LETTERS

Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3

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Oncogenic tyrosine kinases have proved to be promising targets for the development of highly effective anticancer drugs. However, tyrosine kinase inhibitors (TKIs) against the human epidermal growth factor receptor (HER) family show only limited activity against HER2-driven breast cancers, despite effective inhibition of epidermal growth factor receptor (EGFR) and HER2 *in vivo*[1–8]. The reasons for this are unclear. Signalling in *trans* is a key feature of this multimember family and the critically important phosphatidylinositol-3-OH kinase (PI(3)K)/Akt pathway is driven predominantly through transphosphorylation of the kinase-inactive HER3 (refs 9, 10). Here we show that HER3 and consequently PI(3)K/Akt signalling evade inhibition by current HER-family TKIs *in vitro* and in tumours *in vivo*. This is due to a compensatory shift in the HER3 phosphorylation–dephosphorylation equilibrium, driven by increased membrane HER3 expression driving the phosphorylation reaction and by reduced HER3 phosphatase activity impeding the dephosphorylation reaction. These compensatory changes are driven by Akt-mediated negative-feedback signalling. Although HER3 is not a direct target of TKIs, HER3 substrate resistance undermines their efficacy and has thus far gone undetected. The experimental abrogation of HER3 resistance by small interfering RNA knockdown restores potent pro-apoptotic activity to otherwise cytostatic HER TKIs, re-affirming the oncogene-addicted nature of HER2-driven tumours and the therapeutic promise of this oncoprotein target. However, because HER3 signalling is buffered against an incomplete inhibition of HER2 kinase, much more potent TKIs or combination strategies are required to silence oncogenic HER2 signalling effectively. The biologic marker with which to assess the efficacy of HER TKIs should be the transphosphorylation of HER3 rather than autophosphorylation.

Selective inhibitors of the ABL tyrosine kinase are effective in putting nearly all patients with BCR-ABL-driven leukaemia in chronic phase into complete remission[11]. This proof of concept brings new hope to the treatment of other tyrosine-kinase-driven cancers. Another important tyrosine kinase family is the HER family, consisting of EGFR, HER2, HER3 and HER4. A subset of breast cancers are driven by overactive EGFR or HER2 tyrosine kinases and an abundance of data from *in vitro* and mouse models suggests that continued activity of these tyrosine kinases drives cancer progression[12–14]. While the complexities of this tyrosine kinase family are not yet fully understood, their oncogenic signalling functions should, in theory, be amenable to silencing by TKIs. Several orally bioavailable HER-family TKIs are in preclinical and clinical development. Although in *in vitro* biochemical assays these agents differ in their relative activities against individual HER kinase family members, in cell-based assays they are effective at inhibiting both EGFR and HER2 and equally effective at suppressing the growth of EGFR- and HER2-driven tumour cells[15–19]. They are also effective at inhibiting EGFR and HER2 phosphorylation in patients' tissues and tumours[5–8]. But these agents show very limited clinical anti-tumour activity[1–5]. Their clinical development to this point has been driven largely by the detection of modest delays in tumour progression. The failure to reverse cancer progression despite an apparent inhibition of HER kinase function has created an enigma in the concept of TKI cancer therapy that we have been exploring.

It is through heterodimerization and transphosphorylation that the HER family performs its signalling functions. Importantly, downstream PI(3)K/Akt pathway signalling is predominantly mediated through the transphosphorylation of the kinase-inactive member HER3 (refs 9, 10). We have previously reported that sensitivity to HER-family TKI therapy correlates with the inhibition of PI(3)K/Akt pathway signalling[15,20]. We and others have also reported that failure to inhibit PI(3)K/Akt signalling leads to TKI resistance[20–22]. But in contrast to reports from *in vitro* models, Akt activity is not inhibited in most patients on HER TKI therapy[5,6,8]. This led us to look more closely at the inhibition of PI(3)K/Akt signalling.

To investigate this discrepancy, we studied the durability of Akt inhibition by TKI, with surprising results. Although gefitinib inhibits Akt signalling in HER2-driven cancer cells (as previously reported[15]) this inhibition is not durable. Akt signalling resumes after a transient inhibition despite continued drug therapy (Fig. 1a, b). In light of this finding, we looked at the broader HER-family signalling activities over a period of 96 h after continuous exposure of BT474 breast cancer cells to gefitinib at concentrations that non-selectively inhibit EGFR and HER2. TKI treatment effects a sustained inhibition of EGFR and HER2 phosphorylation and a durable inhibition of downstream MAPK (mitogen-activated protein kinase) and JNK (Jun N-terminal kinase) pathway signalling (Fig. 1a). However, dephosphorylation of the kinase-inactive family member HER3 is transient. HER3 signalling resumes and persists despite continued drug exposure and effective suppression of EGFR and HER2 (Fig. 1a, b). The reactivation of HER3 signalling explains the reactivation of Akt signalling, because HER3 is the principal HER-family member that binds PI(3)K and drives Akt signalling in these tumours[9,10]. TKI-refractory Akt signalling remains sensitive to PI(3)K inhibitors as expected (not shown).

These time-dependent findings are not due to drug degradation because the drug is replenished daily in these studies and HER3/Akt signalling resumes despite repeatedly refreshing drug supply up to and beyond the point of resumption of Akt signalling (not shown). There is no significant expression of HER4 before or after drug treatment in these cells (data not shown). These findings are not unique to BT474 and SkBr3 cells and have been confirmed in other HER2-overexpressing breast cancer cells, including MDA-453, AU565, MDA-361 and HCC1954 cells (Supplementary Fig. 1). These findings

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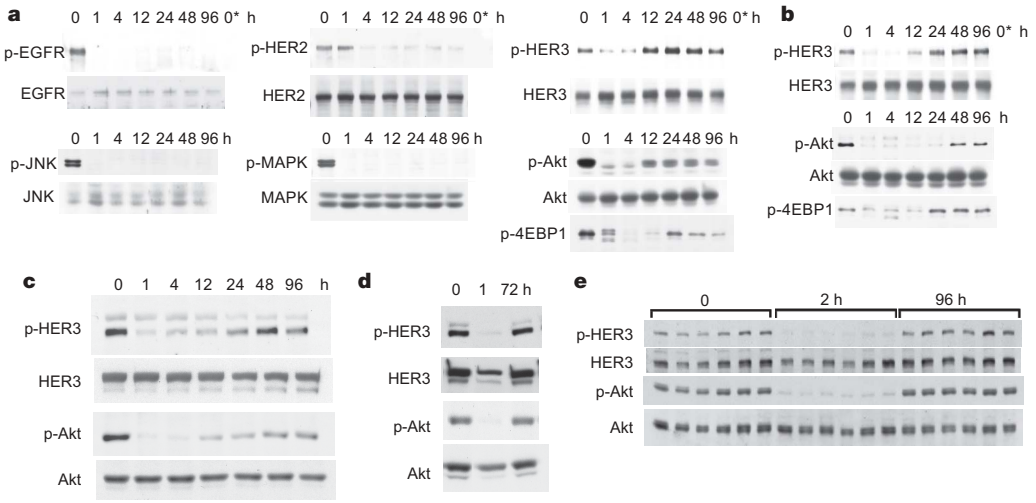

Figure 1 | HER TKIs fail to induce sustained inhibition of HER3 signalling in HER2-driven breast cancer cells. **a**, BT474 cells were treated with 5 μM gefitinib for the indicated times and assayed for expression and phosphorylation of the indicated proteins. Lane 0* is an IgG immunoprecipitation control. **b**, Data of interest shown from the identical experiment done in SkBr3 cells. **c**, SKBr3 cells were treated with 5 μM erlotinib for the indicated times and lysates immunoblotted with the indicated antibodies. **d**, SKBr3 cells were treated with 20 μM AG825 for the indicated times, with the drug being replenished 1 h before the 72 h harvest. **e**, Mice bearing HCC1569 human breast cancer xenografts were treated with gefitinib at 150 mg kg^{-1} once daily. Animals were killed 2 h after the first dose or 2 h after the fourth dose (96 h total treatment) and tumours were rapidly harvested for immunoblotting with the indicated antibodies. The six lanes corresponding to each time point represent six different animals. p, phosphorylated.

are not unique to gefitinib and are seen with other HER TKIs, including agents with *in vitro* selectivity profiles favouring EGFR or HER2, such as erlotinib or AG825 (Fig. 1c, d). Nor are these findings artefacts of the *in vitro* models. Treatment with gefitinib of mice that have various HER2-driven xenograft tumours similarly fails to durably suppress HER3 and Akt signalling, despite a transient suppression (Fig. 1e and Supplementary Fig. 2). This is not due to ineffective drug biodistribution, because in these models gefitinib was dosed three times higher than doses known to achieve sustained xenograft tumour concentrations of above 2–4 μM and averaging 6–10 μM (ref. 23). We had previously established that inactivation of PI(3)K/Akt signalling is mechanistically linked to HER TKI sensitivity in HER-family-driven cancers, so we felt that the failure of these drugs durably to inactivate PI(3)K/Akt signalling was entirely

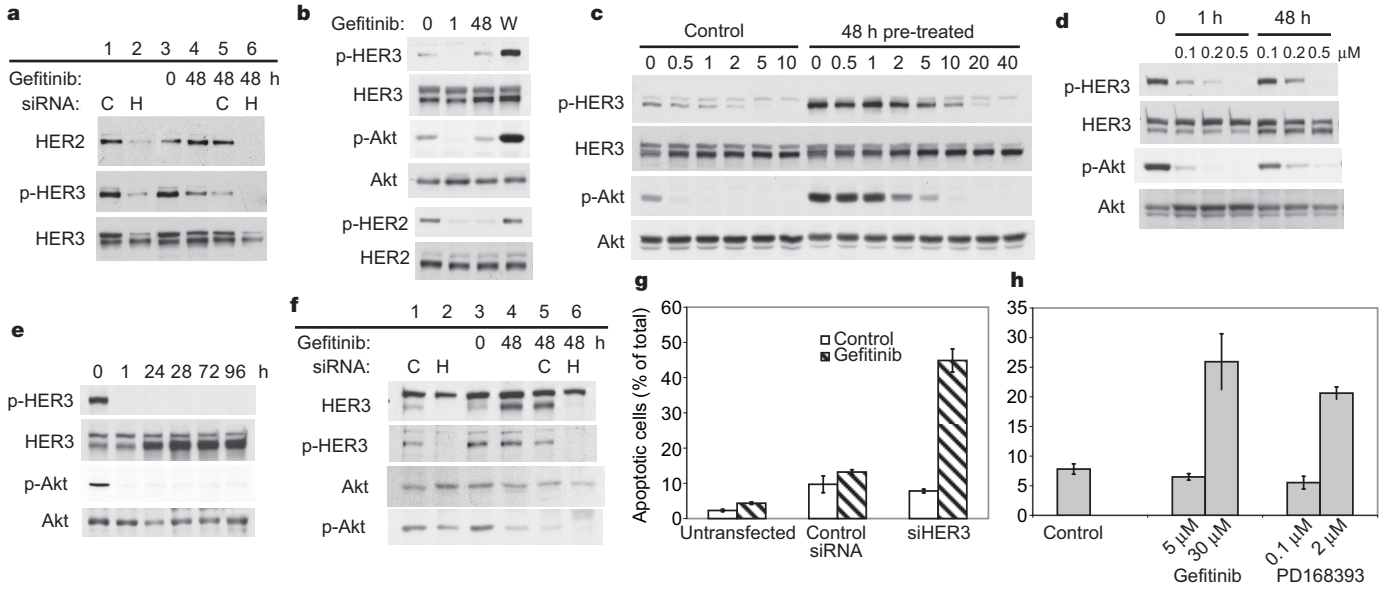

Figure 2 | Forward shift in HER3 phosphorylation–dephosphorylation equilibrium following extended HER TKI treatment. **a**, BT474 cells were transfected with anti-HER2 (H) or control (C) siRNA and harvested 64 h after transfection (lanes 1, 2). Additional arms were treated with 48 h of gefitinib untransfected (lanes 3, 4) or following siRNA transfection (lanes 5, 6). **b**, SKBr3 cells were treated with 5 μM gefitinib for 0, 1 or 48 h. Arm W was treated for 48 h, washed, and incubated in drug-free media for one more hour. **c**, SKBr3 cells were treated with the indicated concentrations of gefitinib for one hour (left side). Additional arms were treated with 5 μM gefitinib for 48 h and subsequently treated with the indicated concentrations of gefitinib for one additional hour (right side). **d**, SKBr3 cells were treated with the indicated concentrations and durations of PD168393. **e**, SKBr3 cells were treated with 2 μM PD168393 for the indicated times. **f**, SKBr3 cells were transfected with anti-HER3 (H) or control (C) siRNA and harvested four days after transfection (lanes 1, 2). Additional arms were treated with gefitinib or control in untransfected cells (lanes 3, 4) or following siRNA transfection (lanes 5, 6). **g**, In parallel with **f**, SkBr3 cells were either left untransfected, or transfected with anti-HER3 or control siRNA followed by 5 μM gefitinib or control for 48 h. Apoptotic cells were identified by their sub-G1 DNA content. **h**, SKBr3 cells were treated as indicated for 48 h. Apoptotic cells were identified by Annexin V expression. Error bars, s.d.

consistent with their limited clinical activities. Therefore we set out to study the molecular mechanism by which HER3 evades TKI therapy.

TKI-refractory HER3 phosphorylation is due to HER2, because it can be suppressed by anti-HER2 siRNA transfection (Fig. 2a). Although cross-talk between receptor families can occur, we have found no evidence for non-HER-family tyrosine kinases mediating TKI-refractory HER3 phosphorylation. The reactivation of HER3 signalling is not associated with the induction of any new tyrosine kinases and remains resistant to the broad-spectrum kinase inhibitor staurosporine (not shown). This apparent desensitization of HER3 signalling to TKIs is due to a forward shift in the equilibrium of the HER3 phosphorylation–dephosphorylation reactions, establishing a new steady-state HER3 phosphorylation level despite significant inhibition of HER2 kinase and autophosphorylation activity by TKIs. This forward shift becomes clearly evident in the form of HER3 and Akt superphosphorylation when drug inhibition is withdrawn during the new steady state (Fig. 2b). HER3 phosphorylation remains suppressible by TKI in the new steady state, but much higher concentrations are required to completely dephosphorylate HER3 because the un-inhibited HER3 phosphorylation state is significantly higher in the new steady state (Fig. 2c, compare left and right). Therefore drug-refractory HER3 phosphorylation is due to resistance at the level of the substrate HER3, and is driven by residual HER2 kinase activity. Similar characteristics apply to the more potent irreversible TKIs[24]. The irreversible TKI PD168393, which inhibits all HER-family kinases, when used at partially or near-maximal inhibitory concentrations, induces a similar desensitization of HER3 to continued drug therapy (Fig. 2d, 0.1–0.2 μM doses). However, at fully inactivating concentrations both reversible (Fig. 2c, 40 μM dose) and irreversible (Fig. 2e) TKI can durably suppress HER3 and Akt signalling.

The biological consequence of drug-refractory HER3 and Akt signalling is tumour cell survival. In fact, the anti-proliferative activity of TKIs is reversible and tumour cells resume proliferative growth after drug withdrawal. If drug-refractory HER3 signalling is averted by anti-HER3 siRNA, TKI treatment of HER2-driven cancer cells leads to apoptotic tumour cell death (Fig. 2f, g, and Supplementary Fig. 4). This is the expected outcome of effective oncoprotein inactivation, and recapitulates the apoptotic fate of oncogene withdrawal seen in reversible transgenic models of HER2 tumorigenesis[14]. Sustained inhibition of HER3 signalling using TKIs at their fully inactivating doses (from Fig. 2c–e) also leads to apoptotic tumour cell death not seen with doses that allow HER3 escape (Fig. 2h).

The TKI-induced forward shift in the HER3 phosphorylation–dephosphorylation steady state is due to increased HER3 substrate concentration driving the forward reaction, and decreased phosphatase activity impeding the reverse reaction. Increased HER3 substrate concentration occurs through a significant increase in HER3 expression at the plasma membrane where the phosphorylation reaction occurs (Fig. 3a, b). Unlike HER2, which is predominantly localized to the plasma membrane, the HER3 pool is largely within intracellular compartments, but there is also some membrane expression[25]. The TKI-induced forward shift in HER3 steady-state phosphorylation is driven by HER3 relocalization to the plasma membrane and can be suppressed by inhibitors of vesicular trafficking (Fig. 3c, d). The HER3 dephosphorylation rate is also slowed after 48 h of TKI exposure (Fig. 3e). The retarded HER3 dephosphorylation rate may be due to reduced access to cytosolic protein tyrosine phosphatases as a result of altered endocytic trafficking, or it may be due to inhibition of protein tyrosine phosphatases. In support of the latter, TKI therapy increases the concentration of cellular reactive oxygen species (Fig. 3f), which are known to inhibit protein tyrosine phosphatases and are thus emerging as an important regulator of their activity[26,27]. Consistent with this, drug-refractory HER3 signalling can be suppressed by concomitant treatment with certain anti-oxidants (Fig. 3g).

The changes in steady-state HER3 signalling that evolve with TKI treatment are driven by the loss of Akt signalling and probably involve Akt-mediated negative feedback signalling. Consistent with this, HER3 signalling does not escape TKI treatment when a constitutively active Akt is transfected (Fig. 4a). Conversely, inhibition of

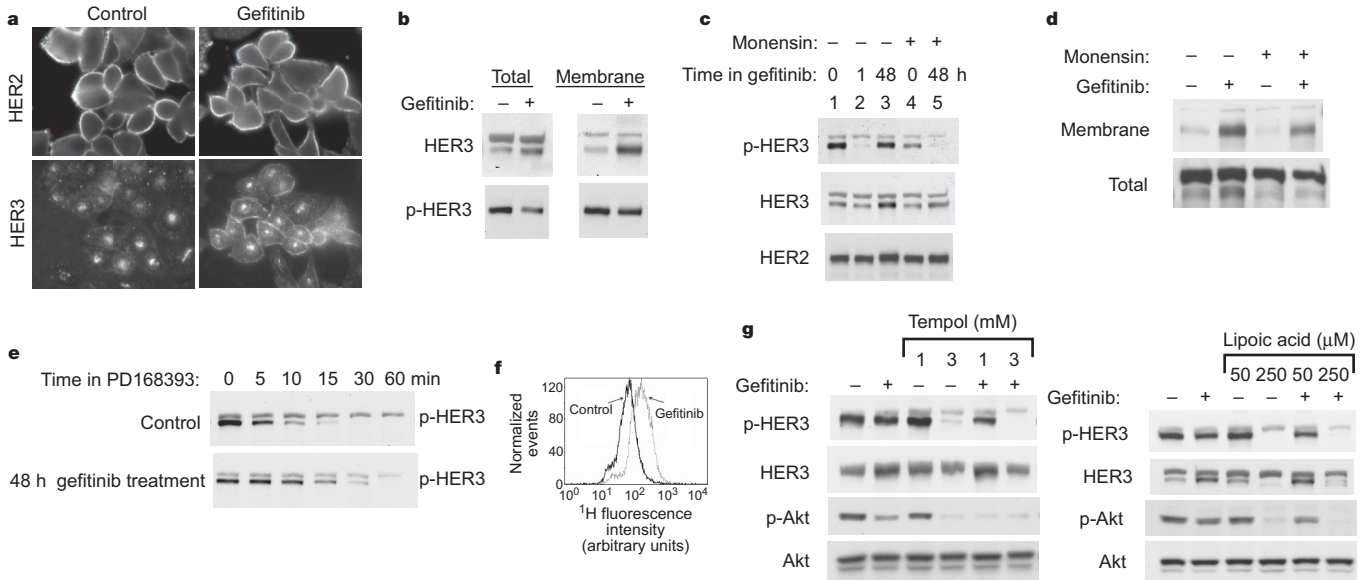

Figure 3 | Mechanism of HER3 reactivation after extended HER TKI treatment. a, SKBr3 cells were treated with 5 μM gefitinib or control for 48 h and stained with anti-HER2 or anti-HER3 antibodies for immunofluorescence microscopy. **b**, In addition, cell surface proteins in control or 48 h pre-treated cells were biotinylated, precipitated and immunoblotted as indicated. **c**, Control or gefitinib pre-treated (5 μM, 48 h) SKBr3 cells were treated with 20 μM monensin for the final 6 h and analysed by western blotting. **d**, SkBr3 cells were treated with gefitinib for 48 h and with 20 μM monensin for the final 6 h. Membrane and total HER3 was immunoblotted from cell surface proteome pulldowns (above) or total lysates (below). **e**, The dephosphorylation rate of p-HER3 following 48 h of gefitinib treatment was determined immediately after initiation of fully inactivating concentrations of the irreversible HER TKI PD168393 (2 μM). **f**, Reactive oxygen species were quantified in control or gefitinib pre-treated (5 μM, 48 h) SKBr3 cells, as described in the Methods. **g**, SKBr3 cells were treated with 5 μM gefitinib or in combination with the indicated concentrations of the anti-oxidants Tempol or α-lipoic acid for 48 h.

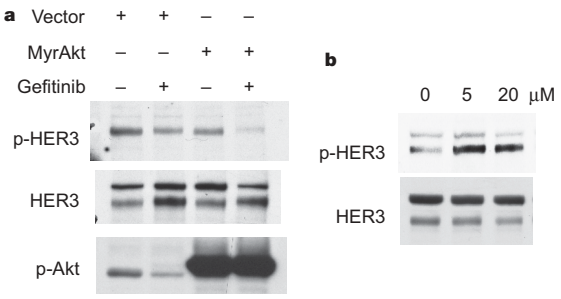

Figure 4 | Akt regulates HER3 signalling via negative feedback signalling. **a**, 2×10^6 SKBr3 cells were transiently transfected with 5 μg of pcDNA3-myristoyl-Akt plasmid or control pcDNA3 plasmid. The following day 5 μM gefitinib was added for an additional 48 h where indicated. Lysates were immunoblotted as indicated. **b**, SKBr3 cells were treated with 5 or 20 μM LY294002 for 8 h and lysates immunoblotted as indicated.

Akt signalling by a PI(3)K inhibitor leads to a compensatory increase in HER3 phosphorylation (Fig. 4b). Complete inactivation of HER2 kinase with high doses of TKIs induces the maximum feedback signalling and HER3 redistribution, but owing to the complete inactivation of HER2 kinase, HER3 signalling cannot be restored and so the feedback loop fails to rescue Akt activity.

Standard preclinical models have notoriously overestimated the clinical potential of HER TKIs, so we challenged the traditional approach to evaluating TKIs in these models. Traditionally, signalling inhibitors are thought to have a continuous suppressive effect through rapid and sustained inhibition of their direct molecular targets and downstream signalling events. This notion of drug therapy may be too simplistic. Clearly, continuous exposure to a growth factor stimulus does not produce continuous high-output downstream signalling. Rather, it leads to a sequence of signalling events, programmed by negative and positive feedback signalling, until establishment of a new steady state in the presence of continued stimulus. We find here that continuous exposure to TKIs similarly leads to a sequence of signalling events that manifest over time, until a new steady state is reached.

With this new perspective, we report that HER3 and PI(3)K/Akt signalling is not effectively inhibited by current TKIs. In particular, the allocation of kinase and signalling functions to different members within the HER family allows the signalling substrate HER3 to restore signalling activity despite significant inhibition of HER2 kinase, in effect buffering HER3-mediated PI(3)K/Akt signalling against an incomplete loss of HER2 kinase function. This inherent signal buffering capacity allows tumour cells to evade the pro-apoptotic effects of TKIs, making such TKI therapy considerably less effective. To treat HER-driven cancers much more effectively may thus require drugs with much higher potency or drugs that completely inactivate HER kinase function. Irreversible TKIs, although more potent, are subject to similar limitations. Owing to their reactive groups and reduced selectivity, many irreversible agents cannot be delivered at completely inactivating doses. Future highly selective irreversible inhibitors may turn out to be more effective. Until highly specific and fully inactivating drugs can be designed, combination treatment strategies designed to undermine the resiliency of HER-family signalling may offer the most promising approach in the near future. In addition, inhibition of autophosphorylation activity deceptively overstates the efficacy of TKIs and is a poor *in vivo* biologic marker. A better biological marker of efficacy with which to guide future therapies should be HER3 transphosphorylation.

The signal buffering capacity endowed by the separation of kinase and signalling functions to different family members in the HER kinase family attests to an evolutionary advantage conferred by the loss of catalytic activity in the HER3 protein kinase. This may be one of the reasons why approximately 10% of human kinases appear to be catalytically inactive[28].

METHODS

Cell culture and reagents. PD168393 was synthesized as previously described[29]. Commercially available gefitinib and erlotinib were purified for *in vitro* use. Reagent sources are detailed in Supplementary Information. For immunofluorescence studies, cells grown on fibronectin-coated cover slips were treated as indicated, fixed in 4% paraformaldehyde, permeabilized, and stained with the indicated primary antibodies and fluoroscein isothiocyanate (FITC)-conjugated secondary antibodies. Cells were visualized using a Zeiss Axioplan 2 fluorescence imaging microscope.

Apoptosis. Cells were seeded at 300,000–500,000 per well in 12-well or 6-well clusters. Apoptotic cells were identified and quantified by analysis of Annexin V binding using the Annexin V-FITC apoptosis detection kit (Calbiochem) according to the manufacturer's instructions, or by their sub-G1 DNA content and quantified by fluorescence-activated cell sorting (FACS) analysis as previously described[30]. All experimental arms were done in duplicate and displayed as averages with standard of deviation (s.d.) error bars.

Transfections. Cells were seeded at a density of 300,000 cells per well in 12-well plates and transfected the following day. For siRNA transfections 100–300 nmol of siRNA (Dharmacon) was premixed with Lipofectamine2000 in Opti-MEM media and then added to each well. For plasmid transfections, 2 μg of plasmid DNA was premixed with Lipofectamine2000 in Opti-MEM media and added to wells for 6 h.

Cell surface biotinylation. Cells were chilled on ice and rinsed twice with ice-cold PBS. Freshly prepared sulpho-NHS-SS-biotin (labelling reagent) was added to the final concentration of 0.5 mg ml^{-1} in PBS. After 45 min incubation at 4 °C, cells were lysed for immunoprecipitation.

Reactive oxidation species assay. Cells were rinsed twice with PBS and incubated with 10 μM of freshly prepared H2DCFDA in phenol-red-free media for 45 min at 37 °C. Cells were then trypsinized and reactive oxygen species levels were detected by flow cytometry.

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1. Winer, E. P. *et al.* Phase II multicenter study to evaluate the efficacy and safety of Tarceva (Erlotinib HCl, OSI-774) in women with previously treated, locally advanced or metastatic breast cancer. *Breast Cancer Res. Treatm.* **76** (suppl. 1), abstr. 445 (2002).
2. Blackwell, K. *et al.* A phase II, open-label, multicenter study of GW572016 in patients with trastuzumab-refractory metastatic breast cancer. *Proc. Am. Soc. Clin. Oncol.* **23**, abstr. 3006 (2004).
3. Dees, E. C. *et al.* Clinical summary of 67 heavily pre-treated patients with metastatic carcinomas treated with GW572016 in a phase Ib study. *Proc. Am. Soc. Clin. Oncol.* **23**, abstr. 3188 (2004).
4. Campos, S. M. *et al.* A phase 2, single agent study of CI-1033 administered at two doses in ovarian cancer patients who failed platinum therapy. *Proc. Am. Soc. Clin. Oncol.* **23**, abstr. 5054 (2004).
5. Baselga, J. *et al.* Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer. *J. Clin. Oncol.* **23**, 5323–5333 (2005).
6. Bacus, S. S., Beresford, P. J., Yarden, Y., Spector, N. & Smith, B. The use of predicting factors and surrogate markers in patients' cancer biopsies treated with targeted antibodies to erbB receptors and erbB tyrosine kinase inhibitors. *Proc. Am. Soc. Clin. Oncol.* **22**, abstr. 3408 (2003).
7. Burris, H. A. *et al.* EGF10004: a randomized, multicenter, phase Ib study of the safety, biologic activity and clinical efficacy of the dual kinase inhibitor GW572016. *Breast Cancer Res. Treatm.* **82** (suppl. 1), abstr. 39 (2003).
8. Spector, N. L. *et al.* Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J. Clin. Oncol.* **23**, 2502–2512 (2005).
9. Soltoff, S. P., Carraway, K. L. III, Prigent, S. A., Gullick, W. G. & Cantley, L. C. ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. *Mol. Cell. Biol.* **14**, 3550–3558 (1994).
10. Kim, H. H., Sierke, S. L. & Koland, J. G. Epidermal growth factor-dependent association of phosphatidylinositol 3-kinase with the erbB3 gene product. *J. Biol. Chem.* **269**, 24747–24755 (1994).
11. Kantarjian, H. *et al.* Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N. Engl. J. Med.* **346**, 645–652 (2002).
12. Muller, W. J., Sinn, E., Pattengale, P. K., Wallace, R. & Leder, P. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-*neu* oncogene. *Cell* **54**, 105–115 (1988).
13. Slamon, D. J. *et al.* Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. *Science* **235**, 177–182 (1987).
14. Moody, S. E. *et al.* Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell* **2**, 451–461 (2002).
15. Moasser, M. M., Basso, A., Averbuch, S. D. & Rosen, N. The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res.* **61**, 7184–7188 (2001).

16. Moulder, S. L. *et al.* Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/*neu* (*erb*B2)-overexpressing breast cancer cells *in vitro* and *in vivo*. *Cancer Res.* **61,** 8887–8895 (2001).
17. Anderson, N. G., Ahmad, T., Chan, K., Dobson, R. & Bundred, N. J. ZD 1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression. *Int. J. Cancer* **94,** 774–782 (2001).
18. Campiglio, M. *et al.* Inhibition of proliferation and induction of apoptosis in breast cancer cells by the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor ZD1839 ('Iressa') is independent of EGFR expression level. *J. Cell. Physiol.* **198,** 259–268 (2004).
19. Akita, R. W. & Sliwkowski, M. X. Preclinical studies with Erlotinib (Tarceva). *Semin. Oncol.* **30** (suppl. 7), 15–24 (2003).
20. She, Q., Solit, D., Basso, A. & Moasser, M. M. Resistance to gefitinib (ZD1839, Iressa) in PTEN null HER overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive PI3K/Akt pathway signaling. *Clin. Cancer Res.* **9,** 4340–4346 (2003).
21. Bianco, R. *et al.* Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene* **22,** 2812–2822 (2003).
22. Haas-Kogan, D. A. *et al.* Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J. Natl Cancer Inst.* **97,** 880–887 (2005).
23. McKillop, D. *et al.* Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor. *Mol. Cancer Ther.* **4,** 641–649 (2005).
24. Fry, D. W. *et al.* Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. *Proc. Natl Acad. Sci. USA* **95,** 12022–12027 (1998).
25. Offterdinger, M., Schofer, C., Weipoltshammer, K. & Grunt, T. W. c-erbB-3: a nuclear protein in mammary epithelial cells. *J. Cell Biol.* **157,** 929–939 (2002).
26. Meng, T. C., Fukada, T. & Tonks, N. K. Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol. Cell* **9,** 387–399 (2002).
27. Tonks, N. K. Redox Redux: Revisiting PTPs and the control of cell signaling. *Cell* **121,** 667–670 (2005).
28. Manning, G., Whyte, D. B., Martinez, R., Hunter, T. & Sudarsanam, S. The protein kinase complement of the human genome. *Science* **298,** 1912–1934 (2002).
29. Tsou, H. R. *et al.* 6-Substituted-4-(3-bromophenylamino)quinazolines as putative irreversible inhibitors of the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2) tyrosine kinases with enhanced antitumor activity. *J. Med. Chem.* **44,** 2719–2734 (2001).
30. Huron, D. R. *et al.* A novel pyridopyrimidine inhibitor of abl kinase is a picomolar inhibitor of Bcr-abl-driven K562 cells and is effective against STI571-resistant Bcr-abl mutants. *Clin. Cancer Res.* **9,** 1267–1273 (2003).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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