

OPINION

Integrating molecular diagnostics into anticancer drug discovery

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Abstract | In the 1990s, the breast cancer drug trastuzumab (Herceptin; Genentech/Roche) — an antibody specific for human epidermal growth factor receptor 2 (HER2; also known as ERBB2) — was approved based on trials in which HER2 expression levels were used to select patients in clinical trials. This provided support for analogous efforts for drugs that target the epidermal growth factor receptor (EGFR). However, the development of these drugs, such as cetuximab (Erbix; Bristol–Myers Squibb/Lilly) and gefitinib (Iressa; AstraZeneca), has revealed that EGFR expression is an insufficient and unreliable biomarker to select patients for EGFR-targeted therapies in both lung and colon cancer. Indeed, evidence on patient populations that are likely to respond to such therapies, on the basis of specific mutations in proteins of the targeted pathway, has only recently been clinically validated and incorporated into some of the drug labels. This article highlights lessons learned from the development of the first drugs targeting the EGFR family and discusses strategies to decrease the risk of failure in clinical development by more effectively integrating molecular diagnostics into anticancer drug discovery.

Anticancer drug discovery and development has been transformed in recent years by the concept that targeting a single protein, often encoded by an oncogene, that has a hypothetical role in tumour biology could be effective in treating cancers that overexpress the target protein. However, with a few notable exceptions, such as the success of imatinib (Glivec/Gleevec; Novartis) in the treatment of chronic myelogenous leukaemia, the first generation of molecularly targeted anticancer drugs were clinically less successful than anticipated, with a number of high-profile failures^{1–4}.

These failures are related to several aspects of anticancer drug discovery and development that are considered in this article, including tumour cell biology, medicinal chemistry, molecular diagnostics and clinical development. First, histopathological tumour classes, particularly epithelial cancers, are heterogeneous at the molecular level. For example, it seems that mutations in the *KRAS* and epidermal growth factor receptor (*EGFR*) oncogenes might be mutually exclusive in lung adenocarcinomas, and cancers associated with these different mutations may have different optimal treatments^{5–7}. Second, many of the first generation of molecularly targeted small-molecule drugs (in particular, kinase inhibitors) were selected for clinical development because

of their specificity for the target protein. However, we now know that they are less selective than desired and that the off-target profile could also be important in their clinical activity. Indeed, multi-target drugs could be more desirable than highly selective agents. This might be because simultaneous inhibition of many signalling pathways is necessary for activity in most tumour types, or because a more relevant target might be among the inhibited ‘off-targets’ of the agent. Third, the ability of diagnostic tests — which have been developed to detect solely the presence of the putative molecular targets — to predict therapeutic response has not been sufficiently validated in preclinical and clinical studies. In particular, tests based on quantitative evaluation of protein expression by immunohistochemistry (IHC) have frequently been unreliable (TABLE 1).

Owing to such issues, there have often been two types of deficiencies in clinical development strategies for molecularly targeted drugs. First, in some cases in which the hypothetical target is known to be present in the majority of the targeted tumour types, there has been no prior plan for biomarker studies in the clinical trials, and so this analysis has had to be done retrospectively in non-optimal circumstances^{8–12}. Second, in some cases, the patient population has been

pre-selected by a single, clinically untested diagnostic method, which hypothetically detects the hypothetical target^{13,14}. Both deficiencies lead to a situation in which the real target patient population cannot be reliably identified, and so some patients (‘false negatives’) cannot access an effective therapy, whereas other patients (‘false positives’) unnecessarily receive the drug.

This article considers lessons learned from the development of several agents targeting the EGFR family that have so far received regulatory approval. It discusses strategies to avoid the deficiencies mentioned above, which will decrease the risk of failure in clinical development and respond to the growing need to demonstrate cost-effectiveness for targeted anticancer drugs.

Lessons from EGFR-targeted drugs

Epithelial cancers (for example, lung cancer, colon cancer and breast cancer) are responsible for the majority of cancer incidence and cancer-related mortality. The EGFR family comprises four closely related tyrosine kinase receptors: *EGFR* (also known as HER1 and ERBB1), *HER2* (also known as ERBB2 and NEU), *HER3* (also known as ERBB3) and *HER4* (also known as ERBB4). They have a crucial role in the physiological regulation of proliferation, differentiation and survival of epithelial cells and have been implicated in oncogenesis in epithelial cancers. The extracellular domain of these cell surface receptors is an ideal target for antibody therapies and the intracellular kinase domain can be targeted by small-molecule drugs¹⁵.

HER2-targeted drugs: trastuzumab. In 1998, trastuzumab (Herceptin; Genentech/Roche), a monoclonal antibody (mAb) specific for HER2, became the first approved drug targeting a member of the EGFR family. Its considerable success has provided encouragement for the development of further targeted drugs. HER2 is over-expressed in 25–30% of invasive ductal breast cancers¹⁶, and high HER2 expression is associated with increased proliferation of tumour cells and poor prognosis¹⁷. The developers of trastuzumab calculated that, without pre-selection of HER2-positive patients, it would have required too many patients and too much follow-up time to demonstrate a statistically significant clinical benefit with a HER2-targeted therapy. To select HER2-positive patients, the obvious and most attractive method was IHC, which directly detects the target protein, is fast and cost-efficient and can be performed in standard pathology laboratories¹⁸.

Table 1 | *In vitro* methods of companion diagnostics for EGFR-targeted drugs

Technique	Principle	Advantages	Disadvantages
Immunohistochemistry (IHC)	Quantitative analysis of the target protein	<ul style="list-style-type: none"> • Simple protocol • Low cost 	<ul style="list-style-type: none"> • EGFR protein expression in epithelial cancers is not tumour-specific • Evaluation is sensitive to variations between laboratory protocols and medical examiners
Fluorescence <i>in situ</i> hybridization	Quantitative analysis of the target gene	<ul style="list-style-type: none"> • Tumour-specific • Allows for objective evaluation 	<ul style="list-style-type: none"> • Complex protocol • Higher costs than IHC
Gene sequencing	Qualitative analysis of the target gene sequence	<ul style="list-style-type: none"> • Tumour-specific • Allows for objective evaluation 	<ul style="list-style-type: none"> • Complex protocol • Higher costs than IHC

EGFR, epithelial growth factor receptor.

Importantly, all normal breast epithelial cells express HER2, and the 'HER2 IHC positivity' is a quantitative rather than a qualitative assessment. Using cell lines, it was possible to establish a standardized IHC procedure and scoring system in which cells containing fewer than 20,000 receptors would show no staining (0), cells containing approximately 100,000 receptors would show partial membrane staining with less than 10% of the cells showing complete membrane staining (1+), cells containing approximately 500,000 receptors would show light-to-moderate complete membrane staining in more than 10% of the cells (2+), and cells containing approximately 2,300,000 receptors would show strong, complete membrane staining in more than 10% of the cells (3+). This scoring system correlates with gene copy number^{18–22}. This also indicates that simply detecting the 'target' protein is not sufficient to prove it is pathologically overexpressed and that the tumour is dependent on the putative drug target. In addition, such a quantitative system is clearly sensitive to the technical details of the IHC protocol.

Trastuzumab is now approved for the first-line treatment of HER2-positive metastatic breast cancer in combination with chemotherapy, on the basis not only of a higher response rate than with chemotherapy alone, but also a significantly longer overall survival²³. As a single agent, trastuzumab achieved 26% (18–35%) clinical response in HER2-positive breast cancer, which was similar to results with chemotherapy²⁴. However, trastuzumab is also approved as a single agent for patients with HER2-positive metastatic breast cancer that has progressed after chemotherapy, for which it showed a 15% response rate²⁵. Although there was no control arm in these

trials to prove that trastuzumab is ineffective in HER2-negative patients²⁴, there was a good correlation between HER2 IHC positivity and clinical response. In patients with 3+ IHC staining, there was an 18–35% response rate, whereas the response rates for 2+ cases was 0–6% (REF. 24).

Some consider that inter-laboratory differences in tissue processing and scoring make the IHC measurement too ambiguous a marker on which to base an important clinical decision, and have proposed that fluorescence *in situ* hybridization (FISH) should be used instead^{26,27}. In addition, *HER2* gene amplification (in contrast to HER2 protein expression) is cancer-specific (TABLE 1). The rationale for the FISH proposal was further reinforced by a retrospective analysis of trastuzumab trials, in which response was seen exclusively in FISH-identified HER2-positive patients²⁸. There is an 89% overlap between the HER2 IHC positive (3+) breast cancers and gene amplification²⁹. However, 24% of 2+ cases were FISH-positive. The clinical response rates of 3+ and FISH-positive patients were very similar²⁹. As FISH is a slower, more expensive method than IHC, the current recommendations of the medical society guidelines (the National Comprehensive Cancer Network and the American Society of Clinical Oncology) on HER2 testing indicate that 3+ IHC-positive results can be the basis of trastuzumab therapy, whereas 2+ cases should be confirmed by FISH analysis^{28,30}. However, given that clinical response has been seen only in patients with gene amplifications, the cost saved in no longer treating the 2–4% of false-positive patients, together with the cost of IHC in all patients, should easily cover the cost of the more expensive FISH method as the exclusive method of HER2 testing,

as has also been suggested by others³¹. In addition, the few patients with false-negative IHC tests but FISH-positive tumours could be identified.

In summary, the success of trastuzumab was partly due to good practice in the drug discovery process, but also due to various more fortunate aspects. The first was that, in breast cancer, the 3+ HER2 protein expression level determined by IHC correlates well with the gene amplification that is a tumour-specific molecular change. The gene level amplification is a reliable sign of pathological deregulation of gene expression, which can lead to the HER2 dependence of the tumour cells.

The second fortunate aspect was that, in a sufficiently high percentage (18–35%) of these HER2-positive tumours, tumour growth was dependent on HER2 activity and responded to the therapy, and this response rate was sufficiently high to achieve statistically significant benefit in the treatment arm of the clinical trials. Interestingly, however, the significance of the high percentage of non-responders does not seem to have been sufficiently considered in the development of other molecularly targeted agents.

The third fortunate aspect was that, although 'HER2-negative' patients were not included in most of the clinical trials, it seems that the response rate of HER2-negative patients is low, and so it is acceptable to exclude them from potentially toxic trastuzumab therapy. Nevertheless, there is evidence that some HER2-negative patients may benefit from trastuzumab owing to a putative anti-angiogenic effect against endothelial cells³², but these observations have not been validated in large numbers of patients. It is also difficult to collect data on such patients, as trastuzumab was approved for routine clinical use only in HER2-positive patients.

A second HER2-targeted drug was approved in 2007: the small-molecule tyrosine kinase inhibitor (TKI) lapatinib (Tyverb; GlaxoSmithKline), which targets both HER2 and EGFR³³. The strategy for its development was to target the same HER2-amplified breast cancer population as trastuzumab, rather than select a smaller but theoretically more responsive population by using predictive diagnostics. The scientific rationale included factors such as the potential advantage of lapatinib in HER2-amplified tumours in which the extracellular domain of the receptor is truncated and therefore resistant to HER2-specific antibodies, and the potential to target brain metastases³⁴.

Overall, the success of trastuzumab, as well as that of imatinib, helped create a false perception that, if there is a validated target for an anticancer drug, all that is needed for success in clinical development is to select patients on the basis of target presence and/or overexpression, or that patient selection is not necessary if the target is present and/or overexpressed in the majority of patients. This perception was a key factor in the failures and controversies associated with several other drugs designed to target EGFR.

EGFR-specific mAbs: cetuximab and panitumumab. EGFR is the major growth factor receptor of epithelial cells and has been a promising target of cancer therapies since the discovery of its growth-stimulating effect on A431 human epithelial cancer cells³⁵. According to several reports, EGFR is 'overexpressed' in 60–80% of colon and lung cancers³⁶. Cetuximab (Erbix; Bristol-Myers Squibb/Lilly) was the first humanized EGFR-specific mAb developed to treat cancer, and was granted accelerated approval by the US Food and Drug Administration (FDA) for the treatment of colorectal cancer in 2004. The preclinical and clinical development of cetuximab followed an analogous approach to that of trastuzumab, in this case selecting patients that had tumours expressing EGFR instead of HER2 (REF. 37). A standardized IHC test was developed (the PharmDx kit, developed by Dako) as the clinical trial assay to select patients with colon carcinoma expressing EGFR in at least 1% of the cancer cells in their tumour tissue sample. So, the marketing approval of cetuximab was based on clinical trials in which the efficacy of the drug was demonstrated in patients with colon carcinomas that stained positive for EGFR with this IHC test. The response rate was 8.8% as a monotherapy in irinotecan-resistant patients and 22.9% in combination with irinotecan^{13,14}.

However, retrospective analysis of the trials showed that the overall response rate did not correlate with the level of EGFR, in contrast to the case with trastuzumab and HER2. Patients with 1% IHC positivity had similar response rates to patients with 100% staining^{13,14}. A control group of patients with negative IHC staining was not included in these trials. Nevertheless, these studies were considered acceptable by many researchers and by the FDA as a basis for cetuximab to be approved only for patients who are positive for this particular IHC test. This is also despite a surprising report indicating that 25% of IHC-negative patients can benefit from cetuximab therapy³⁸.

These controversies — in contrast to the case with trastuzumab — triggered a search for more reliable diagnostic biomarkers. Retrospective analyses have revealed such biomarkers that statistically significantly predict response to cetuximab. Moroni *et al.* found that 8 out of 9 responders had a high *EGFR* copy number determined by FISH compared with only 1 out of 21 non-responders ($p < 0.0001$)³⁹. Lievre *et al.* found high *EGFR* copy number in 3 out of 11 responders compared with none in the non-responders among 30 patients with colon cancer⁴⁰. It seems from these studies that FISH analysis or another molecular diagnostic method that detects high *EGFR* copy number could be suitable for a positive selection of patients who are likely to respond to cetuximab. Data from one study indicated that only 11.5% of colorectal cancers ($n = 147$) have increased gene copy number⁴¹. However, the response rates were higher than 11.5%, and so markers are needed that do not exclude patients who might benefit from cetuximab therapy despite normal *EGFR* copy numbers.

As the level of either the target protein or gene were not sufficient biomarkers for cetuximab response, investigators started to analyse the problem from a signal transduction perspective, focusing on the downstream signalling molecule *KRAS*. Approximately 36–50% of colon carcinomas harbour oncogenic mutant *KRAS*, which keeps *KRAS* constitutively active independently of signals from upstream growth factor receptors such as EGFR⁴². Lievre *et al.* found that, of the 11 out of 30 patients who responded to cetuximab, none had *KRAS* mutations, in contrast to 13 out of 19 of the therapy-resistant tumours ($p = 0.0003$)⁴⁰. Patients with wild-type *KRAS* also lived longer ($p = 0.016$; 16.3 versus 6.9 months). Meta-analysis of the studies of Moroni and Lievre reveals that 91.3% of patients with *KRAS* mutations did not respond to therapy, whereas 50% of patients with wild-type *KRAS* were responders ($p = 0.001$). The only patient with a complete response had wild-type *KRAS* and the highest *EGFR* copy number (greater than 20). In summary, 10% of colon carcinoma patients who have increased *EGFR* copy number had nearly 100% response to cetuximab and 60% of patients who have wild-type *KRAS* had 50% response (FIGS 1, 2, 3).

In a Phase II trial known as OPUS and a Phase III trial known as CRYSTAL, cetuximab was combined with first-line chemotherapy (fluorouracil, leucovorin and oxaliplatin, or irinotecan, fluorouracil and

leucovorin, respectively)^{43,44}. In these trials, clinical benefit was restricted to the wild-type *KRAS* population. Surprisingly, in both trials there was a trend towards a worse response to chemotherapy plus cetuximab than to chemotherapy alone in patients with *KRAS* mutations, which suggests that cetuximab might cause harm in this patient population.

However, the results above indicating the importance of *KRAS* status in response to cetuximab were not generally accepted in clinical use until recently, because they were based on retrospective analysis of clinical samples and preclinical research, and not on prospective randomized clinical trials. Clinicians therefore continued to use a test that was approved by the FDA based on a clinical trial that had design flaws — namely that the diagnostic test itself was not controlled and randomized. Four years after the first reports on the importance of *KRAS* mutations in cetuximab response, this molecular test has now been widely accepted by the scientific community and regulatory authorities. The European Medicines Agency (EMA) integrated *KRAS* testing as a mandatory condition for the administration of cetuximab in May 2008, and the same requirement was introduced by the FDA in July 2009. Unfortunately, IHC testing for EGFR expression is still required, which could continue to unnecessarily exclude patients from treatment.

The efficacy of cetuximab was also investigated in patients with non-small-cell lung cancer (NSCLC). In a Phase III trial known as FLEX, the addition of cetuximab to platinum-based chemotherapy marginally, but significantly, increased the overall survival from 10.1 months to 11.3 months⁴⁵. The patient selection was again only based on EGFR IHC positivity. This small survival advantage was sufficient for the FDA to register cetuximab for this new indication, but the EMA's Committee for Medicinal Products for Human Use adopted a negative opinion on its approval. This decision indicates that regulators may no longer consider mathematically significant but very modest improvements in survival to be sufficient for market approval. The solution is to identify the subgroup of patients in which clinically meaningful benefit can be shown using improved biomarkers.

In the SWOG 0342 Phase II study of cetuximab in patients with NSCLC, both progression-free survival (6 months versus 3 months) and overall survival (16 months versus 8 months) were significantly longer in patients shown to be EGFR-positive by FISH. However, in this trial, all patients received cetuximab in combination with

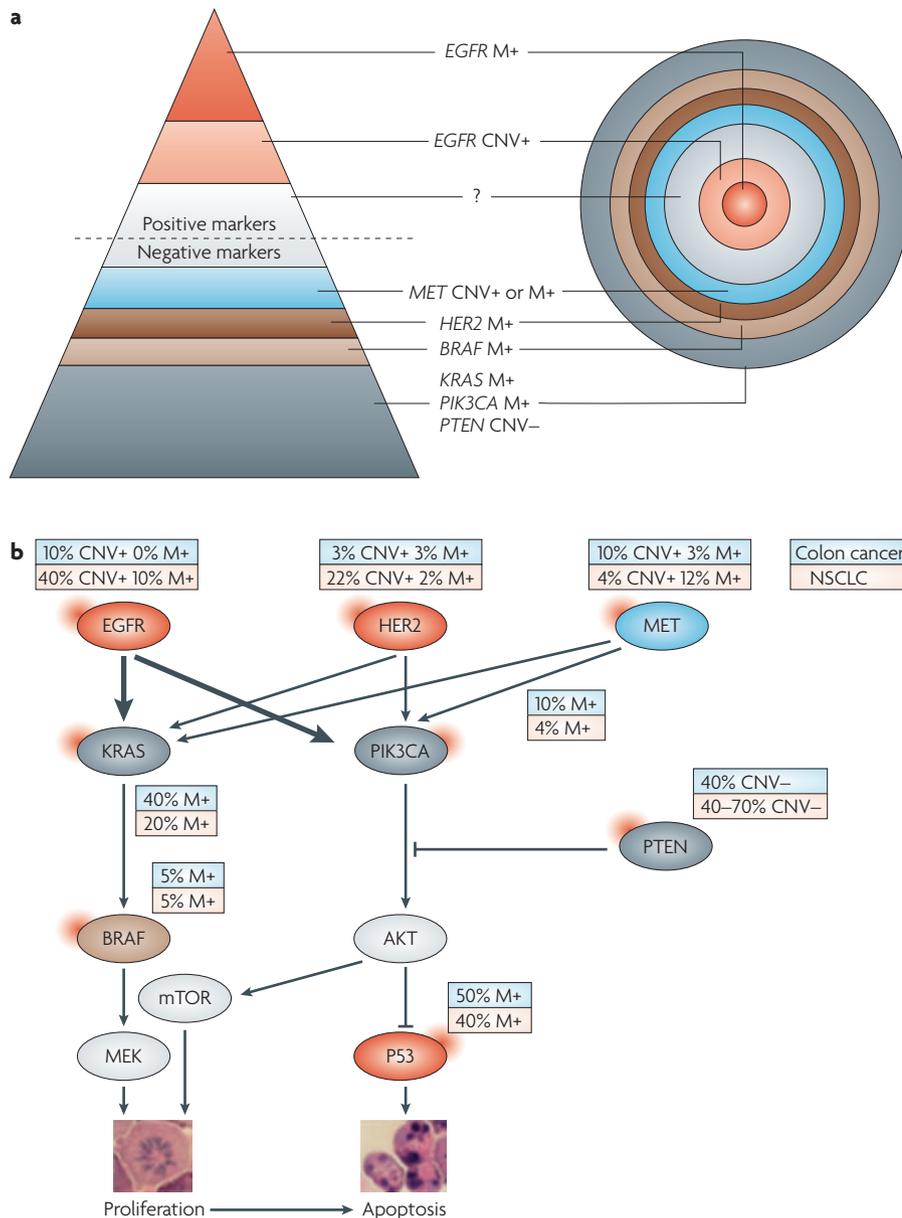


Figure 1 | Molecular mechanism of EGFR signal-dependence in epithelial cancers. Epidermal growth factor receptor (EGFR) inhibitors are only effective if the proliferation and/or survival of the cancer cells are dependent on constitutive EGFR signalling. The level of the oncogene dependence and the predicted efficacy of related targeted therapy can be illustrated by a pyramid (a), in which the upper part shows positive markers and the lower part shows negative markers. Mutations of genes that encode growth factor receptors and components of the downstream signal transduction pathways (b) determine the dependence on EGFR activity. The frequency of copy number variations (increase: 'CNV+' or loss: 'CNV-') or mutations ('M+') of these genes in colon cancer (shown in blue) or in non-small-cell lung cancer (NSCLC; shown in pink) is indicated. Activating mutations of the *EGFR* gene are associated with the highest objective response rate to EGFR-targeted tyrosine kinase inhibitors (TKIs) in lung cancer⁷⁴, but also in one case of ovarian cancer¹⁰⁹. High gene copy number is a predictive marker of good response to EGFR-targeted TKI therapy in lung cancer⁷⁴ and glioblastomas⁹², and EGFR-specific antibody therapy in colon cancer³⁹⁻⁴¹. Alternative signalling from activated receptor tyrosine kinases, such as mutant human epidermal growth factor receptor 2 (HER2; also known as ERBB2)⁹⁷ or amplified MET⁹⁹, decreases the EGFR dependence and therapeutic response. The other important cause of resistance to EGFR inhibitors is the receptor-independent activation of the KRAS-RAF pathway^{40,63,72,86} or the phosphoinositide 3-kinase (PI3K)-phosphatase and tensin homologue (PTEN)-protein kinase B (AKT)⁸⁸⁻⁹¹ pathway by mutations in, or hypermethylation of, the genes coding for the signal transduction proteins downstream of the receptor. Genes responsible for the regulation of apoptosis, such as P53, may also be important in the clinical response to novel therapeutics⁹⁶. MEK, mitogen-activated protein kinase-extracellular signal-regulated kinase kinase; mTOR, mammalian target of rapamycin; PIK3CA, phosphoinositide 3-kinase, catalytic subunit, α -polypeptide.

chemotherapy, and so a potential correlation between cetuximab response and high *EGFR* copy number could not be evaluated⁴⁶. A biomarker-based evaluation of the Phase III BMS 099 trial, which investigated a taxane and carboplatin with or without cetuximab as first-line therapy, did not find longer survival or progression-free survival in patients with FISH-identified EGFR-positive tumours who were treated with cetuximab than in those who were treated with chemotherapy alone⁴⁷. Interestingly, there was also no correlation between cetuximab response and the presence of *KRAS* mutations in this trial, in agreement with preliminary reports on the biomarker analysis of the FLEX study⁴⁸. The failure of the two most logical biomarkers (*EGFR* copy number and *KRAS* mutation) to predict the response to cetuximab in NSCLC might be related to a mechanism that is independent of the EGFR pathway or simply to poor efficacy in the selected patient population.

At the end of 2006, the FDA also approved a fully human EGFR-specific mAb, panitumumab (Vectibix; Amgen) for the treatment of patients with colon carcinoma. The pivotal open-label, randomized study (involving 463 patients) for this drug used the same IHC kit and inclusion criteria of 1% EGFR positivity as was the case with cetuximab⁴⁹. The overall response rate in the trial was 8% as monotherapy in previously chemotherapy-resistant patients, and the mean progression-free survival was 96 days in the panitumumab arm compared with 60 days in the control arm, in which patients received best supportive care. There was no correlation between the clinical benefit and the level of EGFR expression, similarly to cetuximab, but again an EGFR IHC test must be positive for the indication of panitumumab, as there are no data on the efficacy of panitumumab in IHC-identified EGFR-negative patients⁴⁹. Retrospective biomarker analysis revealed that — similarly to cetuximab — patients with *KRAS* mutations do not benefit from panitumumab therapy, and therefore the FDA have now included this test in the drug label. In Europe, given the evolving data on the importance of *KRAS* mutations, the EMA approved panitumumab only for patients with wild-type *KRAS* tumours, in December 2007.

EGFR-specific TKIs: gefitinib and erlotinib. As 50–80% of NSCLC cases reportedly express the EGFR protein³⁷, it was expected that an efficient EGFR inhibitor would have anticancer activity in the majority of patients with NSCLC (with no selection needed), which stimulated considerable efforts to

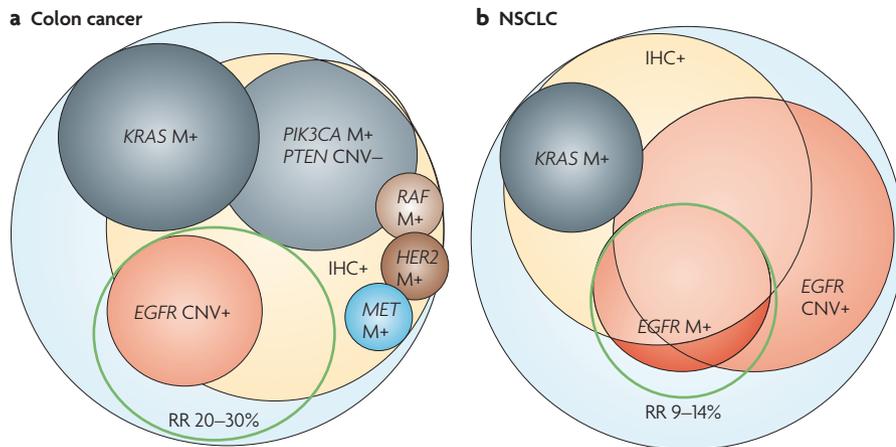


Figure 2 | Prevalence of predictive biomarkers of response to EGFR inhibitors. The epidermal growth factor receptor (EGFR)-specific antibody therapy cetuximab in colon cancer (a) and the EGFR-targeted tyrosine kinase inhibitors erlotinib or gefitinib in lung cancer (b) only induce an objective response in a fraction of patients (green circles). Genetic alterations responsible for differences in drug sensitivity are similar in both tumour types, but the prevalence of these mutations is different, which is indicated by the size of the circles. Mutations ('M+') in *KRAS*^{40,63}, *RAF*⁸⁶, human epidermal growth factor receptor 2 (*HER2*; also known as *ERBB2*)⁹⁷, *MET*⁹⁹ or in the phosphoinositide 3-kinase (PI3K)-phosphatase and tensin homologue (PTEN) pathway predict resistance^{88–91}, whereas high copy numbers of the *EGFR* gene determined by fluorescence *in situ* hybridization (orange circles; 'CNV+') analysis in colon cancer^{39–41} or activating *EGFR* mutations in lung cancer predict objective response in most of the cases^{64,74}. EGFR immunohistochemistry (yellow circles; 'IHC+') is an unreliable predictor of response rate (RR) in both tumour types, as it does not correlate with tumour response^{38,64,74}.

develop such drugs³⁷. The first two EGFR TKIs to reach clinical trials were gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; Roche), which have a similar chemical structure and both compete with ATP for binding to the catalytic domain of EGFR⁵⁰.

Response rates to gefitinib as a second- or third-line monotherapy for patients with NSCLC were only 18.4% and 11.8%, respectively, in the IDEAL1 and IDEAL2 Phase II trials^{51,52}. Nevertheless, the data were sufficient to obtain accelerated approval by the FDA in May 2003. However, gefitinib failed to achieve a significant overall survival benefit in subsequent Phase III trials both in combination with chemotherapy (in a trial known as INTACT I-II)^{2,3} and as monotherapy (in a trial known as ISEL)⁵³. Therefore, gefitinib was withdrawn from the US market except for patients already taking and responding to the therapy, and the application for the European marketing authorization was also withdrawn in 2005 (REF. 54).

Meanwhile, erlotinib also failed to provide a survival benefit as a third-line treatment in combination with chemotherapy for patients with NSCLC in two Phase III trials, TALENT⁵⁵ and TRIBUTE⁵⁶. However, in the placebo-controlled Phase III trial known as BR.21 in patients with locally advanced or metastatic NSCLC who had failed at least one chemotherapy regimen, there was a

moderate but significant (6.7 months versus 4.7 months) survival benefit from erlotinib, although the response rate was also small (8.9%)⁵⁷. On the basis of the significant survival benefit, in November 2004 the FDA approved erlotinib for the second- and third-line therapy of NSCLC patients, without a link to a biomarker.

In summary, both gefitinib and erlotinib produced a treatment response in a surprisingly small population of NSCLC patients, although these patients often experienced complete remission and prolonged tumour-free survival⁵⁷ (see [Supplementary information S1](#) (box)). A search therefore began for a reliable biomarker to identify these patients. A second question was why erlotinib was more successful in the placebo-controlled Phase III BR.21 trial than was gefitinib in the similar ISEL trial.

In the spring of 2004, three independent groups reported that, based on retrospective biomarker analysis, activating mutations in the tyrosine kinase domain of *EGFR* were associated with good clinical response to EGFR-targeted TKIs^{9–11}. All of these papers reported 100% response in *EGFR*-mutant patients, in contrast to only a few percent in the wild-type patients. These reports were followed by numerous independent studies that confirmed these results. Overall, in the 19 reports, the average response rate in the *EGFR*-mutant

patients was 82%, compared with 11.5% in the wild-type patients⁵⁸. Nine of these studies also provided data that overall survival is significantly longer in *EGFR*-mutant patients treated with an EGFR-targeted TKI (average of >2 years) than in wild-type patients (average of 8 months)⁵⁸. The sensitizing mutations clustered in exons 18, 19 and 21 of *EGFR*^{6,59} and were associated with adenocarcinoma histology, non-smoking history, Asian race and female gender^{6,59}. The frequencies of mutations in lung adenocarcinomas were 22–67% in Asia, 3–25% in North America and 10–24% in South Europe^{7,9–11,58,60–62}. Thus, the frequency of mutations corresponds to the frequency of objective tumour responses to EGFR-targeted TKIs.

Retrospective biomarker analysis of the BR.21 trial also found a higher response rate in *EGFR*-mutant patients, although this was only 16% compared with 7% in wild-type patients, and there was no correlation between survival benefit and mutation status¹². However, the tests were not initially confirmed in independent PCR reactions, and a re-analysis reported a response rate of 27% in patients with mutant *EGFR*, although this is still less than the average reported in the literature⁶³.

With regard to other potential biomarkers in BR.21, survival among patients with EGFR protein overexpression (50–55%) was longer in the erlotinib group than in the placebo group, but there was no survival advantage among patients with IHC-identified EGFR-negative tumours. The *p* value, however, for the interaction between IHC and treatment response was 0.25, which indicates a low statistical power. The FISH analysis provided stronger predictive value, but was still not significant (*p* value for interaction = 0.1)¹².

On the basis of these results, as noted above, erlotinib received market authorization in the United States without being linked to a biomarker. Interestingly, in Europe, the market authorization includes a note: "In EGFR-negative cases, the efficacy is not proven". It is not specified, however, how EGFR positivity should be determined. The easiest test is IHC, but this has the lowest predictive value — far from being significant according to the BR.21 study. The concern is that, without patient selection or by favouring the cheaper and more frequently positive biomarker, oncologists are increasingly hesitant to indicate this treatment for their patients owing to the modest clinical responses of IHC-positive patients. Furthermore, there is evidence that patients with activating mutations and gene amplifications are not all IHC positive⁶⁴ (FIGS 1, 2). *In vitro* results also suggest that the

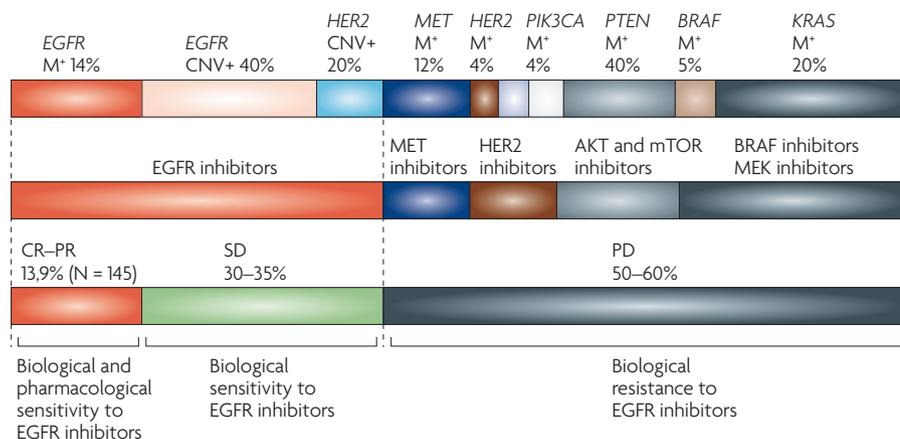


Figure 3 | Personalized therapy for lung adenocarcinomas. The upper horizontal bar represents genes affected by copy number variation (CNV) or mutations and their frequency. The middle bar indicates the target of potential signal transduction therapies associated with the particular molecular subtype. The lower bar indicates the clinical responses to epidermal growth factor receptor (EGFR)-targeted tyrosine kinase inhibitors (TKIs) (as determined in the BR.21 trial) and the possible association with the different biomarkers⁵⁷. Activating mutations of *EGFR* increase the dependence on EGFR and lower the threshold of effective drug concentration of the EGFR-targeted TKI⁷⁴. CNV gain of *EGFR* only increases the biological sensitivity to monoclonal antibodies (mAbs) targeting *EGFR*⁴⁶ and *EGFR*-targeted TKIs¹². CNV gain of *HER2* increases the sensitivity to EGFR-targeted TKIs¹⁰⁰, but decreases response to EGFR-specific mAbs⁹⁷. Activation of downstream signalling molecules confers resistance to EGFR inhibitors, but may provide an ideal target for other therapies^{40,63,72,86,97,99}. Conversely, activation of EGFR decreases the effectiveness of drugs targeting downstream signalling molecules¹⁰². AKT, protein kinase B; CR, complete response; HER2, human epidermal growth factor receptor 2 (also known as ERBB2); MEK, mitogen-activated protein kinase-extracellular signal-regulated kinase kinase; mTOR, mammalian target of rapamycin; PD, progressive disease; PR, partial response; PIK3CA, phosphoinositide 3-kinase, catalytic subunit, α -polypeptide; PTEN, phosphatase and tensin homologue; SD, stable disease.

molecular genetic alterations can sensitize cancer cells to EGFR-targeted TKIs independently of the level of protein expression⁶⁵. Therefore, using IHC-identified EGFR expression to select patients might exclude those who benefit most from erlotinib treatment.

The data described indicated that *EGFR* mutation testing has considerable potential as a biomarker for response to EGFR-targeted TKIs in NSCLC. However, critics noted that these studies were based on retrospective analysis, and furthermore that they compared the clinical outcome of *EGFR*-mutant and wild-type patients all treated with EGFR-targeted TKIs and so could not clearly show whether *EGFR*-mutant patients have a better prognosis regardless of the type of treatment. The breakthrough in the application of *EGFR* mutation analysis to guide treatment in NSCLC did not come until recently. In July 2009, the EMA approved the use of gefitinib for patients with NSCLC carrying activating *EGFR* mutations, in all treatment lines. This decision was based on the biomarker analysis of a randomized Phase III trial known as IPASS⁶⁶.

IPASS evaluated the efficacy, safety and tolerability of gefitinib versus carboplatin and paclitaxel as first-line treatment in a clinically selected population of Asian patients. The trial enrolled 1,217 patients from 9 Asian countries who were light smokers or had never smoked, and had been diagnosed with stage IIIB–IV adenocarcinoma NSCLC. Although the trial reached its target of proving the superiority of gefitinib in this clinically selected population, the biomarker analysis revealed that this advantage is restricted to patients with *EGFR* mutations. *EGFR* mutations were found in 60% of the 437 patients whose tumour samples were available for analysis. The progression-free survival in *EGFR*-mutant patients was 9.5 months with gefitinib treatment versus 6.3 months with carboplatin–paclitaxel treatment, whereas in mutation-negative patients, the progression-free survival in the gefitinib arm was only 1.5 months compared with 5.5 months when treated with chemotherapy⁶⁶.

The approval of gefitinib for *EGFR*-mutant NSCLC patients in all treatment lines was also based on the results of another Phase III

trial known as INTEREST, which showed that gefitinib is not inferior to docetaxel in previously treated patients⁶⁷. The biomarker analysis found greater progression-free survival and response rates in *EGFR*-mutant and in high *EGFR* copy number patients than in patients with wild-type *EGFR*, but this difference between the biomarker subgroups was not observed with regard to overall survival, probably because many patients were subsequently treated with both drugs⁶⁸. Although *EGFR*-mutant patients seemed to have a better prognosis independent of the type of treatment, they benefit much more from gefitinib than chemotherapy. It is also striking that chemotherapy is more effective than gefitinib in patients with wild-type *EGFR*. These data indicate that an *EGFR* mutation test is crucial in optimizing the treatment of NSCLC with gefitinib.

Questions for the future include: why erlotinib provided a survival benefit in an unselected population; whether patients with wild-type *EGFR* benefit more from erlotinib than from chemotherapy (in contrast to gefitinib); whether erlotinib will also be registered for the first-line therapy of *EGFR*-mutant NSCLC; and which drug is better for *EGFR*-mutant patients. One possible explanation for differences between erlotinib and gefitinib is that, although they have similar EGFR-inhibiting activity, their pharmacokinetics are different⁶⁹. In addition, erlotinib is used at the maximum tolerated dose, thereby providing higher plasma levels than with gefitinib, which is used at a dose 4–5 times lower than the maximum tolerated dose⁷⁰. Furthermore, both gefitinib and erlotinib have lower half-maximal inhibitory concentration (IC_{50}) values in biochemical assays with mutant *EGFR* than with wild-type *EGFR*⁷¹. So, considering their *in vivo* free plasma levels and these IC_{50} values, it can be speculated that gefitinib levels are only sufficient to block mutant EGFR, but not wild-type EGFR. By contrast, erlotinib also inhibits wild-type EGFR, which might explain why it could achieve some therapeutic effect (mostly stable disease) in another subgroup of patients carrying wild-type EGFR in whom this protein had a role in disease (for example, owing to gene amplification) (FIG. 3). However, the widespread inhibition of wild-type EGFR could also explain the almost twice as frequent skin and gastrointestinal side effects observed in patients receiving erlotinib compared with those receiving gefitinib³⁷.

Finally, similarly to cetuximab for the treatment of colon carcinomas, the presence of mutant *KRAS* is correlated with a lack of response to EGFR-targeted TKIs in the treatment of lung cancer⁷² (FIGS 1, 2). However, *KRAS* mutation is not a sufficient negative marker for erlotinib use, as its frequency is only 17–20% in adenocarcinomas⁷²; therefore ~80% of patients would in theory be treated based on this criterion, but only 50% of patients in clinical trials showed some evidence of disease control (objective response plus stable disease) due to erlotinib treatment⁵⁷. In addition, a recent study did not find a significant difference in the effect of erlotinib on progression-free survival for NSCLC patients with wild-type *EGFR* and *KRAS*, and those with wild-type *EGFR* and mutant *KRAS*⁷³. This means additional negative markers are needed to exclude patients who do not benefit from EGFR-targeted TKI treatment, which are discussed below (FIG. 3).

Post-marketing studies of EGFR inhibitors.

The IHC tests to detect EGFR and HER2 discussed above were approved based on trials that were uncontrolled from a diagnostics perspective, with response rates of around 9–30% in the selected population. Given that 25 independent reports found an average response rate of 72% and a time to progression of more than 1 year in NSCLC patients with *EGFR* mutations, but only an average 10% response rate and 2-month time to progression in *EGFR* wild-type patients⁵⁸, it is surprising that for 5 years *EGFR* mutation testing was viewed as scientifically interesting but not ready for clinical use⁷⁴. In this case, there seems to be an inconsistency in the standards by which the evidence associated with identification of patients who do or do not respond to a targeted therapy is judged.

For example, a single retrospective study of *EGFR* mutation status in archived tissues from patients from the BR.21 trial of erlotinib was considered sufficient to make the conclusion, “there is no need for mutation testing”¹². However, the importance of many other independent studies that reported that patients with *EGFR* mutations respond well to erlotinib have been downplayed as preliminary and retrospective; the concept of *EGFR* mutation only as an overall good prognostic marker instead of a marker for response is readily accepted from the same study, whereas previous studies that had a different conclusion are disregarded⁶. In response, new studies have been published to counter this report^{75,76}.

Methodological problems in the molecular diagnostic studies in paraffin-embedded tissues have been another pitfall in biomarker studies. This was at least the case in the initial retrospective biomarker analysis of the BR.21 trial of erlotinib^{12,63}. Recent recommendations of the European Association of Pathologists on *KRAS* molecular diagnostics suggested that not only the methods, but most importantly the laboratories, have to be accredited to perform this type of test⁷⁷. Owing to the many potential errors from the sample preparation (for example, lack of changing the blade on the microtome between each sample), the influence of chemical contaminants on the performance of DNA polymerases in PCR reactions and the stringency of quality control in general, even standardized, certified *in vitro* diagnostic assays do not remove the need for ‘clinical’ validation of the laboratories. The question is not only whether the results of a clinical trial can be explained by the biomarker analysis of one central laboratory, but which laboratory can most accurately predict the clinical effectiveness of a targeted anticancer drug in the relevant patient subgroups; for example, 100% prospective association between the clinical response to erlotinib and gefitinib and the presence of activating mutations in *EGFR* detected by a laboratory can provide this type of validation⁶⁴.

In summary, trastuzumab seems to be the most successful of the marketed drugs targeting the EGFR family. However, little is known about its possible off-target effects, and the predictive value of the companion diagnostic is far from perfect. This is an emerging problem, because recent results that have indicated the positive effects of 1-year adjuvant trastuzumab treatment in all HER2-positive patients⁷⁸ could place a large cost burden on health-care systems. There is therefore a need for better diagnostics for more precise patient selection. In the case of cetuximab and panitumumab, *KRAS* testing has now become more widely used in the clinic, but IHC testing for EGFR still has to be performed. IHC testing for EGFR was also used to select NSCLC patients in a trial that showed that adding cetuximab to chemotherapy resulted in a statistically significant improvement in survival, but registration was not approved by the EMA owing to the modest absolute benefit⁴⁵. After a 5-year delay, gefitinib has been approved in Europe for the treatment of *EGFR*-mutant patients. Erlotinib is approved for the second-line treatment of all NSCLC patients in both the United

States and in Europe, although in Hungary, for example, the state-owned health insurance only covers the treatment of patients with wild-type *KRAS* or mutant *EGFR*. This provides a precedent of a payer using a biomarker that is not in the label of the drug to increase the cost-effectiveness of a therapy.

Preclinical biomarker integration

In cancer cells, the expression of hundreds of genes can be different to that in normal cells. The key question is which genes are driving the cancer and promoting cancer cell survival, and which are accompanying signals. If we can identify the factors responsible for cancer cell survival in particular cancers (many of which are kinases), these should be excellent candidates for targeted therapy. Genes that are mutated or amplified in cancer cells are more likely to be survival factors than those which are only overexpressed. The identification of such mutated or amplified genes could form the basis for biomarkers. A combination of target-driven and chemistry-driven drug discovery with biomarker identification and linkage to companion diagnostics could be used to provide more personalized targeted therapy. By “chemistry-driven drug discovery”, we mean selecting drug candidates on the basis of cellular assays, and subsequent selectivity profiling and off-target identification (BOX 1), aided by pharmacophore models and the pool of inhibitors with known selectivity^{79–81}.

For the identification of potential predictive biomarkers that might form the basis of companion diagnostics for both small-molecule inhibitors and mAbs, the lessons learned with the first generation of EGFR inhibitors can be applied. The most important lesson is that the predictive marker of signal transduction therapies (BOX 2) is usually not equivalent to the expression of the target protein. Growth or survival of the particular tumour has to be dependent on the activity of the targeted signal transduction molecule. Recent genome-wide cancer genetics studies have identified hundreds of mutated genes, but only a few important ‘cancer driver’ signal transduction pathways⁸². The challenge for predictive diagnostics is to determine which pathway is the main driver of the malignant phenotype in the particular tumour sample and which drug is able to block this pathway. Activating mutations of *EGFR* seem to be single cancer drivers in the absence of other mutated oncogenes, which explains the high sensitivity of tumours harbouring these mutations to EGFR inhibitors⁸³. Over-activation of

Box 1 | Characterizing the target profile of small-molecule kinase inhibitors

The most obvious way to characterize the target profile of a kinase inhibitor is to perform kinase assays on a large panel of kinases. Other approaches are based on the proteomic identification of kinases to which the drug candidate binds with high affinity.

For example, our recently developed 'target fishing' technology (developed in close collaboration with the Max Planck Institute of Biochemistry (MPI)) relies on the coupling of a small-molecule kinase inhibitor to a matrix through a linker. The aim is to generate an affinity chromatography material that exploits the strong binding affinities between kinase inhibitors and their molecular targets. The technology is straightforward in principle, but requires specialized chemistry to link the kinase inhibitor and maintain its biological activity and a sensitive proteomic technology (provided by the MPI) to identify the bound proteins^{82,113–117}. Once coupled and proven active, immobilized ligands are processed in an affinity chromatography approach to identify all the relevant targets and off-targets from a crude extract, which bind to this matrix. All binders are subsequently cloned, expressed and purified (kinases and non-kinases). The affinities of the free ligands for the various enzymes are determined in relevant *in vitro* assays. Thus, quantitative binding can be determined and even the binding to non-kinases can be verified. As a proof-of-concept experiment for the technology, we used immobilized gefitinib to identify its off-targets and to obtain a better understanding of the pharmacology of gefitinib¹¹⁵. Mass spectrometry (MS) analysis of the identified protein spots and a parallel liquid chromatography–MS/MS analysis not only permitted detection of the internal control, epidermal growth factor receptor, as expected, but identified more than 20 additional gefitinib targets.

Other target fishing technologies have also been described in the literature¹¹⁸, which use a chemical proteomics approach to profile the interaction of small molecules with endogenously expressed protein kinases and purine-binding proteins. This subproteome is captured by immobilized nonselective kinase inhibitors (kinobeads), and the bound proteins are quantified in parallel by mass spectrometry using isobaric tags for relative and absolute quantification (iTRAQ). The technique is based on a competitive binding assay and will identify only the proteins that were pulled down by the broad-spectrum kinase inhibitors on the kinobeads¹¹⁹.

EGFR owing to mutations or increased gene copy number have been proved to be the best positive predictor of EGFR signalling dependence and therapeutic response to EGFR-targeted therapies (mAbs or TKIs) in colon and lung cancer^{38,40,64}.

In the absence of a key single driver oncogene, cancer typically involves the activation of several oncogenes⁸². In the case of inhibitors that specifically target a single receptor tyrosine kinase, activation of alternative growth factor receptors by amplification or activating mutations, or receptor-independent activation of downstream signalling molecules, almost always lead to drug resistance both *in vitro* and in a clinical setting⁸⁴, seemingly regardless of the tumour type and the signal transduction target⁸⁵. For example, EGFR inhibitors are ineffective in *KRAS*-mutant cells^{40,63} and *RAF*-mutant colon cancer⁸⁶, and *KRAS* inhibitors are ineffective in *RAF*-mutant cells⁸⁷. Furthermore, loss of the phosphatase and tensin homologue (*PTEN*) tumour suppressor gene or activating mutations in phosphoinositide 3-kinase, catalytic subunit, α -polypeptide (*PIK3CA*) increases the EGFR-independent activity of the protein kinase B pathway, which predicts resistance to EGFR inhibitors in lung cancer^{88,89}, in colon carcinomas^{90,91} and in glioblastomas⁹², similarly to trastuzumab resistance in

HER2-positive breast cancer⁹³. In addition, not only can anticancer cytotoxicity mediated by DNA-damaging agents be dependent on the executors of active cell death (mostly apoptosis), such as functional tumour protein P53 (REF. 94) and FAS⁹⁵, but so can that mediated by signal transduction therapeutics such as gefitinib⁹⁶.

Activation of alternative growth factor receptors also confers resistance to drugs targeting single tyrosine kinase receptors. For example, *HER2* amplification or overexpression of insulin-like growth factor 1 have been implicated in cetuximab resistance in colon cancer⁹⁷, and activating mutations in *HER2* have been implicated in gefitinib resistance in lung cancer⁹⁸. The amplification of *MET* leads to resistance to gefitinib in lung cancer⁹⁹. However, amplification of *HER2* increases gefitinib sensitivity through the activation of EGFR¹⁰⁰.

The development of predictive biomarkers can be integrated into preclinical anticancer drug discovery by using multiple cell lines to model typical molecular types of cancers. For example, the A431 cell line was first used for the discovery of EGFR inhibitors in bioassays as EGFR was discovered in these cells, in which *EGFR* is amplified and overexpressed¹⁰¹. However, most lung cancer cell lines express much lower *EGFR* levels, and some carry activating mutations and/or

drug resistance mutations in *EGFR* as well as mutations in genes encoding downstream signalling molecules such as *KRAS*, *RAF* and *PTEN*. Therefore, screening against many cell lines of the same histological origin may give information about the general effectiveness of the lead compound while providing a system to model drug sensitivity and resistance and develop molecular diagnostics.

This strategy should also be used for screening other targeted compounds. For example, novel inhibitors of MEK (mitogen-activated protein kinase–extracellular signal-regulated kinase) kinases are ineffective in all cell lines carrying *EGFR* mutations, effective in all cell lines carrying *RAF* mutations, but only effective in half of the cell lines with *KRAS* mutations¹⁰² (FIG. 1). The cancer specificity of the compounds could be determined using normal cell culture as controls, or syngenic synthetic lethality screens to identify compounds that are more effective in the presence of cancer-specific genetic changes¹⁰³.

Clinical biomarker integration

Aspects of clinical trial design can intentionally or unintentionally reveal or obscure the importance of biomarkers. First, increasing the number of patients enrolled in the treatment and control arms can ensure a statistically significant difference between the groups, even if only a subset of patients responds to therapy. For example, if a clinical trial of trastuzumab had been carried out enrolling four times more unselected patients with breast cancer than was necessary to prove efficacy in patients pre-selected by HER2 testing, the results would presumably have been sufficient to support the use of trastuzumab in treating all breast cancer patients without testing for a biomarker. Conversely, even if a novel targeted drug entering clinical trials is well characterized for target specificity and molecular mechanism of action, limiting patient recruitment in clinical trials on the basis of the detection of the target protein by IHC or by a diagnostic test that has not been validated in a controlled preclinical or clinical experiment, and without the information of the full target profile (selectivity panel) of the drug, risks the unjustified restriction of patient access to the drug. This might be more harmful than not selecting patients at all.

Recruitment into the trial of only those patients that have tumour samples for detailed retrospective molecular profiling may provide more useful information to establish a validated companion diagnostic test. If there is a preclinically validated companion diagnostic available, the clinical trial

Box 2 | Signal transduction therapy

The approval of molecularly targeted drugs such as trastuzumab and imatinib is often considered to represent a 'paradigm shift' in anticancer drug development. Nevertheless, it has been argued that classical anticancer agents such as the topoisomerase I inhibitor camptothecin, the topoisomerase II inhibitor etoposide or the thymidylate synthase inhibitor 5-fluorouracil are molecularly targeted, making the distinction artificial. Indeed, individual polymorphisms in these enzymes and enzymes responsible for the metabolism of these drugs can determine individual drug sensitivity and molecular diagnostics can be used to personalize therapy¹¹².

However, it is important to distinguish these therapeutic agents from novel signal transduction therapies. The classical anticancer drugs target the molecular apparatus of the execution of cellular functions such as mitosis or DNA repair, whereas the signal transduction therapies target the pathological signal transduction mechanisms that are responsible for the tumour-specific pathological regulation of these cellular functions. Therefore, for signal transduction therapies, it is often not enough to detect the presence of the drug-sensitive variant of a target protein; the proliferation or the survival of the particular tumour must be dependent on the abnormal activity of the particular target protein (FIG. 1). In other words, the signal transduction therapy targets the signalling pathway. For example, even if the gene encoding epidermal growth factor receptor (*EGFR*) is overexpressed, if downstream mutant *KRAS* maintains the *EGFR*-independent autonomic proliferation signal, *EGFR* inhibitors are not effective. This difference makes the development of companion diagnostics for signal transduction therapies more important and more complicated.

design can be optimized by selecting patients with this diagnostic method, but a biomarker-negative control arm should be included for the clinical validation of the companion diagnostics.

There are major technical and logistical challenges to integrating molecular biomarker tests into clinical trials and into routine clinical work¹⁰⁴. First, molecular pathology laboratories and their diagnostic technologies need to be sufficiently sensitive and robust to give a diagnosis from the majority of tissue samples. A guideline paper from the Society of European Pathologists recommends that only laboratories that can provide results with a success rate of more than 95% and an accuracy of more than 97%, within 2 weeks, should perform *KRAS* mutation testing⁷⁷. Quality control investigations should be carried out regularly to accredit laboratories for companion diagnostics. However, such investigations in Germany and Hungary, for example, have revealed that only a few laboratories can meet these standards at present, and therefore most oncology centres do not have such a laboratory in the same institute (see the [KRAS information](#) website).

A second question is whether the companion diagnostic test should be automatically integrated into the work flow of pathology laboratories. This would shorten the time to obtain the result of companion diagnostics, but may result in many unnecessary tests being performed. For example, the current typical practice in the case of colon carcinoma is that, after diagnostic work that includes staging with computed

tomography scans and histological diagnosis from endoscopic or surgical biopsy, a decision is made as to whether the patient should receive chemotherapy or only surgical treatment. If the patient has only liver metastasis, first-line *EGFR*-targeted (cetuximab) treatment should be considered¹⁰⁵, and the oncologist sends a request to the central molecular pathology laboratory accredited to perform *KRAS* testing. The molecular pathology laboratory then sends a request to the primary pathology department to send the specimen to the laboratory. After the test, the sample is sent back to the pathology laboratory (if there is tissue left) and diagnosis of the *KRAS* test is sent to the oncologist. The challenge is to organize this process within 2 weeks, which is considered the clinically acceptable time frame to start the therapy.

Besides the considerations for biomarker validation, molecular mechanisms of action should be given greater consideration in the clinical trials of future combination therapies. The accepted approach in Phase III trials is to compare the current standard treatment to the new therapy combined with the standard treatment, which theoretically minimizes the patients' risk of receiving less effective treatment than the best treatment that is currently available. However, knowledge of mechanism of action of the drugs and preclinical experiments can predict that some combinations could be antagonistic, thus potentially harming patients and risking the success of the experimental drug. For example, *EGFR* inhibitors induce cell cycle blockade, which increases the resistance

to chemotherapy¹⁰⁶. This effect may have contributed to the failure of erlotinib in combination with gemcitabine–cisplatin⁵⁵ or paclitaxel–carboplatin⁵⁶ and gefitinib in combination with gemcitabine–cisplatin² or paclitaxel–carboplatin³ in Phase III studies.

In addition, only randomized trials with two or more arms can uncover the difference between the prognostic (general clinical behaviour) and predictive (treatment-specific) value of molecular biomarkers. For example, the INTEREST study found a higher response rate to both gefitinib (42.1%) and docetaxel (21.1%) in *EGFR*-mutant NSCLC patients than in NSCLC patients with wild-type *EGFR* (6.6% and 9.8% respectively)⁶⁸. This indicates either that *EGFR* mutations are positive prognostic markers irrespective of the type of treatment or that *EGFR*-mutant tumours are more responsive to both treatments. The design of the trial enabled an important question to be answered — namely, which treatment is most beneficial to *EGFR*-mutant patients — as there was a significantly higher response to gefitinib than to docetaxel, and this was also reflected by significantly longer progression-free survival in gefitinib-treated *EGFR*-mutant patients. Conversely, in accordance with other reports and understanding of the *EGFR* signalling pathway, there was 0% response to gefitinib in *KRAS*-mutant patients. However, the response to docetaxel was also low in this patient population (3.7%) and there was no difference in progression-free survival and overall survival between *KRAS*-mutant patients treated with gefitinib or docetaxel. These results might indicate that *KRAS*-mutant patients do not benefit significantly less from gefitinib than from docetaxel, but potentially also have a worse prognosis and poor response to chemotherapy in general.

Preclinical hypothesis-based combinations and predictive biomarkers might be most effectively investigated in adaptive clinical trials. A good example is the BATTLE trial at the MD Anderson Cancer Center¹⁰⁷. In this trial, patients with NSCLC who had failed at least one prior chemotherapy regimen were enrolled in an umbrella trial in which they gave their consent for a biopsy from their tumour. The tumour sample was analysed for 11 pre-specified biomarkers and, based on the results, patients were classified into four groups (*EGFR* mutant, or high copy number type; *KRAS*–*BRAF* mutants; vascular endothelial growth factor receptor (*VEGFR*)-overexpressing type; and retinoid X receptor (*RXR*)-cyclinD activator). The patients were then randomized into

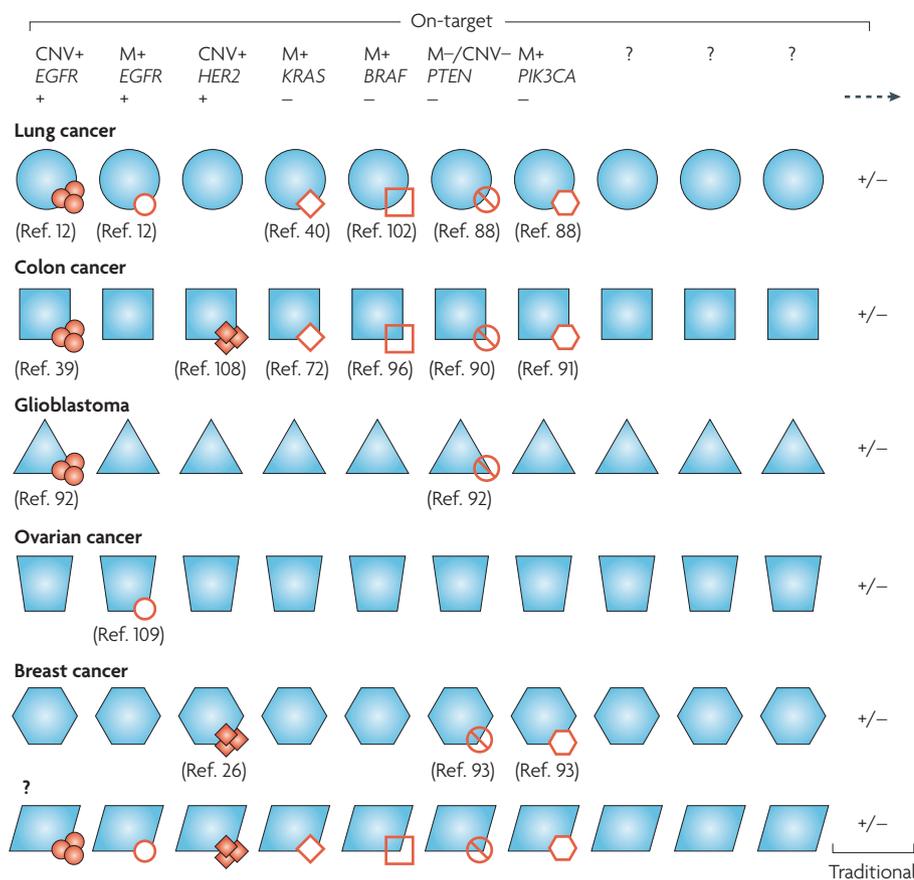


Figure 4 | On-target approach in the clinical development and registration of anticancer signal transduction therapies. In the traditional approach, clinical trials are designed to prove the efficacy of the drug in a certain tumour type. Signal transduction therapies (for example, epidermal growth factor receptor (EGFR) inhibitors) are only effective in a fraction of patients within one tumour type depending on the presence or absence of biomarkers (for example, increased copy number ('CNV+') of *EGFR* (full red circles) or human epidermal growth factor receptor 2 (*HER2*; also known as *ERBB2*) (red squares), or mutations ('M+') of *EGFR*, *KRAS*, *BRAF*, phosphatase and tensin homologue (*PTEN*) and phosphoinositide 3-kinase, catalytic subunit, α -polypeptide (*PIK3CA*). The effectiveness of these new anticancer drugs correlates more strongly with the presence of these biomarkers than with tumour type. Therefore, clinical trials could ultimately aim to validate these biomarkers in multiple tumour types, and drugs could be registered based on the presence of these markers. For example, the EGFR-targeted tyrosine kinase inhibitors erlotinib or gefitinib could be registered for all cancers that have *EGFR*-activating mutations, and the *HER2*-specific antibody trastuzumab could be registered for all cancers with *HER2* amplification. Blue shapes represent different tumour types, and red shapes represent different biomarkers.

four separate Phase II trials, in which they received a particular targeted therapy: erlotinib (inhibitor of EGFR), sorafenib (inhibitor of RAF, VEGFR and platelet-derived growth factor receptor), vandetanib (inhibitor of VEGFR, EGFR and RET), or erlotinib and bexarotene (an RXR activator). The primary end point was disease control at 8 weeks. Tumour responses were monitored in patients who had different biomarkers, and this information was used to validate the biomarker and adapt the randomization of patients subsequently enrolled on the basis of their biomarker profile. This design helps to clinically validate

biomarkers and minimize the number of patients who receive inappropriate treatment. Initial results recently presented at the 101st American Association of Cancer Research meeting (abstract LB-1) confirmed the hypothesis of this trial. For example, patients with *EGFR* mutations or amplification responded best to erlotinib, but did not respond to sorafenib, whereas patients with *KRAS* mutations tended to benefit more from sorafenib than the other three regimens.

Most clinical investigators agree that the best end point for clinical studies is overall survival. However, this is becoming

more difficult to evaluate as patients have more therapeutic choices if they progress on the experimental therapy. Furthermore, there is time pressure to register potentially life-saving drugs. New targeted drugs generally have less serious side effects than previous chemotherapies (which could shorten overall survival despite a good tumour response). However, it should be noted that there are examples in which a statistically significant but very modest survival benefit has been shown despite a low response rate, but this might no longer be sufficient for the registration of these drugs, as illustrated by the FLEX study of cetuximab in NSCLC⁴⁵. In our view, the aim of future drug discovery and development projects should be to identify patient groups in which the response rate to the targeted drug is at least 50% and the progression-free survival benefit is at least 1 year (see [Supplementary information S2](#) (table)). This extent of tumour response in combination with mild side effects might be sufficient to predict overall survival benefit for the patients in trials of novel targeted therapies.

We also consider that regulatory authorities should require the demonstration of a meaningful clinical benefit in a precisely defined patient population, and make their expectations clear to drug developers. Timely and careful preclinical co-development of companion diagnostics and innovative trial design could help reduce the time and cost of clinical trials and, instead of the involvement of large numbers of patients, biomarker-based adaptive randomization could become the standard approach for trials leading to registration. Results of retrospective biomarker analysis should also be accepted if the results are conclusive, as in the case of *EGFR* mutations and *KRAS* mutations.

Finally, the largest paradigm shift and potential solution to some of the challenges discussed above would be if clinical trials investigated the efficacy of a drug against a molecular target (or molecular profile) across several tumour types in which the same molecular profile occurs, rather than on the basis of the tissue of origin (FIG. 4). A plethora of targeted cancer drugs are being developed, and it will be impossible to test all of them in all cancer types in all possible combinations. The molecular target (or the sensitive molecular profile) will be present in a small fraction of cases in each tumour type, and it will be extremely costly and time-consuming to recruit enough patients with each tumour type to provide sufficient evidence of effectiveness for market approval

for each tumour type separately. For example, high *HER2* copy number (FISH positivity) is too rare (3–5%) in colon carcinomas to carry out a prospective clinical trial leading to a market authorization of trastuzumab for this indication, although in the few colon carcinoma patients with high *HER2* copy number, trastuzumab resulted in a good clinical response¹⁰⁸. However, if trastuzumab had been investigated and marketed for the treatment of carcinomas with *HER2* amplification, in theory it could have received market authorization for all *HER2*-positive tumours — not only for *HER2*-positive breast cancer, but also *HER2*-positive colon carcinoma.

The EGFR-targeted TKIs erlotinib and gefitinib have also been consistently effective in the presence of activating *EGFR* mutations regardless of the tumour type^{74,109}, and mutant *KRAS* has been proved to be a negative marker of EGFR-targeted therapies in all tumour types tested so far. An important question is therefore whether we can use the molecular evidence to rationalize the clinical use or clinical trials of current or novel EGFR inhibitors in tumour types for which these drugs have not been registered. The target profile of the signal transduction therapies determines the predictive molecular diagnostics, which are independent of the tumour type and the therapeutic agent. However, to select the appropriate predictive diagnostics, the target profile of these drugs must be known. For example, loss of PTEN in breast cancer does not confer resistance to the EGFR–*HER2* dual inhibitor lapatinib, in contrast to other EGFR inhibitors, which could be due to the multi-target inhibitory profile of this drug¹¹⁰. Molecular target-based registration of anticancer drugs will require screening for mutations in a large number of genes in each patient. Next-generation sequencing technologies might allow cost-effective, large-scale pharmacogenetic analysis of multiple inherited and acquired genetic variations in each individual tumour sample to provide cancer patients with the benefit of personalized genomics^{82,111}.

In conclusion, biomarker studies need to be prospectively integrated into the clinical trials of novel signal transduction therapies in a way that assesses the clinical relevance of the diagnostic test developed during the preclinical phase. In addition, the studies should retrospectively search for new markers. Target analysis can also be performed on the primary tumour samples of responders and non-responders, which might lead to the discovery of new targets by using chemistry-driven strategies. Targeted drugs that have

a properly developed predictive marker for personalized therapy may have a smaller target market, but would have a lower risk of failure owing to a lack of significant therapeutic effect in a diluted patient population, and also a greater competitive advantage for a longer period and a higher chance of coverage by insurance companies. Direct costs would be saved on smaller clinical trials and larger indirect costs could be saved by earlier market entry. Finally, these drugs would better serve the interest of patients by saving them from ineffective therapy and enabling new, more effective therapies to enter clinical development more rapidly to treat this patient population. This could also save health-care systems from the socio-economic and medical–ethical problems of promising but expensive therapies.

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Competing interests statement

The authors declare [competing financial interests](#): see web version for details.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/gene>

EGFR | KRAS

UniProtKB: <http://www.uniprot.org>

EGFR | ERBB2 | ERBB3 | ERBB4 | HER2

FURTHER INFORMATION

István Peták's homepage: <http://www.kps.hu>

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