TIMELINE

Gefitinib — a novel targeted approach to treating cancer

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Abstract | Twenty years after the epidermal growth factor receptor (EGFR) was identified as a potential anticancer target, the EGFR inhibitor gefitinib (Iressa; AstraZeneca) has been approved for the treatment of patients with advanced non-small-cell lung cancer in many countries. Studies have indicated its potential for treating patients with other types of solid tumours. Investigation of gefitinib has not only increased our knowledge about the biology of EGFR signalling, but is contributing to our evolving understanding of which tumours are EGFR dependent.

Greater understanding of the molecular basis of cancer[1] is fostering the development of novel targeted strategies that inhibit specific cancer pathways and key molecules in tumour growth and progression. Such agents, for the most part, spare normal cells and have the potential to be well-tolerated therapies, which will enable patients with cancer to live longer and have an improved quality of life. Growth-factor signalling pathways have been a main focus of research for novel targeted anticancer agents because of their fundamental role in regulating key cellular functions including cell proliferation, differentiation, metastasis and survival. An important mediator of growth-factor signalling pathways is the epidermal growth factor receptor (EGFR) — a 170-kDa glycoprotein[2] that is expressed in most human tissues and is highly expressed in many human solid tumours. It is a member of the human epidermal growth factor receptor (HER) family, and in normal cells it is involved in mediating the signalling pathways related to cell growth and proliferation. Gefitinib (Iressa; AstraZeneca), an inhibitor of EGFR's tyrosine-kinase (EGFR-TK) activity, is the first targeted agent to be approved for the treatment of patients with advanced non-small-cell lung cancer (NSCLC).

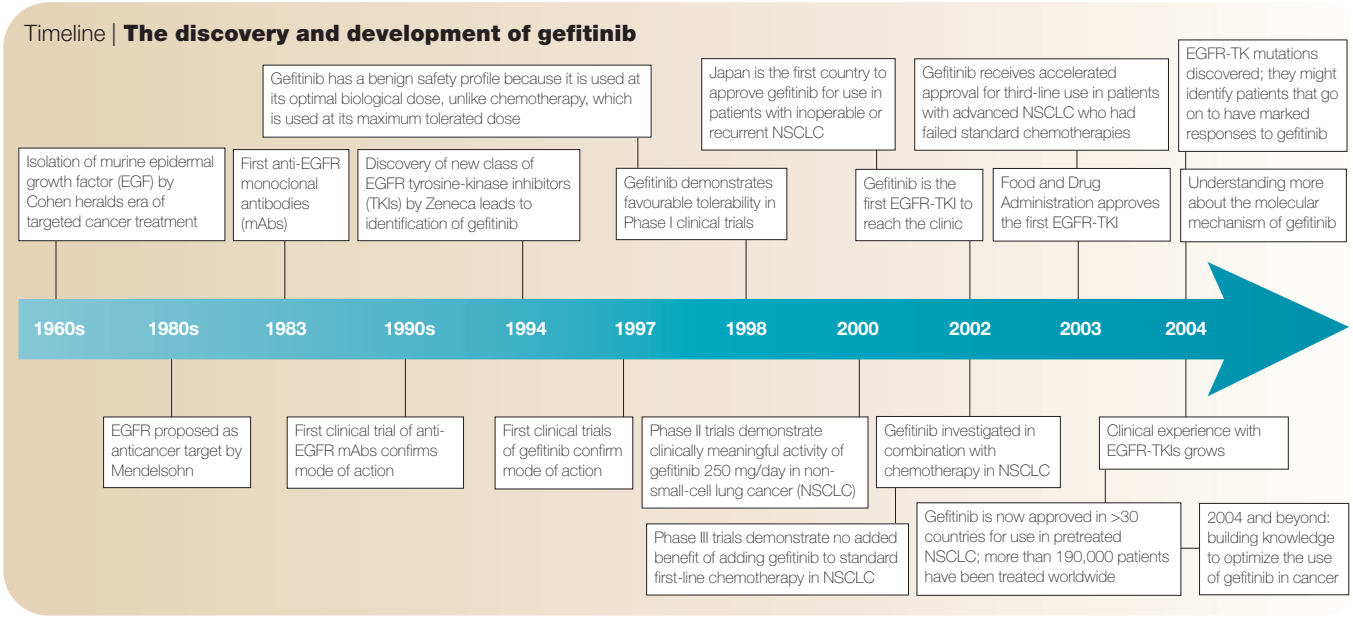
The rationale for EGFR as a potential target for anticancer treatment was based on the work of several research groups. The breakthrough discovery of EGF in mice was made by Stanley Cohen in the early 1960s[3] (TIMELINE). EGF was one of the first growth factors to be isolated and its discovery opened up a research field that has been crucial to the development of both modern-day anticancer and other medical treatments. A decade after his pioneering contribution, Cohen isolated human EGF[4] and Harry Gregory reported the isolation of human urogastrone[5]. Gregory compared the amino-acid composition of the two polypeptides and concluded it was likely that both substances were one and the same[5]. However, another 10 years passed before Cohen cloned and isolated the EGFR[6] and the link between EGFR and the malignant transformation of cells was demonstrated[7]. Research has shown that transformation occurs by an autocrine mechanism, involving autostimulation of EGFR in cancer cells by ligands such as EGF or transforming growth factor-α (TGFα), which are produced by the cancer cells themselves. Other researchers have provided insight into the biochemical consequences of ligand binding to EGFR and suggested that binding stimulates activation of a cyclic-AMP-independent phosphorylation system through an inherent TK located within the receptor[8].

Additional compelling evidence for the role of EGFR in cancer pathogenesis came from reports that many human solid tumours express high levels of EGFR, which frequently correlates with poor prognosis[2,9]. Furthermore, many tumours that express EGFR also produce one or more EGFR ligand, which further supports the hypothesis that autocrine growth-stimulatory mechanisms are involved in EGFR-mediated tumorigenesis[10]. More recent studies have established the EGFR as an anticancer target. Research has shown that EGFR signalling not only increases cell proliferation, but also regulates a range of processes that are essential for tumour progression, including cell motility, cell adhesion, tumour invasion, cell survival and angiogenesis[11].

The magnitude of EGFR signalling is influenced by several cellular mechanisms. These include receptor mutations, heterodimerization with other members of the HER family, increased expression of autocrine ligands and alterations in molecules that control receptor signalling output. A schematic description of the EGFR pathway and its role in tumorigenesis is shown in FIG. 1.

EGFR-targeted therapies

In the early 1980s, Mendelsohn *et al.* proposed that agents designed to block EGFR signalling might be used to treat cancer (TIMELINE). Mendelsohn *et al.* produced two murine monoclonal antibodies (mAbs), 225 and 528, that targeted the EGFR-TK[11–15]. These antibodies inhibited activation of EGFR by competing with EGF or TGFα, binding with equal affinity, thus blocking activation of the receptor TK activity and its downstream signalling. These mAbs were the first anti-EGFR approaches to be developed (TIMELINE). It was anticipated that repeated administration of the mAb would be required to ensure sustained antitumour activity, and that the development of human



anti-mouse antibodies in patients would preclude the use of a murine antibody in the clinic; therefore a human:murine chimeric version of murine mAb 225 was developed. In addition, this mAb had superior binding characteristics and increased antitumour activity over mAbs 225 and 528 (REF. 16). This antibody, known as cetuximab (IMC-C225, Erbitux), has been approved for the treatment of patients with metastatic colorectal cancer (CRC) in the United States and Europe and is undergoing extensive clinical evaluation for the treatment of other cancers. During the 1980s and 1990s, other groups were investigating the potential of anti-EGFR mAbs in the treatment of cancers. In addition to the initial trials with mAb 225 (REF. 17) and mAb 528 (REF. 18), other studies with mAb 425 (REF. 19) and RG 83852 (REF. 20) were conducted.

The demonstration of the potential of EGFR-targeted therapies in the treatment of cancer has prompted the design of several other biological agents that block EGFR signalling. At present, there are more than 20 anti-EGFR agents in development and several are available for use in clinical practice or are at an advanced stage of clinical development (TABLE 1). These agents can be categorized into two main classes. One category comprises the small-molecule EGFR-TK inhibitors that compete with ATP binding to the TK domain of the receptor, which inhibits TK activity and subsequently blocks signal transduction from the EGFR. The other comprises mAbs that are directed at the extracellular portion of the EGFR, which competitively inhibit ligand binding to the receptor.

It is important to review the history of the development of gefitinib for several reasons. As one of the first anti-EGFR agents to enter clinical development, and as the first agent in its class to be approved for clinical use, the development of gefitinib is generating a large body of evidence that provides useful insight for the development of other agents of its class. Although the initial development of gefitinib has focused on its use in patients with NSCLC, investigation of gefitinib in several tumour types, including head and neck cancer, breast cancer and CRC, is ongoing. It has also been tested in combination with conventional chemotherapies in a range of settings, with important implications. Finally, the recent discovery of somatic EGFR-TK mutations in a subset of patients who respond to gefitinib and erlotinib[21–23] is building a greater understanding of the mechanism of action of the EGFR-TK inhibitors. However, many questions still remain, such as why some patients experience stable disease and symptom improvement with gefitinib therapy whereas others experience an objective response, and also how can we better define the use of this drug for patients with NSCLC and other solid tumours.

Characterization of gefitinib

Gefitinib is a novel, low-molecular-weight synthetic anilinoquinazoline — 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)-quinazoline. Its discovery was based on studies designed to characterize the catalytic mechanism of EGFR-TK inhibition and the finding that the 4-anilinoquinazoline class was a promising series of EGFR-TK inhibitors[24,25]. Of several candidate compounds synthesized and tested, gefitinib was identified to have the potential to be a clinically effective drug (TIMELINE). Assessments showed that gefitinib is a potent inhibitor of EGFR-TK activity and demonstrates high and sustained blood levels over 24-hour periods in bioassays[24]. Studies showed that gefitinib demonstrates high enzyme selectivity. Activity against other TKs, such as the structurally closely related HER-family EGFR-TK ERBB2, and the receptors for vascular endothelial cell growth factor (VEGF) FLT1 (also known as VEGFR1) and KDR (also known as VEGFR2) is minimal, as is activity against serine/threonine kinases[26]. Gefitinib inhibited the proliferation of several solid tumour cell lines *in vitro*, including ovarian, breast, colon, NSCLC and head and neck carcinomas, and provided a synergistic enhancement of the inhibitory action of single-agent cytotoxic drugs[27,28]. In addition, dose-dependent antitumour activity was seen in athymic nude mice bearing a range of xenografts[27,28].

Early clinical development

The safety and pharmacokinetics of gefitinib were evaluated in Phase I trials in healthy volunteers and in patients with a range of advanced, refractory, malignant tumours[29–33]. Compared with classical anticancer drug-development strategies, these early studies had two distinct characteristics. First, a large number of patients per dose level (50–1000 mg/day) were entered into these studies, and,

second, they incorporated pharmacodynamic end points to determine the effect of gefitinib on EGFR *in vivo* (BOX 1). Although the large trial populations fuelled some debate[34], they enabled the clinical activity of gefitinib to be studied in a range of tumour types, including NSCLC, and were important to the success of the biomarker programme.

As the basal layers of the epidermis express high levels of activated EGFR, skin biopsies (pre- and on-therapy) were incorporated into the studies to evaluate whether gefitinib could block EGFR activation and EGFR-dependent processes in patients[35]. At doses well below those producing unacceptable toxicity, gefitinib completely prevented EGFR phosphorylation, decreased mitogen-activated protein kinase activity, increased apoptosis and also increased levels of the cyclin-dependent kinase inhibitor p27 (also known as KIP1), which is believed to lead to G1 cell-cycle arrest. In addition, proliferation was reduced, as indicated by a decrease in the proliferation marker Ki67. Gefitinib was well tolerated and showed good bioavailability (60%)[29–33,36]. The most common adverse events reported in these trials were diarrhoea, nausea, rash/acne, vomiting and asthenia. Most of these were transient and mild in severity, according to National Cancer Institute Common Toxicity Criteria[37].

In patients with advanced refractory solid tumours, of whom most had NSCLC, responses were seen across the dose range tested[30–33]. No clear dose response relationship was observed, and pharmacodynamic and pharmacokinetic data showed that gefitinib doses of over 150 mg/day provided antitumour activity. These results highlight the fundamental differences in the dose–toxicity–activity relationships between chemotherapy and biologically targeted therapies. With chemotherapy agents, dose selection is a compromise between the antitumour activity of the agent and its dose-limiting toxicity. This is why these agents are used at their maximum tolerated dose (MTD) — the highest dose of an agent that can be tolerated by a patient. One of the complications of chemotherapy is that the MTD might be lower than the maximum effective dose — the dose that provides maximum cytotoxicity to the tumour and reduces tumour size. Because biologically targeted agents are usually active well below their MTD, they can be administered at their optimal biological dose (the dose that provides optimal efficacy and tolerability) and therefore provide a much-improved risk/benefit ratio, compared with chemotherapy[38].

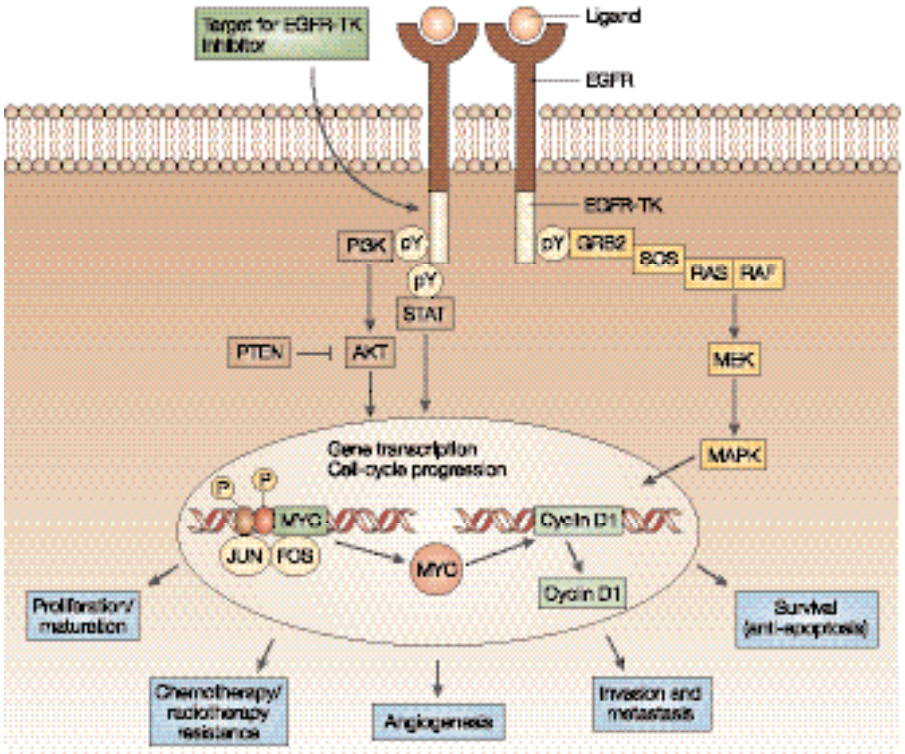


Figure 1 | **The epidermal growth factor receptor signalling pathway.** In response to ligand binding to its extracellular domain, the epidermal growth factor receptor (EGFR) forms homo- or heterodimeric complexes, with either another EGFR or another member of the HER family. This causes structural reorganization within the intracellular portion of the receptor, leading to activation of its kinase activity through autophosphorylation at a tyrosine residue (pY). This, in turn, leads to activation of a range of cell signalling pathways, including the recruitment of the adaptor proteins growth-factor-receptor-bound protein 2 (GRB2) and SOS, leading to activation of the small G proteins RAS and RAF, and signalling through mitogen-activated protein kinase (MAPK) kinase (MEK) and MAPK. EGFR activation also activates the kinase phosphatidylinositol 3-kinase (P13K), which leads to AKT activation, along with the signal transducer and activator of transcription (STAT). Transduction of signals to the nucleus and the activation of gene transcription by factors such as MYC, JUN and FOS leads to the induction of several cellular responses that are required for normal cell growth, including proliferation, survival, differentiation, migration and adhesion. In some tumour cells, EGFR signalling is constitutively active, contributing to the upregulation of many processes that are essential for tumour growth (cell proliferation, survival, angiogenesis, invasion and metastasis)[85–87]. EGFR tyrosine kinase (TK) inhibitors (for example, gefitinib and erlotinib) are small molecules that inhibit ATP binding within the tyrosine-kinase domain of the EGFR, which completely inhibits EGFR autophosphorylation and consequently blocks signal transduction from activated EGFR. As a result, the key mechanisms of tumour growth (blue boxes) are inhibited. Figure modified with permission from REF. 88 © (2000) Adis International Limited.

Clinical development in NSCLC

Following the promising activity at a range of dose levels in patients with advanced NSCLC in Phase I studies, the clinical benefit of gefitinib monotherapy was studied further in this indication in two large, multicentre, Phase II trials. These studies named the Iressa Dose Evaluation in Advanced Lung cancer (IDEAL) 1 and IDEAL 2 (REFS 39,40) involved 210 and 216 participants, respectively. They compared the antitumour activity and safety of two doses of gefitinib, 250 and 500 mg/day, in patients with advanced NSCLC who had relapsed following previous treatment with platinum-based chemotherapy. A summary of results from both trials is shown in TABLE 2 (REFS 39–41).

Disease control. The IDEAL trials reported similar rates of disease control (response and stable disease) for the two doses: 42–54% of patients on the 250 mg/day dose and 36–51% of patients at the 500 mg/day dose. Compassionate use of gefitinib 250 mg/day in the Expanded Access Programme (EAP) in patients with late-stage NSCLC has supported the antitumour activity observed in the IDEAL trials[42]. This programme enrolled patients who either had experienced progression of their disease after chemotherapy or radiotherapy or were unsuitable for such therapies. Such patients were ineligible for gefitinib clinical studies and had no alternative treatment options.

Table 1 | **EGFR-targeted agents**

Anti-EGFR agent*	Drug type	Status	Tumour/cancer type
Gefitinib	Small-molecule EGFR-TKI	Launched, Phase III	NSCLC
		Phase III	Head and neck
		Phase II	CRC, breast, gastrointestinal, prostate and oesophageal
Erlotinib	Small-molecule EGFR-TKI	Pre-registration, Phase III	NSCLC
		Phase III	Pancreatic (trial completed)
		Phase II	Ovarian, head and neck, brain, lung (general), breast, renal, CRC, BAC and HCC
Lapatinib	Small-molecule EGFR-TKI/ERBB2-TKI	Phase III	Breast and renal
		Phase II	CRC, gastric bladder, head and neck, and NSCLC
Cetuximab	Extracellular EGFR mAb	Launched, Phase III	CRC
		Phase III	Pancreatic, head and neck, and NSCLC
		Phase II	NSCLC, breast, renal and prostate
Panitumumab	Extracellular EGFR mAb	Phase III	CRC and lung
		Phase II	NSCLC, renal and prostate

*Table lists agents that have completed or are currently in Phase III trials. BAC, bronchioloalveolar carcinoma; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; HCC, hepatocellular carcinoma; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; TKI, tyrosine-kinase inhibitor.

A Phase III trial called BR21 compared the effects of erlotinib, another EGFR-TK inhibitor, with that of best supportive care (care that prevents or relieves the symptoms of disease or the side effects of treatment, but does not alter the course of the disease) in 731 patients with stage IIIB/IV NSCLC who had received one or two previous chemotherapy regimens[43]. The primary end point of this trial was overall survival, and secondary end points included progression-free survival, quality of life, response to treatment, and safety. This trial reported a disease control rate of 44% in patients on erlotinib versus less than 29% in patients in the placebo group.

Survival. In the IDEAL 1 and IDEAL 2 trials, the 1-year survival rates of pretreated patients with NSCLC who received 250 mg/day gefitinib were 35% and 27%, respectively. Their median duration of overall survival was 7.6 and 7.0 months, respectively[39,40]. Similarly, an analysis of 21,064 patients with locally advanced or metastatic NSCLC who had received gefitinib in the EAP reported a 1-year survival rate of 29.9%[44]. The data reported for gefitinib from both clinical trials and real-life usage compare favourably with the 1-year survival rate of 5.5% and median duration of overall survival of 4.0 months that has been reported in a retrospective analysis of NSCLC patients (n=43) receiving either third- or fourth-line chemotherapy[45].

Erlotinib-treated patients in the BR21 trial also experienced a 1-year survival rate of 31% and median duration of overall survival of 6.7 months[43]. A Phase III placebo-controlled study called Iressa Survival Evaluation in Lung cancer (ISEL), which includes about 1,600 patients with locally advanced or metastatic NSCLC, is underway to determine the effects of treatment with gefitinib 250 mg/day and best supportive care with best supportive care alone. This trial should provide further survival data for the EGFR-TK inhibitor class. The study has completed recruitment and results are expected soon.

Safety. The IDEAL trials showed that gefitinib was generally well tolerated at both 250 and 500 mg/day, and that the most common drug-related adverse events were mild diarrhoea and skin reactions. At both doses, most drug-related adverse events were reversible and caused few patients to discontinue treatment with gefitinib. As the 250 mg/day dose had a better tolerability profile than the 500 mg/day dose, albeit with similar efficacy, 250 mg/day was selected as the optimal biological dose of gefitinib for patients with pretreated advanced NSCLC. Tolerability data from the EAP support the favourable safety profile of gefitinib — in several large case series, most of the adverse drug reactions were mild diarrhoea and skin rash[46].

Recently, there have been reports that interstitial lung disease (ILD) developed in some patients during treatment with EGFR-TK inhibitors. In the IDEAL 1 trial, two Japanese patients who received 500 mg/day gefitinib experienced ILD-type events, but no such cases were reported in the IDEAL 2 trial[39,40]. Similarly, there has been a small number of reports of pulmonary toxicity with erlotinib[47,48]. With gefitinib treatment, the frequency of ILD seems to be higher in Japan (1.9–3% of patients) than in the rest of the world (0.3% of patients), including other South-East-Asian countries (0.3% of patients) (REF. 49, and B. Forsythe and K. Faulkner, personal communication). The mortality rate due to ILD is 0.7% for patients in Japan and 0.1% in the rest of the world (B. Forsythe and K. Faulkner, personal communication), which is approximately one-third of affected patients in each geographical group.

Box 1 | **How gefitinib modified clinical trial objectives**

As an epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitor, gefitinib was expected to reduce the proliferation rate of tumour cells, and thereby lead to disease stabilization rather than tumour regression (an objective response). By contrast, conventional chemotherapy aims to kill tumour cells, thereby producing an objective response. So to assess the full clinical potential of gefitinib as an anticancer agent, the Phase II clinical trials, in addition to measuring its significant antitumour activity, incorporated end points that were generally regarded as being of secondary importance in trials of cytotoxic agents, such as disease control (which incorporates objective response and stabilization of disease) and disease-related symptom improvement. These trials were the first to use the Lung Cancer Subscale of the Functional Assessment of Cancer Therapy-Lung questionnaire to determine the effect of treatment on disease-related symptoms in patients with non-small-cell lung cancer. Similar new approaches to determining the activity of other targeted agents are now being considered by oncologists.

Table 2 | **Summary of results from Phase II IDEAL trials**

Trial	Patient population	Gefitinib dose (mg /day)*	Number of patients	Objective response (%)	Disease control rate (%)	Symptom improvement rate (%)	Potential prognostic factors	Safety	CTC grade 3 or 4 drug-related AEs (%)	Withdrawals due to drug-related AEs (%)
IDEAL 1	PS 0–2; stage IIIA–IV disease; one or two previous CT regimens	250 500	104 106	18.4 19.0	54.4 51.4	40.3 37.0	Female gender; adenocarcinoma tumour histology; Japanese patients	Drug-related AEs generally mild (NCI-CTC grade 1 or 2) and were more common with 500 mg/day; most frequent AEs were mild skin and GI toxicities	8.7 30.2	1.9 9.4
IDEAL 2	PS 0–2; stage IIIB-IV disease; over two previous CT regimens	250 500	102 114	11.8 8.8	42.2 36.0	43.1 35.1	Female gender; adenocarcinoma tumour histology	Drug-related AEs generally mild (NCI-CTC grade 1 or 2) and were more common with 500 mg/day; most frequent AEs were mild skin and GI toxicities	6.9 17.5	1.0 4.0

*Patients were randomized to receive either 250 or 500 mg/day in each trial. AE, adverse event; CT, chemotherapy; GI, gastrointestinal; NCI-CTC, National Cancer Institute Common Toxicity Criteria (version 2.0); PS, performance status. Table compiled from data in REFS 39–41.

The reason for these regional differences in the occurrence of ILD is unknown, although it might be related to an increased susceptibility to ILD within the Japanese population that is independent of treatment with gefitinib. A higher rate of ILD in Japan, compared with other countries, has been reported in patients who were treated with the antirheumatic drug leflunomide[50]. Furthermore, a recent review has identified national differences in the terms used to describe the pulmonary side effects of drugs[51]. A retrospective analysis of 1,976 patients who have received gefitinib in Japan indicates that risk factors for ILD might include smoking status, male gender and pre-existing idiopathic pulmonary fibrosis[49]. Given that ILD is a known complication of lung cancer and has also been associated with chemotherapy and radiotherapy treatment[52], the small risk of developing ILD-type events during treatment with EGFR-TK inhibitors should not prevent patients with NSCLC from receiving these drugs.

Approval of gefitinib. Based on the results of the Phase II IDEAL trials, gefitinib was approved in Japan on July 5, 2002 for the treatment of inoperable or recurrent NSCLC. Subsequently, gefitinib has gained approval for the treatment of previously treated NSCLC in over 30 countries, including the United States.

Understanding responses

Clinical characteristics. Results from the IDEAL trials showed that in an unselected population of patients with pretreated NSCLC, treatment with gefitinib 250 mg/day resulted in clinical benefits (disease stabilization or tumour regression) in about 40–50% of patients, and an objective response in 12–18% of patients[39,40]. However, retrospective analyses of these trial data indicate that certain patient subgroups have a higher probability of achieving an objective tumour response than others. For example, in both IDEAL studies, objective tumour responses were more likely to be seen in female than male patients and in patients with adenocarcinoma NSCLC tumours than tumours of other histological types[39,40]. Furthermore, in the IDEAL 1 study, the response rates in Japanese patients were higher than those observed in non-Japanese patients[39]. Other studies have indicated further demographic factors to add to the list of potential predictive markers of gefitinib response, including patients with tumours of the bronchioloalveolar carcinoma histological subtype and patients with a history of non-smoking[53].

A similar analysis of data from the BR21 study of erlotinib showed that female gender, adenocarcinoma histology and a history of non-smoking could also be predictive of a patient's response to erlotinib[54]. Although tumour EGFR levels and the appearance of rash had initially been postulated as prognostic markers of response, it has become clear during the clinical development of gefitinib that neither of these are effective or reliable predictors of drug response[55,56]. Much research is underway to determine mechanisms of patient responsiveness to EGFR-TK inhibitors. The results of these studies could help in the identification of patients who are likely to benefit most from this class of drugs.

EGFR-TK mutations. Our understanding of why some patient subgroups are more likely to respond to gefitinib than others is limited. However, the recent exciting discovery that some patients with a marked response to gefitinib have somatic EGFR-TK mutations and the finding that the frequency of these mutations is highest in those patient subgroups previously associated with the greatest response to gefitinib[22] (FIG. 2) could provide a partial explanation. The new data indicate that somatic mutations in exons 18–21 in the ATP-binding region of the TK domain of the *EGFR* gene might predict those patients who are likely to have an objective response to gefitinib[21,22]. Whilst investigating whether mutation of receptor TKs has a causal role in the development of NSCLC, Paez *et al.* searched for somatic genetic alterations in NSCLC primary tumour biopsies from 119 unselected patients[22]. Although genes encoding 47 different TK receptors were analysed for mutations, mutations were only observed in the *EGFR* gene. Eighteen different mutations were

found in exons 18–21, which cluster around the TK domain of *EGFR*. These mutations were more frequent in women than in men (20% versus 9%), in adenocarcinomas than in other histologies (21% versus 2%) and in patients from Japan than in patients from the United States (26% versus 2%).

These findings spurred investigation of whether EGFR-TK mutations might be a determinant of gefitinib sensitivity. Paez *et al.* searched for EGFR mutations in tumour samples from five patients who responded to gefitinib (four had achieved a partial response and one had experienced rapid symptom improvement). They found that biopsy samples from all of these tumours had EGFR-TK mutations, whereas none were evident in the tumour samples from four patients who had progressed during gefitinib treatment[22]. Simultaneously with the publication of these results, Lynch *et al.* published the results of their investigation into EGFR-TK mutations in primary tumours from a small number of patients with NSCLC. They identified somatic mutations in the TK domain of EGFR in eight of the nine patients studied who had achieved an objective response with gefitinib and in none of the seven patients studied who had progressed on gefitinib[21]. Following release of these landmark data, analysis of the mutation status of tumours from other patients who have been treated with EGFR-TK inhibitors is being carried out.

So far, three classes of EGFR mutations have been identified — missense mutations, deletions and in-frame insertions (FIG. 2)[21]. Functional analysis in fibroblasts that expressed two mutant forms of EGFR — the L858R missense mutation and L747-P753insS deletion — have provided insight into how these mutations affect the function of gefitinib[21]. These studies showed that activation of mutant EGFR is characteristically more intense and prolonged than that of the activated wild-type receptor, and also that much lower concentrations of gefitinib are needed to completely inhibit this mutant receptor, compared with the wild-type receptor[21]. These studies indicate that these mutations stabilize the interaction between the EGFR-TK domain and ATP or its competitive inhibitor (for example, gefitinib). *In vitro* studies have shown that tumour cell lines that express these mutant forms of EGFR are more susceptible to apoptosis following gefitinib exposure, compared with wild-type cells[57].

Data from Sordella *et al.* also indicate that apoptotic pathways in NSCLC tumours that express mutant forms of EGFR differ from those in wild-type cells[58]. Cells with mutant EGFR preferentially activate the AKT and signal transducer and activator of transcription (STAT) anti-apoptotic signalling pathway, and EGFR inhibition with gefitinib results in rapid cell death. This could underlie the marked responses to gefitinib in patients with mutant EGFR[58]. However, the functional impact of all the EGFR-TK mutations discovered so far, and their clinical significance, is not yet known[57].

These studies raise the possibility of predicting, on the basis of somatic EGFR mutations, which patients are most likely to achieve an objective response with gefitinib and other EGFR-TK inhibitors. However, other mechanisms might be involved in determining sensitivity to gefitinib and other EGFR-TK inhibitors. At least one patient with gefitinib-responsive NSCLC did not have any of these mutations[21], and in one study exploring treatment with erlotinib, one non-responder had EGFR-TK mutations whereas five patients with stable disease did not[57]. Furthermore, the clinical benefits of treatment with gefitinib and other EGFR-TK inhibitors are not restricted to objective response. It is also very important to consider the large proportion of patients who achieve disease stability or symptom improvement that do not seem to be explained by somatic EGFR-TK mutations. Identifying markers of tumour response to EGFR-TK inhibitors is a complex process, and much more research is required to clarify the full clinical implications of the EGFR-TK mutations and to understand how treatment outcome can be predicted.

While these findings raise the possibility of a diagnostic test for EGFR mutation status being developed, there are several practical implications for this. In particular, will one test ever identify all the possible mutations? Although the original publication by Lynch *et al.* described a total of 7 distinct mutations[21], this number continues to grow, with over 40 distinct mutations reported 1 month later at the American Society for Clinical Oncology annual meeting 2004. The effects of the different types of mutations on downstream signalling pathways also differs, making it difficult, at this time, to specify which particular mutations should be screened for. As these EGFR mutations are somatic, rather than germline, any test to determine mutation status will also require direct tumour biopsy material, rather than being performed on more easily accessed tissue such as blood, skin or buccal mucosa. At present, mutation analysis is a complex and time-consuming

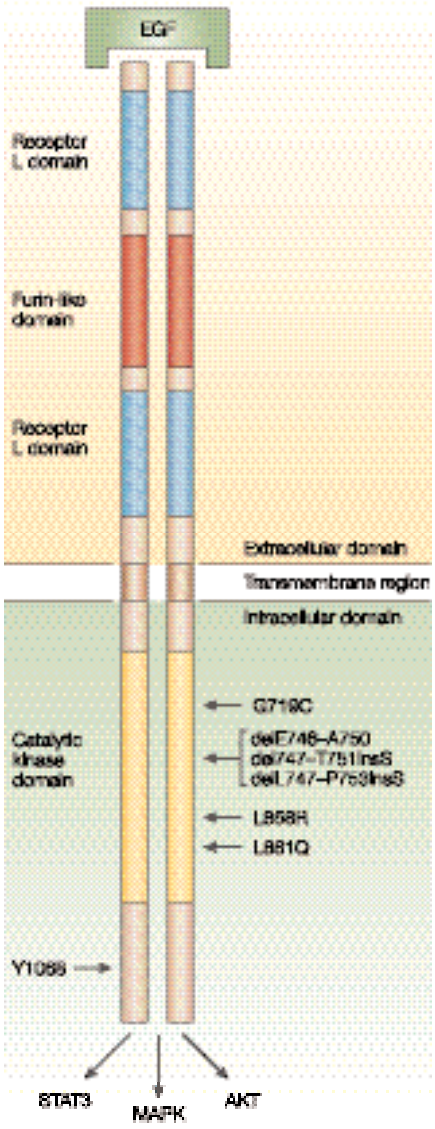


Figure 2 | **The epidermal growth factor receptor.** The epidermal growth factor receptor (EGFR) contains two extracellular L domains, along with a furin-like extracellular domain. These are connected, through the transmembrane region, to an intracellular domain that contains the catalytic kinase domain, along with a tyrosine phosphorylation site (Y1068). This region, when phosphorylated, leads to the activation of signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK) and AKT signalling pathways. The locations of the activating EGFR tyrosine-kinase mutations identified by Lynch *et al.* in tumours from patients with NSCLC who had responded to gefitinib (listed on right side of molecule) are all located within the catalytic kinase domain of the receptor[21]. It is suggested that non-small-cell lung cancer tumour cells that express mutant forms of EGFR preferentially activate the AKT and STAT-mediated anti-apoptotic signalling pathways, so EGFR inhibition with gefitinib results in rapid cell death[58]. Figure modified with permission from REF. 21 © (2004) Massachusetts Medical Society.

procedure, requiring specialist expertise and equipment, and is only available at a limited number of medical research institutions. Furthermore, unless the biopsy sample contains a significant proportion of cancer cells, it is very difficult to establish that a particular tumour does not have cells that express mutant forms of EGFR.

Other factors. Other techniques and biomarkers are being investigated to identify patients with NSCLC who are most likely to respond to certain EGFR inhibitors. These include immunohistochemical assays to evaluate expression levels of EGFR-related proteins, fluorescence *in situ* hybridization (FISH) analysis to identify amplified genes, and gene-expression and proteomic analyses to identify other markers of response to gefitinib. Preliminary evaluation of response, based on *EGFR* mutations and amplification of *EGFR* (as determined by FISH), in a small number of patients (about 20) showed that all responders carried either amplifications or mutations in *EGFR*, or both[59]. Analysis of RNA samples isolated from tumour specimens of 17 patients, of whom 2 had a partial response and 3 had stable disease, revealed that expression levels of several genes, including *STAT5A*, *STAT5B* and gene encoding γ-catenin, is correlated with clinical response[60]. So, EGFR mutations are not the only story in gefitinib sensitivity — other mechanisms are also potentially involved.

Assessing gefitinib response

Results of the IDEAL trials showed that over 40% of symptomatic patients with refractory NSCLC experienced symptom improvement within 8–10 days of starting gefitinib therapy, and that this correlated with response and increased survival (REFS 39,40,61; and R.S.H *et al.*, unpublished observations). Approximately 90% of tumour responses in the trials were seen within the first 2 months. Given the absence of a simple diagnostic test for determining EGFR mutation status, and the fact that EGFR mutations do not seem to account for the full benefit of gefitinib, the most practical way to determine if a patient will benefit from gefitinib currently is to initiate up to 8 weeks of trial therapy.

Combination therapies

In parallel with the IDEAL studies, gefitinib was investigated as a first-line treatment (treatment when a patient has not received any previous therapy for advanced disease) in combination with chemotherapy in two Phase III trials, called Iressa NSCLC Trial Assessing Combination Treatment (INTACT) 1 and INTACT 2. Patients in these trials had either locally advanced stage III disease that was not curable with surgery or radiotherapy, or stage IV disease. Patients in INTACT 1 received gefitinib in combination with gemcitabine and cisplatin[62], whereas patients in INTACT 2 received a combination of gefitinib, paclitaxel and carboplatin[63]. Although preclinical studies[26] had shown synergy among these drugs, and two earlier Phase I studies[64,65] had indicated that first-line combination therapy with gefitinib and platinum-based chemotherapy was feasible, the INTACT trials did not report an increase in survival times among patients who received gefitinib in addition to platinum-based chemotherapy. Similarly, in clinical trials called Tarceva Responses in Conjunction with Paclitaxel and Carboplatin (TRIBUTE), which tested the addition of erlotinib to carboplatin and paclitaxel therapy, and the Tarceva Lung Cancer Investigation Trial (TALENT), which tested erlotinib in combination with cisplatin and gemcitabine, no increases in patient survival time were observed[66,67].

The reasons for these disappointing results are unknown, although the possibility that EGFR-TK inhibitors and chemotherapy have antagonistic effects has been proposed[68]. Antagonism between cytostatic and cytotoxic agents has been demonstrated between tamoxifen and chemotherapy in patients with breast cancer receiving adjuvant therapy[69]. Gefitinib has both antiproliferative and pro-apoptotic effects. Its antiproliferative effects are the result of p27-mediated G1 cell-cycle arrest of EGFR-dependent tumour cells that, in a similar way to tamoxifen, could render tumour cells less sensitive to cytotoxic agents. Conversely, the pro-apoptotic effects of gefitinib could increase the antitumour effects of chemotherapy. The challenge is to dissociate the antiproliferative effects from the apoptotic effects of gefitinib when it is used in combination with chemotherapy. Early preclinical studies in human tumour xenograft models involving the combination of gefitinib and chemotherapy indicated that intermittent gefitinib administration was significantly superior to continuous dosing[70]. The antiproliferative effects of gefitinib could require continuous kinase inhibition to maintain cell-cycle arrest, whereas sensitization to apoptosis might require temporary inhibition of the survival (anti-apoptotic) pathways.

In the INTACT 2 trial, a subset of patients with adenocarcinoma histology who had received chemotherapy for over 90 days seemed to have a slightly better survival outcome if they also received gefitinib. This indicates the possible efficacy of gefitinib monotherapy in maintenance therapy[63] — as a cytostatic agent that maintains tumour regression after chemotherapy. So, instead of concomitant administration, scheduling gefitinib after chemotherapy might benefit patients with NSCLC. This sequential approach is now being investigated in a US Cooperative Group Phase III study, in which patients with inoperable stage III NSCLC receive gefitinib or placebo following treatment with chemoradiation and consolidation docetaxel.

Several key classes of agents that target specific cellular mechanisms are in different phases of clinical development in combination with EGFR inhibitors. As these agents have the potential to target different signalling pathways involved in cancer pathogenesis, they have the potential to be used in combination. For example, antitumour activity of erlotinib in combination with the angiogenesis inhibitor bevacizumab has been reported in a Phase I/II study of patients with recurrent NSCLC who had received one or more chemotherapy regimen[71]. An example of an agent that selectively targets two key pathways in tumour growth (the EGFR and VEGFR pathways) is ZD6474, which is now in Phase II development[72].

Ongoing development in NSCLC

How can the use of gefitinib in patients with NSCLC be optimized, both as a monotherapy and in sequence with chemotherapy? Although in most countries where gefitinib is approved the licence is for use only in pretreated patients with advanced NSCLC, the drug is now being investigated in patients with all stages of lung cancer. In one Phase II trial conducted in Japan, first-line gefitinib 250 mg/day therapy resulted in an overall tumour response rate of 30%, but 4 of the 40 patients in this trial developed ILD[73]. Another Phase II study evaluated gefitinib 250 mg/day as first-line treatment in patients with NSCLC and poor performance status. Treatment was well tolerated, resulted in a disease control rate (the proportion of patients with partial or complete tumour regression or stable disease) of 48.3%, and a tumour response rate of 5.2%[74]. These data support the further investigation of gefitinib as a first-line therapy. Large trials are underway to define its full potential in patients with NSCLC, which might include use as adjuvant, first-, second- and third-line treatment and as maintenance therapy (TABLE 3).

Table 3 | **Gefitinib trials underway in patients with non-small-cell lung cancer**

Name	Phase	Design	Gefitinib therapy*	Number of patients	Patient status	Drug compared with	Primary end point	Sponsor
BR19	III	Double blind	Adjuvant	1,160	Tumour surgically removed, with stage IB, II and IIIA (N2) NSCLC	Placebo	Overall survival	NCIC, EORTC
EORTC 08021-ILCP	III	Double blind	Adjuvant	736	Stage IIIB/IV NSCLC, PS 0–2, first-line CT	Placebo	Survival, progression-free survival, toxicity	EORTC, ILCP
INVITE	II	Open label	First line	192	Age ≥70 years, PS ≤2, stage IIIB/IV NSCLC	Vinorelbine	Progression-free survival	AstraZeneca
INSTEP	II	Double blind	First line plus BSC	200	PS 2 or 3, stage IIIB/IV NSCLC	Placebo plus BSC	Progression-free survival	AstraZeneca
INTEREST	III	Open label	Second/third line	1,440	Locally advanced or metastatic NSCLC	Docetaxel	Overall survival	AstraZeneca
ISEL	III	Double blind	Second/third line plus BSC	1,692	Stage IIIB/IV NSCLC, PS 0–2	Placebo plus BSC	Overall survival	AstraZeneca
V15-32	III	Open label	Second/third line	484	Stage IIB/IV NSCLC, PS 0–2	Docetaxel	Survival	AstraZeneca
SWOG 0023	III	Double blind	Maintenance	840	Patients with stage III NSCLC who have received CT/RT with consolidation docetaxel	Placebo	Overall survival, progression-free survival	SWOG

*Patients receive 250 mg/day. BSC, best supportive care; CT, chemotherapy; EORTC, European Organisation for Research and Treatment of Cancer; ILCP, Italian Lung Cancer Project; NCIC, National Cancer Institute of Canada; NSCLC, non-small-cell lung cancer; PS, performance status; RT, radiotherapy; SWOG, Southwest Oncology Group.

Using gefitinib to treat other cancers

Studies are also underway to evaluate the ability of gefitinib to treat patients with other solid tumours, such as head and neck cancer, breast cancer and CRC. Gefitinib 500 mg/day has shown encouraging single-agent activity and favourable tolerability as first- or second-line therapy in a Phase II study of 52 patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SCCHN)[75]. Of the 47 patients evaluable for response, 10.6% had an observed response and a substantial number had disease control (53%). Median time to progression was 3.4 months and overall survival was 8.1 months. Gefitinib was well tolerated and the study findings support further investigation of gefitinib in patients with SCCHN. Two key international trials are now recruiting patients and will assess the potential of gefitinib as a first- and second-/third-line therapy in patients with head and neck cancer. In a Phase II study, first-line treatment with gefitinib will be combined with chemoradiotherapy, and in the Phase III trial, gefitinib monotherapy (250 and 500 mg/day) is being compared with methotrexate as a second- or third-line treatment. Data from a Phase I study in patients with metastatic and/or locally recurrent SCCHN indicate that the cyclooxygenase-2 inhibitor celecoxib increases the antitumour activity of gefitinib, with a response rate of 33.3%[76].

Although results from trials investigating the clinical benefit of gefitinib monotherapy in patients with breast cancer have shown few objective responses[77,78], alternative approaches, such as combining gefitinib with endocrine treatment, seem more promising. Many oestrogen-receptor-positive breast tumours initially respond to antihormone therapy. These responses, however, are often incomplete and prolonged treatment results in resistance, induction of EGFR expression and the emergence of highly proliferative cells. Recent preclinical data in oestrogen-receptor-positive breast cancer cells indicate that combining gefitinib with tamoxifen or fulvestrant, either as cotreatment[79,80] or pretreatment[81], induces an additive antitumour effect and prevents the emergence of EGFR-positive antihormone resistance. Gefitinib is believed to overcome antihormone resistance by eliminating crosstalk between the oestrogen receptor and ERBB2 (also known as HER2) signalling pathways[81]. Recent pharmacokinetic data from patients with breast cancer who were treated with gefitinib show that concentrations of gefitinib in tumours (2.3–25.8 μg/g) were much higher than in plasma (0.10–0.42 μg/g)[82]. This tumour/plasma ratio (54-fold) was much higher than that observed in animal models, and confirms that gefitinib is extensively distributed to breast tumour tissue. The trial is designed to identify molecular alterations in human breast cancer tissue after short-term exposure to gefitinib. Clinical trials are now being designed to further investigate the effects of gefitinib on breast tumours.

Gefitinib 500 mg/day therapy has also shown activity in patients with metastatic CRC, when administered in combination with FOLFOX-4, a combination of three chemotherapy drugs — oxaliplatin, leucovorin and fluorouracil. In a Phase II trial, patients with advanced CRC who had received previous therapy or no previous therapy received FOLFOX-4 for 14 days, and thereafter gefitinib was added to the treatment regimen[83]. Although the trial is ongoing, results are available from 50 patients. Patients who had not been treated with chemotherapy had a response rate of 78%, and patients who did not respond to previous chemotherapy had a response rate of 36%. These data are encouraging compared with those usually observed with FOLFOX-4 therapy alone in patients with metastatic CRC.

Results have also been reported in patients with gastric cancer. In a Phase II trial of 75 patients with advanced metastatic gastric cancer, gefitinib therapy resulted in disease control in 13 patients (13.9%), of whom 1 had a partial response after receiving 250 mg/day and 12 had stable disease after receiving either 250 mg/day or 500 mg/day of gefitinib. Again, the drug was generally well tolerated at both doses, although the lower dose was associated with fewer drug-related adverse events[84].

Future directions

Over 190,000 patients have been treated with gefitinib worldwide. Since their onset, studies of gefitinib have generated a large body of data that have contributed to the ongoing clinical advancement of this drug and provides useful knowledge that could assist the development of other EGFR-TK inhibitors. Initially, clinical trials with gefitinib focused primarily on patients with advanced NSCLC, but ongoing trials are providing encouraging evidence for its potential in treating earlier-stage disease and several other tumour types. Investigations are also underway to find out how gefitinib can be combined with chemotherapy and other novel agents. The discovery of EGFR mutations and the potential identification of other markers that predict patient response could help to optimize the use of gefitinib in the future. Nonetheless, understanding the basis of stable disease and symptom improvement remains an important challenge.

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Competing interests statement

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

Online links

DATABASES

The following terms in this article are linked online to:

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FURTHER INFORMATION

Epidermal growth factor receptor information and resources: http://www.egfr-info.com/
Information on gefitinib: http://www.iressa.com/
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