ERBB RECEPTORS AND CANCER: THE COMPLEXITY OF TARGETED INHIBITORS

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Abstract | ERBB receptor tyrosine kinases have important roles in human cancer. In particular, the expression or activation of epidermal growth factor receptor and ERBB2 are altered in many epithelial tumours, and clinical studies indicate that they have important roles in tumour aetiology and progression. Accordingly, these receptors have been intensely studied to understand their importance in cancer biology and as therapeutic targets, and many ERBB inhibitors are now used in the clinic. We will discuss the significance of these receptors as clinical targets, in particular the molecular mechanisms underlying response.

Subclass I of the receptor tyrosine kinase (RTK) superfamily consists of the ERBB or epidermal growth factor (EGF) receptors and comprises four members: EGFR/ERBB1, ERBB2, ERBB3 and ERBB4. All members have an extracellular ligand-binding region, a single membrane-spanning region and a cytoplasmic tyrosine-kinase-containing domain. The ERBB receptors are expressed in various tissues of epithelial, mesenchymal and neuronal origin. Under normal physiological conditions, activation of the ERBB receptors is controlled by the spatial and temporal expression of their ligands, which are members of the EGF family of growth factors (reviewed in REFS 1,2) (FIG. 1). Ligand binding to ERBB receptors induces the formation of receptor homo- and heterodimers and activation of the intrinsic kinase domain, resulting in phosphorylation on specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which leads to the activation of intracellular signalling pathways (reviewed in REFS 2–4).

The importance of ERBB receptors during development and in normal adult physiology is evident from analyses of genetically modified mice (BOX 1). Furthermore, EGFR and ERBB2 have been implicated in the development of many human cancers. Patients with cancer whose tumours have alterations in ERBB receptors tend to have a more aggressive disease, and one that is associated with factors that predict a poor clinical outcome, so ERBB receptors have been intensely pursued as therapeutic targets (reviewed in REF. 5). There are two major classes of anti-ERBB therapeutics: ectodomain-binding antibodies and small-molecule tyrosine-kinase inhibitors (TKIs) that compete with ATP in the tyrosine-kinase domain (BOX 2). Many of these therapies are either in clinical use or in advanced clinical development and these will be a main topic of this review.

We will outline our understanding of how ERBB receptors contribute to cancer and, in particular, how targeted therapeutics affect the transformed phenotype. Using data gleaned from preclinical models, and where possible from the clinic, we will discuss potential molecular mechanisms that underlie a successful response to the blockade of ERBB signalling. We will also discuss mechanisms that allow tumour cells to escape from anti-ERBB therapies and suggest alternative strategies that might lead to more effective treatment in the clinic.

The ERBB receptors and their ligands

With respect to ERBB-receptor binding, the EGF family of ligands can be divided into three groups: the first includes EGF, transforming growth factor-α and

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Summary

- The family of ERBB or epidermal growth factor (EGF) receptors includes four members: EGFR/ERBB1, ERBB2, ERBB3 and ERBB4. EGFR and ERBB2 are involved in development of numerous types of human cancer and they have been intensely pursued as therapeutic targets.
- Two important types of ERBB inhibitor are in clinical use: humanized antibodies directed against the extracellular domain of EGFR or ERBB2, and small-molecule tyrosine-kinase inhibitors (TKIs) that compete with ATP in the tyrosine-kinase domain of the receptor.
- In preclinical models, treatment of tumour cells with ERBB-directed TKIs and antibodies rapidly downregulates phosphatidylinositol-3-kinase–AKT, mitogen-activated protein kinase, SRC, and signal transducer and activator of transcription (STAT) signalling, and blocks the proliferation of tumour cells. In the clinic, skin biopsies (surrogate tissue), and to a limited extent tumours, have been analysed for the molecular consequences of treatment with ERBB inhibitors.
- ERBB-directed therapeutics have demonstrated clinical efficacy; however, the antitumour effects are often not as strong as predicted from preclinical studies. There are likely to be various reasons why this is so, an important one being that other tumour-cell alterations influence the tumour response to ERBB-targeted inhibitors. Therefore, rational drug-combination strategies have great potential to combat the complexity of tumour biology.

amphiregulin, which bind specifically to EGFR; and the second includes betacellulin, heparin-binding EGF (HB-EGF) and epiregulin, which show dual specificity, binding both EGFR and ERBB4. The third group is composed of the neuregulins (NRGs) and forms two subgroups based on their capacity to bind ERBB3 and ERBB4 (NRG1 and NRG2) or only ERBB4 (NRG3 and NRG4) (FIG. 1a). None of the EGF family of peptides bind ERBB2; however, MUC4, a member of the mucin family, acts as an intramembrane modulator of ERBB2 activity[6]. Despite having no soluble ligand, ERBB2 is important because it is the preferred heterodimerization partner of the other ligand-bound family members[7] (FIG. 1a).

Activated ERBBs stimulate many intracellular signalling pathways and, despite extensive overlap in the molecules that are recruited to the different active receptors, different ERBBs preferentially modulate certain signalling pathways, owing to the ability of individual ERBBs to bind specific effector proteins (FIG. 1b). Two of the main pathways activated by the receptors are the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K)–AKT pathways (reviewed in REFS 2–4). Other important ERBB signalling effectors are the signal transducer and activator of transcription proteins (STATs; reviewed in REF. 8), which, in cancer, have often been associated with EGFR activation[9]; SRC tyrosine kinase, the activity of which is increased in response to EGFR and ERBB2 signalling (reviewed in REF. 10); and mammalian target of rapamycin (mTOR), a serine/threonine kinase activated downstream of PI3K–AKT and other growth regulators (reviewed in REF. 11) (FIG. 2).

G-PROTEIN-COUPLED RECEPTORS
A large family of receptors that span the membrane seven times and couple to G proteins, which are composed of α-, β- and γ-subunits. The α-subunit contains the nucleotide (GTP or GDP) binding site, and the β- and γ-subunits behave as a single entity.

ERBB receptors and cancer

The ERBB receptors are implicated in the development of many types of cancer, and EGFR was the first tyrosine-kinase receptor to be linked directly to human tumours (for a timeline on EGFR and cancer see REF. 12). ERBB receptors undergo various types of alteration in human tumours.

Gene amplification leading to EGFR overexpression is often found in human cancers[13,14]. Furthermore, in many tumours EGF-related growth factors are produced either by the tumour cells themselves or are available from surrounding stromal cells, leading to constitutive EGFR activation (FIG. 2) (reviewed in REF. 15). In gliomas, *EGFR* amplification is often accompanied by structural rearrangements that cause in-frame deletions in the extracellular domain of the receptor, the most frequent being the EGFRvIII variant[16]. Carcinomas of the breast, lung and ovaries have also been reported to express this variant[17], although these data await further confirmation. Somatic mutations in the tyrosine-kinase domain of EGFR were recently identified in non-small-cell lung cancers (NSCLCs) in a subgroup of patients that showed clinical responses to treatment with the TKIs gefitinib[18,19] and erlotinib[20]. The functional properties of these mutant receptors will be discussed below.

Amplification of *ERBB2* leading to overexpression of the receptor, originally detected in a subset of breast tumours[21], occurs in other human cancers such as ovarian, gastric and salivary cancers (reviewed in REFS 5,22). Intriguingly, mutations in the kinase domain of *ERBB2* have been identified in a small number of NSCLCs[23]. The impact of these mutations on ERBB2 activity remains to be explored.

Structural studies on ERBB receptors

Publications describing the crystal structure of the EGFR, ERBB2 and ERBB3 ectodomains (reviewed in REF. 24) have led to new insights into some intriguing questions concerning the process of ligand-induced receptor dimerization and biological activity of ERBB2-targeted antibodies. The extracellular region of each ERBB receptor consists of four domains (I–IV; FIG. 3). Determination of the structure of ligand-bound EGFR has confirmed earlier studies (reviewed in REF. 24) that show the importance of domains I and III in peptide binding. Moreover, these studies also revealed that there is a direct receptor–receptor interaction promoted by the domain II dimerization arm; the ligands are not involved in the receptor–receptor interaction[25,26]. In unliganded ERBB3 (REF. 27) or ligand-bound inactive EGFR[28] the receptors assume the so-called tethered structure, in which the domain II dimerization interface is blocked by intramolecular interactions between domains II–IV. The EGFRvIII variant is missing exons 1–7 (REF. 16) and, consequently, the domain II dimerization arm, and cannot assume the closed tethered structure, perhaps explaining its constitutive activation[29].

The structure of ERBB2's extracellular region is radically different from the others. ERBB2 has a fixed conformation that resembles the ligand-activated state: the domain II–IV interaction is absent and the dimerization loop in domain II is exposed[30,31]. This

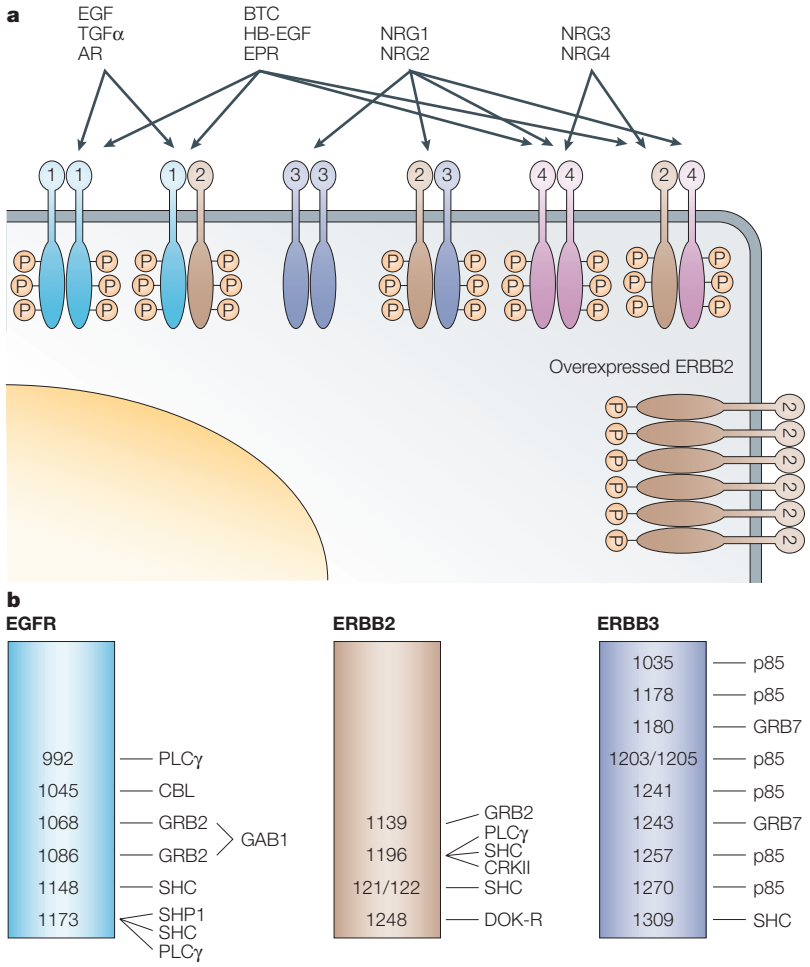

Figure 1 | **ERBB receptors, ligands, dimers and downstream signalling pathways.** **a** | Members of the epidermal growth factor (EGF) family of growth factors are ligands for the ERBB receptors. Ligand binding to ERBB receptors induces the formation of receptor homo- and heterodimers and the activation of the intrinsic kinase domain, resulting in phosphorylation on specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which leads to the activation of intracellular signalling pathways. None of the ligands bind ERBB2, but ERBB2 is the preferred dimerization partner for all the other ERBB receptors. ERBB3 has impaired kinase activity and only acquires signalling potential when it is dimerized with another ERBB receptor, such as ERBB2. Overexpression of ERBB2 in tumours leads to constitutive activation of ERBB2, presumably because of increased receptor concentrations at the plasma membrane. Many of these tumours contain phosphorylated ERBB3, which couples ERBB2 to the phosphatidylinositol 3-kinase (PI3K)–AKT pathway[128]. **b** | Schematic representation of the main autophosphorylation sites in EGF receptor (EGFR), ERBB2 and ERBB3 and of the signalling molecules associated with these sites. Despite extensive overlap in the molecules recruited to the active receptors, there is some preferential modulation of signalling pathways. Tumour cells that express EGFR with kinase-domain mutations preferentially activate the pro-survival PI3K–AKT and signal transducer and activator of transcription (STAT) pathways[67]. Although EGFR has no consensus sequence for the p85 adaptor subunit of PI3K, it couples to this pathway through GAB1, which binds growth-factor-receptor-bound protein 2 (GRB2). Although no direct binding data have been published, STATs have been proposed to couple to EGFR through tyrosine-1068 and tyrosine-1086 (REF. 137). Additional EGFR binding partners are discussed in a recent review[137]. ERBB2 couples to the mitogen-activated protein kinase pathway through GRB2, SHC, downstream of kinase related (DOK-R)[138] and CRK; phospholipase Cγ (PLCγ) binding has recently been described[139]. Although ERBB3 is able to bind neuregulins (NRGs), it has impaired kinase activity owing to substitutions in crucial residues in the tyrosine-kinase domain. Therefore, ERBB3 only becomes phosphorylated and functions as a signalling entity when it is dimerized with another ERBB receptor[140], ERBB2 being its preferred partner[7]. ERBB3 contains six docking sites for the p85 adaptor subunit of PI3K and couples very efficiently to this pathway (reviewed in REF. 3). AR, amphiregulin; BTC, betacellulin; EPR, epiregulin; HB-EGF, heparin-binding EGF; NRGs, neuregulins; TGFα, transforming growth factor-α.

structure is consistent with the data that indicate that ERBB2 is the preferred partner for the other activated ERBBs, as it is permanently poised for interaction with another ligand-bound receptor. Furthermore, this structure explains why no soluble EGF-related ligand has been found. It predicts that ERBB2 possesses a unique subdomain I–III interaction that makes ligand binding impossible because the site is buried and not accessible for interaction.

ERBB-receptor transactivation in cancer

The EGF family of growth factors are produced as transmembrane precursors that can be cleaved by cell-surface proteases (reviewed in REFS 32,33), a step that leads to the release of soluble ligands. This cleavage, referred to as ectodomain shedding, is an important step in the control of ligand availability and receptor activation[34] (reviewed in REF. 32). ERBB receptors are often constitutively stimulated in cancer owing to the presence of EGF ligands in the tumours[15] (FIG. 2). Therefore, it is essential to understand the mechanisms that control ligand processing, as novel therapeutic targets might be discovered.

The proteases involved in ectodomain shedding belong to the metalloproteinase family, in particular the ADAM (a disintigrin and metalloprotease) family and matrix metalloproteinases (MMPs). The production of soluble EGF family ligands through ectodomain shedding occurs in response to diverse stimuli and was first described following activation of G-PROTEIN-COUPLED RECEPTORS (GPCRs)[35]. In cells treated with a receptor agonist, GPCR stimulates a batimastat-sensitive metalloproteinase that induces cleavage and release of HB-EGF, leading to the rapid phosphorylation of EGFR[36]. This process, termed EGFR transactivation, has important biological implications, as it leads to stimulation of intracellular pathways such as MAPK signalling[37]. The proteases involved in ectodomain shedding have also been examined in tumour cells. ADAMs, including ADAM9, ADAM10, ADAM15 and ADAM17 (REFS 38,39) have been associated with the shedding of distinct EGF-related ligands in cancer cells. In primary breast tumours, there is a correlation between high EGFR activity and high ADAM17 levels[34].

It is now widely accepted that diverse GPCR agonists transactivate ERBBs in both normal and cancer cells. Although EGFR and ERBB2 have usually been monitored following GPCR stimulation, it is important to keep in mind that NRGs — the ligands for ERBB3 and/or ERBB4 — are processed by the same metalloproteinases[40] (reviewed in REF. 33). For certain cancer types, such as prostate cancer, the deregulated expression of GPCRs and their ligands has been linked to tumour development (reviewed in REF. 41), and chronic EGFR activation is well described in prostate tumours[42], indicating a potential link between the two receptor classes.

More recently, ERBB transactivation has been shown to involve other physiological ligands (FIG. 2). The binding of WNT to its seven-pass membrane receptor Frizzled (FZD) transactivates EGFR[43]. The

Box 1 | The toxicity of ERBB-directed therapeutics is related to their physiological roles

Mice lacking epidermal growth factor receptor (EGFR) usually die during the first postnatal week owing to respiratory problems. They also show gastrointestinal phenotypes, thin skin, and hair-follicle defects that result in brittle hair[145–147]. These observations help explain the most common side effects associated with EGFR inhibition in the clinic: rash and acneic skin reactions (including folliculitis) and diarrhoea[58,148]. Indeed, cutaneous skin rash has been proposed as a surrogate marker of clinical benefit for many EGFR-targeted agents[149]. Although infrequent (1% globally[150]), interstitial lung disease (interstitial pneumonia) has also been associated with gefitinib treatment in patients with non-small-cell lung cancer; patients with lung comorbidities, such as idiopathic pulmonary fibrosis, seem to be particularly at risk[150,151]. This is consistent with the demonstration that gefitinib augments bleomycin-induced pulmonary fibrosis in a murine model, supporting a role for EGFR in the regenerative epithelial-cell proliferation associated with pulmonary fibrosis[152].

ERBB2 has an essential role in the developing heart[153]. Embryos that lack the receptor die owing to improper formation of the ventricular trabeculea, the myocardium responsible for maintaining blood flow. Conditional ablation of ERBB2 in postnatal cardiac-muscle cell lineages revealed a role for ERBB2 in the adult heart. In its absence, ventricular enlargement of both chambers was observed, which is consistent with dilated cardiomyopathy[154]. In the clinic, some trastuzumab-treated breast cancer patients displayed cardiac phenotypes, including cardiomyopathy, congestive heart failure and decreased left ventricular ejection fraction. This was particularly true for patients treated concurrently with anthracyclines[155]. Considering that ERBB2-null cardiomyocytes showed an increased sensitivity to adriamycin-induced toxicity[154], it is possible that in the clinical setting trastuzumab-mediated effects on cardiac ERBB2 signalling might aggravate anthracycline-induced toxicity. The heart phenotype observed in *Erbb2*-knockout mice is identical to that observed in mice lacking ERBB4 (REF. 156) or for neuregulin-1 (NRG1)[57], demonstrating the importance of the ligand-induced ERBB2–ERBB4 heterodimer in heart development. As both ERBB2 and ERBB4 are expressed in adult cardiomyocytes[158] and NRG1 promotes survival of isolated cardiomyocytes[159], it is possible that ERBB4 has a role in trastuzumab-induced cardiotoxicity. It should be noted, however, that the antibody does not interfere with the NRG1-induced ERBB2–ERBB4 heterodimererization (see main text).

mechanism seems to be similar to that described for GPCRs, as it is rapid and blocked by metalloproteinase inhibitors; however, the target ligand has not been identified. WNT–FZD-mediated transactivation has been observed in normal mammary cells[43] and in breast cancer cells (T. Schlange and N.E.H., unpublished observations). Oestradiol (E2) binding to plasma-membrane-associated oestrogen receptor (ER) has also been shown to rapidly transactivate ERBBs. According to one report, E2-stimulated activation of MMP2 and MMP9 leads to the release of HB-EGF[44]. Tamoxifen, a selective ER modifier (SERM) was shown to transactivate EGFR and ERBB2, and in ERBB2-overexpressing breast cancer cells this reduced the antiproliferative activity of the SERM[45]. This has important clinical implications that will be discussed below.

Box 2 | Background on ERBB-targeted antibodies and kinase inhibitors

The first epidermal growth factor receptor (EGFR)-specific monoclonal antibodies (mAbs) were isolated using partially purified receptor[160] and A431-EGFR-overexpressing cancer cells[161]. Specific antibodies were detected by ^{125}I-EGF-binding inhibition. Cetuximab is a chimeric human:murine derivative of mAb225, isolated by Mendelsohn and colleagues[160], and is a potent inhibitor of cancer cells that have autocrine EGFR activation and human tumour xenografts that overexpress the receptor[162]. Cetuximab was approved for treatment of patients with advanced colorectal cancer in 2003. Turning to ERBB2, mAb4D5, isolated by Ullrich and colleagues[68], and trastuzumab — its humanized (human IgG1 backbone, murine complementary-determining regions) variant[163] — block proliferation of ERBB2-overexpressing breast cancer cells. Trastuzumab was approved for the treatment of ERBB2-overexpressing metastatic breast cancer in 1998. The mechanism underlying trastuzumab's clinical efficacy is still under debate and seems to be multifaceted[51].

Mutation of the kinase domain of EGFR blocks its biological activity[164], providing a rationale for developing tyrosine-kinase inhibitors for cancer treatment. Many years of medicinal chemistry, together with progress in protein-kinase crystallization, has subsequently proven that the ATP-binding domains of kinases are attractive targets for rational drug design. Therefore, the development of ATP-site-directed, low-molecular-weight tyrosine-kinase inhibitors (TKIs) has taken centre stage in modern cancer therapy[165]. Levitzki and colleagues did some of the pioneering work in designing EGFR TKIs, which they named tyrphostins[166]. Subsequently, optimization of various lead structures (including quinazolines, pyrrolopyrimidines, phenylaminopyrimidines) led to the development of several ERBB-directed TKIs, some of which are already registered for the treatment of cancer patients or are well advanced in clinical development[165] (TABLE 1).

Considering that many GPCR agonists stimulate protein kinase C (PKC) and SRC (reviewed in REF. 46), these kinase families might have widespread functions in ERBB transactivation, by providing the link between, for example, GPCR agonists, metalloproteinases and ligand processing. It has been observed that PKCδ is recruited to and phosphorylates ADAM9, resulting in proHB-EGF processing[38]. The SH3 domain of SRC and other family members has been shown to interact with proline-rich motifs in the cytoplasmic tail of ADAMs[47]. Once recruited, SRC might phosphorylate specific tyrosine residues in the cytoplasmic domain of ADAMs, thereby influencing the ability of the ADAM to cleave proEGF-related peptides. How PKC or SRC direct specific metalloproteinases to cleave their substrates remains to be explored. For example, it might involve the relocalization of a protease to specialized membrane regions, as recently described for ADAM19 and NRGβ1 (REF. 40), or the clustering of a protease with its substrate[48]. A consistent increase in the level of SRC kinase activity in primary tumours of the colon[49] and breast[50] was described several years ago. It will be interesting to explore the effects of SRC inhibition

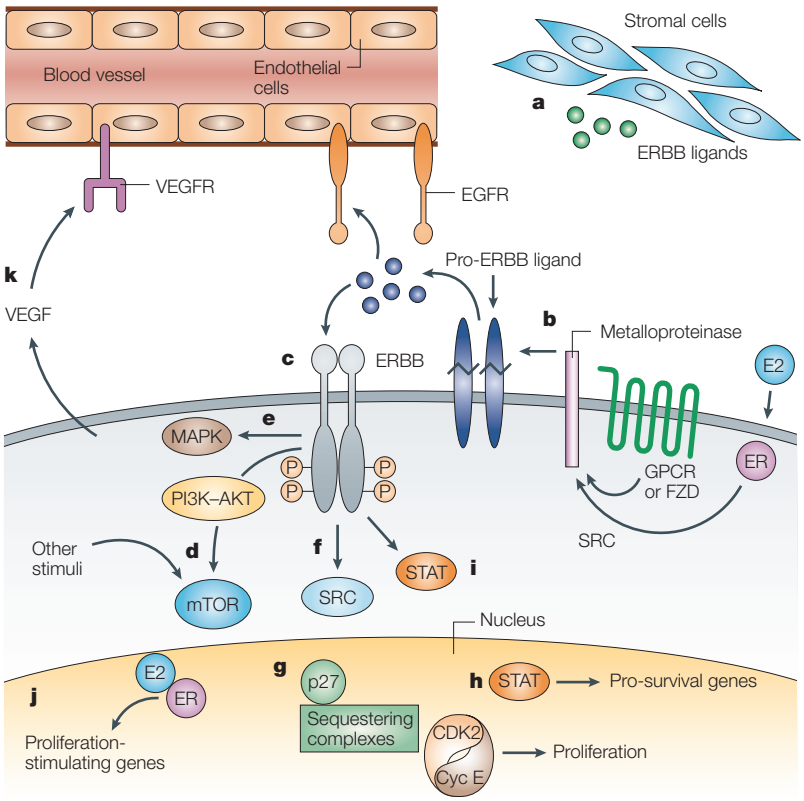

Figure 2 | **Active ERBB receptors and downstream signalling pathways in a tumour setting.** In tumour cells, ERBB receptor tyrosine kinases are activated by various mechanisms, including mutation, overexpression, and autocrine or paracrine production of epidermal growth factor (EGF) family ligands. **a** | Paracrine ERBB ligands (green circles) are released from stromal cells. **b** | Autocrine ligand (blue circles) production results from the activation of G-protein-coupled receptors (GPCRs), Frizzled (FZD) or oestrogen receptor (ER), which causes the metalloproteinase-mediated cleavage and release of pro-EGF-related ligands (a process known as ectodomain shedding). The mechanisms controlling ectodomain shedding are still largely unknown, although SRC kinase has been implicated. **c** | Active ERBB receptors stimulate numerous signalling pathways by recruiting proteins to specific phosphorylated tyrosine residues in their carboxy-terminal domain. **d** | The phosphatidylinositol 3-kinase (PI3K)–AKT pathway is stimulated through recruitment of the p85 adaptor subunit of PI3K to the receptor. Mammalian target of rapamycin (mTOR) acts as a central sensor for nutrient/energy availability, and can also be modulated by PI3K–AKT-dependent mechanisms[11,92]. **e** | The mitogen-activated protein kinase (MAPK) pathway is activated by recruitment of growth-factor-receptor-bound protein 2 (GRB2) or SHC to the receptor. **f** | SRC kinase is activated by ERBB receptors and by GPCRs (**b**) and ER. There are many nuclear effectors of ERBBs in tumour cells. **g** | One of these is the cyclin-dependent kinase inhibitor p27 (also known as KIP1), which has an important role in the control of proliferation. In tumour cells with overexpressed ERBB2, p27 is sequestered from cyclin E (Cyc E)–CDK2 complexes and cells progress through the cell cycle[54]. **h** | Signal transducer and activator of transcription (STAT) is another nuclear effector. **i** | Binding of STAT to ERBB leads to its tyrosine phosphorylation, dimerization and nuclear entry, resulting in STAT binding to specific DNA sequences in promoter regions of target genes encoding, for example, pro-survival factors (**h**). **j** | Nuclear ER and oestradiol (E2) controls transcription of cell-cycle regulators that are particularly important for breast cancer cell proliferation[102]. **k** | ERBB receptors also stimulate transcription of vascular endothelial growth factor (VEGF) through the MAPK pathway[141]. VEGF has a role in induction of tumour-associated angiogenesis. Active EGFR receptors have been detected on tumour-associated endothelial cells, which has been proposed to result from tumour release of ERBB ligands[142]. EGFR, EGF receptor; VEGFR, VEGR receptor.

on ectodomain shedding. A detailed discussion of metalloproteinases and ectodomain shedding is beyond the scope of this article. However, it is evident that the process is complex; several proteases can process an individual pro-ligand and a specific protease has several substrates (reviewed in REF. 48).

XENOGRAFT
Commonly refers to the growth of tumour cells as tumours in immunocomprised mice.

ERBB receptors as targets for cancer therapy

The ERBB receptors are aberrantly activated in a wide range of human tumours, and as such they are excellent candidates for selective anticancer therapies. Several antibodies directed against the extracellular domain of ERBBs and TKIs that target the kinase domain are in clinical use or at advanced developmental stages (TABLE 1). The treatment of tumour cells with these agents affects many of the intracellular pathways that are essential for cancer development and progression (FIG. 2). In preclinical models, treatment of tumour cells with ERBB-targeted TKIs and antibodies rapidly downregulates PI3K–AKT, MAPK, SRC and STAT signalling and, as a consequence, blocks the proliferation of tumour cell lines and XENOGRAFTS in nude mice[8,10,51–57].

Is there any evidence that in the clinical setting anti-ERBB drugs function by decreasing the activity of signalling pathways? Current clinical practice concentrates on the use of SURROGATE TISSUE to analyse the molecular consequences of treatment with EGFR inhibitors. In surrogate tissue, downregulation of EGFR phosphorylation is associated with the downregulation of MAPK signalling; the increased expression of the cyclin-dependent kinase (CDK) inhibitor p27 (also known as KIP1); changes in STAT3 activity; and a decreased proliferation index, associated in some cases with increased apoptosis[58–61]. Of course, the ideal tissue to use in these pharmacodynamic studies is the tumour[62] and a few studies have shown that these pathways are downregulated in tumours from treated patients[63,64]. In this context, it is important to note that the toxicity reported for ERBB-targeted drugs is correlated with known functions of EGFR and ERBB2 in normal physiology (BOX 1).

Response to ERBB-targeted therapeutics

When considering how ERBB-targeted therapeutics function, it is important to mention that, in contrast to the TKIs, antibodies targeting EGFR and ERBB2 have the inherent ability to recruit immune effector cells such as macrophages and monocytes to the tumour through the binding of the antibody constant Fc domain to specific receptors on these cells. In xenograft models at least, this mechanism is relevant for the anti-tumour activity of ERBB2-targeted trastuzumab[65]. Whether this mechanism has a role in clinical efficacy in cancer patients remains unclear.

Most NSCLC patients who showed clinical responses to treatment with gefitinib and erlotinib (both of which are TKIs) had tumours with somatic mutations in the EGFR kinase domain[18–20]. However, it should be noted that some responding patients had tumours with no kinase-domain mutation. Therefore, although this observation is very exciting, the clinical significance of wild-type EGFR versus mutated EGFR for response to TKIs needs further examination and is discussed in detail in a recent review[66]. *In vitro* analyses of tumour cells that express EGFRs with kinase-domain mutations have indicated that these mutations increase the sensitivity of the receptor to activation by ligands[18,19,67]. Moreover, tumour cells with mutant EGFR preferentially activate the pro-survival PI3K–AKT and

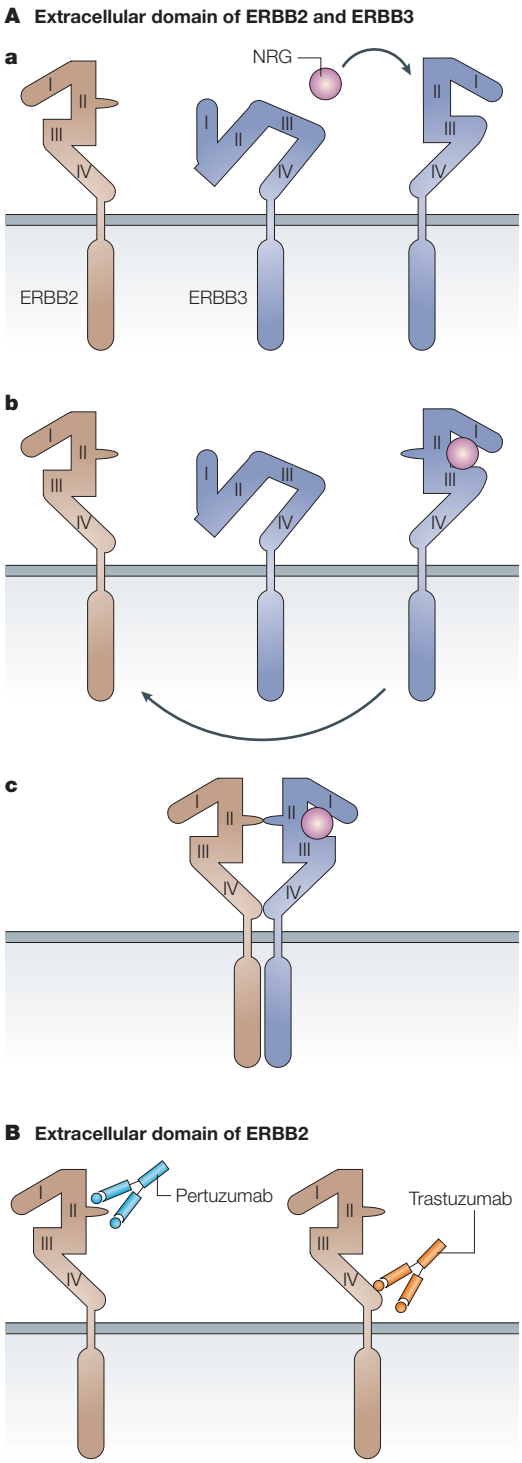

Figure 3 | **ERBB-receptor ectodomain structures. A** | The extracellular region of each ERBB receptor consists of four domains (I–IV). It has been proposed that in the absence of ligand, ERBB3 and epidermal growth factor receptor (EGFR; not shown) assume a tethered structure[27,28] (**a**). Domains I and III are involved in neuregulin (NRG) binding and, following this, the dimerization arm in domain II is exposed (**b**) and promotes receptor–receptor interaction (**c**)[25,26]. ERBB2 has a fixed conformation that resembles the ligand-activated state of EGFR and ERBB3 (REFS. 30,31). **B** | The ERBB2-directed antibodies trastuzumab and pertuzumab bind domains IV and II, respectively[31,77].

SURROGATE TISSUE
To examine the *in vivo* efficacy of tyrosine-kinase inhibitors targeted at epidermal growth factor receptor (EGFR) in cancer patients, skin biopsies of treated patients have been examined for downregulation of EGFR phosphorylation.

AUTOCRINE
A form of bioregulation in which a secreted peptide affects only the cell from which it is secreted.

STAT pathways, and treatment of these cells with a TKI induces apoptosis[67]. AUTOCRINE EGFR activation is an early event in the development of head and neck squamous-cell carcinoma (HNSCC)[9]. Considering the documented role of STAT3-mediated survival in preclincal models of HNSCC, this tumour type might be particularly susceptible to anti-EGFR therapies.

Preclinical studies showed that the murine precursor of trastuzumab 4D5 blocks tumour cells that overexpress ERBB2, but not those expressing low levels of the receptor[51,68]. Accordingly, trastuzumab is prescribed to breast cancer patients whose tumours overexpress that receptor. Clinical trials showed that the addition of trastuzumab to standard chemotherapy prolonged relapse-free survival, leading to the approval of the drug for treatment of ERBB2-overexpressing metastatic breast cancer patients. The mechanism underlying trastuzumab's clinical efficacy is likely to be multifacted[51]. In addition to the Fc-mediated functions mentioned above, preclinical studies have shown that the antibody downregulates ERBB2 levels[68] and ERBB2-mediated signalling pathways[54,55]. Furthermore, metalloproteinase-mediated ERBB2 ectodomain shedding has been proposed to cause constitutive ERBB2 signalling and trastuzumab also blocks this process[70].

Resistance to ERBB-directed therapeutics

During the process of cancer development, cells acquire multiple mutations, each of which contribute to and are necessary for full malignancy (reviewed in REF. 71). Therefore, it is unlikely that targeting only one alteration will be sufficient to kill metastatic tumour cells. For example, for ERBB2-overexpressing metastatic breast cancer, response rates of approximately 35% were observed in the trastuzumab-treated patients[72]. This is likely to be for various reasons, including resistance to the targeted therapy or, more broadly, because the malignant phenotype is unlikely to be due to ERBB2 activation alone. We will discuss the data indicating that other tumour-cell alterations do impact on response to ERBB inhibitors and present rational strategies for combining ERBB-targeted agents with other signal-transduction inhibitors or with cytotoxics.

Acquired resistance to EGFR-targeted TKIs. As discussed above, tumours of lung cancer patients who responded to gefitinib and erlotinib expressed EGFRs that had gain-of-function mutations in the kinase domain[18–20]. The recent identification of additional mutations in NSCLC patients whose tumours displayed drug-sensitive mutations and who initially responded to TKI treatment might explain some of the acquired resistance to EGFR inhibitors[73]. Resistance to TKIs has emerged as a significant clinical problem, initially in the context of chronic myelogenous leukaemia (CML), a disease that is associated with the BCR–ABL oncoprotein. CML patients treated with the BCL–ABL-targeted TKI imatinib often experience complete remission. However, imatinib-resistance can arise and has been associated with acquired mutations in the BCR–ABL kinase domain[74].

Table 1 | **ERBB-targeted therapeutics in clinical use**

Compound	Type	Target	Company	Status and comments
Trastuzumab (Herceptin)	Humanized mAb	ERBB2	Genentech/Roche	Approved for the treatment of ERBB2-overexpressing breast cancer; ongoing trials for use in combination with various other drugs
Pertuzumab (Omnitarg)	Humanized mAb	ERBB2	Genentech	Phase II trials to treat ovarian cancer, breast cancer, prostate cancer and NSCLC; based on its ability to block ERBB2 dimerization, trials are ongoing in cancer that express low ERBB2 levels
Cetuximab (Erbitux)	Chimeric mAb	EGFR	ImClone/Merck KgaA Bristol-Myers Squibb	Approved for the treatment of CRC; ongoing trials in combination with various drugs for treatment of pancreatic cancer, HNSCC and NSCLC
Matuzumab	Humanized mAb	EGFR	Merck KGaA	Phase II trials for NSCLC, gynaecological cancer, pancreatic cancer and oesophageal cancer
Panitumumab	Humanized mAb	EGFR	Abgenix	Trials are ongoing for CRC, RCC and NSCLC
Gefitinib (Iressa)	TKI	EGFR	AstraZeneca	Approved for the treatment of NSCLC after failure on other available treatments; ongoing trials in HNSCC, gastrointestinal cancer and breast cancer
Erlotinib (Tarceva)	TKI	EGFR	Genentech/OSI Pharmaceuticals	Approved for the treatment of NSCLC after failure on other available treatments; ongoing trials in many cancer types
Lapatinib	TKI	EGFR/ERBB2	GlaxoSmithKline	Phase III trial underway on breast cancer patients who are refractory to trastuzumab and chemotherapy
AEE788	TKI	EGFR/ERBB2/VEGFR	Novartis	Phase I trials underway — first multifunction EGFR/ERBB2/VEGFR inhibitor, and there are many potential indications
CI-1033	Irreversible TKI	EGFR/ERBB2	Pfizer	Phase II trials underway in breast and NSCLC
EKB-569	Irreversible TKI	EGFR/ERBB2	Wyeth-Ayerst	Phase II trials underway in NSCLC
EXEL 7647/EXEL 0999	TKI	EGFR/ERBB2/VEGFR	EXELIXIS	Phase I trials underway

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; HNSCC, nead and neck squamous-cell cancer; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; RCC, renal-cell cancer; TKI, tyrosine-kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

How do ERBB2-overexpressing cancer cells escape from trastuzumab? Only approximately one-third of patients with ERBB2-overexpressing metastatic breast cancer respond to trastuzumab[69,72,75]. These clinical results indicate that many ERBB2-overexpressing tumours are resistant to this agent. Several theories, ranging from the existence of compensatory pathways to signalling aberrations downstream of ERBB2, have been proposed to explain the clinical results. Considering the first, ERBB ligands, either PARACRINE or autocrine, might facilitate escape from trastuzumab through the activation of alternative ERBB receptor homo- and heterodimers. In fact, it has been shown experimentally that trastuzumab cannot block the proliferation of tumour cells that have autocrine EGFR activation[54], and it cannot prevent the ligand-induced formation of ERBB2-containing heterodimers or the activation of downstream signalling pathways[55,76] (FIG. 4a). As trastuzumab binds to domain IV of ERBB2, a region not involved in receptor dimerization[31] (FIG. 3), this explains why ERBB ligands can induce the formation of ERBB2-containing heterodimers in the presence of the antibody. By contrast, pertuzumab binds ERBB2 near the centre of the domain II dimerization arm[77] (FIG. 3), thereby preventing the formation of ligand-induced ERBB2-containing heterodimers[78]. This characteristic might partly explain why pertuzumab inhibits the growth of tumours that express low ERBB2 levels, whereas trastuzumab does not[76]. The impact that the different characteristics of pertuzumab have on its clinical efficacy remains to be uncovered.

The potential for compensatory pathways to confer resistance to anti-ERBB therapeutics is not restricted to ERBB2 inhibitors, as bypassing the effects of an EGFR-directed TKI through ligand-mediated activation of other ERBBs has also been observed and was circumvented by the use of TKIs that target many different ERBB receptors[55]. Clearly, the relevance of these observations to the trastuzumab- or TKI-treated patient will only become apparent when more detailed epidemiology has been carried out, correlating the molecular characteristics of a tumour with clinical response. However, considering that many tumours express multiple ERBB receptors and co-express one or more ERBB ligand[15,79], the potential for their involvement in resistance should be kept in mind.

Resistance to ERBB-directed therapeutics through activation of other RTKs. Aberrant activation of other RTKs, for example, insulin like growth factor-1

PARACRINE
A form of bioregulation in which a secreted peptide affects a neighbouring cell.

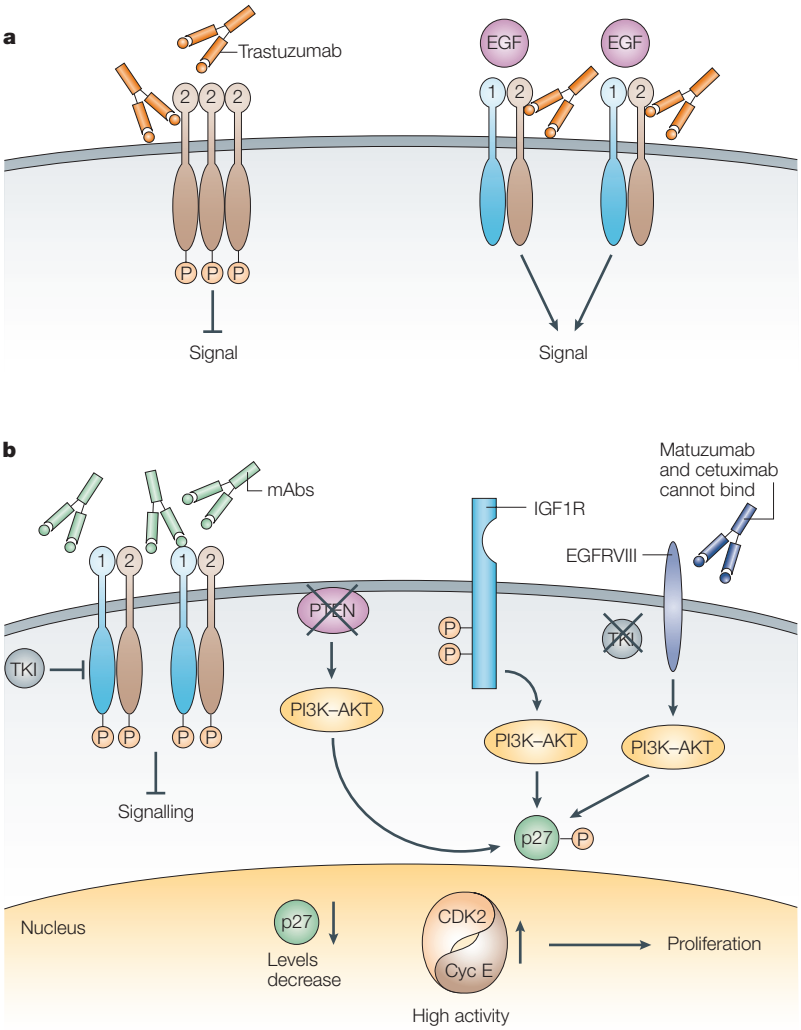

Figure 4 | **Mechanisms of resistance to anti-ERBB therapeutics. a** | Resistance of tumour cells to trastuzumab through ligand-induced activation of ERBB2 dimers. Binding of trastuzumab to overexpressed ERBB2 leads to downregulation of receptor signalling potential, resulting in a block in tumour-cell proliferation. Preclinical results have shown that ERBB ligands can circumvent trastuzumab's ability to block downstream signalling and proliferation of ERBB2-overexpressing tumour cells. There are several likely explanations including the inability of trastuzumab to prevent the formation of ligand-induced ERBB2-containing heterodimers[55,76]. **b** | Multiple mechanisms have the potential to allow tumour cells to escape from ERBB-targeted therapeutics. Treatment of tumour cells with monoclonal antibodies (mAbs) or tyrosine-kinase inhibitors (TKIs), the two classes of ERBB-targeted therapeutics discussed in the main text, interferes with ERBB-receptor signalling. PTEN dephosphorylates position D3 of phosphatidylinositol-3,4,5 triphosphate, and thereby antagonizes phosphatidylinositol 3-kinase (PI3K)–AKT pathway signalling. Loss or mutation of PTEN might cause tumour-cell resistance to ERBB therapeutics, because in cells with low PTEN levels activation of the PI3K–AKT pathway becomes independent of ERBB-receptor activation. Other classes of receptor tyrosine kinases, such as the insulin-like growth factor 1 receptor (IGF1R) might be constitutively active in tumour cells. As IGF1R signalling potential is not blocked by ERBB-targeted therapeutics, constitutive activation of this receptor can promote strong activation of intracellular signalling pathways (such as PI3K–AKT), even in the presence of ERBB-targeted therapeutics. The epidermal growth factor receptor variant III (EGFRvarIII) cannot bind the EGFR-targeted monoclonal antibodies cetuximab or matuzumab, and has been reported to be resistant to gefitinib[143]. Strong PI3K–AKT signalling interferes with ability of the cyclin-dependent kinase inhibitor (CKI) p27 (also known as KIP1) to block tumour proliferation in response to ERBB-targeted therapeutics. AKT phosphorylates p27 on Thr157, leading to its cytoplasmic retention[144]. Furthermore, low levels of p27 and concomitant upregulation of cyclin E (Cyc E)–CDK2 kinase activity in the nucleus have been reported in cells with high PI3K–AKT signalling[84]. In each case, p27 would not be able to function as a negative regulator of Cyc-E–CDK2 and tumour cells would proliferate.

receptor (IGF1R)[80] or fibroblast growth factor receptor family members[81], occurs in various types of cancer. These alterations might also impact on response to ERBB-targeted agents (FIG. 4b). Indeed, the trastuzumab-sensitive ERBB2-overexpressing SKBR3 human breast cancer cell line was rendered resistant to the antibody following ectopic IGF1R expression[82]. In comparison to parental cells, these cells expressed low levels of the CDK inhibitor p27 (REF. 83). The antiproliferative effect of trastuzumab has been linked to an increased association of p27 with the cyclin-E–CDK2 complex, resulting in decreased kinase activity and a G1 block[54]. So the IGF1R-expressing cells might be trastuzumab insensitive because of downregulation of this important negative regulator of cyclin-E–CDK2. A link to p27 has also been established in trastuzumab-resistant SKBR3 cells, in which it was shown that continuous growth in trastuzumab resulted in cells with low p27 levels and high CDK2 kinase activity. Reintroduction of p27 into these cells restored trastuzumab sensitivity[84]. It is interesting that co-targeting ERBB2 and IGF1R revealed a synergistic effect on cell growth in ERBB2-overexpressing MCF7 breast cancer cells[85], an observation that prompts further investigation.

Loss of PTEN and resistance to ERBB-targeted therapeutics. The antiproliferative effect of ERBB-targeted therapeutics often correlates with the downregulation of MAPK and PI3K–AKT pathways. It has been suggested that persistent activation of these pathways caused by aberrations downstream of the receptors might also have a role in resistance to trastuzumab, as well as EGFR-directed inhibitors[86–88]. In fact, activation of AKT, or loss or mutation of the dual-specificity protein and lipid phosphatase PTEN, the negative regulator of PI3K, have been found to be important causes of tumour-cell resistance[87] (FIG. 4b). The main role of PTEN is to dephosphorylate position D3 of phosphatidylinositol-3,4,5 triphosphate, and thereby antagonize PI3K function, leading to downregulation of AKT activity. In a small panel of ERBB2-overexpressing primary breast tumours it was shown that the expression level of PTEN was positively correlated with trastuzumab's clinical efficacy[56]. In this respect, constitutive PI3K–AKT signalling through loss of PTEN expression[89], amplification of chromosomal loci encoding AKT or PI3K (reviewed in REF. 90), or gain-of-function mutations in *PIK3CA*[91] are common in solid tumours and might have an important role in modulating the efficacy of ERBB-directed therapies. Consequently, logical combination strategies to alleviate this potential resistance mechanism might be required.

Drug combinations: the key to success?

During the course of tumour development, genetic alterations arise that contribute to the processes linked to metastatic cancer[71]. Aberrantly activated ERBB receptors contribute to many of the processes[5]. However, it is very unlikely that inhibiting only these receptors will block the malignant process.

A combination of signal-transduction inhibitors will probably have a stronger inhibitory effect.

Rationale for combination of ERBB and mTOR inhibitors. An important mediator of the PI3K–AKT pathway with respect to tumour-cell growth and proliferation is the mTOR kinase. mTOR is a member of the phosphoinositide-kinase-related kinase family, which also includes PI3K[11]. The mTOR pathway acts as a central sensor for nutrient/energy availability, and can also be modulated by PI3K–AKT-dependent mechanisms[11,92] (FIG. 2). In the presence of mitogenic stimuli and sufficient nutrients and energy, mTOR relays a positive signal to the translational machinery, facilitating events that drive cell growth[11]. The importance of mTOR signalling in tumour biology is now widely accepted.

Consequently, several agents that selectively target mTOR (that is, the rapamycin derivatives RAD001 and CCI-779) are being developed as oncological treatments[11,93]. Considering that ERBB receptors signal through the PI3K–AKT pathway, it is not surprising that mTOR activity can be influenced by ERBB activation[94,95]. However, there is accumulating evidence that mTOR also signals independently from these RTKs[96,97]. This indicates that targeting mTOR in combination with anti-ERBB therapeutics might lead to more profound effects on tumour-cell biology than could be achieved through individual targeting of the proteins (FIG. 5A). As mentioned above, loss of PTEN has been demonstrated to counteract the antitumour action of gefitinib[87,88]. There is also a clear association between PI3K–AKT activation and ERBB2 overexpression in breast cancer[98] and PTEN loss or activation of the PI3K–AKT pathway has been associated with a poorer response and resistance to trastuzumab[56,82,83,99]. Bearing in mind that loss of PTEN or hyperactivation of AKT has been suggested to sensitize tumours to the effects of mTOR inhibition[11,100,101], clinical investigation into combination treatment with mTOR and ERBB inhibitors is warranted.

Rationale for combining anti-oestrogens or aromatase inhibitors with ERBB inhibitors. Oestrogen-bound ER interacts with oestrogen-responsive elements to stimulate the transcription of target genes involved in cell-cycle progression and survival, a process implicated in the deregulated cell proliferation associated with breast cancer[102]. Although therapeutics that interfere with ER function, including the SERMs tamoxifen and fulvestrant (a partial ER agonist and a complete ER antagonist, respectively) have significantly contributed to a reduction in breast cancer mortality, at best 50–60% of ER-positive breast cancers respond to anti-oestrogen therapy[103] (reviewed in REF. 104). Consequently, several aromatase inhibitors that reduce oestrogen biosynthesis, such as letrozole and anastrozole, have also been developed as part of a therapeutic strategy aimed at expanding on the clinical success of anti-oestrogens (reviewed in REF. 105).

Recently, it has become evident that oestrogen–ER signalling is far more complex than was initially anticipated, and has pleiotropic effects through non-genomic interactions with growth-factor signalling pathways[106]. Several levels of interaction between the ER and ERBB RTKs have been documented (FIG. 5B). As mentioned above, *in vitro* E2 treatment transactivates EGFR and ERBB2. Moreover, ER physically interacts with ERBB2 (REF. 107). Strikingly, in many preclinical studies, upregulation of EGFR and ERBB2 expression has been associated with resistance to endocrine therapies (reviewed in REFS 108,109). Indeed, tamoxifen can act as an oestrogen agonist in breast cancer cells that have increased ERBB2 levels[45] and reduction in tamoxifen resistance is associated with ERBB2 downregulation[110]. E2 treatment also activates the PI3K–AKT and MAPK pathways in oestrogen-sensitive breast cancer cells[111] and ER directly interacts with the p85 regulatory subunit of PI3K[112]. Furthermore, the converse occurs: AKT, MAPK and p38 MAPK phosphorylate ER on key residues that are involved in the induction of ligand-independent activation by growth-factor receptors (reviewed in REF. 113). The observation that long-term oestrogen-deprived MCF7 cells (with increased sensitivity to oestrogen) show upregulation of ERBB2 (REF. 114) indicates that ERBB-receptor signalling is also fundamental to the adaptation of cultured breast cancer cells to low oestrogen levels, a situation that could be said to mimic therapy with aromatase inhibitors.

Based on the extensive crosstalk between the oestrogen–ER and ERBB signalling pathways, drug-combination approaches targeting both pathways would seem to be a rational clinical strategy to improve the efficacy of endocrine therapies, as well as to potentially circumvent or delay the development of resistance. Indeed, NEOADJUVANT studies showed that primary breast tumours derived from ER-positive patients exhibiting ERBB2-overexpression had an impeded antiproliferative response to endocrine therapy[115], and tamoxifen treatment resulted in increased ERBB2 activation in tumours at relapse[116]. Moreover, although the use of a single prognostic factor should be viewed with caution in the heterogeneous setting of cancer[117], in both the advanced and adjuvant setting, ER-positive patients with ERBB2-overexpressing breast tumours do seem to have a poorer clinical outcome to endocrine therapy[118] (reviewed in REF. 108). Interestingly, in a neoadjuvant study, advanced-disease patients with EGFR- or ERBB2-positive breast tumours responded well to the aromatase inhibitor letrozole but poorly to the SERM tamoxifen[119]. This observation indicates that oestrogen deprivation might be a more effective neoadjuvant therapy than SERMs in this patient population, and is presumably related to the different mode of action of aromatase inhibitors as opposed to SERMs. However, whether oestrogen deprivation would be the preferred strategy in this patient population awaits further clarification.

Preclinical efforts to examine combinations of tamoxifen with the EGFR inhibitor gefitinib *in vitro* and in experimental tumour models have demonstrated the potential for improved antitumour effects even in the

NEOADJUVANT
A therapy that is given before the main treatment, which could be, for example, surgery.

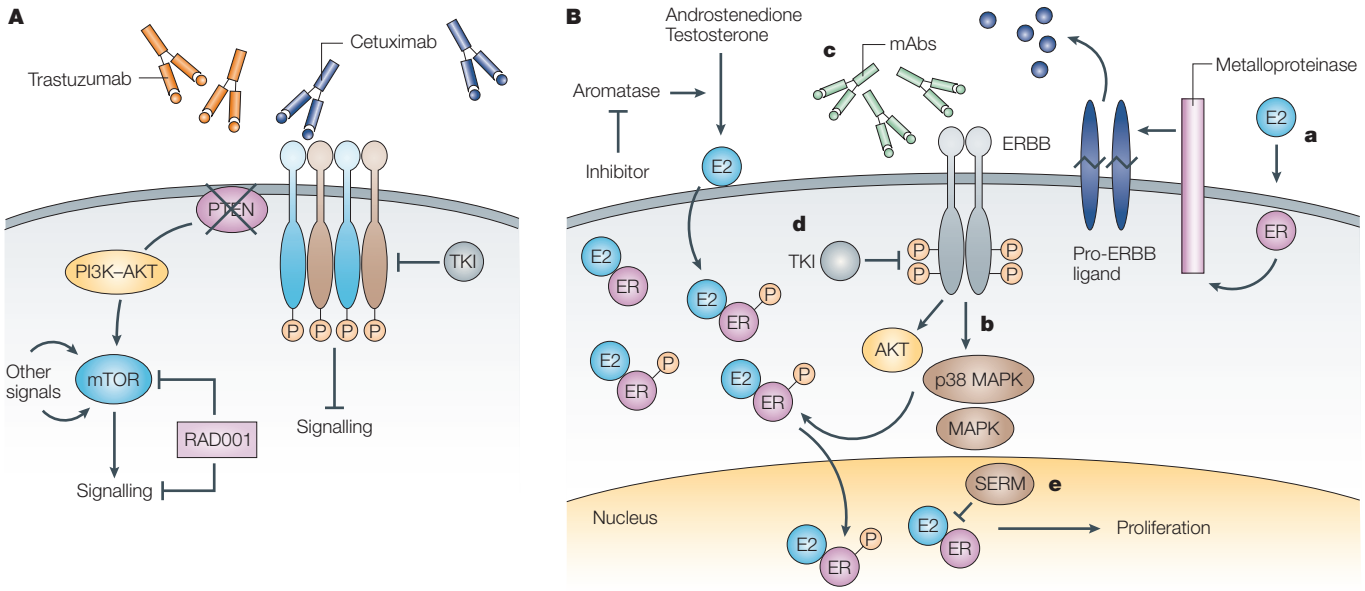

Figure 5 | **Combination strategies to potentiate cellular response and overcome resistance. A** | Mammalian target of rapamycin (mTOR) and ERBB inhibitors. An important mediator of the phosphatidylinositol 3-kinase (PI3K)–AKT pathway, with respect to tumour-cell growth and proliferation, is mTOR. mTOR acts as a central sensor for nutrient/energy availability, and is also modulated by the PI3K–AKT pathway. Although it has been shown that ERBB receptors signal through mTOR in cancer cells[94,95], there is accumulating evidence that mTOR can signal independently from these receptor tyrosine kinases (RTKs)[96,97], for example in tumour cells with low levels of PTEN. This indicates that targeting mTOR with a specific inhibitor such as RAD001 in combination with anti-ERBB therapeutics such as tyrosine-kinase inhibitors (TKIs) or antibodies (such as trastuzumab or cetuximab), might have more profound antitumour activity than could be achieved through individual targeting of the proteins. **B** | Anti-oestrogens, aromatase inhibitors and ERBB inhibitors. Several levels of interaction between ERBB receptors and oestrogen receptor (ER) have been documented. First (**a**), oestradiol (E2) treatment transactivates ERBB receptors (mainly EGFR and ERBB2 have been studied), very likely mediated through metalloproteinase activation leading to pro-ERBB ligand cleavage and ectodomain shedding (**a**). ERBB RTK-induced signalling to downstream effectors (**b**), in particular AKT, mitogen-activated protein kinase (MAPK) and p38 MAPK, leads to direct phosphorylation of ER on key residues involved in ligand-independent activation of the steroid-hormone receptor. Based on this extensive crosstalk, drug combinations targeting both pathways would seem to be a rational clinical strategy. These could include ERBB-targeted monoclonal antibodies (**c**) or TKIs (**d**), in combination with selective ER modifiers (SERMs; **e**) such as tamoxifen, which acts as an antagonist in breast cancer cells, or aromatase inhibitors, which lower the content of E2 available to the tumour cell.

background of tamoxifen resistance[45,109]. However, a study showing antagonism when using trastuzumab in combination with tamoxifen in the ERBB2-overexpressing BT474 breast cell line[120] indicates that more experiments will be needed to determine the feasibility of this combination approach. But it should be noted that promising preliminary data, which indicate an increased objective response to the combination, are emerging from a Phase II clinical trial of trastuzumab combined with letrozole in patients with ER- and ERBB2-positive advanced breast cancer. Moreover, Phase III clinical trials have been initiated with trastuzumab or lapatinib (TABLE 1) in combination with anastrozole, as well as a randomized Phase II study evaluating gefitinib with either fulvestrant or anastrozole (reviewed in REF. 108). Clearly, the results of these clinical studies, together with more in-depth preclinical analyses, will help to define the future of this promising combination strategy.

Chemotherapy and ERBB inhibitors. In preclinical experiments it was shown that the antitumour effect of EGFR-targeted monoclonal antibodies was strengthened when combined with the DNA-crosslinking drug cisplatin[121]. The activity of ERBB2-targeted trastuzumab was also enhanced when combined with cisplatin[122] or docetaxel[123]; the latter was registered with trastuzumab for cancer treatment. Mechanistic studies revealed that in the presence of the ERBB2-targeted antibody, cancer cells treated with platinating agents showed a reduction in unscheduled DNA synthesis, a sign of DNA repair[122,124]. Therefore, downregulation of ERBB2 interferes with the ability of tumour cells to repair DNA adducts, thereby causing tumour-cell death. More recently, larger studies revealed synergistic interactions between trastuzumab and carboplatin, 4-hydroxycyclophosphamide, docetaxel or vinorelbine in a panel of ERBB2-overexpressing breast cancer cells[124]. At present, the combination of trastuzumab, docetaxel and platinum salts is being analysed in clinical trials[125]. Preclinical studies with EGFR-directed TKIs in combination with chemotherapeutics looked promising[126]. Regrettably, Phase III trials evaluating the addition of the TKIs gefitinib or erlotinib to chemotherapy as first-line therapy in patients with metastatic NSCLC failed to show an advantage in response rate, progression-free survival

or overall survival compared with standard treatment[66,127]. Elucidating why this combination approach was unsuccessful might provide valuable information pertinent to the design of drug-combination trials in the future.

Perspectives and future directions

Our increased understanding of the molecular, structural and biological characteristics of the ERBB RTK family has been essential for the rational development of ERBB-targeted inhibitors. As we discuss here, ectodomain-targeted antibodies and TKIs are in clinical use and show efficacy. Nevertheless, one of the key goals for future work will be the development of accurate predictors of response to ERBB-targeted therapies. Considering, for example, that only about one-third of the pre-selected group of ERBB2-overexpressing breast cancer patients respond to trastuzumab[75], it becomes obvious that other factors must be considered before choosing a patient for this treatment. These predictors should help in the design of better clinical trials for drug testing, thereby allowing the more rapid approval of novel therapeutics.

The recent discovery of kinase-domain mutations in EGFR and ERBB2 and their impact on response to ERBB-targeted therapeutics awaits further clinical and basic research. It will be important to understand how the mutated receptors contribute to tumour biology. Moreover, the intriguing results indicating that mutant EGFR couples to pro-survival pathways more efficiently than the wild-type receptor needs to be confirmed in patients. Finally, it will be essential to determine if kinase-domain mutations will be a useful tool for patient selection. In particular, are mutations in the ERBB2 kinase domain predictive for clinical response? Considering the proven molecular role of the ERBB2–ERBB3 heterodimer in breast tumour cell lines that overexpress ERBB2 (REF. 128), it will be interesting to see if activating ERBB3 mutations are uncovered in tumours that have low ERBB2 levels. The role of ERBB4 in cancer biology needs more study, one important reason being to determine how blockade of ERBB4 by multitargeted ERBB-kinase inhibitors impacts on clinical response. In this context, it will be essential to determine whether targeting multiple ERBB receptors will lead to unacceptable toxicity. Lastly, although there is evidence supporting the use of skin as a surrogate tissue to evaluate molecular responses to ERBB inhibitors[62], the development of biomarker analyses to directly assess tumour response should be given high priority. This will become especially important when attempting to evaluate the effects of drug-combination strategies and for establishing effective criteria for patient selection.

Turning to combination strategies, the importance of IGF1R in maintaining strong activation of the PI3K–AKT pathway, and its potential to interfere with ERBB-targeted inhibitors, indicates that it is logical to consider combining anti-ERBB agents with an IGF1R inhibitor[129]. More generally, the PI3K–AKT pathway can be activated by many different mechanisms and these could also be targeted together with ERBB RTKs. As discussed above, mTOR inhibitors are very appealing. However, there are inhibitors targeting other kinases on the PI3K–AKT pathway (reviewed in REFS 130,131) that might also be effective in combination. Furthermore, targeting the MAPK pathway (reviewed in REF. 132), specifically the RAF kinase (reviewed in REF. 133), would also seem to be appropriate.

Initially, the development of a TKI with a very specific target was an important goal in the field. Considering our increased understanding of how the tumour microenvironment impacts on the progression of an initially well-encapsulated tumour into metastatic cancer, the concept of targeting several key kinases important in this progress has emerged. Based on the importance of tumour vasculature in the process of cancer growth and spread, inhibitors that block endothelial-cell survival have gained in importance. Preclinical studies have demonstrated encouraging combination effects with ERBB- and vascular endothelial growth factor receptor (VEGFR)-directed inhibitors in experimental animal tumour models[134,135]. It will be very interesting to see how multi-targeted inhibitors such as AEE788 and EXEL7647 (TABLE 1) that block both ERBB and VEGFRs fare in the clinic in comparison to other ERBB inhibitors. In several preclinical models, AEE788 was as effective as the combination of the ERBB inhibitor PKI166 and the VEGFR inhibitor PTK787/ZK22584 (REF. 136). Clearly, agents that have dual activity in one molecule present a 'combination strategy in one', which could provide more flexibility in terms of the range of potential tumour indications and facilitate advanced combination strategies.

In the future, we are confident that by continuing the exchange of information between basic and clinical studies we will uncover further factors that underlie clinical response to ERBB-targeted therapeutics. We also hope that the continued translation of knowledge that is emerging from the field of signal transduction will contribute not only to the development of novel therapeutics, but also allow us to optimally use those already in the clinic.

1. Riese, D. J. & Stern, D. F. Specificity within the EGF family/ErbB receptor family signaling network. *Bioessays* **20**, 41–48 (1998).
2. Yarden, Y. & Sliwkowski, M. X. Untangling the ErbB signalling network. *Nature Rev. Mol. Cell Biol.* **2**, 127–137 (2001).
3. Olayioye, M. A., Neve, R. M., Lane, H. A. & Hynes, N. E. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.* **19**, 3159–3167 (2000).
4. Schlessinger, J. Common and distinct elements in cellular signaling via EGF and FGF receptors. *Science* **306**, 1506–1507 (2004).
5. Holbro, T. & Hynes, N. E. ErbB receptors: directing key signaling networks throughout life. *Annu. Rev. Pharmacol. Toxicol.* **44**, 195–217 (2004).
6. Ramsauer, V. P., Carraway, C. A., Salas, P. J. & Carraway, K. L. Muc4/sialomucin complex, the intramembrane ErbB2 ligand, translocates ErbB2 to the apical surface in polarized epithelial cells. *J. Biol. Chem.* **278**, 30142–30147 (2003).

7. Graus-Porta, D., Beerli, R. R., Daly, J. M. & Hynes, N. E. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling. *EMBO J.* **16**, 1647–1655 (1997).
8. Yu, H. & Jove, R. The STATs of cancer — new molecular targets come of age. *Nature Rev. Cancer* **4**, 97–105 (2004).
9. Grandis, J. R. *et al.* Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis *in vivo*. *Proc. Natl Acad. Sci. USA* **97**, 4227–4232 (2000).
10. Ishizawar, R. & Parsons, S. J. c-Src and cooperating partners in human cancer. *Cancer Cell* **6**, 209–214 (2004).
11. Bjornsti, M. A. & Houghton, P. J. The TOR pathway: a target for cancer therapy. *Nature Rev. Cancer* **4**, 335–348 (2004).
12. Gschwind, A., Fischer, O. M. & Ullrich, A. The discovery of receptor tyrosine kinases: targets for cancer therapy. *Nature Rev. Cancer* **4**, 361–370 (2004).
13. Ohgaki, H. *et al.* Genetic pathways to glioblastoma: a population-based study. *Cancer Res.* **64**, 6892–6899 (2004).
14. Sunpaweravong, P. *et al.* Epidermal growth factor receptor and cyclin D1 are independently amplified and overexpressed in esophageal squamous cell carcinoma. *J. Cancer Res. Clin. Oncol.* **131**, 111–119 (2005).
15. Salomon, D. S., Brandt, R., Ciardiello, F. & Normanno, N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit. Rev. Oncol. Hematol.* **19**, 183–232 (1995).
16. Ekstrand, A. J., Sugawa, N., James, C. D. & Collins, V. P. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proc. Natl Acad. Sci. USA* **89**, 4309–4313 (1992).
17. Moscatello, D. K. *et al.* Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer Res.* **55**, 5536–5539 (1995).
18. Lynch, T. J. *et al.* 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).
19. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
20. Pao, W. *et al.* EGF 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. USA* **101**, 13306–13311 (2004).
References 18–20 are the first reports to describe the presence of cancer-specific mutations in the EGFR kinase domain. Patients with non-small-cell lung tumours containing these mutations had a higher chance of responding to EGFR tyrosine-kinase inhibitors than patients expressing wild-type EGFR in their tumours.
21. 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).
This paper was the first to show that *ERBB2* gene amplification is associated with an increased risk of relapse and death for patients with early-stage breast cancer.
22. Hynes, N. E. & Stern, D. F. The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim. Biophys. Acta* **1198**, 165–184 (1994).
23. Stephens, P. *et al.* Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* **431**, 525–526 (2004).
24. Burgess, A. W. *et al.* An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors. *Mol. Cell* **12**, 541–552 (2003).
25. Garrett, T. P. *et al.* Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor α. *Cell* **110**, 763–773 (2002).
26. Ogiso, H. *et al.* Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains. *Cell* **110**, 775–787 (2002).
References 25 and 26 are the first to show the crystal structure of the EGFR ectodomain in complex with a ligand. Although each ligand simultaneously contacts two binding sites in the ectodomain, the ligand does not span the ectodomain dimer; EGFR dimerization is entirely receptor mediated. In the latter publication, this unique receptor-mediated dimerization was verified by mutagenesis.
27. Cho, H. S. & Leahy, D. J. Structure of the extracellular region of HER3 reveals an interdomain tether. *Science* **297**, 1330–1333 (2002).
28. Ferguson, K. M. *et al.* EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization. *Mol. Cell* **11**, 507–517 (2003).
29. Batra, S. K. *et al.* Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant *EGFRvIII* gene. *Cell Growth Differ.* **6**, 1251–1259 (1995).
30. Garrett, T. P. *et al.* The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Mol. Cell* **11**, 495–505 (2003).
31. Cho, H. S. *et al.* Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature* **421**, 756–760 (2003).
32. Harris, R. C., Chung, E. & Coffey, R. J. EGF receptor ligands. *Exp. Cell Res.* **284**, 2–13 (2003).
33. Falls, D. L. Neuregulins: functions, forms, and signaling strategies. *Exp. Cell Res.* **284**, 14–30 (2003).
34. Borrell-Pages, M., Rojo, F., Albanell, J., Baselga, J. & Arribas, J. TACE is required for the activation of the EGFR by TGF-α in tumors. *EMBO J.* **22**, 1114–1124 (2003).
35. Daub, H., Weiss, F. U., Wallasch, C. & Ullrich, A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* **379**, 557–560 (1996).
Treatment of cells with GPCR agonists induces rapid EGFR tyrosine phosphorylation. This has been termed EGFR transactivation and was shown in this paper to result from metalloproteinase activation leading to the cleavage and release of HB-EGF.
36. Prenzel, N. *et al.* EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**, 884–888 (1999).
37. Luttrell, L. M., Daaka, Y. & Lefkowitz, R. J. Regulation of tyrosine kinase cascades by G-protein-coupled receptors. *Curr. Opin. Cell Biol.* **11**, 177–183 (1999).
38. Izumi, Y. *et al.* A metalloprotease-disintegrin, MDC9/meltrin-γ/ADAM9 and PKCδ are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J.* **17**, 7260–7272 (1998).
39. Gschwind, A., Hart, S., Fischer, O. M. & Ullrich, A. TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells. *EMBO J.* **22**, 2411–2421 (2003).
40. Wakatsuki, S., Kurisaki, T. & Sehara-Fujisawa, A. Lipid rafts identified as locations of ectodomain shedding mediated by Meltrin β/ADAM19. *J. Neurochem.* **89**, 119–123 (2004).
41. Daaka, Y. G proteins in cancer: the prostate cancer paradigm. *Sci. STKE* **216**, re2 (2004).
42. Scher, H. I. *et al.* Changing pattern of expression of the epidermal growth factor receptor and transforming growth factor α in the progression of prostatic neoplasms. *Clin. Cancer Res.* **1**, 545–550 (1995).
43. Civenni, G., Holbro, T. & Hynes, N. E. Wnt1 and Wnt5a induce cyclin D1 expression through ErbB1 transactivation in HC11 mammary epithelial cells. *EMBO Rep.* **4**, 166–171 (2003).
44. Razandi, M., Pedram, A., Park, S. T. & Levin, E. R. Proximal events in signaling by plasma membrane estrogen receptors. *J. Biol. Chem.* **278**, 2701–2712 (2003).
45. Shou, J. *et al.* Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J. Natl Cancer Inst.* **96**, 926–935 (2004).
46. Luttrell, D. K. & Luttrell, L. M. Not so strange bedfellows: G-protein-coupled receptors and Src family kinases. *Oncogene* **23**, 7969–7978 (2004).
47. Poghosyan, Z. *et al.* Phosphorylation-dependent interactions between ADAM15 cytoplasmic domain and Src family protein-tyrosine kinases. *J. Biol. Chem.* **277**, 4999–5007 (2002).
48. Seals, D. F. & Courtneidge, S. A. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes Dev.* **17**, 7–30 (2003).
49. Bolen, J. B., Veillette, A., Schwartz, A. M., DeSeau, V. & Rosen, N. Activation of pp60c-src protein kinase activity in human colon carcinoma. *Proc. Natl Acad. Sci. USA* **84**, 2251–2255 (1987).
50. Ottenhoff-Kalff, A. E. *et al.* Characterization of protein tyrosine kinases from human breast cancer: involvement of the c-src oncogene product. *Cancer Res.* **52**, 4773–4778 (1992).
51. Sliwkowski, M. X. *et al.* Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin. Oncol.* **26**, 60–70 (1999).
The different mechanisms that have been proposed to contribute to trastuzumab's clinical efficacy are discussed in this paper.
52. Petit, A. M. *et al.* Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: angiogenic implications for signal transduction therapy of solid tumors. *Am. J. Pathol.* **151**, 1523–1530 (1997).
53. Overholser, J. P., Prewett, M. C., Hooper, A. T., Waksal, H. W. & Hicklin, D. J. Epidermal growth factor receptor blockade by antibody IMC-C225 inhibits growth of a human pancreatic carcinoma xenograft in nude mice. *Cancer* **89**, 74–82 (2000).
54. Lane, H. A. *et al.* ErbB2 potentiates breast tumor proliferation through modulation of $p27^{Kip1}$–Cdk2 complex formation: receptor overexpression does not determine growth dependency. *Mol. Cell. Biol.* **20**, 3210–3223 (2000).
55. Motoyama, A. B., Hynes, N. E. & Lane, H. A. The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. *Cancer Res.* **62**, 3151–3158 (2002).
Trastuzumab-treated breast cancer cells escape from the antiproliferative effects of the monoclonal antibody in the presence of ERBB ligands owing to the fact that trastuzumab cannot block the homo/heterodimerization of other ERBB receptors.
56. Nagata, Y. *et al.* PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* **6**, 117–127 (2004).
57. Kerbel, R. & Folkman, J. Clinical translation of angiogenesis inhibitors. *Nature Rev. Cancer* **2**, 727–739 (2002).
58. Baselga, J. *et al.* 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).
59. Albanell, J. *et al.* Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. *J. Clin. Oncol.* **20**, 110–124 (2002).
60. Malik, S. N. *et al.* Pharmacodynamic evaluation of the epidermal growth factor receptor inhibitor OSI-774 in human epidermis of cancer patients. *Clin. Cancer Res.* **9**, 2478–2486 (2003).
61. Vanhoefer, U. *et al.* Phase I study of the humanized antiepidermal growth factor receptor monoclonal antibody EMD72000 in patients with advanced solid tumors that express the epidermal growth factor receptor. *J. Clin. Oncol.* **22**, 175–184 (2004).
62. Baselga, J. Skin as a surrogate tissue for pharmacodynamic end points: is it deep enough? *Clin. Cancer Res.* **9**, 2389–2390 (2003).
63. Daneshmand, M. *et al.* A pharmacodynamic study of the epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 in metastatic colorectal cancer patients. *Clin. Cancer Res.* **9**, 2457–2464 (2003).
64. Tabernero, J. *et al.* A phase I pharmacokinetic (PK) and serial tumor and skin pharmacodynamic (PD) study of weekly, every 2 weeks or every 3 weeks 1-hour (h) infusion EMD72000, an humanized monoclonal anti-epidermal growth factor receptor (EGFR) antibody, in patients (p) with advanced tumors known to overexpress the EGFR. *Eur. J. Cancer* **38** (Suppl. 7), 69 (2002).
65. Clynes, R. A., Towers, T. L., Presta, L. G. & Ravetch, J. V. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nature Med.* **6**, 443–446 (2000).
66. Herbst, R. S., Fukuoka, M. & Baselga, J. Timeline: Gefitinib-a novel targeted approach to treating cancer. *Nature Rev. Cancer* **4**, 956–965 (2004).
67. Sordella, R., Bell, D. W., Haber, D. A. & Settleman, J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* **305**, 1163–1167 (2004).
68. Hudziak, R. M. *et al.* p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. *Mol. Cell. Biol.* **9**, 1165–1172 (1989).
69. Cobleigh, M. A. *et al.* Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.* **17**, 2639–2648 (1999).
70. Molina, M. A. *et al.* Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res.* **61**, 4744–4749 (2001).
71. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
72. Vogel, C. L. *et al.* Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.* **20**, 719–726 (2002).
73. Pao, W. *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2**, 1–11 (2005).

This paper describes the identification of acquired mutations in the EGFR kinase domain of cancer patients who have become resistant to gefitinib or erlotinib.

74. Gorre, M. E. *et al.* Clinical resistance to STI-571 cancer therapy caused by *BCR–ABL* gene mutation or amplification. *Science* **293**, 876–880 (2001).
75. Harries, M. & Smith, I. The development and clinical use of trastuzumab (Herceptin). *Endocr. Relat. Cancer* **9**, 75–85 (2002).
76. Agus, D. B. *et al.* Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell* **2**, 127–137 (2002).
77. Franklin, M. C. *et al.* Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* **5**, 317–328 (2004).
78. Jackson, J. G., St Clair, P., Sliwkowski, M. X. & Brattain, M. G. Blockade of epidermal growth factor- or heregulin-dependent ErbB2 activation with the anti-ErbB2 monoclonal antibody 2C4 has divergent downstream signaling and growth effects. *Cancer Res.* **64**, 2601–2609 (2004).
79. Rubin Grandis, J. *et al.* Levels of TGF-α and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J. Natl Cancer Inst.* **90**, 824–832 (1998).
80. Laban, C., Bustin, S. A. & Jenkins, P. J. The GH-IGF-I axis and breast cancer. *Trends Endocrinol Metab.* **14**, 28–34 (2003).
81. Adnane, J. *et al.* BEK and FLG, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. *Oncogene* **6**, 659–663 (1991).
82. Lu, Y., Zi, X., Zhao, Y., Mascarenhas, D. & Pollak, M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J. Natl Cancer Inst.* **93**, 1852–1857 (2001).
Results in this paper show that activation of the IGF1R in ERBB2-overexpressing breast cancer cells renders initially trastuzumab-sensitive cells resistant to the antibody.
83. Lu, Y., Zi, X. & Pollak, M. Molecular mechanisms underlying IGF-I-induced attenuation of the growth-inhibitory activity of trastuzumab (Herceptin) on SKBR3 breast cancer cells. *Int. J. Cancer* **108**, 334–341 (2004).
84. Nahta, R., Takahashi, T., Ueno, N. T., Hung, M. C. & Esteva, F. J. $P27^{kip1}$ down-regulation is associated with trastuzumab resistance in breast cancer cells. *Cancer Res.* **64**, 3981–3986 (2004).
85. Camirand, A., Lu, Y. & Pollak, M. Co-targeting HER2/ErbB2 and insulin-like growth factor-1 receptors causes synergistic inhibition of growth in HER2-overexpressing breast cancer cells. *Med. Sci. Monit.* **8**, BR521–BR526 (2002).
86. Janmaat, M. L., Kruyt, F. A., Rodriguez, J. A. & Giaccone, G. Response to epidermal growth factor receptor inhibitors in non-small cell lung cancer cells: limited antiproliferative effects and absence of apoptosis associated with persistent activity of extracellular signal-regulated kinase or Akt kinase pathways. *Clin. Cancer Res.* **9**, 2316–2326 (2003).
87. She, Q. B., Solit, D., Basso, A. & Moasser, M. M. Resistance to gefitinib in PTEN-null HER-overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3'-kinase/Akt pathway signaling. *Clin. Cancer Res.* **9**, 4340–4346 (2003).
88. 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).
This paper shows that tumour cells with low PTEN levels are resistance to ERBB-targeted inhibitors.
89. Eng, C. *PTEN*: one gene, many syndromes. *Hum. Mutat.* **22**, 183–198 (2003).
90. Thompson, J. E. & Thompson, C. B. Putting the rap on Akt. *J. Clin. Oncol.* **22**, 4217–4226 (2004).
91. Samuels, Y. *et al.* High frequency of mutations of the *PIK3CA* gene in human cancers. *Science* **304**, 554 (2004).
92. Krymskaya, V. P. Tumour suppressors hamartin and tuberin: intracellular signalling. *Cell Signal.* **15**, 729–739 (2003).
93. Dutcher, J. P. Mammalian target of rapamycin (mTOR) inhibitors. *Curr. Oncol. Rep.* **6**, 111–115 (2004).
94. Koziczak, M. & Hynes, N. E. Cooperation between fibroblast growth factor receptor-4 and ErbB2 in regulation of cyclin D1 translation. *J. Biol. Chem.* **279**, 50004–50011 (2004).
95. Zhou, X. *et al.* Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. *Clin. Cancer Res.* **10**, 6779–6788 (2004).
96. Aoki, M., Blazek, E. & Vogt, P. K. A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. *Proc. Natl Acad. Sci. USA* **98**, 136–1341 (2001).
Results presented in this paper justify combining mTOR inhibitors with inhibitors blocking other signalling entities such as the ERBB receptors, as the data demonstrate that cellular transformation can be independently driven by non-overlapping signalling pathways.
97. Venkateswarlu, S. *et al.* Autocrine heregulin generates growth factor independence and blocks apoptosis in colon cancer cells. *Oncogene* **21**, 78–86 (2002).
98. Stal, O. *et al.* Akt kinases in breast cancer and the results of adjuvant therapy. *Breast Cancer Res.* **5**, R37–R44 (2003).
99. Clark, A. S., West, K., Streicher, S. & Dennis, P. A. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. *Mol. Cancer Ther.* **1**, 707–717 (2002).
100. Majumder, P. K. *et al.* mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1-dependent pathways. *Nature Med.* **10**, 594–601 (2004).
101. Neshat, M. S. *et al.* Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc. Natl Acad. Sci. USA* **98**, 10314–10319 (2001).
This paper shows that PTEN-deficient tumour cells are particularly sensitive to mTOR inhibition. These results are important because they indicate that activation of the PI3K pathway could affect response to mTOR inhibition.
102. Doisneau-Sixou, S. F. *et al.* Estrogen and antiestrogen regulation of cell cycle progression in breast cancer cells. *Endocr. Relat. Cancer* **10**, 179–186 (2003).
103. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group. *Lancet* **339**, 1–15 (1992).
104. Robertson, J. F. Selective oestrogen receptor modulators/new antioestrogens: a clinical perspective. *Cancer Treat. Rev.* **30**, 695–706 (2004).
105. Smith, I. E. & Dowsett, M. Aromatase inhibitors in breast cancer. *N. Engl. J. Med.* **348**, 2431–2442 (2003).
106. Schiff, R. *et al.* Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. *Clin. Cancer Res.* **10**, 331S–336S (2004).
107. Matsuda, S. *et al.* 17β-estradiol mimics ligand activity of the c-erbB2 protooncogene product. *Proc. Natl Acad. Sci. USA* **90**, 10803–10807 (1993).
108. Ellis, M. Overcoming endocrine therapy resistance by signal transduction inhibition. *Oncologist* **9** (Suppl. 3), 20–26 (2004).
109. Gee, J. M. *et al.* The antiepidermal growth factor receptor agent gefitinib (ZD1839/Iressa) improves antihormone response and prevents development of resistance in breast cancer *in vitro*. *Endocrinology* **144**, 5105–5117 (2003).
110. Chung, Y. L., Sheu, M. L., Yang, S. C., Lin, C. H. & Yen, S. H. Resistance to tamoxifen-induced apoptosis is associated with direct interaction between Her2/neu and cell membrane estrogen receptor in breast cancer. *Int. J. Cancer* **97**, 306–312 (2002).
111. Keshamouni, V. G., Mattingly, R. R. & Reddy, K. B. Mechanism of 17-β-estradiol-induced Erk1/2 activation in breast cancer cells. A role for HER2 AND PKC-δ. *J. Biol. Chem.* **277**, 22558–22565 (2002).
112. Simoncini, T. *et al.* Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature* **407**, 538–541 (2000).
113. Ali, S. & Coombes, R. C. Endocrine-responsive breast cancer and strategies for combating resistance. *Nature Rev. Cancer* **2**, 101–112 (2002).
114. Martin, L. A. *et al.* Enhanced estrogen receptor (ER) α, ERBB2, and MAPK signal transduction pathways operate during the adaptation of MCF-7 cells to long term estrogen deprivation. *J. Biol. Chem.* **278**, 30458–30468 (2003).
115. Dowsett, M. *et al.* HER-2 amplification impedes the antiproliferative effects of hormone therapy in estrogen receptor-positive primary breast cancer. *Cancer Res.* **61**, 8452–8458 (2001).
116. Dowsett, M. Molecular changes in tamoxifen-relapsed breast cancer: relationship between ER, HER2 and p38-MAP-kinase. *Proc. Am. Soc. Clin. Oncol.* **22**, 3 (2003).
117. Arpino, G. *et al.* HER-2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a southwest oncology group study. *Clin. Cancer Res.* **10**, 5670–5676 (2004).
118. De Placido, S. *et al.* Twenty-year results of the Naples GUN randomized trial: predictive factors of adjuvant tamoxifen efficacy in early breast cancer. *Clin. Cancer Res.* **9**, 1039–1046 (2003).
119. Ellis, M. J. *et al.* Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J. Clin. Oncol.* **19**, 3808–3816 (2001).
This clinical study revealed that patients with EGFR- or ERBB2-positive breast tumours responded well to the aromatase inhibitor letrozole but poorly to the SERM tamoxifen. These results demonstrate the value of molecular profiling to aid the selection of appropriate targeted therapies.
120. Ropero, S. *et al.* Trastuzumab plus tamoxifen: anti-proliferative and molecular interactions in breast carcinoma. *Breast Cancer Res. Treat.* **86**, 125–137 (2004).
121. Aboud-Pirak, E. *et al.* Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma *in vitro* and in nude mice. *J. Natl Cancer Inst.* **80**, 1605–1611 (1988).
122. Pietras, R. J. *et al.* Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* **9**, 1829–1838 (1994).
This paper provides a mechanism of the synergistic activity of trastuzumab and cisplatin in ERBB2-overexpressing cancer cells. Downregulation of ERBB2 signalling activity interferes with the ability of cancer cells to repair DNA adducts, leading to death of tumour cells.
123. Pegram, M. D., Lopez, A., Konecny, G. & Slamon, D. J. Trastuzumab and chemotherapeutics: drug interactions and synergies. *Semin. Oncol.* **27**, 21–25 (2000).
124. Pegram, M. D. *et al.* Rational combinations of trastuzumab with chemotherapeutic drugs used in the treatment of breast cancer. *J. Natl Cancer Inst.* **96**, 739–749 (2004).
125. Pegram, M. D. *et al.* Results of two open-label, multicenter phase II studies of docetaxel, platinum salts, and trastuzumab in HER2-positive advanced breast cancer. *J. Natl Cancer Inst.* **96**, 759–769 (2004).
126. Ciardiello, F. *et al.* Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.* **6**, 2053–2063 (2000).
127. Dancey, J. E. Predictive factors for epidermal growth factor receptor inhibitors — the bull's-eye hits the arrow. *Cancer Cell* **5**, 411–415 (2004).
128. Holbro, T. *et al.* The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc. Natl Acad. Sci. USA* **100**, 8933–8938 (2003).
This paper uses elegant technology to demonstrate that ERBB2-overexpressing breast cancer cells use ERBB3 to activate the PI3K pathway. Both downregulation of ERBB3 expression and targeting ERBB2 directly with TKIs block proliferation of tumour cells.
129. Mitsiades, C. S. *et al.* Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. *Cancer Cell* **5**, 221–230 (2004).
130. Ward, S. G. & Finan, P. Isoform-specific phosphoinositide 3-kinase inhibitors as therapeutic agents. *Curr. Opin. Pharmacol.* **3**, 426–434 (2003).
131. Mills, G. B. *et al.* Linking molecular diagnostics to molecular therapeutics: targeting the PI3K pathway in breast cancer. *Semin. Oncol.* **30**, 93–104 (2003).
132. Sebolt-Leopold, J. S. & Herrera, R. Targeting the mitogen-activated protein kinase cascade to treat cancer. *Nature Rev. Cancer* **4**, 937–947 (2004).
133. Dibb, N. J., Dilworth, S. M. & Mol, C. D. Switching on kinases: oncogenic activation of BRAF and the PDGFR family. *Nature Rev. Cancer* **4**, 718–727 (2004).
134. Wood, J. M. *et al.* PTK787/ZK 222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res.* **60**, 2178–2189 (2000).
135. Shaheen, R. M. *et al.* Inhibited growth of colon cancer carcinomatosis by antibodies to vascular endothelial and epidermal growth factor receptors. *Br. J. Cancer* **85**, 584–589 (2001).
136. Traxler, P. *et al.* AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res.* **64**, 4931–4941 (2004).
This paper presents an extensive *in vitro* and *in vivo* analysis of the activity of the multifunction inhibitor AEE788, which targets both ERBB and VEGFRs. A direct comparison with ERBB- and VEGFR-specific TKIs used in combination is performed.
137. Jorissen, R. N. *et al.* Epidermal growth factor receptor: mechanisms of activation and signalling. *Exp. Cell Res.* **284**, 31–53 (2003).

138. Dankort, D., Jeyabalan, N., Jones, N., Dumont, D. J. & Muller, W. J. Multiple ErbB-2/Neu phosphorylation sites mediate transformation through distinct effector proteins. *J. Biol. Chem.* **276**, 38921–38928 (2001).
139. Marone, R. *et al.* Memo mediates ErbB2-driven cell motility. *Nature Cell Biol.* **6**, 515–522 (2004).
140. Kim, H. H., Vijapurkar, U., Hellyer, N. J., Bravo, D. & Koland, J. G. Signal transduction by epidermal growth factor and heregulin via the kinase-deficient ErbB3 protein. *Biochem. J.* **334** (Pt 1), 189–195 (1998).
141. Yen, L. *et al.* Differential regulation of tumor angiogenesis by distinct ErbB homo- and heterodimers. *Mol. Biol. Cell* **13**, 4029–4044 (2002).
142. Baker, C. H. *et al.* Blockade of epidermal growth factor receptor signaling on tumor cells and tumor-associated endothelial cells for therapy of human carcinomas. *Am. J. Pathol.* **161**, 929–938 (2002).
143. Heimberger, A. B. *et al.* Brain tumors in mice are susceptible to blockade of epidermal growth factor receptor (EGFR) with the oral, specific, EGFR-tyrosine kinase inhibitor ZD1839 (iressa). *Clin. Cancer Res.* **8**, 3496–3502 (2002).
144. Shin, I. *et al.* PKB/Akt mediates cell-cycle progression by phosphorylation of $p27^{Kip1}$ at threonine 157 and modulation of its cellular localization. *Nature Med.* **8**, 1145–1152 (2002).
145. Miettinen, P. J. *et al.* Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* **376**, 337–341 (1995).
146. Sibilia, M. & Wagner, E. F. Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* **269**, 234–238 (1995).
147. Threadgill, D. W. *et al.* Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* **269**, 230–234 (1995).
148. Tan, A. R. *et al.* Evaluation of biologic end points and pharmacokinetics in patients with metastatic breast cancer after treatment with erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor. *J. Clin. Oncol.* **22**, 3080–3090 (2004).
149. Cohen, E. E. *et al.* Phase II trial of ZD1839 in recurrent or metastatic squamous cell carcinoma of the head and neck. *J. Clin. Oncol.* **21**, 1980–1987 (2003).
150. Camus, P., Kudoh, S. & Ebina, M. Interstitial lung disease associated with drug therapy. *Br. J. Cancer* **91** (Suppl. 2), S18–S23 (2004).
151. Sumpter, K., Harper-Wynne, C., O'Brien, M. & Congleton, J. Severe acute interstitial pnuemonia and gefitinib. *Lung Cancer* **43**, 367–368 (2004).
152. Suzuki, H., Aoshiba, K., Yokohori, N. & Nagai, A. Epidermal growth factor receptor tyrosine kinase inhibition augments a murine model of pulmonary fibrosis. *Cancer Res.* **63**, 5054–5059 (2003).
153. Lee, K. F. *et al.* Requirement for neuregulin receptor erbB2 in neural and cardiac development. *Nature* **378**, 394–398 (1995).
154. Crone, S. A. *et al.* ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nature Med.* **8**, 459–465 (2002).
155. Slamon, D. J. *et al.* Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* **344**, 783–792 (2001).
156. Gassmann, M. *et al.* Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* **378**, 390–394 (1995).
157. Meyer, D. & Birchmeier, C. Multiple essential functions of neuregulin in development. *Nature* **378**, 386–390 (1995).
158. Fuchs, I. B. *et al.* Analysis of HER2 and HER4 in human myocardium to clarify the cardiotoxicity of trastuzumab (Herceptin). *Breast Cancer Res. Treat.* **82**, 23–28 (2003).
159. Zhao, Y. Y. *et al.* Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. *J. Biol. Chem.* **273**, 10261–10269 (1998).
160. Sato, J. D. *et al.* Biological effects *in vitro* of monoclonal antibodies to human epidermal growth factor receptors. *Mol. Biol. Med.* **1**, 511–529 (1983).
161. Schreiber, A. B., Lax, I., Yarden, Y., Eshhar, Z. & Schlessinger, J. Monoclonal antibodies against receptor for epidermal growth factor induce early and delayed effects of epidermal growth factor. *Proc. Natl Acad. Sci. USA* **78**, 7535–7539 (1981).
162. Goldstein, N. I., Prewett, M., Zuklys, K., Rockwell, P. & Mendelsohn, J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin. Cancer Res.* **1**, 1311–1318 (1995).
163. Carter, P. *et al.* Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl Acad. Sci. USA* **89**, 4285–4289 (1992).
164. Honegger, A. M. *et al.* A mutant epidermal growth factor receptor with defective protein tyrosine kinase is unable to stimulate proto-oncogene expression and DNA synthesis. *Mol. Cell. Biol.* **7**, 4568–4571 (1987).
165. Traxler, P. Tyrosine kinases as targets in cancer therapy-successes and failures. *Expert Opin. Ther. Targets* **7**, 215–234 (2003).
166. Gazit, A., Yaish, P., Gilon, C. & Levitzki, A. Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. *J. Med. Chem.* **32**, 2344–2352 (1989).

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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