were obtained with gefitinib (table S2), and a similar pattern of insensitivity was noted with the more potent irreversible EGFR TKI afatinib (table S2). Transient transfection and overexpression of these EGFR constructs in Cos-7 cells led to comparable results (fig. S2). Examination of EGFR signaling revealed that phosphorylation of EGFR, AKT, and ERK was not fully inhibited by 1  $\mu$ M erlotinib in cells with the inhibitor-insensitive EGFR exon 20 insertion mutations (Fig. 1D). A dose-response experiment disclosed that an inhibitor-insensitive EGFR exon 20 insertion (A767\_V769dupASV) lacked deactivation of phosphorylated EGFR, downstream targets (PI3K/AKT and MAPK/ERK), and up-regulation of the apoptotic cascade, as measured by levels of BIM [a marker of EGFR TKI-induced cell killing (13)], with 1  $\mu$ M or submicromolar concentrations of erlotinib (Fig. 1E). In stark contrast, the inhibition of these downstream phosphorylation events and up-regulation of BIM were seen with EGFR-A763\_Y764insFQEA in a similar fashion as with inhibitor-sensitive L858R (Fig. 1, D and E).

### Enzyme kinetic analysis of representative EGFR exon 20 insertion mutants

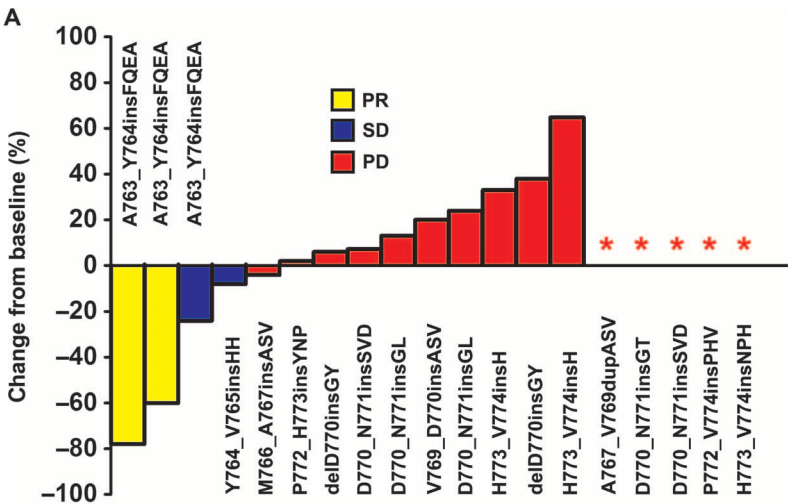
On the basis of our cell line studies, we selected a representative inhibitor-resistant exon 20 insertion mutant, D770\_N771insNPG, and the inhibitor-sensitive A763\_Y764insFQEA for detailed enzyme kinetic studies. We expressed and purified the kinase domain of these mutants (residues 696 to 1022 of EGFR) and of the L858R mutant and

wild-type EGFR. Consistent with their transforming activity (fig. S1), both insertion mutants exhibited higher catalytic activity than wild-type EGFR, although they were not as active as EGFR-L858R (Table 1).

The exquisite sensitivity of EGFR-L858R and exon 19 deletions to EGFR TKIs stems from two effects: their enhanced intrinsic affinity for these TKIs and their markedly decreased affinity for substrate ATP (38, 39). Thus, we measured the Michaelis constant for ATP ( $K_{m[ATP]}$ ) for D770\_N771insNPG and A763\_Y764insFQEA (Table 1 and fig. S3), as well as the inhibition constant ( $K_i$ ) for gefitinib for these mutants (Table 1 and fig. S4). Notably, the inhibitor-sensitive A763\_Y764insFQEA mutant bound gefitinib ~10-fold more tightly than the insensitive D770\_N771insNPG mutant (Table 1). The D770\_N771insNPG mutant bound gefitinib with a  $K_i$  similar to that of wild-type EGFR ( $K_i = 25.7$  nM for D770\_N771insNPG and  $K_i = 16.4$  nM for wild-type EGFR). The  $K_i$  value for A763\_Y764insFQEA (2.5 nM) was comparable to that we measured for the L858R mutant (6.4 nM). Because inhibitors must compete with substrate ATP for binding, the ratio of  $K_i/K_{m[ATP]}$  provides a better estimate of potency than

**Table 1. Enzyme kinetic parameters of wild-type (WT) and mutant EGFR kinases.** All experiments were performed in triplicate ( $n = 3$ ).  $K_{m[ATP]}$ , Michaelis constant for ATP;  $K_i$ , inhibition constant; SD, standard deviation.

EGFR kinase	Fold activity versus WT	$K_{m[ATP]}$ ( $\mu$ M), average (SD)	$K_{i[gefitinib]}$ (nM), average (SD)	$K_i/K_m$ ( $\times 10^{-3}$ )
WT	1.00	4.98 ( $\pm 1.2$ )	16.4 ( $\pm 0.2$ )	3.29
L858R	33.12	68.5 ( $\pm 3.4$ )	6.4 ( $\pm 0.8$ )	0.09
D770_N771insNPG	4.93	36.8 ( $\pm 7.4$ )	25.7 ( $\pm 4.3$ )	0.69
A763_Y764insFQEA	8.96	19.2 ( $\pm 0.9$ )	2.5 ( $\pm 0.3$ )	0.13



**Fig. 2. Response to EGFR TKIs of NSCLCs harboring EGFR exon 20 insertion mutations.** (A) Waterfall plot of best responses of target tumor lesions after exposure to gefitinib or erlotinib in relation to baseline measurements for each patient. The plot highlights that all A763\_Y764insFQEA-bearing tumors decreased in size after exposure to erlotinib, whereas other

the  $K_i$  alone. On the basis of this measure, A763\_Y764insFQEA is predicted to be almost as sensitive to gefitinib as the L858R mutant, whereas D770\_N771insNPG is expected to be many fold less sensitive (Table 1). The practical effect of these alterations in kinetic parameters can be seen in inhibition curves measured at a range of ATP concentrations; like L858R, the A763\_Y764insFQEA mutant is effectively inhibited by gefitinib in the presence of 1 mM ATP (which approximates the concentration of ATP in cells), whereas D770\_N771insNPG is not (fig. S4).

**EGFR exon 20 insertion mutations and response to reversible EGFR inhibitors in patients with NSCLC**

To determine whether clinical responses matched the pattern of sensitivity to EGFR TKIs that we observed in vitro, we assembled data from 19 NSCLC patients with diverse EGFR exon 20 insertion mutations who were treated with reversible EGFR TKIs (Fig. 2, A and B, and table S3).

Consistent with previous reports (35, 40–44), almost all 19 NSCLC patients from our centers displayed progressive disease in the course of treatment with gefitinib or erlotinib (Fig. 2B, table S3, and fig. S5). However, three patients with A763\_Y764insFQEA EGFR-mutated NSCLCs achieved either stable disease or partial response when treated with erlotinib (Fig. 2, A and B, fig. S5, and table S3). Even in this small case series, the RR of gefitinib or erlotinib was significantly higher for A763\_Y764insFQEA (2 of 3, 66.6%) when compared to all other mutations within or following the C helix (0 of 16, 0%;  $P = 0.0175$ ); furthermore, the progression-free survival of these patients exceed that of patients with other exon 20 mutations (fig. S6). Thus, as predicted by our preclinical studies, NSCLCs harboring EGFR A763\_Y764insFQEA appear to respond to gefitinib and erlotinib.

**Characterization of an A763\_Y764insFQEA mutated lung cancer cell line**

To further study the properties and inhibitor sensitivity of the EGFR-A763\_Y764insFQEA insertion, we derived a lung cell line from the

EGFR mutation	Best response to reversible EGFR TKI				
	Drug	PR	SD	PD	RR [%]
A763_Y764insFQEA	Erlotinib	2	1	—	66.6
Y764_V765insHH	Gefitinib	—	1	—	0
M766_A767insASV	Erlotinib	—	—	1	0
A767_V769dupASV	Gefitinib	—	—	1	0
V769_D770insASV	Erlotinib	—	—	1	0
D770_N771insGL	Erlotinib	—	—	2	0
D770_N771insGT	Erlotinib	—	—	1	0
D770_N771insSVD	Erlotinib	—	1	1	0
delD770insGY	Erlotinib	—	—	2	0
P772_H773insYNP	Gefitinib	—	—	1	0
P772_V774insPHV	Erlotinib	—	—	1	0
H773_V774insH	Gefitinib/erlotinib	—	—	2	0
H773_V774insNPH	Erlotinib	—	—	1	0

mutations had either minimal changes or increase in target lesions. Yellow bars, partial response (PR); blue bars, stable disease (SD); red bars, progressive disease (PD). Asterisk indicates patients who displayed nonmeasurable progressive disease. (B) Detailed response of each individual mutation type analyzed.



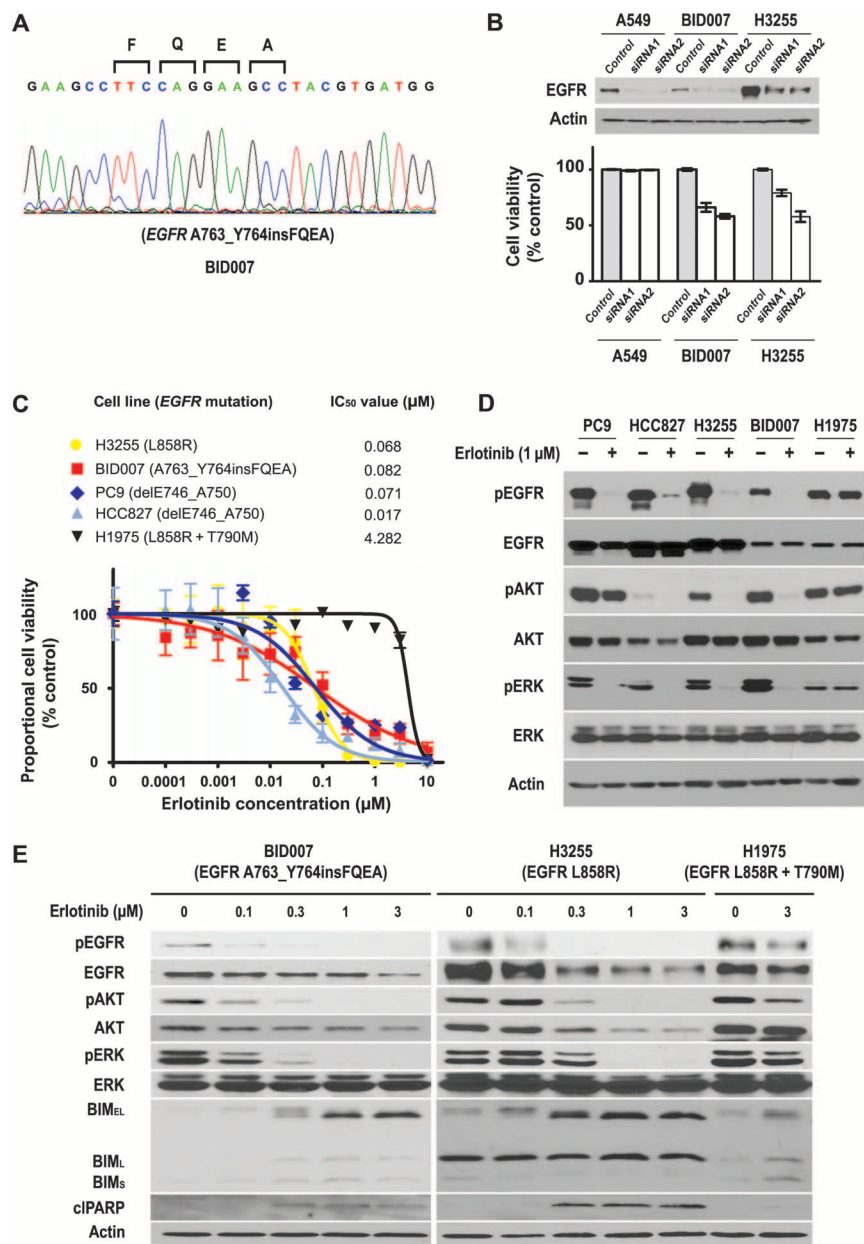
malignant pleural effusion of a patient with an NSCLC containing *EGFR* A763\_Y764insFQEA (Fig. 3A, table S3, and fig. S5). The original biopsy disclosed an adenocarcinoma (fig. S7), and the derived cell line (named BID007) was adherent to the tissue culture plates (fig. S8). Sequence of exons 18 to 21 of *EGFR* from DNA isolated from BID007 confirmed the presence of the 12 nucleotides corresponding to the A763\_Y764insFQEA insertion (Fig. 3A). DNA was also analyzed for additional hotspot mutations in 113 known oncogenes and tumor suppressor genes (table S4), and no additional mutation was identified. A detailed genome-wide single-nucleotide polymorphism analysis to determine copy number variations in BID007's DNA showed that many regions of the cells' chromosomes had broad and focal copy gains or losses (fig. S9A). The region around chromosome 7, corresponding to *EGFR*, demonstrated slight amplification (fig. S9B). In addition, BID007 had a focal area of deletion encompassing the *CDKN2A* tumor suppressor gene (fig. S9C), an area commonly deleted in cancers (45).

To determine whether BID007 requires mutant *EGFR* for its viability, we used siRNAs against *EGFR* in BID007, as well as in lung cancer cell lines H3255 (*EGFR*-L858R) and known to undergo cell proliferation arrest and cell death upon inactivation of *EGFR* and A549 (*KRAS*-mutated). In both BID007 and H3255, *EGFR*-specific siRNAs decreased expression of *EGFR* and suppressed cell growth (Fig. 3B). As expected, *EGFR* siRNAs had no effect on the growth of *KRAS*-mutated A549 cells (Fig. 3B). These results demonstrate that BID007 cells, similar to other *EGFR*-mutated cell lines, are dependent on aberrant *EGFR* signaling.

The sensitivity of BID007 cells to *EGFR* TKIs was compared to that of a group of three *EGFR* TKI-sensitive *EGFR*-mutated NSCLC lines [PC9 (*EGFR*-delE746\_A750), HCC827 (delE746\_A750), and H3255] and one erlotinib-resistant line [H1975 (L858R + T790M)]. Proliferation assays with increasing concentrations of *EGFR* TKIs disclosed that growth of BID007, PC9, HCC827, and H3255 cells was inhibited by submicromolar concentrations of erlotinib (Fig. 3C and table S2), gefitinib, and afatinib (table S2), indicating augmented sensitivity relative to the inhibitor-resistant H1975 cell line.

The levels of phosphorylated *EGFR*, *AKT*, and *ERK* in BID007, PC9, HCC827, and H3255 were diminished by 1  $\mu$ M erlotinib (Fig. 3D). A dose-response experiment highlighted that only BID007 and H3255 had deactivation of phosphorylated *EGFR*, *AKT*, and *ERK* and up-regulation of *BIM* with submicromolar concentrations of erlotinib (Fig. 3E), and underwent apoptosis with submicromolar doses of erlotinib, as shown by *PARP* cleavage (Fig. 3E). These results supported our

siRNA experiments and further detailed that BID007 cells were not only dependent on *EGFR* signaling but also sensitive to clinically achievable submicromolar concentrations of gefitinib and erlotinib.



**Fig. 3. BID007, a cell line expressing *EGFR*-A763\_Y764insFQEA.** (A) Sequence of BID007's DNA confirms *EGFR*-A763\_Y764insFQEA. (B) Small interfering RNA (siRNA) knockdown of *EGFR* in NSCLC cell lines A549, BID007, and H3255 cells for 72 hours ( $n = 3$ ). *EGFR* protein was detected by immunoblotting. Compared to A549 cells, siRNA1 and siRNA2 inhibited proliferation in BID007 ( $P = 0.005$  and  $P = 0.001$ , respectively) and H3255 ( $P = 0.003$  and  $P = 0.003$ , respectively). (C) Dose-dependent cell growth inhibition of H3255, BID007, HCC827, H1975, and PC9 cells. The cells were treated with the indicated doses of erlotinib for 72 hours. Error bars indicate SD ( $n = 3$ ). Calculated average  $IC_{50}$  values of H3255, BID007, HCC827, H1975, and PC9 cells are shown ( $n = 3$ ). (D) Inhibition of *EGFR* signaling by erlotinib in NSCLC cell lines. PC9, HCC827, H3255, BID007, and H1975 cells were treated with or without 1  $\mu$ M erlotinib for 6 hours. Phosphorylation of *EGFR*, *AKT*, and *ERK* proteins was detected by immunoblotting. (E) Dose response of erlotinib in BID007, H3255, and H1975 cells. The cells were treated with indicated doses of erlotinib for 24 hours. Immunoblotting was done against the indicated proteins (*EGFR*, *AKT*, *ERK*, *cIPAR*, and *BIM*).

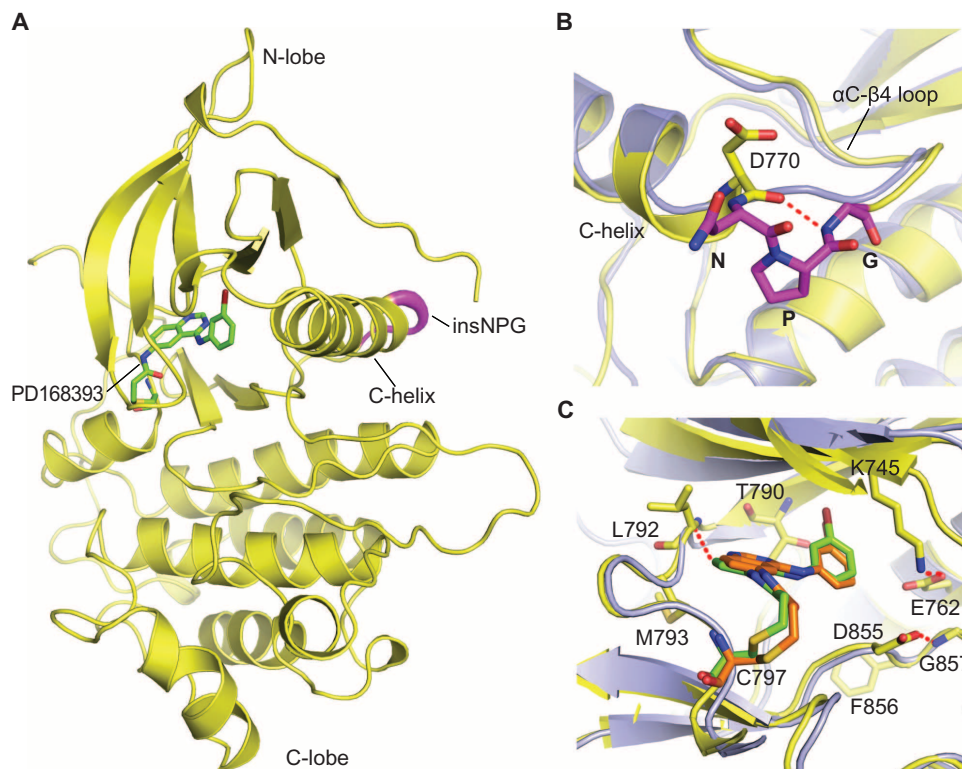
## Structural analysis of EGFR TKI-resistant and TKI-sensitive EGFR exon 20 mutants

To better understand the mechanism of activation of TKI-resistant EGFR exon 20 insertion mutations, we determined the structure of D770\_N771insNPG in complex with the inhibitor PD168393 at 3.5-Å resolution (table S5). For comparison, we also determined the structure of EGFR-L858R in complex with the same compound (table S5). PD168393 is a preclinical irreversible anilinoquinazoline inhibitor that is structurally related to afatinib and dacomitinib (46, 47). Preparation of a covalent complex with PD168393 stabilized the protein during final stages of purification and was required for crystallization. The D770\_N771insNPG mutant adopts an active conformation with the C helix in its inward position (Fig. 4A). The structure contains five molecules in the crystallographic asymmetric unit arranged in a continuous chain of “asymmetric dimer” interactions in which the C lobe of one molecule interacts with the N lobe of the next (fig. S10A). This mode of interaction mediates EGFR activation by ligand-induced dimerization (48), and is typically recapitulated in crystals of the EGFR kinase in its active conformation. The D770\_N771insNPG insertion lies at the C-terminal end of the C helix, immediately following D770 (Fig. 4A). Together with D770, the three inserted residues form a tight turn, with a hydrogen bond formed between the main-chain carbonyl of D770 and the amide of the inserted glycine (Fig. 4B and fig. S10, B and C). Beyond this glycine, the conformation of the polypeptide chain is quite similar to that of the wild-type and L858R kinases (38), and overall, the D770\_N771insNPG structure superimposes well with the L858R structure in complex with the same inhibitor (Fig. 4, B and C). Differences, which likely arise from the divergent crystal packing in D770\_N771insNPG, include a small change in the relative orientation of the N and C lobes, and the conformation of the activation loop, which is fully ordered in the D770\_N771insNPG structure but not in the present L858R structure. Within the ATP site, the conformation of the covalently bound inhibitor is essentially the same as in the L858R structure, and although the resolution of this study is relatively low (3.5 Å), there is no evidence that the D770\_N771insNPG mutation alters interactions with the inhibitor (Fig. 4C). Likewise, we expect that binding of gefitinib and erlotinib would be unperturbed in this mutant, consistent with the closely similar  $K_i$  values for gefitinib we measure for D770\_N771insNPG and wild-type EGFR (Table 1).

D770\_N771insNPG may activate EGFR by blocking the conformational rearrangements required for the inactive conformation of the kinase. In the inactive state, the C helix pivots outward and shifts by ~3.5 Å toward the insertion site. The insertion is at the “pivot point” of the C helix; thus, the inserted residues may steri-

cally inhibit the reorientation of the C helix that is characteristic of the inactive state. This mutation may also interfere with an adjacent “electrostatic switch” interaction that is proposed to stabilize an inactive EGFR dimer (49). Irrespective of the detailed mechanism of activation, our structure and kinetic data show that D770\_N771insNPG promotes the active conformation, as reflected by its enhanced catalytic activity (Table 1) and a propensity to form the asymmetric dimer as seen in the crystal structure (fig. S10A) and in solution (fig. S11).

Because we have been unable to crystallize the A763\_Y764insFQEA mutant EGFR, we studied it further using homology modeling. The four-residue FQEA insertion occurs just C terminal to A763, in the middle of the C helix. From a structural perspective, the four inserted residues are expected to form about one turn of helix and shift the register of adjacent residues in the helix, either toward the N terminus or toward the C terminus (Fig. 5A). The C helix contains a key active-site residue, E762, just N terminal to the site of the insertion (50). A shift in register toward the C terminus would leave this catalytically important residue unaltered. Alternatively, a shift toward the N terminus would displace this residue by one turn, but effectively replace it with a glutamic acid residue introduced by the insertion (FQEA, Fig. 5A). To distinguish between these two possibilities, we prepared two altered versions of this mutant: one in which the endogenous E762 is



**Fig. 4. Implications of the crystal structure of the EGFR exon 20 insertion D770\_N771insNPG (insNPG).** (A) Crystal structure of the insNPG mutant. The inhibitor PD168393, covalently bound to Cys<sup>797</sup> (C797), is shown in stick form with carbon atoms colored green. The inserted NPG sequence is highlighted in magenta. (B) Detailed view of the NPG insertion. The insNPG structure is shown in yellow with the inserted residues in magenta in stick form and is superimposed on the L858R structure (blue ribbon). (C) Superposition of the active-site region of the insNPG and L858R mutants bound to the inhibitor PD168393. The compound binds in an essentially identical manner in both structures, forming a covalent bond with C797.

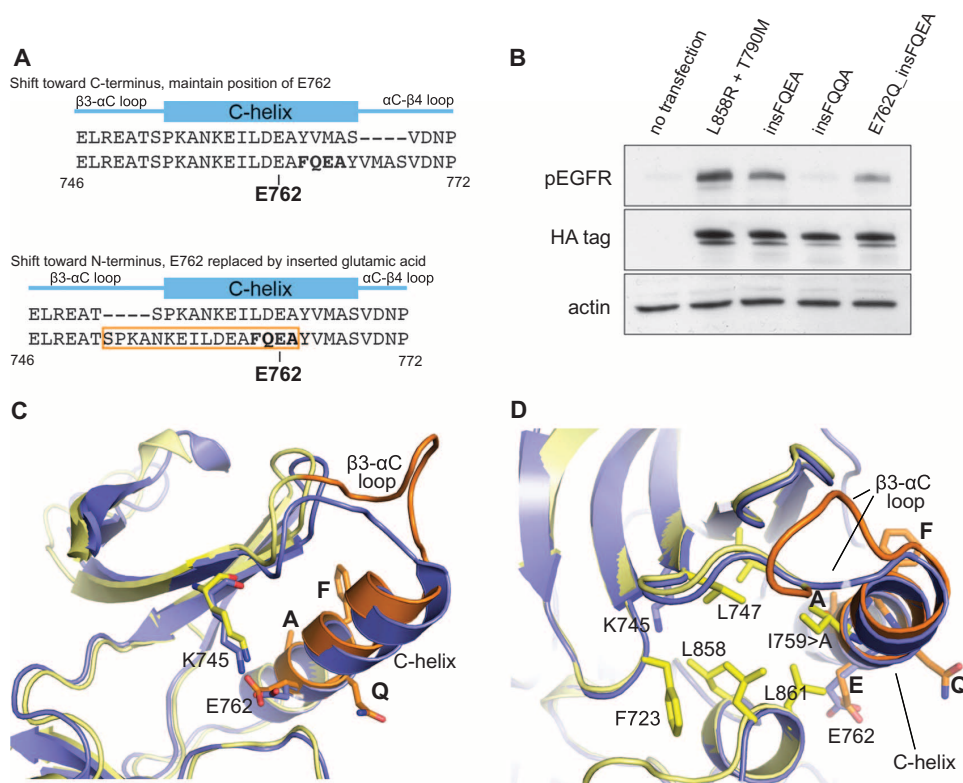


mutated to glutamine (E762Q\_insFQEA) and a second in which the inserted glutamic acid residue is mutated to glutamine (A763\_Y764insFQQA). We found that E762Q\_insFQEA, like A763\_Y764insFQEA, was highly active in a ligand-independent manner, but A763\_Y764insFQQA was inactive (Fig. 5B). Thus, we conclude that the insertion shifts the register of the helix in the N-terminal direction and that the glutamic acid residue in the FQEA insertion assumes the active-site role of E762 in the wild-type kinase. On the basis of these findings, we prepared homology models of the A763\_Y764insFQEA mutant using both the active and inactive conformations of the kinase (Fig. 5, C and D). In both models, the insertion was modeled in a helical conformation, shifting the register of the C helix toward its N terminus, and resulting in a longer loop at its N-terminal end (the  $\beta$ 3- $\alpha$ C loop). Examination of the homology model of the inactive state suggests a mechanism of activation related to that of L858R (38). The shift in register in the C helix effectively replaces I759 with alanine (Fig. 5D). Isoleucine at position 759, together with L747, L858, and L861, is part of a cluster of interacting hydrophobic residues that helps to stabilize the inactive conformation. The L858R and L861Q mutations within this cluster are well-characterized activating mutations (28), and mutation of L747 in this cluster to proline or serine is believed to underlie the activity of EGFR exon 19 insertions (25). Thus, we hypothesize that the de facto I759A replacement caused by the FQEA insertion may activate EGFR via a similar mechanism (destabilization of the inactive conformation). Furthermore, we note that like both exon 19 deletion and insertion mutants (25), A763\_Y764insFQEA must alter the structure of the  $\beta$ 3- $\alpha$ C loop. The model based on the active state supports the hypothesis that the glutamic acid residue in the A763\_Y764insFQEA insertion can effectively replace E762 in the active site (Fig. 4, A and C). Finally, inspection of the model indicates that the FQEA insertion is compatible with formation of the asymmetric dimer, and, consistent with this, analysis of the A763\_Y764insFQEA mutant using size-exclusion chromatography revealed a monomer-dimer equilibrium in solution (fig. S11).

## DISCUSSION

EGFR exon 20 insertion mutations comprise a unique set of EGFR-activating mutations. These have been previously described as insensitive to clinically available reversible EGFR TKIs, gefitinib and erlotinib (31, 32, 35, 51). In the present report, we

provide detailed characterization of a broad spectrum of these mutations and their correlation with patient response to EGFR TKIs. Our study was limited by a number of factors, including a paucity of available NSCLC cell lines with EGFR exon 20 insertion mutations, a lack of in vivo models, and the small number of exon 20 insertion-mutated NSCLC cases that were treated with reversible EGFR TKIs in our centers. Within these constraints, our data confirm that most exon 20 insertions are not inhibited by submicromolar concentrations of reversible EGFR TKIs. The most common EGFR exon 20 insertions that have



**Fig. 5. Homology modeling of the TKI-sensitive EGFR-A763\_Y764insFQEA mutation (insFQEA).** (A) Two plausible alignments of the wild-type and insFQEA sequences in the region of the C helix. The inserted FQEA residues (bold) could shift the register of the C helix in the C-terminal direction (upper panel) or in the N-terminal direction (lower panel) and maintain a glutamic acid in the position of Glu<sup>762</sup>, a key active-site residue. A C-terminal shift would be expected to lengthen the loop following the C helix ( $\alpha$ C- $\beta$ 4), whereas an N-terminal shift would lengthen the loop preceding it ( $\beta$ 3- $\alpha$ C). The region that would be altered relative to wild type is boxed in orange (lower panel). (B) EGFR mutant constructs introduced into Cos-7 cells. EGFR phosphorylation and the presence of transfected constructs [as measured by hemagglutinin (HA) tag levels] are depicted. Four different constructs—insFQEA, insFQQA (E→Q mutation in the inserted sequence), E762Q\_insFQEA (E→Q mutation in the endogenous E762), and L858R + T790M—were transiently transfected into Cos-7 cells. Phosphorylation of EGFR and HA tag was detected by immunoblotting. Mutagenesis of the respective glutamic acid residues indicates a shift in the N-terminal direction. (C) Homology model of the insFQEA mutant (yellow) superimposed on the wild-type EGFR structure (blue ribbon) in the active conformation. The inserted residues are labeled in bold, and the shifted sequence is colored orange [corresponding to the boxed region in (A)]; the glutamic acid residue in the FQEA insertion assumes the position of E762 and is positioned to form a salt bridge with K745. (D) Homology model of the insFQEA mutant in the inactive conformation (yellow, with the structurally altered region in orange) superimposed on the wild-type EGFR structure in the inactive conformation (drawn from PDB ID: 1XKK). A cluster of hydrophobic residues (yellow side chains) is important for the stability of the inactive state; L858 and L861 are part of this cluster. The insFQEA insertion will shift an alanine residue into the position of I759 in this cluster (I759>A). The insertion is also expected to alter the length and conformation of the  $\beta$ 3- $\alpha$ C loop, which is the site of exon 19 deletion mutations.

been reported in NSCLC samples are post-C helix insertions of one to four amino acids, which, in aggregate, account for 80 to 90% of all exon 20 insertions (31, 32, 35). We characterized Y764\_V765insHH, M766\_A767insAI, A767\_V769dupASV, D770\_N771insNPG, D770\_N771insSVD, and H773\_V774insH using in vitro models and found that cells bearing these mutant EGFR proteins are not inhibited by clinically achievable doses of gefitinib, erlotinib (12, 35, 52–54), or the irreversible EGFR inhibitor afatinib (35, 55). These results are consistent with our observations that tumors harboring these mutations did not achieve clinical or radiographic responses to usual doses of gefitinib (250 mg/day) or erlotinib (150 mg/day) in NSCLC patients. Therefore, on the basis of our data and previously published reports (31, 32, 35), the aforementioned mutations affecting amino acids Y764 to V774 should be classified as nonsensitizing to the reversible EGFR TKIs gefitinib and erlotinib.

Unexpectedly, we identified an EGFR exon 20 insertion mutation that was inhibited by gefitinib and erlotinib at submicromolar concentrations: EGFR A763\_Y764insFQEA. This sequence alteration accounts for 10 to 20% of all reported *EGFR* exon 20 insertions (31, 32, 35). Our kinetic characterization of this mutant revealed that it is activated, relative to wild-type EGFR, and that it is much more potently inhibited by gefitinib than is wild-type EGFR. In vitro characterization of A763\_Y764insFQEA demonstrated that it had an  $IC_{50}$  of  $<0.05 \mu M$  to erlotinib or gefitinib, which is more than 10-fold lower than the clinically achievable serum concentrations of these EGFR TKIs [gefitinib (250 mg/day) has serum troughs of  $\sim 0.5 \mu M$ , and erlotinib (100 to 150 mg/day) has serum troughs of  $\sim 1$  to  $2 \mu M$ ] at their clinical dosing schemes (12, 52). In addition, submicromolar concentrations of erlotinib inhibited phosphorylation of EGFR in A763\_Y764insFQEA mutant cells, led to deactivation of the PI3K/AKT and MAPK/ERK pathways, and subsequently induced apoptosis in our models. These results were also confirmed in BID007, an NSCLC-derived cell line bearing the *EGFR* A763\_Y764insFQEA mutation. Three patients with NSCLCs harboring *EGFR* A763\_Y764insFQEA had clinical and radiographic regressions or stable disease on erlotinib (150 mg/day). Consequently, we conclude that *EGFR* A763\_Y764insFQEA is an EGFR TKI-sensitizing mutation. Structurally, *EGFR*-A763\_Y764insFQEA is very different from other exon 20 insertions. Notwithstanding the limitations inherent in homology modeling, our structural analysis and supporting mutagenesis indicate that the inserted FQEA sequence shifts the register of the C helix toward its N terminus, altering the length of the  $\beta 3$ - $\alpha C$  loop, leading into the helix, and effectively leading to an I759A replacement. The  $\beta 3$ - $\alpha C$  loop is the site of exon 19 deletion mutations, and the I759A alteration is immediately adjacent to L858R and L861Q, and we hypothesize that it leads to catalytic activation in a related manner. Thus, at a structural and enzyme kinetic level, A763\_Y764insFQEA may more closely resemble the L858R and exon 19 deletion mutants than it does other exon 20 insertion mutations. *EGFR*-A763\_Y764insFQEA and the identical D761\_E762insEAFQ amino acid sequence appear to be unique among reported *EGFR* exon 20 insertions in having the ability to induce an N-terminal shift in the C helix while maintaining or replacing active-site residue E762.

The structure of D770\_N771insNPG, which we studied as a representative EGFR TKI-insensitive exon 20 mutation, shows how the three-amino acid insertion forms a “wedge” at the end of the C helix that may effectively lock the helix in its inward, active position. Although each will differ in detail, we expect that other EGFR exon 20 insertion mutants that insert one to three residues at or near the end of the C helix will have a similar structural effect. The D770\_N771insNPG structure, together

with our enzyme kinetic studies, shows that this insertion mutant binds EGFR TKIs with a binding mode and apparent affinity similar to that of wild-type EGFR. Thus, the clinical resistance of this mutant does not stem from steric interference with inhibitor binding. Rather, D770\_N771insNPG simply does not sensitize to gefitinib and erlotinib inhibition. L858R and exon 19 deletion mutants exhibit increased  $K_{m[ATP]}$  and higher affinity (5- to 10-fold) for gefitinib or erlotinib than does the wild-type receptor (39, 50, 56). Together with the oncogene-addicted state of tumors bearing these mutations (39, 50, 56), these factors explain their sensitivity to EGFR TKIs (50). Because the dose-limiting toxicities of gefitinib and erlotinib arise from inhibition of wild-type EGFR in normal tissues, the enhanced sensitivity of these mutants relative to wild-type EGFR effectively creates the “therapeutic window” that makes gefitinib and erlotinib useful drugs (39, 50, 56). In these sensitive EGFR mutants, serum concentrations can be achieved that functionally shut down the oncogenic mutant EGFR, but relatively spare the wild-type receptor in the skin and gastrointestinal tract. Unlike EGFR-L858R and exon 19 deletion mutants, the D770\_N771insNPG mutation lacks this “Achilles’ heel,” because it activates EGFR without markedly diminishing its affinity for ATP or increasing its affinity for EGFR TKIs.

Our findings have important implications for efforts to develop EGFR TKIs effective against D770\_N771insNPG and other “insensitive” exon 20 insertion mutants. The EGFR-T790M mutation confers resistance by reversing the sensitization conferred by primary activating mutations, but it does so by altering the gatekeeper residue in the ATP site that is an important determinant of inhibitor specificity (50, 57). Thus, the T790M structural alteration in essence “enabled” discovery of inhibitors that selectively inhibit T790M-bearing mutants, when in association with L858R or exon 19 deletions, relative to wild-type EGFR (58, 59). By contrast, there are no structural alterations proximate to the ATP-binding cleft in D770\_N771insNPG. Additionally, the similar ATP- and TKI-binding properties of this mutant, compared with wild-type EGFR, suggest that it will be difficult to achieve the requisite selectivity versus the wild-type receptor (39, 50, 56, 58, 59). Indeed, it seems that EGFR monoclonal antibodies (60) and EGFR wild-type kinase-sparing covalent T790M selective TKIs (61) are ineffective in typical EGFR exon 20 insertion mutants. Biochemical screening of large compound libraries to identify potent inhibitors of the D770\_N771insNPG mutant, with counter-screening against wild-type EGFR to identify mutant-selective compounds or scaffolds, may be the most rational approach to what appears to be a very challenging drug discovery problem. The close homology of EGFR and ERBB2 insertion mutations (46, 62) may indicate that future therapeutic options for EGFR exon 20 insertion mutations may also be applicable to other cohorts of NSCLC.

In summary, our results not only explain the intricate interplay between different EGFR mutations and their response to EGFR TKIs but also provide guidance for the selection (in the case of A763\_Y764insFQEA) or omission (in the case of other exon 20 insertion mutations) of clinically available TKIs for the treatment of *EGFR* exon 20 insertion-mutated NSCLCs.

## MATERIALS AND METHODS

See Supplementary Materials.

## SUPPLEMENTARY MATERIALS

[www.sciencetranslationalmedicine.org/cgi/content/full/5/216/216ra177/DC1](http://www.sciencetranslationalmedicine.org/cgi/content/full/5/216/216ra177/DC1)

Materials and Methods

Fig. S1. Transforming ability of *EGFR* mutant constructs introduced into Ba/F3 cells.

Fig. S2. EGFR mutant constructs introduced into Cos-7 cells.

Fig. S3. Determination of the Michaelis constant for ATP ( $K_{m(ATP)}$ ) for EGFR mutants.  
 Fig. S4. Inhibition of EGFR mutant kinases by gefitinib at a range of ATP concentrations.  
 Fig. S5. Examples of computed tomography (CT) images of patients with NSCLC harboring EGFR exon 20 insertion mutations.  
 Fig. S6. Kaplan-Meier progression-free survival (PFS) curve estimates.  
 Fig. S7. Pathology from a never smoker with an adenocarcinoma of the lung harboring EGFR A763\_Y764insFQEA.  
 Fig. S8. Different morphologic features of EGFR-mutated NSCLC cell lines.  
 Fig. S9. Copy number changes in BID007.  
 Fig. S10. Additional implications of the crystal structure of the prototypical post-C helix EGFR exon 20 insertion D770\_N771insNPG (insNPG).  
 Fig. S11. Size-exclusion chromatography of EGFR exon 20 insertion kinase domains.  
 Table S2. Amino acid sequence of EGFR exon 20 insertion mutations designed for in vitro studies.  
 Table S2. Summary of IC<sub>50</sub>s obtained with different EGFR mutations.  
 Table S3. Clinical, pathological, and molecular characteristics and response to reversible EGFR TKIs for patients with tumors harboring EGFR exon 20 insertions.  
 Table S4. Genes analyzed for hotspot mutations using OncoMap version 3 extended analysis.  
 Table S5. Crystallographic data collection and refinement statistics for EGFR-D770\_N771insNPG and EGFR-L858R bound to the irreversible EGFR TKI PD168393.  
 Table S6. Oligonucleotides used to derive EGFR exon 20 insertion mutations.  
 References (63–70)

## REFERENCES AND NOTES

- R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013. *CA Cancer J. Clin.* **63**, 11–30 (2013).
- T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman, D. A. Haber, Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
- J. G. Paez, P. A. Jänne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson, M. Meyerson, EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
- W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, B. Singh, R. Heelan, V. Rusch, L. Fulton, E. Mardis, D. Kupfer, R. Wilson, M. Kris, H. Varmus, EGFR receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 13306–13311 (2004).
- H. Shigematsu, L. Lin, T. Takahashi, M. Nomura, M. Suzuki, I. I. Wistuba, K. M. Fong, H. Lee, S. Toyooka, N. Shimizu, T. Fujisawa, Z. Feng, J. A. Roth, J. Herz, J. D. Minna, A. F. Gazdar, Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J. Natl. Cancer Inst.* **97**, 339–346 (2005).
- L. V. Sequist, D. W. Bell, T. J. Lynch, D. A. Haber, Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. *J. Clin. Oncol.* **25**, 587–595 (2007).
- G. J. Riely, K. A. Politi, V. A. Miller, W. Pao, Update on epidermal growth factor receptor mutations in non-small cell lung cancer. *Clin. Cancer Res.* **12**, 7232–7241 (2006).
- M. Tokumo, S. Toyooka, K. Kiura, H. Shigematsu, K. Tomii, M. Aoe, K. Ichimura, T. Tsuda, M. Yano, K. Tsukuda, M. Tabata, H. Ueoka, M. Tanimoto, H. Date, A. F. Gazdar, N. Shimizu, The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin. Cancer Res.* **11**, 1167–1173 (2005).
- K. Politi, M. F. Zakowski, P. D. Fan, E. A. Schonfeld, W. Pao, H. E. Varmus, Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. *Genes Dev.* **20**, 1496–1510 (2006).
- H. Ji, D. Li, L. Chen, T. Shimamura, S. Kobayashi, K. McNamara, U. Mahmood, A. Mitchell, Y. Sun, R. Al-Hashem, L. R. Chirieac, R. Padera, R. T. Bronson, W. Kim, P. A. Jänne, G. I. Shapiro, D. Tenen, B. E. Johnson, R. Weissleder, N. E. Sharpless, K. K. Wong, The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. *Cancer Cell* **9**, 485–495 (2006).
- S. V. Sharma, D. W. Bell, J. Settleman, D. A. Haber, Epidermal growth factor receptor mutations in lung cancer. *Nat. Rev. Cancer* **7**, 169–181 (2007).
- K. S. Nguyen, S. Kobayashi, D. B. Costa, Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancers dependent on the epidermal growth factor receptor pathway. *Clin. Lung Cancer* **10**, 281–289 (2009).
- D. B. Costa, B. Halmos, A. Kumar, S. T. Schumer, M. S. Huberman, T. J. Boggon, D. G. Tenen, S. Kobayashi, BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLOS Med.* **4**, 1669–1679 (2007).
- M. S. Cragg, J. Kuroda, H. Puthalakath, D. C. Huang, A. Strasser, Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLOS Med.* **4**, 1681–1689 (2007).
- Y. Gong, R. Somwar, K. Politi, M. Balak, J. Chmielecki, X. Jiang, W. Pao, Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. *PLOS Med.* **4**, e294 (2007).
- J. Deng, T. Shimamura, S. Perera, N. E. Carlson, D. Cai, G. I. Shapiro, K. K. Wong, A. Letai, Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. *Cancer Res.* **67**, 11867–11875 (2007).
- L. V. Sequist, R. G. Martins, D. Spigel, S. M. Grunberg, A. Spira, P. A. Jänne, V. A. Joshi, D. McCollum, T. L. Evans, A. Muzikansky, G. L. Kuhlmann, M. Han, J. S. Goldberg, J. Settleman, A. J. Iafrate, J. A. Engelman, D. A. Haber, B. E. Johnson, T. J. Lynch, First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J. Clin. Oncol.* **26**, 2442–2449 (2008).
- D. B. Costa, S. Kobayashi, D. G. Tenen, M. S. Huberman, Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. *Lung Cancer* **58**, 95–103 (2007).
- R. Rosell, T. Moran, C. Queralt, R. Porta, F. Cardenal, C. Camps, M. Majem, G. Lopez-Vivanco, D. Isla, M. Provencio, A. Insa, B. Massuti, J. L. Gonzalez-Larriba, L. Paz-Ares, I. Bover, R. Garcia-Campelo, M. A. Moreno, S. Catot, C. Rolfo, N. Reguart, R. Palmero, J. M. Sánchez, R. Bastus, C. Mayo, J. Bertran-Alamillo, M. A. Molina, J. J. Sanchez, M. Taron; Spanish Lung Cancer Group, Screening for epidermal growth factor receptor mutations in lung cancer. *N. Engl. J. Med.* **361**, 958–967 (2009).
- M. Maemondo, A. Inoue, K. Kobayashi, S. Sugawara, S. Oizumi, H. Isobe, A. Gemma, M. Harada, H. Yoshizawa, I. Kinoshita, Y. Fujita, S. Okinaga, H. Hirano, K. Yoshimori, T. Harada, T. Ogura, M. Ando, H. Miyazawa, T. Tanaka, Y. Saijo, K. Hagiwara, S. Morita, T. Nukiwa; North-East Japan Study Group, Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* **362**, 2380–2388 (2010).
- T. Mitsudomi, S. Morita, Y. Yatabe, S. Negoro, I. Okamoto, J. Tsurutani, T. Seto, M. Satouchi, H. Tada, T. Hirashima, K. Asami, N. Katakami, M. Takada, H. Yoshioka, K. Shibata, S. Kudoh, E. Shimizu, H. Saito, S. Toyooka, K. Nakagawa, M. Fukuoka; West Japan Oncology Group, Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomised phase 3 trial. *Lancet Oncol.* **11**, 121–128 (2010).
- T. S. Mok, Y. L. Wu, S. Thongprasert, C. H. Yang, D. T. Chu, N. Saijo, P. Sunpaweravong, B. Han, B. Margono, Y. Ichinose, Y. Nishiaki, Y. Ohe, J. J. Yang, B. Chewaskulyong, H. Jiang, E. L. Duffield, C. L. Watkins, A. A. Armour, M. Fukuoka, Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **361**, 947–957 (2009).
- C. Zhou, Y. L. Wu, G. Chen, J. Feng, X. Q. Liu, C. Wang, S. Zhang, J. Wang, S. Zhou, S. Ren, S. Lu, L. Zhang, C. Hu, C. Hu, Y. Luo, L. Chen, M. Ye, J. Huang, X. Zhi, Y. Zhang, Q. Xiu, J. Ma, L. Zhang, C. You, Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. *Lancet Oncol.* **12**, 735–742 (2011).
- L. V. Sequist, J. C. Yang, N. Yamamoto, K. O’Byrne, V. Hirsh, T. Mok, S. L. Geater, S. Orlov, C. M. Tsai, M. Boyer, W. C. Su, J. Bennouna, T. Kato, V. Gorbunova, K. H. Lee, R. Shah, D. Massey, V. Zazulina, M. Shahidi, M. Schuler, Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J. Clin. Oncol.* **31**, 3327–3334 (2013).
- M. He, M. Capelletti, K. Nafa, C. H. Yun, M. E. Arcila, V. A. Miller, M. S. Ginsberg, B. Zhao, M. G. Kris, M. J. Eck, P. A. Jänne, M. Ladanyi, G. R. Oxnard, EGFR exon 19 insertions: A new family of sensitizing EGFR mutations in lung adenocarcinoma. *Clin. Cancer Res.* **18**, 1790–1797 (2012).
- T. Mitsudomi, Y. Yatabe, Epidermal growth factor receptor in relation to tumor development: EGFR gene and cancer. *FEBS J.* **277**, 301–308 (2010).
- T. Mitsudomi, Y. Yatabe, Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. *Cancer Sci.* **98**, 1817–1824 (2007).
- P. Yeh, H. Chen, J. Andrews, R. Naser, W. Pao, L. Horn, DNA-mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT): A catalog of clinically relevant cancer mutations to enable genome-directed anticancer therapy. *Clin. Cancer Res.* **19**, 1894–1901 (2013).
- Y. L. Choi, M. Soda, Y. Yamashita, T. Ueno, J. Takashima, T. Nakajima, Y. Yatabe, K. Takeuchi, T. Hamada, H. Haruta, Y. Ishikawa, H. Kimura, T. Mitsudomi, Y. Taniai, H. Mano; ALK Lung Cancer Study Group, EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. *N. Engl. J. Med.* **363**, 1734–1739 (2010).
- W. Pao, J. Chmielecki, Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat. Rev. Cancer* **10**, 760–774 (2010).
- G. R. Oxnard, P. C. Lo, M. Nishino, S. E. Dahlberg, N. I. Lindeman, M. Butaney, D. M. Jackman, B. E. Johnson, P. A. Jänne, Natural history and molecular characteristics of lung cancers harboring EGFR exon 20 insertions. *J. Thorac. Oncol.* **8**, 179–184 (2013).
- M. E. Arcila, K. Nafa, J. E. Chaft, N. Rekhtman, C. Lau, B. A. Reva, M. F. Zakowski, M. G. Kris, M. Ladanyi, EGFR exon 20 insertion mutations in lung adenocarcinomas: Prevalence, molecular heterogeneity, and clinicopathologic characteristics. *Mol. Cancer Ther.* **12**, 220–229 (2013).



33. S. Murray, I. J. Dahabreh, H. Linardou, M. Manoloukos, D. Bafaloukos, P. Kosmidis, Somatic mutations of the tyrosine kinase domain of epidermal growth factor receptor and tyrosine kinase inhibitor response to TKIs in non-small cell lung cancer: An analytical database. *J. Thorac. Oncol.* **3**, 832–839 (2008).
34. H. Linardou, I. J. Dahabreh, D. Bafaloukos, P. Kosmidis, S. Murray, Somatic *EGFR* mutations and efficacy of tyrosine kinase inhibitors in NSCLC. *Nat. Rev. Clin. Oncol.* **6**, 352–366 (2009).
35. H. Yasuda, S. Kobayashi, D. B. Costa, *EGFR* exon 20 insertion mutations in non-small-cell lung cancer: Preclinical data and clinical implications. *Lancet Oncol.* **13**, e23–e31 (2012).
36. S. Kobayashi, T. J. Boggon, T. Dayaram, P. A. Jänne, O. Kocher, M. Meyerson, B. E. Johnson, M. J. Eck, D. G. Tenen, B. Halmos, *EGFR* mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **352**, 786–792 (2005).
37. S. Kobayashi, H. Ji, Y. Yuza, M. Meyerson, K. K. Wong, D. G. Tenen, B. Halmos, An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor. *Cancer Res.* **65**, 7096–7101 (2005).
38. C. H. Yun, T. J. Boggon, Y. Li, M. S. Woo, H. Greulich, M. Meyerson, M. J. Eck, Structures of lung cancer-derived *EGFR* mutants and inhibitor complexes: Mechanism of activation and insights into differential inhibitor sensitivity. *Cancer Cell* **11**, 217–227 (2007).
39. K. D. Carey, A. J. Garton, M. S. Romero, J. Kahler, S. Thomson, S. Ross, F. Park, J. D. Haley, N. Gibson, M. X. Sliwkowski, Kinetic analysis of epidermal growth factor receptor somatic mutant proteins shows increased sensitivity to the epidermal growth factor receptor tyrosine kinase inhibitor, erlotinib. *Cancer Res.* **66**, 8163–8171 (2006).
40. L. V. Sequist, B. Besse, T. J. Lynch, V. A. Miller, K. K. Wong, B. Gitlitz, K. Eaton, C. Zacharchuk, A. Freyman, C. Powell, R. Ananthakrishnan, S. Quinn, J. C. Soria, Neratinib, an irreversible pan-ERbB receptor tyrosine kinase inhibitor: Results of a phase II trial in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **28**, 3076–3083 (2010).
41. D. A. Eberhard, B. E. Johnson, L. C. Amler, A. D. Goddard, S. L. Heldens, R. S. Herbst, W. L. Ince, P. A. Jänne, T. Januario, D. H. Johnson, P. Klein, V. A. Miller, M. A. Ostland, D. A. Ramies, D. Sebanovic, J. A. Stinson, Y. R. Zhang, S. Seshagiri, K. J. Hillan, Mutations in the epidermal growth factor receptor and in *KRAS* are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J. Clin. Oncol.* **23**, 5900–5909 (2005).
42. J. Y. Wu, S. G. Wu, C. H. Yang, C. H. Gow, Y. L. Chang, C. J. Yu, J. Y. Shih, P. C. Yang, Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin. Cancer Res.* **14**, 4877–4882 (2008).
43. T. Harada, A. Lopez-Chavez, L. Xi, M. Raffeld, Y. Wang, G. Giaccone, Characterization of epidermal growth factor receptor mutations in non-small-cell lung cancer patients of African-American ancestry. *Oncogene* **30**, 1744–1752 (2011).
44. H. Sasaki, K. Endo, M. Takada, M. Kawahara, N. Kitahara, H. Tanaka, M. Okumura, A. Matsumura, K. Iuchi, T. Kawaguchi, O. Kawano, H. Yukiue, T. Yokoyama, M. Yano, Y. Fujii, *EGFR* exon 20 insertion mutation in Japanese lung cancer. *Lung Cancer* **58**, 324–328 (2007).
45. B. A. Weir, M. S. Woo, G. Getz, S. Perner, L. Ding, R. Beroukhi, W. M. Lin, M. A. Province, A. Kraja, L. A. Johnson, K. Shah, M. Sato, R. K. Thomas, J. A. Barletta, I. B. Borecki, S. Broderick, A. C. Chang, D. Y. Chiang, L. R. Chirieac, J. Cho, Y. Fujii, A. F. Gazdar, T. Giordano, H. Greulich, M. Hanna, B. E. Johnson, M. G. Kris, A. Lash, L. Lin, N. Lindeman, E. R. Mardis, J. D. McPherson, J. D. Minna, M. B. Morgan, M. Nadel, M. B. Orringer, J. R. Osborne, B. Ozenberger, A. H. Ramos, J. Robinson, J. A. Roth, V. Rusch, H. Sasaki, F. Shepherd, C. Sougnez, M. R. Spitz, M. S. Tsao, D. Twomey, R. G. Verhaak, G. M. Weinstock, D. A. Wheeler, W. Winckler, A. Yoshizawa, S. Yu, M. F. Zakowski, Q. Zhang, D. G. Beer, I. I. Wistuba, M. A. Watson, L. A. Garraway, M. Ladanyi, W. D. Travis, W. Pao, M. A. Rubin, S. B. Gabriel, R. A. Gibbs, H. E. Varmus, R. K. Wilson, E. S. Lander, M. Meyerson, Characterizing the cancer genome in lung adenocarcinoma. *Nature* **450**, 893–898 (2007).
46. J. A. Engelman, K. Zejnullahu, C. M. Gale, E. Lifshits, A. J. Gonzales, T. Shimamura, F. Zhao, P. W. Vincent, G. N. Naumov, J. E. Bradner, I. W. Althaus, L. Gandhi, G. I. Shapiro, J. M. Nelson, J. V. Heymach, M. Meyerson, K. K. Wong, P. A. Jänne, PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with *EGFR* and *ERBB2* mutations that are resistant to gefitinib. *Cancer Res.* **67**, 11924–11932 (2007).
47. D. Li, L. Ambrogio, T. Shimamura, S. Kubo, M. Takahashi, L. R. Chirieac, R. F. Padera, G. I. Shapiro, A. Baum, F. Himmelsbach, W. J. Rettig, M. Meyerson, F. Solca, H. Greulich, K. K. Wong, BIBW2992, an irreversible *EGFR/HER2* inhibitor highly effective in preclinical lung cancer models. *Oncogene* **27**, 4702–4711 (2008).
48. X. Zhang, J. Gureasko, K. Shen, P. A. Cole, J. Kuriyan, An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125**, 1137–1149 (2006).
49. N. Jura, Y. Shan, X. Cao, D. E. Shaw, J. Kuriyan, Structural analysis of the catalytically inactive kinase domain of the human EGF receptor 3. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 21608–21613 (2009).
50. M. J. Eck, C. H. Yun, Structural and mechanistic underpinnings of the differential drug sensitivity of *EGFR* mutations in non-small cell lung cancer. *Biochim. Biophys. Acta* **1804**, 559–566 (2010).
51. J. Y. Wu, C. J. Yu, Y. C. Chang, C. H. Yang, J. Y. Shih, P. C. Yang, Effectiveness of tyrosine kinase inhibitors on “uncommon” epidermal growth factor receptor mutations of unknown clinical significance in non-small cell lung cancer. *Clin. Cancer Res.* **17**, 3812–3821 (2011).
52. D. B. Costa, K. S. Nguyen, B. C. Cho, L. V. Sequist, D. M. Jackman, G. J. Riely, B. Y. Yeap, B. Halmos, J. H. Kim, P. A. Jänne, M. S. Huberman, W. Pao, D. G. Tenen, S. Kobayashi, Effects of erlotinib in *EGFR* mutated non-small cell lung cancers with resistance to gefitinib. *Clin. Cancer Res.* **14**, 7060–7067 (2008).
53. J. Baselga, D. Rischin, M. Ranson, H. Calvert, E. Raymond, D. G. Kieback, S. B. Kaye, L. Gianni, A. Harris, T. Bjork, S. D. Averbuch, A. Feyereislova, H. Swaisland, F. Rojo, J. Albanell, Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J. Clin. Oncol.* **20**, 4292–4302 (2002).
54. M. Hidalgo, L. L. Siu, J. Nemunaitis, J. Rizzo, L. A. Hammond, C. Takimoto, S. G. Eckhardt, A. Tolcher, C. D. Britten, L. Denis, K. Ferrante, D. D. Von Hoff, S. Silberman, E. K. Rowinsky, Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J. Clin. Oncol.* **19**, 3267–3279 (2001).
55. T. A. Yap, L. Vidal, J. Adam, P. Stephens, J. Spicer, H. Shaw, J. Ang, G. Temple, S. Bell, M. Shahidi, M. Uttenreuther-Fischer, P. Stopfer, A. Futreal, H. Calvert, J. S. de Bono, R. Plummer, Phase I trial of the irreversible *EGFR* and *HER2* kinase inhibitor BIBW 2992 in patients with advanced solid tumors. *J. Clin. Oncol.* **28**, 3965–3972 (2010).
56. R. Mulloy, A. Ferrand, Y. Kim, R. Sordella, D. W. Bell, D. A. Haber, K. S. Anderson, J. Settleman, Epidermal growth factor receptor mutants from human lung cancers exhibit enhanced catalytic activity and increased sensitivity to gefitinib. *Cancer Res.* **67**, 2325–2330 (2007).
57. C. H. Yun, K. E. Mengwasser, A. V. Toms, M. S. Woo, H. Greulich, K. K. Wong, M. Meyerson, M. J. Eck, The T790M mutation in *EGFR* kinase causes drug resistance by increasing the affinity for ATP. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2070–2075 (2008).
58. W. Zhou, D. Ercan, L. Chen, C. H. Yun, D. Li, M. Capelletti, A. B. Cortot, L. Chirieac, R. E. Jacob, R. Padera, J. R. Engen, K. K. Wong, M. J. Eck, N. S. Gray, P. A. Jänne, Novel mutant-selective *EGFR* kinase inhibitors against *EGFR* T790M. *Nature* **462**, 1070–1074 (2009).
59. H. J. Lee, G. Schaefer, T. P. Heffron, L. Shao, X. Ye, S. Sideris, S. Malek, E. Chan, M. Merchant, H. La, S. Ubhayakar, R. L. Yauch, V. Pirazzoli, K. Politi, J. Settleman, Noncovalent wild-type-sparing inhibitors of *EGFR* T790M. *Cancer Discov.* **3**, 168–181 (2013).
60. J. Cho, L. Chen, N. Sangji, T. Okabe, K. Yonesaka, J. M. Francis, R. J. Flavin, W. Johnson, J. Kwon, S. Yu, H. E. Greulich, B. E. Johnson, M. J. Eck, P. A. Janne, K. K. Wong, M. Meyerson, Cetuximab response of lung cancer-derived EGF receptor mutants is associated with asymmetric dimerization. *Cancer Res.* **73**, 6770–6779 (2013).
61. A. O. Walter, S. R. Tjin Tham, H. J. Haringsma, K. Ohashi, J. Sun, K. Lee, A. Dubrovskiy, M. Labenski, Z. Zhu, Z. Wang, M. Sheets, T. St Martin, R. Karp, D. van Kalken, P. Chaturvedi, D. Niu, M. Nacht, R. C. Petter, W. Westlin, K. Lin, S. Jaw-Tsai, M. Raponi, T. Van Dyke, J. Etter, Z. Weaver, W. Pao, J. Singh, A. D. Simmons, T. C. Harding, A. Allen, Discovery of a mutant-selective covalent inhibitor of *EGFR* that overcomes T790M-mediated resistance in NSCLC. *Cancer Discov.* 10.1158/2159-8290.CD-13-0314 (2013).
62. Y. Minami, T. Shimamura, K. Shah, T. LaFramboise, K. A. Glatt, E. Liniker, C. L. Borgman, H. J. Haringsma, W. Feng, B. A. Weir, A. M. Lowell, J. C. Lee, J. Wolf, G. I. Shapiro, K. K. Wong, M. Meyerson, R. K. Thomas, The major lung cancer-derived mutants of *ERBB2* are oncogenic and are associated with sensitivity to the irreversible *EGFR/ERBB2* inhibitor HKI-272. *Oncogene* **26**, 5023–5027 (2007).
63. L. V. Sequist, J. von Pawel, E. G. Garmey, W. L. Akerley, W. Brugger, D. Ferrari, Y. Chen, D. B. Costa, D. E. Gerber, S. Orlov, R. Ramla, S. Arthur, I. Gorbachevsky, B. Schwartz, J. H. Schiller, Randomized phase II study of erlotinib plus vandetanib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. *J. Clin. Oncol.* **29**, 3307–3315 (2011).
64. W. L. Yeo, G. J. Riely, B. Y. Yeap, M. W. Lau, J. L. Warner, K. Bodio, M. S. Huberman, M. G. Kris, D. G. Tenen, W. Pao, S. Kobayashi, D. B. Costa, Erlotinib at a dose of 25 mg daily for non-small cell lung cancers with *EGFR* mutations. *J. Thorac. Oncol.* **5**, 1048–1053 (2010).
65. R. K. Thomas, A. C. Baker, R. M. Debiasi, W. Winckler, T. LaFramboise, W. M. Lin, M. Wang, W. Feng, T. Zander, L. MacConaill, J. C. Lee, R. Nicoletti, C. Hattom, M. Goyette, L. Girard, K. Majumdar, L. Ziaugra, K. K. Wong, S. Gabriel, R. Beroukhi, M. Peyton, J. Barretina, A. Dutt, C. Emery, H. Greulich, K. Shah, H. Sasaki, A. Gazdar, J. Minna, S. A. Armstrong, I. K. Mellingerhoff, F. S. Hodi, G. Dranoff, P. S. Mischel, T. F. Cloughesy, S. F. Nelson, L. M. Liao, K. Mertz, M. A. Rubin, H. Moch, M. Loda, W. Catalona, J. Fletcher, S. Signoretti, F. Kaye, K. C. Anderson, G. D. Demetri, R. Dummer, S. Wagner, M. Herlyn, W. R. Sellers, M. Meyerson, L. A. Garraway, High-throughput oncogene mutation profiling in human cancer. *Nat. Genet.* **39**, 347–351 (2007).
66. A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni, R. J. Read, Phaser crystallographic software. *J. Appl. Crystallogr.* **40**, 658–674 (2007).
67. A. T. Brünger, P. D. Adams, G. M. Clore, W. L. DeLano, P. Gros, R. W. Grosse-Kunstleve, J. S. Jiang, J. Kuszewski, M. Nilges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonson, G. L. Warren, Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr. D Biol. Crystallogr.* **54**, 905–921 (1998).
68. P. Emsley, K. Cowtan, Coot: Model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 (2004).
69. G. N. Murshudov, A. A. Vagin, E. J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallogr. D Biol. Crystallogr.* **53**, 240–255 (1997).
70. A. W. Schüttelkopf, D. M. van Aalten, PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 1355–1363 (2004).

**Acknowledgments:** We thank the members of the Costa, Kobayashi, and Eck laboratories for their technical support and all patients who were involved in this study. **Funding:** This work was funded in part through fellowships from the American Society of Clinical Oncology Conquer Cancer Foundation (D.B.C.), a Lung Cancer Foundation of America–International Association for the Study of Lung Cancer grant (D.B.C.), American Cancer Society grant RSG 11-186 (D.B.C.), an American Lung Association grant (M.M.), a Uniting Against Lung Cancer grant (M.M.), a Saunders Family research fund (M.M.), National Natural Science Foundation of China grant 31270769 (C.-H.Y.), and NIH grants CA090578 (D.B.C., S.S.K., and M.M.), CA116020 (M.J.E. and M.M.), CA154303 (M.J.E.), CA126026 (S.S.K.), and CA169259 (S.S.K.). **Author contributions:** H.Y., E.P., C.-H.Y., M.J.E., S.S.K., and D.B.C. were involved in the conception of this study; H.Y., N.J.S., W.-L.Y., E.P., C.-H.Y., D.W.C., G.R.O., N.Y., A.R.L.-A., S.N., K.I., C.S.L., T.M., L.V.S., M.S.H., J.E.C., G.J.R., M.E.A., R.A.S., M.M., M.J.E., S.S.K., and D.B.C. were involved in data acquisition; H.Y., E.P., C.-H.Y., T.M., N.Y., A.R.L.-A., S.N., K.I., E.P., M.J.E., M.M., S.S.K., and D.B.C. were involved in data analysis and interpretation; M.J.E., M.M., S.S.K., and D.B.C. provided administrative and funding support; H.Y., E.P., C.-H.Y., M.J.E., S.S.K., and D.B.C. were involved in writing the report; and all authors approved the final version. **Competing interests:** D.B.C. has received consulting fees from Roche, Pfizer, and AstraZeneca. M.M. has received consulting fees from Novartis, Bayer, and Foundation Medicine, and patent royalties from LabCorp (M.M. is a co-inventor on a patent for the use of EGFR mutations for lung cancer diagnosis, assigned to Dana-Farber Cancer Institute and licensed to LabCorp). M.M. is also the founder of and equity holder in Foundation Medicine. M.J.E. has received consulting fees and

research support from Novartis. L.V.S. has received consulting fees from Boehringer-Ingelheim, Clovis Oncology, AstraZeneca, Merrimack Pharmaceuticals, and GlaxoSmithKline. G.J.R. has received consulting fees from AstraZeneca, Boehringer-Ingelheim, Celgene, Foundation Medicine, Abbott, Novartis, Daiichi, Tragara, Ariad, and Genentech. G.R.O. has received consulting fees from Celgene, Foundation Medicine, Abbott, Novartis, Daiichi, Tragara, AstraZeneca, Boehringer-Ingelheim, Genentech, and Ariad. No other conflict of interest is stated. **Data and materials availability:** The following protein structures were deposited into the PDB: ID 4LRM (EGFR kinase of D770\_N771insNPG + PD168393) and ID 4LQM (EGFR kinase of L858R + PD168393).

Submitted 31 July 2013

Accepted 12 November 2013

Published 18 December 2013

10.1126/scitranslmed.3007205

**Citation:** H. Yasuda, E. Park, C.-H. Yun, N. J. Sng, A. R. Lucena-Araujo, W.-L. Yeo, M. S. Huberman, D. W. Cohen, S. Nakayama, K. Ishioka, N. Yamaguchi, M. Hanna, G. R. Oxnard, C. S. Lathan, T. Moran, L. V. Sequist, J. E. Chaft, G. J. Riely, M. E. Arcila, R. A. Soo, M. Meyerson, M. J. Eck, S. S. Kobayashi, D. B. Costa, Structural, biochemical, and clinical characterization of epidermal growth factor receptor (EGFR) exon 20 insertion mutations in lung cancer. *Sci. Transl. Med.* **5**, 216ra177 (2013).

## Structural, Biochemical, and Clinical Characterization of Epidermal Growth Factor Receptor (EGFR) Exon 20 Insertion Mutations in Lung Cancer

Hiroyuki Yasuda, Eunyong Park, Cai-Hong Yun, Natasha J. Sng, Antonio R. Lucena-Araujo, Wee-Lee Yeo, Mark S. Huberman, David W. Cohen, Sohei Nakayama, Kota Ishioka, Norihiro Yamaguchi, Megan Hanna, Geoffrey R. Oxnard, Christopher S. Lathan, Teresa Moran, Lecia V. Sequist, Jamie E. Chaft, Gregory J. Riely, Maria E. Arcila, Ross A. Soo, Matthew Meyerson, Michael J. Eck, Susumu S. Kobayashi, and Daniel B. Costa

*Sci. Transl. Med.*, **5** (216), .

DOI: 10.1126/scitranslmed.3007205

### A Crystal Clear Cause of Drug Resistance

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are used to treat a variety of cancers, including non-small cell lung cancer. EGFR mutations have a wide range of effects on the success of TKI treatment in this cancer type, with some sensitizing the tumors to TKI inhibitors and others making them resistant to targeted therapy. For example, most of the mutations in exon 20, a relatively common mutation site, prevent cancer cells from responding to EGFR inhibitors.

Here, Yasuda and co-workers determined the crystal structure of EGFR with an exon 20 mutation and used a combination of kinetic studies and structural analysis to elucidate the mechanism for these mutants' differential sensitivity to TKIs. The findings of Yasuda *et al.* clarify the reasons for the drug resistance of most exon 20 mutations and show the mechanism for the rare mutation in the same exon that increases tumors' sensitivity to treatment.

In addition to explaining which of the mutants are resistant to targeted inhibition of EGFR and the reasons for this phenomenon, this work could help with the development of future therapeutics. By taking advantage of the crystal structure and detailed insights into the function of mutant EGFR, researchers may be able to design drugs that exploit the unique structural features of resistant mutants and specifically target them for treatment.

### View the article online

<https://www.science.org/doi/10.1126/scitranslmed.3007205>

### Permissions

<https://www.science.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of service](#)