Comparing antibody and small-molecule therapies for cancer

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Abstract | The 'magic bullet' concept of specifically targeting cancer cells at the same time as sparing normal tissues is now proven, as several monoclonal antibodies and targeted small-molecule compounds have been approved for cancer treatment. Both antibodies and small-molecule compounds are therefore promising tools for target-protein-based cancer therapy. We discuss and compare the distinctive properties of these two therapeutic strategies so as to provide a better view for the development of new drugs and the future direction of cancer therapy.

The term 'magic bullet', coined by bacteriologist Paul Ehrlich in the late 1800s, originally described a chemical with the ability to specifically target microorganisms. His concept (specific targeting) was expanded thereafter to include cancer treatments, and has been successfully applied to the development of innovative cancer-treatment strategies with different, more specific mechanisms of action than conventional chemotherapeutic agents[1]. Such molecular targeting techniques[2] include monoclonal antibodies (mAbs), small molecules, peptide mimetics and antisense oligonucleotides. With the advances in understanding of aberrant signalling pathways in various types of cancer cells, many pivotal regulators of malignant behaviour in cancer cells have emerged as candidates for molecular target-based cancer therapy. Such strategies have improved the management of cancers[3]. A crucial challenge in the development of targeted agents is to choose an appropriate approach. The two main approaches discussed here are therapeutic mAbs and small-molecule inhibitors (TABLE 1).

Key signalling molecules, such as protein tyrosine kinases, have proven to be good targets for small-molecule inhibitors that compete with ATP and inhibite kinase activity[4]. Such inhibitors have clinically effective responses in chronic myeloid leukaemia (CML), gastrointestinal stromal tumours (GISTs)[5] and non-small-cell lung cancer (NSCLC)[6]. Another group of targets is represented by tumour-selective cell-surface proteins, which can be recognized by antibodies. The therapeutic application of mAbs has improved response rates in patients with malignant lymphomas and is currently being assessed in other tumour types[7].

Many small-molecule agents and mAbs that target growth-factor receptors and their signalling pathways have been developed and subjected to clinical trials. Some molecules are targeted by both types of inhibitors, including members of the ErbB family of receptor tyrosine kinases (RTKs). The ErbB family comprises four members: epidermal growth factor receptor (EGFR, also known as ERBB1), ERBB2 (also known as HER2), ERBB3 and ERBB4 (REFS 8,9). Both gene amplification and overexpression of EGFR and ERBB2 are frequently observed in breast, lung and colorectal cancers, and the deregulated activation of intracellular mitogenic signalling by the ErbB family has been implicated in various cancers[9]. Therefore, these receptors have been a focus of molecular-targeting therapy[10]. To compare mAbs and small-molecule inhibitors, this Review will highlight EGFR-targeted agents that have shown clinical success.

Accumulating clinical-trial results are showing that monotherapy with a target-specific agent might need to be reassessed. Most tumours, particularly solid tumours, are multifactorial and are frequently linked to defects in more than one signalling pathway[3]. Therefore, a dual-targeting or multi-targeting therapy might be more rational, not only to efficiently eliminate cancer cells, but also to limit the emergence of drug resistance. Which class of targeted agent will provide the best solution to this problem? Considering the differences in specificity or selectivity between mAbs and small-molecule inhibitors might lead to the further improvement of targeting strategies for cancer therapy.

In this Review we will describe the development of mAbs and small-molecule inhibitors, and then compare and contrast these two strategies using EGFR-targeted agents.

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At a glance

- The concept of specific molecular targeting has been applied to the development of innovative cancer-treatment strategies. At present, two main approaches are available for use in clinical practice: therapeutic monoclonal antibodies (mAbs) and small-molecule agents.
- We focus on the ErbB receptor family, particularly epidermal growth factor receptor (EGFR, also known as ERBB1) as an example of a target in our comparison of mAbs and small-molecule inhibitors. Cetuximab, a mAb, and gefitinib and erlotinib, which are small-molecule inhibitors, differ markedly in their basic properties and their underlying mechanisms of action.
- The presence of activating mutations within the ATP-binding cleft of the EGFR kinase domain is associated with the sensitivity of non-small-cell lung cancer (NSCLC) to gefitinib, but not to cetuximab. By contrast, cetuximab shows a clinical benefit for colorectal cancers that overexpress EGFR in a manner independent of EGFR mutations. In malignant glioma, the sensitivity to gefitinib is closely related to deletions within the ectodomain of EGFR. In contrast to these drug-sensitivity mutations, the appearance of the T790M mutation confers resistance to gefitinib in NSCLC.
- There are unique immune-effector mechanisms that are only triggered by therapeutic mAbs, such as antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and complement-dependent cell-mediated cytotoxicity. By contrast, the effects of small-molecule agents are not directly linked to the activation of an immune response against tumour cells.
- In general, mild adverse effects such as dermatological complications are commonly observed with these two classes of EGFR inhibitors. Although interstitial lung diseases or diarrhoea are more commonly associated with small-molecule therapies, therapeutic murine mAbs or chimeric mAbs can cause immunogenicity, leading to the production of human anti-mouse antibodies or human antichimeric antibodies, respectively.
- It has been shown that mAbs such as trastuzumab and cetuximab exert synergistic anti-tumour effects in combination with chemotherapeutic agents more frequently than small-molecule inhibitors.
- The combination of distinct classes of EGFR inhibitors could not only increase their efficacy, but also contribute to overcoming resistance to one class of EGFR inhibitor.
- Further investigation into the distinct properties of these two classes of targeted agents should not only contribute to the development of new targeted agents but also provide an optimal therapeutic strategy for cancer treatment, thereby leading to the improvement of dual-targeted or multi-targeted therapy.

Monoclonal antibodies for cancer therapy

The 'magic bullet' concept became a reality a quarter of a century after the discovery of somatic cell hybridization, a technique for generating mAbs pioneered by Milstein and Köhler in 1975 (REF. 11). Early clinical trials with murine mAbs failed owing to their short half-life, xenogenicity and limited activity[12]. During this intervening period, the application of genetic recombination for humanizing rodent mAbs[7] made large-scale production feasible, and enabled mAbs to be designed with better affinities, efficient selection, decreased immunogenicity and optimized effector functions. Furthermore, proteomics and genomics combined with bacteriophage display enabled the rapid selection of high-affinity mAbs. Genetic engineering has made it possible to design chimeric mouse–human mAbs, among which the anti-CD20 mAb rituximab (Rituxan) has revolutionized lymphoma treatment[13] (TABLE 1 and FIG. 1). A humanized mAb has provided new prospects for the treatment of breast cancer. Trastuzumab (Herceptin) is the first clinically approved mAb against an ErbB family member (ERBB2)[14] (TABLE 1 and FIG. 1). It has excellent anti-tumour activity, particularly when combined with the cytotoxic agents doxorubicin and paclitaxel[15].

Trastuzumab is approved for the treatment of patients with metastatic breast cancer who carry an increased *ERBB2* copy number. Another anti-ERBB2 mAb, pertuzumab (Omnitarg), is also under evaluation in phase II trials[16]. Unlike trastuzumab, which affects ERBB2 shedding[17], pertuzumab sterically interferes with ERBB2 homo- and heterodimerization and subsequent signalling events[18]. On the other hand, trastuzumab cannot prevent the formation of ligand-induced ERBB2-containing heterodimers[16]. So, pertuzumab is effective against trastuzumab-insensitive tumours that do not have *ERBB2* amplification[18,19]. Therefore, pertuzumab might be effective over a broad range of cancers with either normal or increased ERBB2 levels.

In parallel with the development of trastuzumab, our group also developed CH401, a mouse–human chimeric mAb directed against ERBB2[20], by a unique procedure that used a mouse-mutant hybridoma with no mouse immunoglobulin (Ig) heavy chains and a human Ig expression vector. CH401 has been evaluated in a preclinical study, and it significantly reduced the *in vivo* growth of various ERBB2-expressing tumour cells[21,22]. Of note, CH401 has shown an apoptosis-inducing effect, presumably through the activation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)[21,22]. Our results showed that it is significantly more effective than trastuzumab[23].

These ERBB2-targeted therapeutic mAbs have used three distinct strategies for signal blockade including interference with ligand interactions and receptor downregulation (trastuzumab), inhibition of receptor dimerization (pertuzumab), and induction of apoptosis (CH401).

EGFR is also overexpressed in various cancers, including colon and breast, and mAbs directed against EGFR have also been developed[24]. Cetuximab (also known as

Bacteriophage display
A display method for identifying proteins or peptides that recognize and bind to a target molecule(s). Bacteriophages that display the antibody of interest are selected by antigen binding and are propagated in bacteria. This helps identify therapeutic antibodies with high binding affinity.

Shedding
The release of the extracellular domain of a cell-membrane protein, such as a growth-factor receptor, from the cell surface. ERBB2 is proteolytically cleaved, possibly by a matrix metalloproteinase activator, although this proteolysis does not seem to be mediated by a general shedding system that can be activated by protein kinase C. ERBB2 cleavage generates a membrane-associated receptor fragment with potentially increased tyrosine kinase activity.

Table 1 | **Two classes of FDA-approved targeted agents and the spectrum of targeted cancers**

		Targeted cancer												
		Solid tumours							Haematological tumours					
Agent	**Target for agent**	**NSCLC**	**Breast cancer**	**CRC**	**GIST**	**Renal cancer**	**Pancreatic cancer**	**HNSCC**	**AML**	**B-cell CLL**	**CML**	**B-cell lymphoma**	**Multiple myeloma**
mAbs													
Cetuximab (Erbitux)	EGFR			✓‡				✓§					
Trastuzumab (Herceptin)¶	ERBB2		✓										
Bevacizumab (Avastin)#	VEGF			✓									
Rituximab (Rituxan)**	CD20											✓	
Ibritumomab tiuxetan (Zevalin)*	CD20											✓	
Tositumomab-I^{131} (Bexxar)*	CD20											✓	
Gemtuzumab ozogamicin (Mylotarg)‡‡	CD33								✓				
Alemtuzumab (Campath)	CD52									✓			
Small-molecule inhibitors													
Imatinib mesylate (Glivec)	TKs (BCR-ABL, KIT, PDGFR)				✓						✓		
Gefitinib (Iressa)	TK (EGFR)	✓											
Erlotinib (Tarceva)	TK (EGFR)	✓					✓§§						
Sunitinib (Sutent)	TKs (VEGFR, PDGFR, KIT, FLT3)				✓	✓							
Sorafenib (Nexavar)	Kinases (B-Raf, VEGFR2, EGFR, PDGFR)					✓							
Bortezomib (Velcade)	28S protease												✓

Agents are shown as generic names with trade names in parentheses. The table lists cancers to which each targeted agent is approved. *Radiolabelled with Yttrium^{90} or Iodine^{131}. ‡In combination with irinotecan or administered as a single agent. §In combination with radiation therapy or administered as a single agent. ¶In combination with paclitaxel or administered as a single agent. #In combination with 5-fluorouracil-based chemotherapy. ** In combination with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) or other anthracycline-based chemotherapy regimens. ‡‡This mAb is linked to *N*-acetyl-γ calicheamicin, a bacterial toxin. After internalization of the mAb, the released toxin binds to DNA and causes double-strand DNA breaks. §§In combination with gemcitabine. AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FLT3, Fms-like tyrosine kinase 3; GIST, gastrointestinal stromal tumour; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; HNSCC, head and neck squamous-cell carcinoma; TK, tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

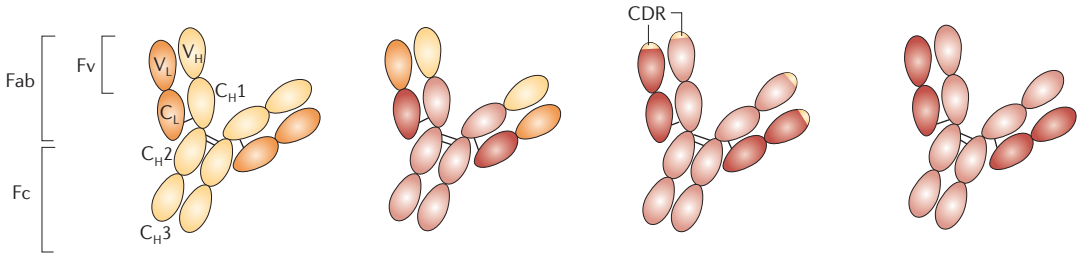
C225; Erbitux) is a chimeric IgG1-isotype mAb that binds to EGFR with high affinity and abrogates ligand-induced EGFR phosphorylation[25,26]. In addition, panitumumab (ABX-EGF) was developed as a fully human IgG2-isotype mAb against EGFR, and a recent randomized phase III trial has shown that panitumumab monotherapy improved the progression-free survival of patients with previously treated metastatic colorectal cancer[27].

Putative mechanisms of mAb-based cancer therapy can be classified into two categories. The first is direct action, which can be further subcategorized into three modes of action. One mode of action is blocking the function of target signalling molecules or receptors. This can occur by blocking ligand binding, inhibiting cell-cycle progression or DNA repair[28], inducing the regression of angiogenesis[29], increasing the internalization of receptors[30,31] or reducing proteolytic cleavage of receptors[17]. Other modes of direct action are stimulating function, which induces apoptosis, and targeting function. In the case of targeting function, mAbs can be conjugated with toxins, radioisotopes, cytokines, DNA molecules or even small-molecule agents[7,32,33] to selectively target tumour cells (TABLE 1 and FIG. 1). The second mechanism of mAb therapy is indirect action mediated by the immune system. The elimination of tumour cells using mAbs depends on Ig-mediated mechanisms, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC), to activate immune-effector cells (FIG. 2).

Complement-dependent cytotoxicity
This is one of the antigen-elimination processes that is mediated by immunoglobulins (Ig). When IgM and certain IgG subclasses (IgG1 and IgG3) bind to an antigen, one of the complement factors is strongly activated. Then, a sequence of cleavage reactions of other complement factors (classical pathway of complement activation) is triggered to activate their cytotoxic function, which leads to the destruction of the target cells.

Small-molecule agents for cancer therapy

RTKs and non-RTKs are crucial mediators in signalling pathways of cell proliferation, differentiation, migration, angiogenesis, cell-cycle regulation and others[4,34,35], and many are deregulated during tumorigenesis. Small-molecule inhibitors target these kinases by direct effects on tumour cells, rather than by causing immune responses as mAbs do. Most small-molecule inhibitors of tyrosine kinases are ATP mimetics. Imatinib mesylate (Glivec), one of the first successful small-molecule inhibitors, inactivates the kinase activity of the BCR–ABL fusion protein in CML[36,37] (TABLE 1). It has shown



Type of mAb	Murine	Chimeric	Humanized	Human
	Ibritumomab tiuxetan (CD20); IgG1κ*	Cetuximab (EGFR); IgG1κ	Trastuzumab (ERBB2); IgG1κ	Panitumumab (EGFR); IgG2
	Tositumomab-I^{131} (CD20); IgG2aλ*	Rituximab (CD20); IgG1κ	Bevacizumab (VEGF); IgG1	
			Alemtuzumab (CD52); IgG1κ	
			Gemtuzumab ozogamicin (CD33); IgG4κ*	

Figure 1 | **The classification of therapeutic monoclonal antibodies (mAbs) by the different antibody types — murine, chimeric, humanized and human.** Advances in genetic engineering techniques have contributed to the development of humanized therapeutic mAbs. The fundamental structure of an intact, single immunoglobulin G (IgG) molecule has a pair of light chains (orange/red) and a pair of heavy chains (yellow/pink). Light chains are composed of two separate regions (one variable region (V_L) and one constant region (C_L)), whereas heavy chains are composed of four regions (V_H, C_H1, C_H2 and C_H3). The complementarity-determining regions (CDRs) are found in the variable fragment (Fv) portion of the antigen-binding fragment (Fab). Chimeric mAbs such as cetuximab and rituximab are constructed with variable regions (V_L and V_H) derived from a murine source and constant regions derived from a human source. Humanized therapeutic mAbs are predominantly derived from a human source except for the CDRs, which are murine. There are currently four approved humanized mAbs. Both murine and human mAbs are entirely derived from mouse and human sources, respectively. Panitumumab (ABX–EGF) is a fully human anti-epidermal growth factor receptor (EGFR) mAb, but has not yet been approved. Furthermore, several mAbs (marked with an asterisk) are armed with cytotoxins including radionucleotides or a bacterial toxin (see text for further details). There is a significant difference between the IgG subclasses in terms of their half-lives in the blood (IgG1, IgG2 and IgG4 approximately 21 days; IgG3 approximately 7 days) and in terms of their capability to activate the classical complement pathway and to bind Fcγ-receptors (see the legend of FIG. 2). The choice of an IgG subclass is a key factor in determining the efficacy of therapeutic mAbs. Most of the approved mAbs shown here belong to the IgG1 subclass, which has a long half-life and triggers potent immune-effector functions such as complement-dependent cytotoxicity (CDC), complement-dependent cell-mediated cytotoxicity (CDCC) and antibody-dependent cellular cytotoxicity (ADCC). On the other hand, panitumumab is an IgG2 subclass that does not show potent CDC and ADCC, but it has recently shown its efficacy in a phase III trial as a monotherapy for the treatment of metastatic colorectal cancer. VEGF, vascular endothelial growth factor.

remarkable efficacy for the treatment of patients with Philadelphia chromosome-positive CML[38]. It is also a multi-targeted inhibitor of other tyrosine kinases, including KIT, which is key to the pathogenesis of metastatic GISTs, and the platelet-derived growth factor receptors PDGFRα and PDGFRβ, which are key to the pathogenesis of PDGF-driven tumours such as glioblastoma and dermatofibrosarcoma protuberans[39].

EGFR is also a rational target for small-molecule inhibitors[40]. Gefitinib (Iressa)[6] and erlotinib (Tarceva)[41] selectively inhibit EGFR, and both are efficacious against EGFR-expressing cancers such as NSCLC and head and neck squamous-cell carcinoma (HNSCC) (TABLE 1). Phase II studies of these agents have also shown their efficacy with or without concurrent chemotherapy in HNSCC, and several phase III trials of gefitinib are ongoing[42]. Erlotinib in combination with an anti-metabolite, gemcitabine, is also approved for treating advanced pancreatic cancer.

Unlike mAbs, small-molecule agents can translocate through plasma membranes and interact with the cytoplasmic domain of cell-surface receptors and intracellular signalling molecules. Therefore, various small-molecule inhibitors have been generated to target cancer-cell proliferation and survival by inhibiting Ras prenylation[43], Raf–MEK kinase[44], phosphatidylinositol 3-kinase (PI3K), the mammalian target of rapamycin (mTOR) pathway or heat shock protein 90 (HSP90) (REF. 45); cancer-cell adhesion and invasion by inhibiting SRC kinase[46] or matrix metalloproteinases (MMPs)[47]; or neovascularization by inhibiting the vascular endothelial growth factor RTK (VEGFR).

As a new type of small-molecule agent, sorafenib (Nexavar) is known to exert its inhibitory effect on not only different isoforms of Raf serine kinase but also various RTKs such as VEGFR, EGFR and PDGFR[34]. This dual-action kinase inihibitor shows broad-spectrum anti-tumour activity by inhibiting tumour proliferation and angiogenesis[48]. Another new anti-angiogenesis small-molecule drug, sunitinib malate (Sutent), is also a multi-targeted tyrosine kinase inhibitor of VEGFR, PDGFR, KIT and Fms-like tyrosine kinase 3 (FLT3)[48]. Potential targets for the development of small-molecule agents have also been identified in the ubiquitin–proteasome

Antibody-dependent cellular cytotoxicity
This reaction can be initiated by the Fc portion of immunoglobulins (Ig). Phagocytes such as monocytes/macrophages, dendritic cells, natural killer cells and neutrophils take up IgG-coated target cells through binding with Fcγ-receptors on the surface of the phagocytes. This is eventually followed by the elimination of target cells.

ATP mimetics
These small-molecule inhibitors competitively bind to the ATP-binding cleft at the activation loop of target kinases, thereby inhibiting their kinase activity.

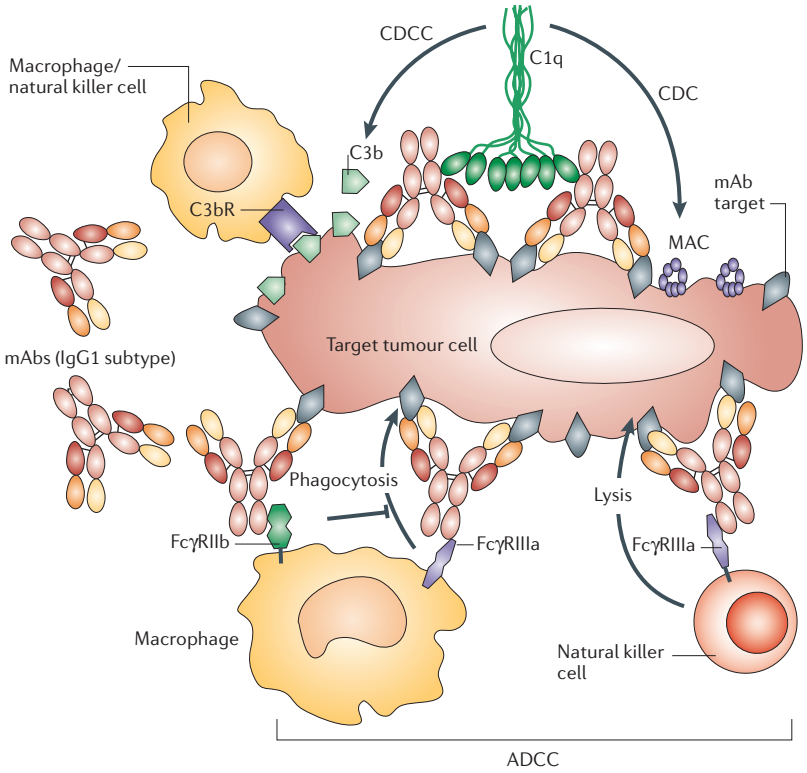


Figure 2 | **Schematic model of antibody action by immune mechanisms.** Following the binding of monoclonal antibodies (mAbs) to a specific target on a tumour cell, C1q complement factor interacts with the C_H2 constant region of the mAb, which leads to the activation of a proteolytic cascade of the complement classical pathway and consequently induces the formation of a membrane-attack complex (MAC) for the lysis of tumour cells; this effect is termed complement-dependent cytotoxicity (CDC). C3b, which is generated during this cascade reaction, functions as an opsonin to facilitate phagocytosis and cytolysis through its interaction with the C3b receptor (C3bR) on a macrophage or natural killer (NK) cell[118]; this activity is termed complement-dependent cell-mediated cytotoxicity (CDCC). In addition, mAb-binding to tumour cells induces antibody-dependent cellular cytotoxicity (ADCC); immune-effector cells such as macrophages and NK cells are recruited and interact with the C_H3 region of the mAbs through FcγRIIIa expressed by both effector cells. Then, mAb-coated tumour cells are phagocytosed by macrophages or undergo cytolysis by NK cells. On the other hand, there is a negative regulation to modulate the cytotoxic response against tumours through FcγRIIb, which is expressed on the cell surface of macrophages. Immunoglobulin G1 (IgG1) and IgG3 can activate the classical complement pathway and interact with Fcγ receptors more potently than IgG2 or IgG4. In particular, IgG4 cannot activate the classical complement pathway.

pathway, which is crucial in processes including cell-cycle arrest and apoptosis. Bortezomib (Velcade), which was first developed as a selective, reversible inhibitor of the chymotryptic protease in the 26S proteasome, has been reported to be effective against various cancers, particularly haematological malignancies (TABLE 1).

Chymotryptic protease in the 26S proteasome
The 26S proteasome is a multicatalytic complex, which is composed of the 20S catalytic core subunit and the 19S regulatory subunit that recognize and degrade ubiquitylated proteins. A chymotrypsin-like proteolytic activity is one of the catalytic activities of this core subunit for the hydrolysis of peptide substrates.

Comparison between mAbs and small-molecules

Many preclinical and clinical studies have indicated that targeting EGFR could represent a significant contribution to cancer therapy. Because both mAb and small-molecule EGFR inhibitors have been approved as cancer therapies, we will use them as our primary example to compare mAbs and small-molecule inhibitors. There is no clear difference in the spectrum of cancers targeted by the one mAb and the two small-molecule inhibitors that are approved by the US Food and Drug Administration (FDA) and specifically target EGFR (TABLE 1). Further comparison between these two classes of targeted agents will be discussed below.

Basic drug properties and development. The timelines for the development of mAbs versus small-molecule inhibitors seem to differ. Following the establishment of mouse hybridoma technology, the mAb approach was first applied to block EGFR-mediated signalling for cancer treatment in the early 1980s. About 10 years behind this, the potential of EGFR-targeted therapy contributed to the development of small-molecule EGFR tyrosine kinase inhibitors (TKIs)[6].

Although therapeutic mAb development requires relatively complex processes with huge monetary costs compared with small-molecule inhibitors, many biotech and pharmaceutical firms are vying to develop therapeutic mAbs after the advent of humanization techniques and human antibodies[49]. Furthermore, chimeric and humanized mAbs, which have been the predominant mAbs entering clinical studies, have higher approval success rates (18% and 24%, respectively)[50] than new chemical entities (NCEs) including small-molecule agents (5%)[51], especially in the field of oncology[50]. On the other hand, small-molecule agents are less expensive and more convenient to administer than mAbs.

mAbs and small-molecule inhibitors differ in several pharmacological properties. Anti-EGFR mAbs are large proteins (around 150 kDa) and are generally intravenously administered, whereas EGFR TKIs are orally available, synthetic chemicals (approximately 500 Da). The large molecular weight of mAbs is probably the cause of their inefficient delivery into brain tissues because of the blood–brain barrier, so therapeutic mAbs for brain cancer are usually delivered intra-tumorally[52]. In addition, we speculate that owing to the difference in molecular size, intact Igs such as IgG subclasses might be less efficient for tissue penetration, tumour retention and blood clearance than small-molecule agents. In fact, there are marked differences between these two classes of agents in several pharmacokinetic properties. According to FDA labelling, the mAb half-lives (that is, cetuximab: 3.1–7.8 days, allowing for once-weekly dosing) are much longer than those of small-molecule agents (that is, gefitinib, approximately 48 hrs; erlotinib, approximately 36 hrs; allowing for once-daily dosing). Also, pharmacokinetic studies showed that plasma concentrations of small-molecule agents can vary at a given dose between patients[53]. This might be explained by the oral administration of small-molecule agents versus the intravenous administration of mAbs. Furthermore, it might also be speculated that the degradation system for small-molecule agents (chemicals) might vary more in individuals than that for mAbs (proteins).

Because of their inability to pass through the cellular membrane, mAbs can only act on molecules that are expressed on the cell surface or secreted[54]. Bevacizumab

(Avastin) is the main mAb agent to have been developed against the secreted pro-angiogenic protein VEGF, and it improves survival when combined with 5-fluorourocil (5-FU)-based chemotherapy in patients with metastatic colorectal cancer (TABLE 1 and FIG. 1). However, small-molecule inhibitors can pass into the cytoplasm, and can therefore be developed to target any molecules regardless of their cellular location[53]. So, mAbs possess biological activities that are not shared by small-molecule inhibitors, and *vice versa*.

Typically, the advantage of therapeutic mAbs in cancer treatment is thought to depend on their capability to bind antigens expressed on the tumour-cell surface with a highly specific selectivity. The antigen-binding affinity of an antibody is also associated with its biological potency[54]. Therefore, it is presumed that mAbs might

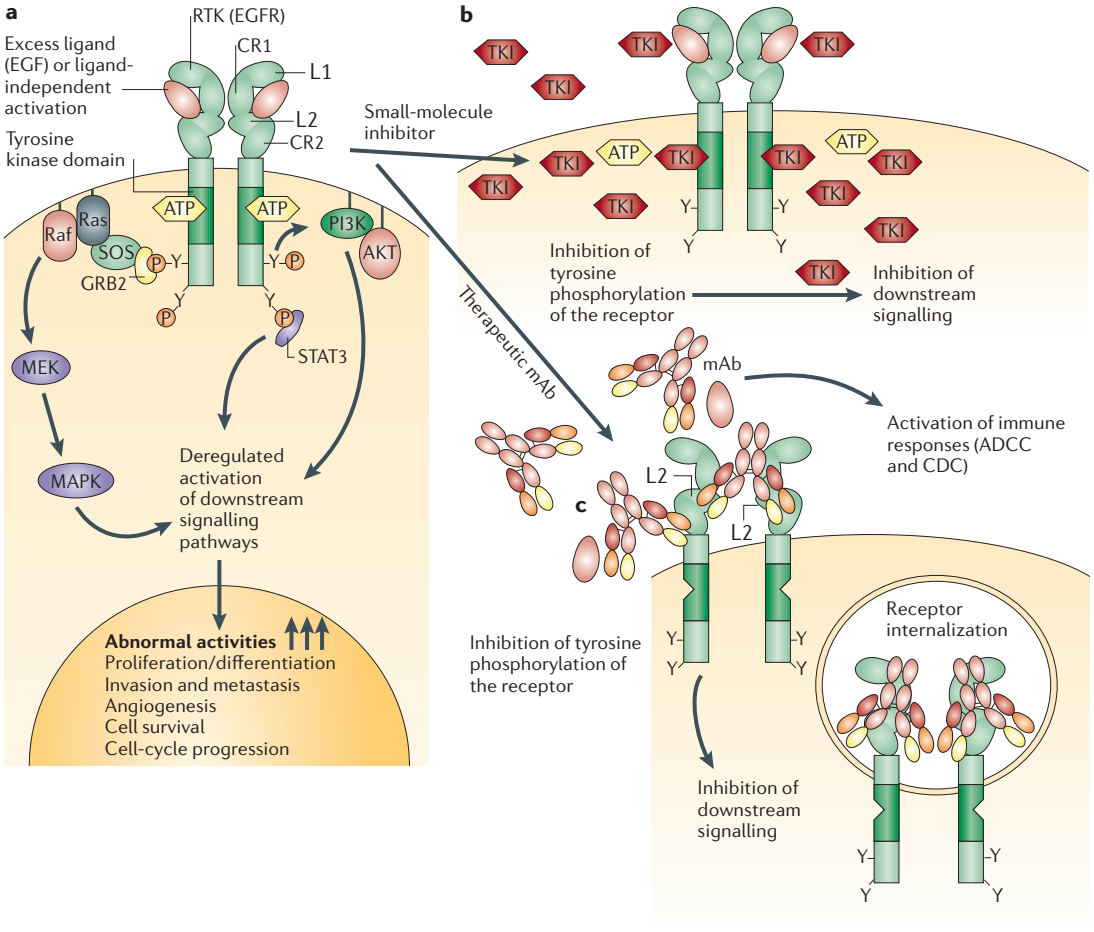


Figure 3 | **Distinct mechanisms of small-molecule inhibitors and monoclonal antibodies for targeting receptor tyrosine kinases in cancer cells. a** | Epidermal growth factor receptor (EGFR) and receptor tyrosine kinase (RTK)-dependent growth signalling in cancer cells. The extracellular region of EGFR consists of four domains, the ligand-binding domains (L1 and L2) and the cysteine-rich domains (CR1 and CR2), and the C-terminal domain of EGFR contains six tyrosine residues (Y; only two are depicted here for simplicity). Following the activation of EGFR by ligand binding or ligand-independent dimerization, the Ras–Raf–MEK–MAPK pathway is activated through the growth factor receptor-bound protein 2 (GRB2)–SOS complex. EGFR-mediated signalling also activates the phosphatidylinositol 3-kinase (PI3K)–AKT pathway, which contributes to anti-apoptotic effects of EGFR activation. Additionally, signal transducer and activator of transcription (Stat) proteins (STAT1, STAT3 and STAT5) are also activated. The coordinated effects of these EGFR downstream signalling pathways lead to the induction of cellular responses including proliferation, differentiation, cell motility, adhesion and angiogenesis. The deregulation of EGFR-mediated signalling in some cancer cells leads to aberrant proliferation, invasion, metastasis and neovascularization[9]. **b** | Small-molecule tyrosine kinase inhibitors (TKIs) such as gefitinib function as ATP analogues and inhibit EGFR signalling by competing with ATP binding within the catalytic kinase domain of RTKs. As a result, the activation of various downstream signalling pathways is blocked. Each TKI has a different selectivity for RTKs, and some are dual- or multi-selective, which might provide a therapeutic advantage. **c** | By contrast, therapeutic monoclonal antibodies (mAbs) bind to the ectodomain of the RTK with high specificity (for example, cetuximab binds to the L2 domain of EGFR, and thereby inhibits its downstream signalling by triggering receptor internalization and hindering ligand–receptor interaction. Unlike small-molecule inhibitors, mAbs also activate Fcγ-receptor-dependent phagocytosis or cytolysis by immune-effector cells such as neutrophils, macrophages and natural killer cells by inducing complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)[107]. MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase.

Complement-dependent cellular cytotoxicity
This is a cell-mediated effector mechanism for target cell killing. As similarly observed in CDC, complement activation is triggered in CDCC by the interaction of C1 q to the Fc regions of antibodies bound to target antigens. During this process, several complement components, such as C3b, are generated and recognized by effector immune cells through their complementary receptors, which leads to phagocytosis and cytotoxicity.

Opsonins
Opsonins are any molecules with which antigens are coated, such as IgG and components of complement factors (C1 q, C3b, iC3b, and C4b), to become more susceptible to phagocytosis by macrophages or neutrophils.These phagocytes bind opsonin molecules through Fcγ receptors or complement receptors that are expressed on their surface membrane.

be more effective against circulating cancer cells than against solid tumours, possibly because of their poor ability to penetrate into tissues and tumours, although there might be other contributing factors such as the availability of effector cells. This might be partly linked to the high approval rates and marketing successes of both armed and unarmed mAbs for haematological malignancies (TABLE 1). However, three mAbs have been approved by the FDA for the treatment of solid tumours. Most of the FDA-approved small-molecule agents are more frequently used for the treatment of solid tumours, whereas only two small-molecule agents are indicated for use against haematological tumours.

Anti-EGFR mAbs and EGFR TKIs target distinct domains of EGFR, the extracellular ligand-binding domain and intracellular tyrosine kinase domain of the receptor, respectively (FIG. 3). Following interaction with the receptor, the small-molecule TKIs gefitinib and erlotinib specifically inhibit EGFR phosphorylation and downstream signalling pathways. By contrast, recent structural analysis by Li *et al.* showed that the interaction of the mAb cetuximab with EGFR results in the partial occlusion of the ligand-binding region (L2) and steric hindrance preventing the receptor from adopting the extended conformation required for dimerization[55]. In another example, trastuzumab, the mAb directed against ERBB2, distinctively binds to the juxtamembrane domain (CR2) of ERBB2, eventually leading to the inhibition of downstream signalling[56].

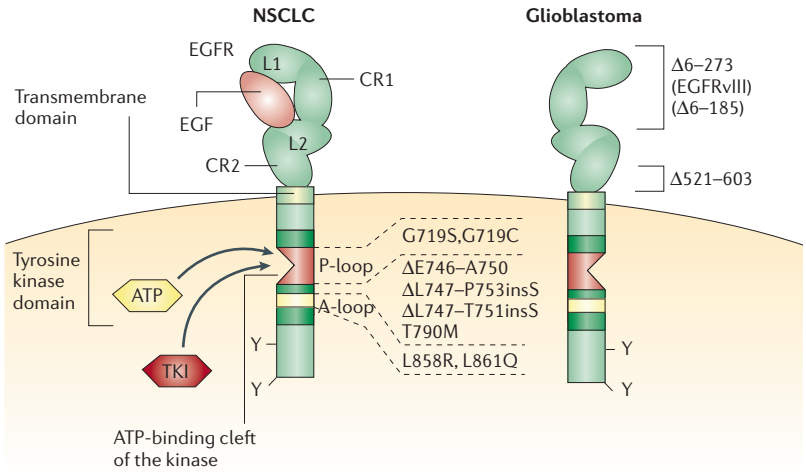


Figure 4 | **EGFR mutations correlated with clinical response to EGFR inhibitors.** Two types of EGFR (epidermal growth factor receptor) mutations have been reported so far in relation to the sensitivity and resistance to gefitinib of non-small-cell lung cancer (NSCLC; left)[75,76,86], both of which occur in the ATP-binding cleft. First, missense mutations that are detected within the nucleotide triphosphate binding domain (P-loop, exon 18; red) of the tyrosine kinase (G719S and G719C); or within the activating loop (A-loop, exon 21; yellow) (L858R and L861Q). Second, in-frame deletions with or without the insertion of a serine residue (exon 19), which are clustered in the region between codon 746–759; for example, ΔE746–A750, ΔL747–T751insS, ΔL747–P753insS. Mutations clustered within the ATP-binding cleft would be predicted to stabilize the interaction of ATP or an inhibitor molecule with this pocket, consequently leading to the more intense and sustained activation or inhibition of EGFR than that of the wild-type receptor. However, a recent report[163] has shown that such mutations of EGFR do not affect the binding affinity of gefitinib or erlotinib to the ATP-binding pocket of the receptor, which contrasts with other activating catalytic domain mutations that have a profound effect on the interaction with imatinib mesylate, another small-molecule inhibitor. On the other hand, a resistance-related mutation, T790M, was also found within the ATP-binding cleft of the EGFR kinase domain. This mutation leads to steric hindrance to the accessibility of an inhibitor into the cleft due to the bulkiness of the methionine side chain. Unlike NSCLC, glioblastomas (right) do not frequently have mutations in the EGFR kinase domain but rather in the extracellular domain of EGFR[87]. A recent study showed that in glioblastomas, EGFRvIII, a constitutively active genomic-deletion variant of EGFR (Δ6–273), preferentially activates the phosphatidylinositol 3-kinase (PI3K)– AKT pathway and, in tumours with intact PTEN expression, confers sensitivity to EGFR kinase inhibitors[88]. Other EGFR mutations reported in glioblastomas include the deletion of exons 14–15, which leads to the expression of a short-form mutant partly lacking the CR2 domain (Δ521–603)[87]. However, the functional role of this mutant form remains unknown. CR1, cysteine-rich domain 1; L1, ligand-binding domain 1; TKI, tyrosine kinase inhibitor.

Specificity. Small-molecule inhibitors are generally thought to be less specific than therapeutic mAbs[57]. However, this lower specificity is potentially advantageous, albeit with some risk of increased toxicity, in that it confers the ability to inhibit several signalling pathways at plasma concentrations that are clinically possible[58]. In particular, small-molecule EGFR TKIs show varying degrees of cross-reactivity for the ErbB family members, which might account for their potent anti-tumour effects when used in combination with a more selective mAb against EGFR[57]. Supporting this, Huang *et al.*[57] showed significant tumour regression following treatment with cetuximab plus gefitinib or erlotinib in a xenograft model with a human NSCLC cell line. Both combinations reduced tumour volume by approximately 75%, whereas monotherapy with cetuximab or the EGFR TKIs reduced tumour volume by approximately 50% or 20%. Similarly, another study by Matar *et al.*[59] with an epithelial carcinoma cell line showed that combination treatment increased the inhibition of cell and tumour xenograft growth, possibly through shared and complementary mechanisms of action with gefitinib and cetuximab.

Although gefitinib is relatively mono-selective, with a 200-fold greater affinity for EGFR than for ERBB2[34,60], several multi-selective EGFR inhibitors have been developed. Canertinib (CI-1033)[61] is a multi-selective EGFR inhibitor that rapidly and irreversibly inhibits all ErbB family members. Another multi-selective EGFR inhibitor is lapatinib (GW-572016)[62], which reversibly and specifically inhibits both EGFR and ERBB2. A phase III study in patients with advanced trastuzumab-resistant breast cancer indicated that lapatinib might offer significant benefits in combination with capecitabine. The median progression-free survival was twice as long (36.9 weeks) with combination therapy than with capecitabine monotherapy[63]. Based on the acceptable tolerability and efficacy of this combination therapy, a Biologics License Application (BLA) submission is currently pending[64]. The efficacy of lapatinib has also been reported in advanced renal cancer (phase III study)[65] and HNSCC (phase I study)[66]. The cooperative inhibitory effects of multi-targeting might enable broader anti-tumour activity and improve efficacy. In addition, it might follow that the development of resistance is less likely. On the other hand, no therapeutic mAbs with such cross-reactivity have yet been reported.

Sensitivity and resistance mechanisms. An important issue remains whether a relationship exists between EGFR expression and clinical outcome with EGFR-targeted agents. Several preclinical studies with cetuximab and gefitinib showed that both were potent in human cancer cells with highly variable EGFR levels[67–69]. In a retrospective evaluation, there was no significant association between EGFR expression and clinical response to gefitinib in NSCLC[70]. In addition, the results of a randomized, placebo-controlled phase III study in patients with advanced NSCLC showed that EGFR expression did not predict survival benefit with erlotinib[71]. Several factors other than the level of EGFR expression have therefore been shown to be involved in predicting the clinical response to EGFR-targeted therapeutics[72–74]. Certain subsets of patients also seem to be refractory to EGFR-inhibitor treatment despite high levels of EGFR expression in their tumours. Furthermore, cancer cells often acquire resistance to EGFR inhibitors, but different mechanisms seem to underlie sensitivity to mAbs and EGFR TKIs.

Recent clinical studies have shown that mutations in EGFR significantly affect, with a positive or negative correlation, clinical responses to small-molecule TKIs in patients with NSCLC[75,76]. Highly responsive NSCLC contains somatic mutations of EGFR, including small deletions (amino acids 747–750) or point mutations (most commonly a L858R replacement)[75–82] (FIG. 4). These mutations seem to result in the repositioning of crucial residues that surround the ATP-binding cleft of the EGFR tyrosine kinase domain, thereby stabilizing the interactions of the inhibitor with the kinase domain[75]. Therefore, these mutation types increase the sensitivity of tumour cells to gefitinib; the autophosphorylation of mutant EGFR is inhibited at gefitinib concentrations 10–100-fold lower than those necessary to inhibit wild-type EGFR[76]. Furthermore, NSCLC cells with the L858R mutation undergo apoptosis following gefitinib treatment, whereas cells that contain wild-type EGFR undergo cell-cycle arrest[83]. In addition, more recent reports have indicated that other factors have a role in determining responsiveness to gefitinib in patients with NSCLC, including amplifications of *EGFR* and *ERBB2* (REFS 84,85), as the ERBB2 status (determined by the use of fluorescence *in situ* hybridization (FISH)) is a validated marker for the clinical benefit of trastuzumab for breast cancer[16].

Despite the positive correlation between EGFR mutations and sensitivity to TKIs, it seems that most patients with NSCLC who are treated with these compounds develop resistance, in part because of additional EGFR mutations, particularly the T790M mutation, which leads to the steric hindrance of gefitinib or erlotinib binding due to the presence of the bulkier methionine in the catalytic cleft[86] (FIG. 4). By contrast, malignant glioma frequently shows deletions within the extracellular domain of EGFR but infrequent mutations in the kinase domain. The presence of these deletions might increase the sensitivity of gliomas to gefitinib therapy[87], wherein the co-expression of EGFR deletion mutant variant III and the tumour-suppressor protein PTEN affect sensitivity[88].

It is unclear whether mutations in the intracellular domains of EGFR affect the response to therapeutic mAbs. Mukohara *et al.*[89] compared the efficacy of gefitinib and cetuximab on NSCLC with EGFR mutations. Gefitinib was more effective than cetuximab at inhibiting not only *in vitro* growth, but also the induction of apoptosis in EGFR-mutant NSCLC cell lines. Gefitinib consistently suppressed EGFR phosphorylation in EGFR-mutant cell lines, whereas cetuximab had less of an inhibitory effect. Of note, even high concentrations of cetuximab failed to show any inhibitory effect on EGFR phosphorylation in EGFR-mutant cells[89, 90]. Clinical data indicate that mutant EGFRs are more sensitive to gefitinib than to cetuximab, which suggests that EGFR mutations in NSCLC cells are associated with gefitinib, but not cetuximab, sensitivity.

In colorectal cancers it has been reported that EGFR mAbs are more effective than small-molecule inhibitors[91–94]. The difference in the effectiveness of the two classes of agents on colorectal cancer might therefore be partially explained by the lower frequency of activating EGFR mutations[95] such as those found in NSCLC. However, the efficacy of therapeutic mAbs in colorectal cancer does not seem to correlate with EGFR expression[96]. Cetuximab has been shown to be effective even in patients with EGFR-negative colorectal cancer, as determined by immunohistochemistry[91]. In fact, this remains an emerging issue for cetuximab-based therapy for colorectal cancer; there are currently no adequate markers that can efficiently predict the benefit from EGFR-targeted therapy. This issue might be partly related to the limited ability of the immunohistochemical detection method. Moroni *et al.*[97] showed that eight out of nine panitumumab or cetuximab responders with colorectal cancer had an increased *EGFR* copy number. Therefore, the evaluation of *EGFR* amplification status by FISH could help select patients for cetuximab therapy in colorectal cancer.

EGFR phosphorylation does not seem to correlate exactly with the effect of cetuximab on tumour-cell growth. Despite no inhibitory effect on EGFR phosphorylation[89,90], cetuximab potently inhibited the growth of HCC827 NSCLC cells, which contain a deletion mutation in exon 19 of *EGFR*. By contrast, the growth of three different EGFR-mutant NSCLC cell lines was not inhibited by cetuximab[89]. Therefore, factors other than the modification of EGFR phosphorylation by mutations might affect the anti-tumour efficacy of mAbs in some types of NSCLC. If EGFR phosphorylation is not always coupled with the sensitivity of these inhibitors, then it is possible that cetuximab could have an inhibitory effect on the activation of downstream pathways mediated by ERK1/2 and AKT, thereby producing anti-tumour effects. Several lines of evidence support the important role of AKT in EGFR-mediated cell survival[98–100]. Furthermore, Amann *et al.*[90] suggest that in addition to EGFR mutations, other factors in NSCLC cells such as high expression levels of other ErbB family members might contribute to the sensitivity to both types of EGFR inhibitors, possibly through the deregulated activation of the AKT pathway downstream of EGFR. The possible

involvement of determinants other than EGFR mutations needs to be addressed to clarify the mechanism(s) that underlie resistance to EGFR inhibitors, as supported by several recent reports[98,99,101,102].

Cancer stem cells could be a source of tumour relapse and drug resistance during treatment with targeted therapies[103,104]. Recent CML studies quantitatively validate the model whereby imatinib affects differentiated leukaemic cells but not leukaemic stem cells, which are eventually linked to relapse[105]. No such stem-cell-related resistance has been reported for mAb-based therapies. On the other hand, the resistance mechanisms to mAbs not shared by TKIs are intrinsically host related. For instance, the impairment of ADCC, possibly through a defective immune system or other mechanisms, could result in resistance to treatment with mAbs[106] because ADCC is a unique *in vivo* mechanism of action for these agents.

Immune mechanisms. There are important differences between the effects of mAbs and small-molecule inhibitors on immune responses. The mechanisms that underlie the therapeutic effects of small-molecule agents are not directly linked to the activation of the immune response against tumour cells, whereas mAbs exert not only direct inhibitory effects on tumour growth but also have the ability to activate indirect accessory antitumour activities such as ADCC and CDC[107] (FIG. 2). Because of these properties, one can envisage that *in vitro* growth inhibition by mAbs might not accurately reflect the *in vivo* efficacy of mAb treatment compared with small-molecule agents. In fact, cetuximab is less effective at inhibiting the proliferation of NSCLC cell lines than gefitinib, whereas the inhibitory effect of cetuximab on *in vivo* tumour growth seems to be more significant than that of gefitinib[57,89]. Although no effect of gefitinib on immunological responses has, to our knowledge, been described, the engagement of the activation antibody receptor (FcγRIII) on effector cells such as natural killer (NK) cells or monocytes/macrophages (FIG. 2) is a dominant component of *in vivo* cytotoxic activity mediated by cetuximab against tumours. There have also been reports on the pharmacogenetic association of FcγR polymorphisms and the clinical response to rituximab in patients with follicular non-Hodgkin lymphoma[108,109], which supports the contribution of FcγR-mediated ADCC to the clinical effect of mAbs. However, an $F(ab')_2$ form of cetuximab that lacks FcRγ-chain interaction still has an inhibitory effect on *in vivo* tumour growth, although half of the activity is induced by native cetuximab[110]. A partially reduced response was also observed in FcRγ-chain-deficient mice[106]. By contrast, a regulatory mechanism by the inhibitory antibody receptor (FcγRIIb) was also reported (FIG. 2). In syngeneic and xenograft models with three different tumours, Clynes *et al.* clearly showed more robust antitumour effects of the therapeutic mAbs trastuzumab and rituximab in FcγRIIb-deficient mice[106]. Therefore, Fc-receptor-dependent mechanisms contribute substantially to the anti-tumour activities of mAbs, but their interference with signalling pathways and the engagement of other immune-effector mechanisms including CDC are also putatively involved.

Regarding the contribution of CDC to immune mechanisms, the role of complement factors as an effector mechanism is still controversial. The observation that at least 10 times more mAbs are required to trigger CDC on the cell surface than to trigger ADCC[111] suggests that most mAbs are engaged in an ADCC event during treatment, whereas mAbs are unlikely to reach the surface density on target cells sufficient to activate the classical complement pathway. In support of this, the therapeutic activity of rituximab does not correlate with either the susceptibility of lymphoma cells to *in vitro* complement-mediated lysis induced by rituximab or the expression levels of the complement-regulatory proteins[112]. On the other hand, some evidence supports the involvement of CDC in mAb-mediated immune mechanisms[113–115]. *In vivo* data showed that rituximab, which redistributes CD20 into membrane rafts[116], is bound efficiently by C1q and deposits C3b, which activates CDC[117]. In addition, the *in vivo* role of CDC in the action of rituximab is suggested by evidence that complement depletion[115] or C1q-deficient mice[114] showed reduced or abolished efficacy of rituximab in lymphoma models. Complement-dependent cellular cytotoxicity (CDCC) might also be a mechanism of tumour-cell killing[118] (FIG. 2). During the complement activation cascade, C3b generation triggers phagocytosis and cellular lysis through the engagement with C3b-receptor macrophages, NK cells and polymorphonuclear leukocytes. Other activated complement factors such as CD3a and C5a might also facilitate inflammatory responses to efficiently eliminate tumour cells.

Several strategies have been explored to increase antibody-mediated effector functions and optimize efficacy[54]. To increase FcγR-mediated ADCC activity, the amino-acid sequence or glycosylation of the C_H2 region of mAbs has been manipulated by computational design or mutational analysis to improve its interaction with FcγRs[119–121]. New CD20 mAbs with strikingly potent CDC activity have also been developed using human Ig transgenic mice[122] or through engineering the amino-acid sequence of the C1q-binding site[123].

Adverse effects. In general, the adverse effects associated with small-molecule inhibitors are mild. The most frequently observed adverse effects of gefitinib are cutaneous (for example, rash, acne, dry skin and pruritus) and gastrointestinal symptoms (for example, diarrhoea, nausea, vomiting and anorexia)[34, 124]. Similar to small-molecule agents, most of the observed adverse effects of mAb therapies are mild, including dermatological (for example, acne, rash, dry skin and pruritus) and other manifestations (for example, fever, chill and asthenia), without the bone-marrow suppressive properties of chemotherapy.

The most common symptom associated with both classes of anti-EGFR agents is an acneiform skin rash resulting from the effects of EGFR inhibition, not from a drug-related allergic reaction[125], possibly due to the expression of EGFRs in the epidermis. Interestingly, a

Cancer stem cells
A small subpopulation of quiescent tumour cells within a tumour that have properties similar to normal stem cells, such as the capability to undergo self-renewal and to maintain tumour growth and heterogeneity. According to the stem-cell-based model, conventional therapies typically target actively proliferating cells but spare drug-resistant cancer stem cells, which might contribute to therapeutic failure and eventual relapses.

Pruritus
A dermatological symptom (itching) that is often observed in cutaneous lesions caused by allergy and infections.

Asthenia
A general feeling of weakness or lack of vigour, which can be associated with various diseases.

growing number of reports show a positive correlation between skin rash and clinical outcome in EGFR-targeted therapies with cetuximab and erlotinib, although this effect is less consistent for gefitinib[126]. Therefore, skin rash might be a possible marker for evaluating and monitoring the efficacy of anti-EGFR agents. This skin rash is not thought to be dose-limiting, and completely resolves following treatment cessation[25,60]. Dermatological toxicity is not significantly different between both types of inhibitors. On the other hand, diarrhoea is not common in patients treated with mAbs but is in patients treated with small-molecule inhibitors[71,127,128], and it can be dose-limiting[34,53]. This might be linked to the oral administration of small-molecule inhibitors, although direct evidence has not been provided for such an association. Unlike small-molecule inhibitors, mAbs can trigger allergic reactions such as anaphylactoid reactions and urticaria[129], but these are manageable by conventional treatments and are not clinically limiting[25].

The only severe toxicity reported to date with any of these agents is gefitinib-related interstitial pneumonitis, the highest incidence of which was observed in Japanese patients at 1–2% (3–4 times higher than that for patients worldwide)[130]. Over 170 patients died from this pulmonary disease after treatment with gefitinib[34]. Recent analyses of chest radiographic and computer tomography (CT) findings showed that the imaging of gefitinib-related interstitial lung disease is similar to that of pulmonary damage caused by conventional antineoplastic agents[131]. We speculate that pulmonary toxicity with gefitinib might be due to a direct cytotoxic effect, although its aetiology is not yet clear. Japanese patients with NSCLC also show a higher response to gefitinib, which is associated with a more frequent detection of EGFR mutations[132]. Therefore, differences in genetic background could underlie the high incidence of gefitinib-induced interstitial lung disease among Japanese patients. Furthermore, gefitinib interacts with the ATP-binding cassette transporter ABCG2, which might be involved in the efflux of gefitinib from cells[133]. Therefore, the genetic alteration of *ABCG2* might affect the absorption, tissue distribution and toxicities of gefitinib. The development of new inhibitors that can discriminate between wild-type and tumour-specific mutant EGFRs might provide a solution to the adverse effects described above.

Distinct from small-molecule agents, any protein therapeutic is potentially immunogenic. Previously, the development of therapeutic murine mAbs was hindered by problems such as a lack of efficacy and rapid clearance by human anti-mouse antibodies (HAMAs). Such an immunogenicity problem does not disappear by using chimeric or humanized mAbs, and even human mAbs pose this problem. As cetuximab is a mouse–human chimeric mAb containing 5–10% murine protein it has, although less frequently than fully murine mAbs[25,134], the potential to induce the production of human anti-chimeric antibodies (HACAs), which might interfere with its efficacy. However, the generation of HACAs occurs in only a small fraction (3%) of patients treated with cetuximab, so HACA responses are not thought to be clinically limiting[25].

Response rates. In a series of clinical trials, gefitinib and erlotinib caused objective responses in 10–20% of previously treated patients with NSCLC[135–138]. In a recent placebo-controlled phase III clinical trial[71,128], erlotinib significantly prolonged the survival of patients with NSCLC, whereas gefitinib did not significantly improve survival. As for monotherapy with therapeutic mAbs, both preclinical and clinical studies have shown efficacy in some patients with colorectal cancer, NSCLC and other solid tumours[139,140]. No remarkable difference in the overall rate of response to monotherapy is apparent between these two classes of agents, which is supported by previous preclinical data that show that the induction of cell-cycle arrest and cytotoxic activity is almost the same between small-molecule inhibitors and mAbs. To improve the efficacy of these agents, therapeutic strategies in combination with chemotherapy or radiotherapy have been investigated.

Combination with chemotherapy or radiotherapy. Clinical trials using mAbs or small-molecule inhibitors combined with chemotherapy have shown a paradoxical distinction between these two classes of agents in lung cancer. The combination of gefitinib with two different chemotherapy regimens in advanced NSCLC did not result in any additive effects over chemotherapy alone in two large randomized studies[141,142]. By contrast, anti-tumour effects were increased by the addition of cetuximab to chemotherapy in advanced NSCLC[143,144]. We think that the underlying mechanisms for this synergy might include the interruption of EGFR-activated survival and proliferation signalling[145], which makes tumour cells more vulnerable to chemotherapy, but this cannot account for the distinction between these two classes of targeted agents. The discrepancy might be explained partly by some positive, direct action of mAbs on apoptotic pathways. In addition, some *in vivo*, specific role of therapeutic mAbs might also contribute to a synergistic effect with cytotoxic chemotherapeutic agents. In this regard, we presume that mAbs but not small-molecule inhibitors show advantageous activity because of their indirect actions, for example, the activation of immune responses such as ADCC. This activity might be further increased by some immunostimulatory process, such as the activation of macrophages, in response to cytotoxic-agent-induced cell death.

A difference in responsiveness to these two types of inhibitors is not observed in every type of cancer. Several clinical trials have shown the effectiveness of cetuximab combined with irinotecan-based chemotherapy in metastatic colorectal cancer[92,94,145]. However, in contrast to the lack of synergy in NSCLC, it has been reported that gefitinib has a synergistic effect in combination with chemotherapy in metastatic colorectal cancer[146]. Kuo and Fisher argued that the differences between NSCLC and colorectal cancer with respect to EGFR expression and mutation status do not completely explain this dichotomy[146]. Therefore, the mechanism that underlies the synergistic effects of these EGFR inhibitors seems to be multifactorial.

Anaphylactoid reactions
Systemic immunological hyper-responses that mimic anaphylaxis. In contrast to IgE-mediated anaphylactic reactions, these are triggered by an IgE-independent mechanism, frequently appear as allergic reactions to drugs, foods and exercise, and manifest as potentially life-threatening symptoms such as hypotension, bronchospasm and laryngeal oedema.

Urticaria
A cutaneous symptom that primarily manifests as a rash and pruritus. This manifestation is caused by IgE- or non-IgE hypersensitivity with histamine and other vasoactive chemicals released from mast cells as a result of exposure to drugs and foods.

Interstitial pneumonitis
A form of pneumonia that is characterized by non-infectious inflammation and fibrosis in the space between the epithelial and endothelial basement membranes of the lower respiratory tract. This is caused by unknown and known factors such as drugs (gefitinib, lefluomide or irinotecan) or environmental factors, and can be observed in association with other diseases (for example, connective tissue diseases). Patients with this disorder typically present with cough and shortness of breath.

Human anti-mouse antibodies
HAMAs are antibodies that are produced by the human immune system against therapeutic murine monoclonal antibodies (mAbs)

Human anti-chimeric antibodies
HACAs are antibodies that are produced against murine components of chimeric or humanized mAbs. HAMAs and HACAs are often related to immunogenicity problems associated with a lack of efficacy and rapid clearance during mAb therapy.

In HNSCC, accumulating preclinical and clinical studies have shown an increased effect of cetuximab in combination with radiotherapy[147], therefore contributing to its approval by the FDA. In addition, a recent early-phase trial has also shown encouraging data for the combination of gefitinib with chemoradiation[148]. In metastatic colorectal cancer, another FDA-approved mAb, bevacizumab, also significantly improved response rates and overall survival of patients in combination with 5-FU-based chemotherapy[149]. Although the underlying mechanism is still unclear, we speculate that these augmentative effects of mAbs might be partially due to their possible role in increasing p53-dependent apoptosis, which is an important apoptotic pathway activated by genotoxic agents[150]. Analogous to this, we reported a similar mechanism for the synergistic effect of interferon-α (IFNα) and IFNβ with genotoxic stresses such as 5-FU or γ-irradiation: IFNα and IFNβ treatment contributes to the increase of DNA-damage-induced apoptosis by activating *TP53* expression[151]. Nevertheless, the association of *TP53* status with responsiveness to the combination of bevacizumab and 5-FU-based chemotherapy in colorectal cancer remains controversial[152,153], whereas p53 loss of function seems to predict resistance to the combination of gefitinib with chemotherapy, particularly in colorectal cancers with intact p21 expression[95].

Synergistic effects of the combination of monoclonal antibodies with small-molecule inhibitors. When one envisages potential synergism of the non-redundant properties of targeted mAbs and small-molecule inhibitors, another interesting question is raised: can the combination of distinct classes of inhibitors to the same target molecule, for example, anti-EGFR mAbs and EGFR TKIs, augment their efficacy for cancer therapy compared with using a single EGFR inhibitor? Huang *et al.* studied the effect of combination treatment with cetuximab and either gefitinib or erlotinib[57]. They found that the phosphorylation of EGFR and its downstream signalling molecules, ERK and AKT, is more severely inhibited by combined treatment, which induced apoptosis in HNSCC cell lines. In addition, gefitinib or erlotinib still retained the capacity to inhibit EGFR-mediated signalling and *in vitro* proliferation of lung and HNSCC cells, which are highly resistant to cetuximab. Furthermore, combined treatment with cetuximab and gefitinib or erlotinib significantly inhibited the growth of human tumour xenografts, whereas treatment with a single agent produced only modest growth inhibition. Their findings suggest that the combination of distinct classes of EGFR inhibitors might not only increase their efficacy through non-overlapping mechanisms of action, but also assist in overcoming resistance to one class of EGFR inhibitor[57]. Consistent with this, other groups have shown that therapeutic mAbs can lower the effective dose of small-molecule inhibitors such as gefitinib or lapatinib, which might contribute to the reduction of toxicity without compromising efficacy[154,155]. Preclinical studies[58,156] have shown increased efficacy when trastuzumab is combined with lapatinib in ERBB2-positive breast cancer cells, which might support the encouraging phase I study results of these agents in a combined regimen[157]. Although antibody-related immune activation might explain this synergy, several reports showed direct actions against cancer cells. Treatment with lapatinib and trastuzumab increased apoptosis of ERBB2-overexpressing breast cancer cells[58], and trastuzumab might sensitize cancer cells to treatment with lapatinib during combination therapy[156]. Further clarification of the mechanism of action of each class of agents will be required to validate the efficacy of combinations.

Conclusion and future directions

The recent clinical successes of therapeutic mAbs and small molecules in cancer treatment have established these agents as the first cornerstone of molecular targeting therapy for cancers. However, the issues that have arisen during the development of targeted agents must be addressed, and on the basis of these data an appropriate approach should be chosen to develop targeted drugs with greater efficacy and safety. In particular, during preclinical drug development it is crucial to predict how potent and selectively targeted drugs will function in eventual clinical applications. However, the biochemical criteria for target validation[158] have yet to be decided. Knight *et al.* have recently used a systematic approach for parallel evaluation using a chemically diverse panel of small-molecule inhibitors that target the PI3K family[159]. Such integrated approaches should be useful for the mapping of drug targets.

The activation of anti-tumour immunity is probably crucial for efficiently eliminating tumour cells. In this regard, small-molecule agents that do not directly act on the immune system should be combined with drugs with immunostimulatory activities to maximize therapeutic effects. As such, efforts have been made to target a molecule with combinations of different classes of agents, and several reports have provided evidence for the potential synergistic effects of mAb therapies and small-molecule inhibitors for cancer treatment[57,59,154,160]. Although the efficient doses or schedules for combination therapies need to be optimized, and the predictive criteria for the selection of patients that might benefit from dual-agent therapy need to be established, a role for therapeutic mAbs and small-molecule inhibitors in combination therapies is emerging. Therefore, the simultaneous use of distinct classes of agents that target one specific molecule could be thought of as one of the promising strategies for maximally inhibiting target molecule(s) and overcoming the limitations of any single blockade.

However, in most solid tumours oncogenic progression is a multistep process and molecular pathogenesis is not linked to the defect of a single target. In this context, a single targeted therapy seems theoretically to be an unfavourable strategy and cannot be expected to yield optimal outcomes, which is paradoxical to the original concept that a single targeted therapy would be ideal, with fewer side effects due to its high specificity. Therefore, the establishment of multi-targeted therapies

through the combination of agents targeted to several distinct molecules might be one of the goals for cancer treatment in the immediate future. This could overcome issues of tumour heterogeneity at the same time as maintaining the selectivity of treatment. In addition, monotherapy is further evolving with new single inhibitors that target several molecules simultaneously. Several bi- or multi-selective ErbB inhibitors are now in clinical trials and await further comparative analyses with mono-selective ErbB inhibitors.

Furthermore, a new aspect of cancer-targeted therapies has been provided by recent findings with cancer stem cells that show that mTOR inhibition by rapamycin selectively eliminates leukaemic stem cells without affecting normal haematopoietic stem cells[161,162]. Therefore, targeting cancer stem cells or aberrant signalling pathway(s) in those cells might offer a rational, effective approach of targeting therapies.

Targeted mAb and small-molecule inhibitor combinations should be further studied so that the advantageous properties of both classes of agent can be exploited to maximize their efficacy. A better understanding of targeted therapeutics in the context of a cancer-stem-cell-directed strategy might lead to the design of new, effective combitation therapy protocols, which will hopefully improve the prognoses of cancer patients. Further investigations and the development of small-molecule inhibitors and mAbs will be required to optimize the next generation of both molecular and cellular target-directed therapies.

1. Chabner, B. A. & Roberts, T. G., Jr. Timeline: chemotherapy and the war on cancer. *Nature Rev. Cancer* **5**, 65–72 (2005).
2. Sawyers, C. Targeted cancer therapy. *Nature* **432**, 294–297 (2004).
A concise review of the molecular basis of targeted cancer therapy with a particular focus on protein kinase targets.
3. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
4. Baselga, J. Targeting tyrosine kinases in cancer: the second wave. *Science* **312**, 1175–1178 (2006).
This is a very recent review of tyrosine kinase inhibitors in cancer therapy, which includes their brief histories and current issues that will affect the future development of new molecularly targeted agents.
5. Savage, D. G. & Antman, K. H. Imatinib mesylate-a new oral targeted therapy. *N. Engl. J. Med.* **346**, 683–693 (2002).
6. Herbst, R. S., Fukuoka, M. & Baselga, J. Gefitinib-a novel targeted approach to treating cancer. *Nature Rev. Cancer* **4**, 956–965 (2004).
A comprehensive review of the EGFR inhibitor gefitinib, from the molecular mechanism of its inhibitory effects on EGFR signalling to its clinical development in NSCLC.
7. Carter, P. Improving the efficacy of antibody-based cancer therapies. *Nature Rev. Cancer* **1**, 118–129 (2001).
8. 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).
9. Yarden, Y. & Sliwkowski, M. X. Untangling the ErbB signalling network. *Nature Rev. Mol. Cell Biol.* **2**, 127–137 (2001).
10. Mendelsohn, J. & Baselga, J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J. Clin. Oncol.* **21**, 2787–2799 (2003).
11. Kohler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
12. Green, L. L. *et al.* Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. *Nature Genet.* **7**, 13–21 (1994).
13. Maloney, D. G. *et al.* IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* **90**, 2188–2195 (1997).
14. Carter, P. *et al.* Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl Acad. Sci. USA* **89**, 4285–4289 (1992).
15. Baselga, J., Norton, L., Albanell, J., Kim, Y. M. & Mendelsohn, J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res.* **58**, 2825–2831 (1998).
16. Krejsa, C., Rogge, M. & Sadee, W. Protein therapeutics: new applications for pharmacogenetics. *Nature Rev. Drug Discov.* **5**, 507–521 (2006).
17. 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).
18. Franklin, M. C. *et al.* Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* **5**, 317–328 (2004).
19. Agus, D. B. *et al.* Phase I clinical study of pertuzumab, a novel HER dimerization inhibitor, in patients with advanced cancer. *J. Clin. Oncol.* **23**, 2534–2543 (2005).
20. Ishida, T., Tsujisaki, M., Hinoda, Y., Imai, K. & Yachi, A. Establishment and characterization of mouse-human chimeric monoclonal antibody to erbB-2 product. *Jpn. J. Cancer Res.* **85**, 172–178 (1994).
21. Hinoda, Y., Sasaki, S., Ishida, T. & Imai, K. Monoclonal antibodies as effective therapeutic agents for solid tumors. *Cancer Sci.* **95**, 621–625 (2004).
22. Sasaki, S. *et al.* Human tumor growth suppression by apoptosis induced with anti-ErbB-2 chimeric monoclonal antibody. *Jpn. J. Cancer Res.* **89**, 562–570 (1998).
23. Nahta, R., Hung, M. C. & Esteva, F. J. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res.* **64**, 2343–2346 (2004).
24. Huang, S. M. & Harari, P. M. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. *Invest. New Drugs* **17**, 259–269 (1999).
25. Baselga, J. The EGFR as a target for anticancer therapy--focus on cetuximab. *Eur. J. Cancer* **37** (Suppl. 4), S16–S22 (2001).
26. Goldberg, R. M. Cetuximab. *Nature Rev. Drug Discov.* Suppl., S10–S11 (2005).
27. Gibson, T. B., Ranganathan, A. & Grothey, A. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. *Clin. Colorectal Cancer* **6**, 29–31 (2006).
28. 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).
29. Izumi, Y., Xu, L., di Tomaso, E., Fukumura, D. & Jain, R. K. Tumour biology: herceptin acts as an anti-angiogenic cocktail. *Nature* **416**, 279–280 (2002).
30. Harding, J. & Burtness, B. Cetuximab: an epidermal growth factor receptor chemeric human-murine monoclonal antibody. *Drugs Today (Barc.)* **41**, 107–127 (2005).
31. Maier, L. A. *et al.* Requirements for the internalization of a murine monoclonal antibody directed against the *HER-2/neu* gene product c-erbB-2. *Cancer Res.* **51**, 5361–5369 (1991).
32. Brekke, O. H. & Sandlie, I. Therapeutic antibodies for human diseases at the dawn of the twenty-first century. *Nature Rev. Drug Discov.* **2**, 52–62 (2003).
A review that describes the fundamental properties of antibodies and antibody engineering for their therapeutic applications, as well as antibody-mediated effector mechanisms such as ADCC or CDC.
33. Hudson, P. J. & Souriau, C. Engineered antibodies. *Nature Med.* **9**, 129–134 (2003).
34. Arora, A. & Scholar, E. M. Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.* **315**, 971–979 (2005).
35. Krause, D. S. & Van Etten, R. A. Tyrosine kinases as targets for cancer therapy. *N. Engl. J. Med.* **353**, 172–187 (2005).
36. Druker, B. J. STI571 (Gleevec) as a paradigm for cancer therapy. *Trends Mol. Med.* **8**, S14–S18 (2002).
37. Druker, B. J. Imatinib as a paradigm of targeted therapies. *Adv. Cancer Res.* **91**, 1–30 (2004).
38. O'Dwyer, M. E., Mauro, M. J. & Druker, B. J. STI571 as a targeted therapy for CML. *Cancer Invest.* **21**, 429–438 (2003).
39. Buchdunger, E., O'Reilly, T. & Wood, J. Pharmacology of imatinib (STI571). *Eur. J. Cancer* **38** Suppl. 5, S28–S36 (2002).
40. Mendelsohn, J. The epidermal growth factor receptor as a target for cancer therapy. *Endocr. Relat. Cancer* **8**, 3–9 (2001).
41. Minna, J. D. & Dowell, J. Erlotinib hydrochloride. *Nature Rev. Drug Discov.* Suppl., S14–S15 (2005).
42. Chai, R. L. & Grandis, J. R. Advances in molecular diagnostics and therapeutics in head and neck cancer. *Curr. Treat. Options Oncol.* **7**, 3–11 (2006).
43. Cohen, L. H. *et al.* Inhibitors of prenylation of Ras and other G-proteins and their application as therapeutics. *Biochem. Pharmacol.* **60**, 1061–1068 (2000).
44. Sridhar, S. S., Hedley, D. & Siu, L. L. Raf kinase as a target for anticancer therapeutics. *Mol. Cancer Ther.* **4**, 677–685 (2005).
45. Neckers, L. & Neckers, K. Heat-shock protein 90 inhibitors as novel cancer chemotherapeutics- an update. *Expert Opin. Emerg. Drugs* **10**, 137–149 (2005).
46. Sawyer, T. K. Cancer metastasis therapeutic targets and drug discovery: emerging small-molecule protein kinase inhibitors. *Expert Opin. Investig. Drugs* **13**, 1–19 (2004).
47. Mannello, F., Tonti, G. & Papa, S. Matrix metalloproteinase inhibitors as anticancer therapeutics. *Curr. Cancer Drug Targets* **5**, 285–298 (2005).
48. Marx, J. Cancer. Encouraging results for second-generation antiangiogenesis drugs. *Science* **308**, 1248–1249 (2005).
49. Baker, M. Upping the ante on antibodies. *Nature Biotechnol.* **23**, 1065–1072 (2005).
50. Reichert, J. M., Rosensweig, C. J., Faden, L. B. & Dewitz, M. C. Monoclonal antibody successes in the clinic. *Nature Biotechnol.* **23**, 1073–1078 (2005).
51. Kola, I. & Landis, J. Can the pharmaceutical industry reduce attrition rates? *Nature Rev. Drug Discov.* **3**, 711–715 (2004).
52. Butowski, N. & Chang, S. M. Small molecule and monoclonal antibody therapies in neurooncology. *Cancer Control* **12**, 116–124 (2005).
53. Dancey, J. & Sausville, E. A. Issues and progress with protein kinase inhibitors for cancer treatment. *Nature Rev. Drug Discov.* **2**, 296–313 (2003).
A thorough review that gives an overview of the clinical development of various protein kinase inhibitors as targets of molecular-based cancer therapies.
54. Carter, P. J. Potent antibody therapeutics by design. *Nature Rev. Immunol.* **6**, 343–357 (2006).

55. Li, S. *et al.* Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. *Cancer Cell.* **7**, 301–311 (2005).
56. 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).
57. Huang, S., Armstrong, E. A., Benavente, S., Chinnaiyan, P. & Harari, P. M. Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. *Cancer Res.* **64**, 5355–5362 (2004).
58. Xia, W. *et al.* Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. *Oncogene* **24**, 6213–6221 (2005).
59. Matar, P. *et al.* Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. *Clin. Cancer Res.* **10**, 6487–6501 (2004).
60. Thomas, S. M. & Grandis, J. R. Pharmacokinetic and pharmacodynamic properties of EGFR inhibitors under clinical investigation. *Cancer Treat. Rev.* **30**, 255–268 (2004).
61. Ranson, M. Epidermal growth factor receptor tyrosine kinase inhibitors. *Br. J. Cancer* **90**, 2250–2255 (2004).
62. Rusnak, D. W. *et al.* The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines *in vitro* and *in vivo*. *Mol. Cancer Ther.* **1**, 85–94 (2001).
63. Geyer, C. E. *et al.* Scientific Special Session: Docetaxel added to Induction Therapy in Head and Neck Cancer. *ASCO web site* [online], http://www.asco.org/portal/site/ASCO/menuitem.34d60f5624ba07fd506fe310ee37a01d/?vgnextoid=76f8201eb61a7010VgnVCM100000ed730ad1RCRD&vmview=abst_detail_view&confID=40&abstractID=90002 (2006).
64. GlaxoSmithKline. GlaxoSmithKline receives positive data and halts enrolment in Phase III trial of Tykerb® (Lapatinib) in advanced breast cancer. *GlaxoSmithKline web site* [online], http://www.gsk.com/ControllerServlet?appId=4&pageId=402&newsid=784 (2006).
65. Ravaud, A. *et al.* Efficacy of lapatinib in patients with high tumor EGFR expression: results of a phase III trial in advanced renal cell carcinoma (RCC). *J. Clin. Oncol.* **24**, 4502 (2006).
66. Harrington, K. J. *et al.* A phase I, open-label study of lapatinib plus chemoradiation in patients with locally advanced squamous cell carcinoma of the head and neck (SCCHN). *J. Clin. Oncol.* **24**, 5553 (2006).
67. 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).
68. Moasser, M. M., Basso, A., Averbuch, S. D. & Rosen, N. The tyrosine kinase inhibitor ZD1839 ('Iressa') inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. *Cancer Res.* **61**, 7184–7188 (2001).
69. Saltz, L. *et al.* Cetuximab (IMC-225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer that expresses epidermal growth factor receptors. *Proc. Am. Soc. Clin. Oncol.* **20**, 3 (2001).
70. Bailey, L. R. *et al.* Tumor EGFR membrane staining is not clinically relevant for predicting response in patients receiving gefitinib ('Iressa', ZD1839) monotherapy for pretreated advanced non-small-cell lung cancer: IDEAL 1 and 2. *Proc. Am. Assoc. Cancer Res.* **44**, 1362 (2003).
71. Shepherd, F. A. *et al.* Erlotinib in previously treated non-small-cell lung cancer. *N. Engl. J. Med.* **353**, 123–132 (2005).
72. Arteaga, C. L. & Baselga, J. Clinical trial design and end points for epidermal growth factor receptor-targeted therapies: implications for drug development and practice. *Clin. Cancer Res.* **9**, 1579–1589 (2003).
73. Bianco, R., Troiani, T., Tortora, G. & Ciardiello, F. Intrinsic and acquired resistance to EGFR inhibitors in human cancer therapy. *Endocr. Relat. Cancer* **12** Suppl. 1, S159–S171 (2005).
74. Bishop, P. C. *et al.* Differential sensitivity of cancer cells to inhibitors of the epidermal growth factor receptor family. *Oncogene* **21**, 119–127 (2002).
75. 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).
76. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
The above two reports (references 75 and 76) show the identification of somatic mutations in exons 18–21 of the *EGFR* gene in NSCLC. These results indicated these *EGFR* mutations as possible determinants of gefitinib sensitivity to NSCLC.
77. Han, S. W. *et al.* Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J. Clin. Oncol.* **23**, 2493–2501 (2005).
78. Huang, S. F. *et al.* High frequency of epidermal growth factor receptor mutations with complex patterns in non-small cell lung cancers related to gefitinib responsiveness in Taiwan. *Clin. Cancer Res.* **10**, 8195–8203 (2004).
79. Kosaka, T. *et al.* Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res.* **64**, 8919–8923 (2004).
80. Miller, V. A. *et al.* Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. *J. Clin. Oncol.* **22**, 1103–1109 (2004).
81. 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).
82. Shigematsu, H. *et al.* Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J. Natl Cancer Inst.* **97**, 339–346 (2005).
83. Tracy, S. *et al.* Gefitinib induces apoptosis in the EGFRL858R non-small-cell lung cancer cell line H3255. *Cancer Res.* **64**, 7241–7244 (2004).
84. Cappuzzo, F. *et al.* Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J. Natl Cancer. Inst.* **97**, 643–655 (2005).
85. Cappuzzo, F. *et al.* Increased *HER2* gene copy number is associated with response to gefitinib therapy in epidermal growth factor receptor-positive non-small-cell lung cancer patients. *J. Clin. Oncol.* **23**, 5007–5018 (2005).
86. Kobayashi, S. *et al.* EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **352**, 786–792 (2005).
This paper documented the identification of a second mutation (T790M) in NSCLC bearing an activating EGFR mutation, which might be related to the resistance of NSCLC to gefitinib.
87. Frederick, L., Wang, X. Y., Eley, G. & James, C. D. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res.* **60**, 1383–1387 (2000).
88. Mellinghoff, I. K. *et al.* Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N. Engl. J. Med.* **353**, 2012–2024 (2005).
89. Mukohara, T. *et al.* Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. *J. Natl Cancer Inst.* **97**, 1185–1194 (2005).
An interesting report of a comparative evaluation of the sensitivites of gefitinib and cetuximab to NSCLC cell lines that habour EGFR mutations, and consistent data by retrospective analysis of NSCLC patients with EGFR mutations treated with both gefitinib and cetuximab.
90. Amann, J. *et al.* Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res.* **65**, 226–235 (2005).
91. Chung, K. Y. *et al.* Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J. Clin. Oncol.* **23**, 1803–1810 (2005).
92. Cunningham, D. *et al.* Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* **351**, 337–345 (2004).
93. Mackenzie, M. J. *et al.* A phase II trial of ZD1839 (Iressa) 750 mg per day, an oral epidermal growth factor receptor-tyrosine kinase inhibitor, in patients with metastatic colorectal cancer. *Invest. New Drugs* **23**, 165–170 (2005).
94. Saltz, L. B. *et al.* Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J. Clin. Oncol.* **22**, 1201–1208 (2004).
95. Ogino, S. *et al.* Molecular alterations in tumors and response to combination chemotherapy with gefitinib for advanced colorectal cancer. *Clin. Cancer Res.* **11**, 6650–6656 (2005).
96. Italiano, A. Targeting the epidermal growth factor receptor in colorectal cancer: advances and controversies. *Oncology* **70**, 161–167 (2006).
97. Moroni, M. *et al.* Gene copy number for epidermal growth factor receptor (*EGFR*) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol.* **6**, 279–286 (2005).
98. 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).
99. 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).
100. 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).
Describes the essential role of AKT and STAT signalling pathways in mutant EGFR-mediated cell survival, which provide a putative mechanism underlying the therapeutic effect of gefitinib in NSCLC.
101. 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).
102. Li, B., Chang, C. M., Yuan, M., McKenna, W. G. & Shu, H. K. Resistance to small molecule inhibitors of epidermal growth factor receptor in malignant gliomas. *Cancer Res.* **63**, 7443–7450 (2003).
103. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
104. Dean, M., Fojo, T. & Bates, S. Tumour stem cells and drug resistance. *Na ture Rev. Cancer* **5**, 275–284 (2005).
105. Michor, F. *et al.* Dynamics of chronic myeloid leukaemia. *Nature* **435**, 1267–1270 (2005).
106. 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).
107. Iannello, A. & Ahmad, A. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. *Cancer Metastasis Rev.* **24**, 487–499 (2005).
108. Cartron, G. *et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood* **99**, 754–758 (2002).
109. Weng, W. K. & Levy, R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J. Clin. Oncol.* **21**, 3940–3947 (2003).
110. Fan, Z., Masui, H., Altas, I. & Mendelsohn, J. Blockade of epidermal growth factor receptor function by bivalent and monovalent fragments of 225 anti-epidermal growth factor receptor monoclonal antibodies. *Cancer Res.* **53**, 4322–4328 (1993).
111. Hale, G., Clark, M. & Waldmann, H. Therapeutic potential of rat monoclonal antibodies: isotype specificity of antibody-dependent cell-mediated cytotoxicity with human lymphocytes. *J. Immunol.* **134**, 3056–3061 (1985).
112. Weng, W. K. & Levy, R. Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. *Blood* **98**, 1352–1357 (2001).
113. Manches, O. *et al. In vitro* mechanisms of action of rituximab on primary non-Hodgkin lymphomas. *Blood* **101**, 949–954 (2003).

114. Di Gaetano, N. *et al.* Complement activation determines the therapeutic activity of rituximab *in vivo*. *J. Immunol.* **171**, 1581–1587 (2003).
115. Cragg, M. S. & Glennie, M. J. Antibody specificity controls *in vivo* effector mechanisms of anti-CD20 reagents. *Blood* **103**, 2738–2743 (2004).
116. Chan, H. T. *et al.* CD20-induced lymphoma cell death is independent of both caspases and its redistribution into triton X-100 insoluble membrane rafts. *Cancer Res.* **63**, 5480–5489 (2003).
117. Cragg, M. S. *et al.* Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. *Blood* **101**, 1045–1052 (2003).
118. Gorter, A. & Meri, S. Immune evasion of tumor cells using membrane-bound complement regulatory proteins. *Immunol. Today* **20**, 576–582 (1999).
119. Lazar, G. A. *et al.* Engineered antibody Fc variants with enhanced effector function. *Proc. Natl Acad. Sci. USA* **103**, 4005–4010 (2006).
120. Li, H. *et al.* Optimization of humanized IgGs in glycoengineered Pichia pastoris. *Nature Biotechnol.* **24**, 210–215 (2006).
121. Umana, P., Jean-Mairet, J., Moudry, R., Amstutz, H. & Bailey, J. E. Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity. *Nature Biotechnol.* **17**, 176–180 (1999).
122. Teeling, J. L. *et al.* Characterization of new human CD20 monoclonal antibodies with potent cytolytic activity against non-Hodgkin lymphomas. *Blood* **104**, 1793–1800 (2004).
123. Idusogie, E. E. *et al.* Engineered antibodies with increased activity to recruit complement. *J. Immunol.* **166**, 2571–2575 (2001).
124. Dancey, J. E. & Freidlin, B. Targeting epidermal growth factor receptor — are we missing the mark? *Lancet* **362**, 62–64 (2003).
125. Herbst, R. S., LoRusso, P. M., Purdom, M. & Ward, D. Dermatologic side effects associated with gefitinib therapy: clinical experience and management. *Clin. Lung Cancer* **4**, 366–369 (2003).
126. Perez-Soler, R. & Saltz, L. Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining? *J. Clin. Oncol.* **23**, 5235–5246 (2005). **This review describes the association between EGFR-targeted agents and skin rash, a common adverse effect, and considers the possibility that this observation could be an indicator of the efficacy of EGFR inhibition.**
127. Buter, J. & Giaccone, G. Medical treatment of non-small-cell lung cancer. *Ann. Oncol.* **16** Suppl. 2, ii229–ii232 (2005).
128. Thatcher, N. *et al.* Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* **366**, 1527–1537 (2005).
129. Baselga, J. *et al.* Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J. Clin. Oncol.* **18**, 904–914 (2000).
130. Inoue, A. *et al.* Severe acute interstitial pneumonia and gefitinib. *Lancet* **361**, 137–139 (2003).
131. Endo, M., Johkoh, T., Kimura, K. & Yamamoto, N. Imaging of gefitinib-related interstitial lung disease: multi-institutional analysis by the West Japan Thoracic Oncology Group. *Lung Cancer* **52**, 135–140 (2006).
132. Calvo, E. & Baselga, J. Ethnic differences in response to epidermal growth factor receptor tyrosine kinase inhibitors. *J. Clin. Oncol.* **24**, 2158–2163 (2006).
133. Elkind, N. B. *et al.* Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). *Cancer Res.* **65**, 1770–1777 (2005).
134. Khazaeli, A. L., Falcey, J., Paulter, V., Fetzer, M. & Waksal, H. Low immunogenicity of a chimeric monoclonal antibody (MoAb), IMC-C225, used to treat epidermal growth factor receptor-positive tumors. *Proc. Am. Soc. Clin. Oncol.* abstr 808 (2000).
135. Fukuoka, M. *et al.* Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J. Clin. Oncol.* **21**, 2237–2246 (2003).
136. Kris, M. G. *et al.* A phase II trial of ZD1839 (Iressa) in advanced non-small cell lung cancer (NSCLC) patients who had failed platinum- and docetaxel-based regimens (IDEAL 2). *Proc. Am. Soc. Clin. Oncol.* **21**, 292a (2002).
137. Perez-Soler, R. *et al.* Determinants of tumor response and survival with erlotinib in patients with non-small-cell lung cancer. *J. Clin. Oncol.* **22**, 3238–3247 (2004).
138. Shepherd, F. A., Pereira, J. & Ciuleanu, T. E. A randomized placebo-controlled trial of erlotinib in patients with advanced non-small cell lung cancer (NSCLC) following failure of 1st line or 2nd line chemotherapy: A National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) trial. *J. Clin. Oncol.* **22**, 622s (2004).
139. Mendelsohn, J. Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. *Clin. Cancer Res.* **3**, 2703–2707 (1997).
140. Lilenbaum, R. *et al.* A phase II trial of cetuximab as therapy for recurrent non-small cell lung cancer (NSCLC). *ASCO Meeting Proc.* **23**, 7036 (2005).
141. Giaccone, G. *et al.* Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial — INTACT 1. *J. Clin. Oncol.* **22**, 777–784 (2004).
142. Herbst, R. S. *et al.* Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial — INTACT 2. *J. Clin. Oncol.* **22**, 785–794 (2004).
143. Rosell, R. *et al.* Randomized phase II study of cetuximab in combination with cisplatin (C) and vinorelbine (V) vs. CV alone in the first-line treatment of patients (pts) with epidermal growth factor receptor (EGFR)-expressing advanced non-small-cell lung cancer (NSCLC). *J. Clin. Oncol.* **22**, 618s (2004).
144. Thienelt, C. D. *et al.* Multicenter phase I/II study of cetuximab with paclitaxel and carboplatin in untreated patients with stage IV non-small-cell lung cancer. *J. Clin. Oncol.* **23**, 8786–8793 (2005).
145. Wong, S. F. Cetuximab: an epidermal growth factor receptor monoclonal antibody for the treatment of colorectal cancer. *Clin. Ther.* **27**, 684–694 (2005).
146. Kuo, T. & Fisher, G. A. Current status of small-molecule tyrosine kinase inhibitors targeting epidermal growth factor receptor in colorectal cancer. *Clin. Colorectal Cancer* **5** Suppl. 2, S62–S70 (2005).
147. Bonner, J. A., & Harari, M. Cetuximab prolongs survival in patients with locoregionally advanced squamous cell carcinoma of head and neck: A phase III study of high dose radiation therapy with or without cetuximab. *J. Clin. Oncol.* **22**, 489s (2004).
148. Doss, H. H. *et al.* Induction chemotherapy + gefitinib followed by concurrent chemotherapy/radiation therapy/gefitinib for patients (pts) with locally advanced squamous carcinoma of the head and neck: a phase I/II trial of the Minnie Pearl Cancer Research Network. *J. Clin. Oncol.* **24**, 5543 (2006).
149. Hurwitz, H. *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* **350**, 2335–2342 (2004).
150. Lowe, S. W., Ruley, H. E., Jacks, T. & Housman, D. E. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **74**, 957–967 (1993).
151. Takaoka, A. *et al.* Integration of interferon-a/b signalling to p53 responses in tumour suppression and antiviral defence. *Nature* **424**, 516–523 (2003).
152. Huether, A., Hopfner, M., Baradari, V., Schuppan, D. & Scherubl, H. EGFR blockade by cetuximab alone or as combination therapy for growth control of hepatocellular cancer. *Biochem. Pharmacol.* **70**, 1568–1578 (2005).
153. Ince, W. L. *et al.* Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J. Natl Cancer Inst.* **97**, 981–989 (2005).
154. Minna, J. D., Peyton, M. J. & Gazdar, A. F. Gefitinib versus cetuximab in lung cancer: round one. *J. Natl Cancer Inst.* **97**, 1168–1169 (2005).
155. Xia, W., Liu, L. H., Ho, P. & Spector, N. L. Truncated ErbB2 receptor (p95ErbB2) is regulated by heregulin through heterodimer formation with ErbB3 yet remains sensitive to the dual EGFR/ErbB2 kinase inhibitor GW572016. *Oncogene* **23**, 646–653 (2004).
156. Konecny, G. E. *et al.* Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res.* **66**, 1630–1639 (2006).
157. Storniolo, A. *et al.* A phase I, open-label study of lapatinib (GW572016) plus trastuzumab; a clinically active regimen. *J. Clin. Oncol.* **23**, 559 (2005).
158. Williams, M. Target validation. *Curr. Opin. Pharmacol.* **3**, 571–577 (2003).
159. Knight, Z. A. *et al.* A pharmacological map of the PI3-K family defines a role for p110α in insulin signaling. *Cell* **125**, 733–747 (2006).
160. Raben, D. *et al.* The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer. *Clin. Cancer Res.* **11**, 795–805 (2005).
161. Yilmaz, O. H. *et al.* Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature* **441**, 475–482 (2006).
162. Zhang, J. *et al.* PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature* **441**, 518–522 (2006).
163. Fabian, M. A. *et al.* A small molecule-kinase interaction map for clinical kinase inhibitors. *Nature Biotechnol.* **23**, 329–336 (2005).

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Competing interests statement

The authors declare no competing financial interests.

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