Peer reviewed article

Targeting the epidermal growth factor receptor (EGFR) – a new therapeutic option in oncology?

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Summary

The epidermal growth factor receptor (EGFR) is commonly overexpressed in a variety of solid tumours, and clinical trials indicate that this antigen has important roles in cancer aetiology and progression. EGFR thus provides a rational target for cancer therapies and a number of strategies influencing this receptor, and its downstream signal cascades, including monoclonal antibodies, tyrosine-kinase inhibitors, antisense oligonucleotides inhibiting EGFR synthesis and antibody-based immunoconjugates, have been evaluated. In particular, monoclonal antibodies targeting the receptor's extracellular domain and small molecules blocking tyrosine-kinase activation intracellularly have already shown some activity in clinical phase I–III trials. These two major classes of anti-EGFR therapeutics will be the main topic of this review. In the case of tyrosine-kinase inhibitors, amplification, high polysomy of the EGFR gene, high protein expression and mutations of the receptor were found to be significantly associated with better response to such treatment. However, many questions remain unanswered and future issues in the development of EGFR inhibitors will include the identification of biological predictors of response, combination with other therapies and also their use in earlier stages of cancer.

Key words: EGFR; monoclonal antibodies; tyrosine-kinase inhibitors; targeted therapies

Introduction

No financial support declared.

The epidermal growth factor receptor (EGFR) is the prototypic member of the class I superfamily of receptor tyrosine kinases (RTKs), which includes HER1 (EGFR, ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). All members have a ligand-binding region, a single membrane-spanning region and a cytoplasmic intracellular tyrosine-kinase-containing domain (fig. 1). Receptors of this superfamily are expressed in various tissues of epithelial, mesenchymal and neuronal origin. Binding of a ligand to ErbB receptors induces the formation of receptor homo- and heterodimers followed by activation of the intrinsic tyrosine-kinase domain by phosphorylation. These phosphorylated intracellular sites serve as docking stations for a range of proteins which trigger the activation of intracellular signalling pathways. So far three major intracellular signalling pathways have been identified which mediate the downstream effects of ErbB receptor activation. The first pathway involves the mitogen-activated protein kinase (MAPK) and the second the phosphatidyl-inositol 3-kinase (PI3K)-AKT [1]. The third important receptor-signalling effectors are the signal transducer and activator of transcription proteins (STATs) [2].

In normal as well as in tumour tissue the ErbB

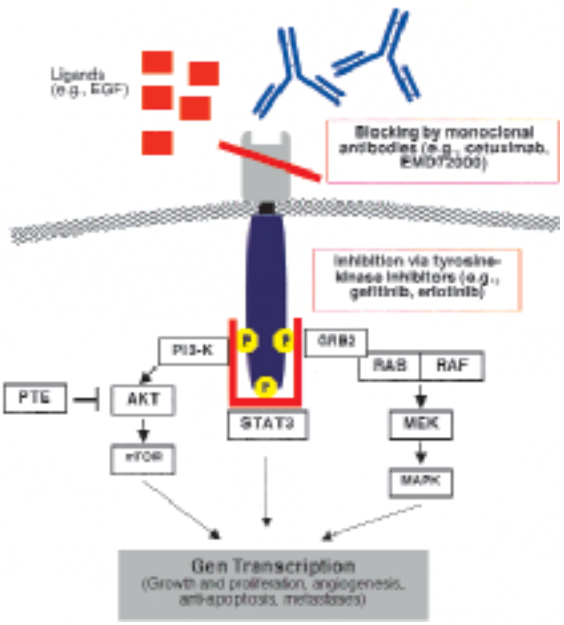

Figure 1

Inhibition of EGFR on different levels. EGF, "epidermal growth factor"; PI3-K, "phosphatidylinositol 3-OH kinase"; AKT, serine-threonine kinase; mTOR, "rapamycin protein kinase"; PTEN, "phosphatase and tensin homologue deleted from chromoson 10"; GRB2, "growth factor receptor-bound protein 2"; MAPK, "mitogen-activated protein kinase"; MEK, "MAPK kinase"; STAT3, "signal transducer and activator of transcription 3"

receptors are activated by a variety of receptor-specific ligands. In the case of EGFR, ligands specific for this receptor are the epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) [3].

The rationale for use of the EGF receptor as a target antigen for specific anticancer therapies is based on its role in cancer cell growth through cellular proliferation, invasion, angiogenesis, metastasis and inhibition of apoptosis (for review, [4]). EGFR is dysregulated in several malignant disorders including lung, breast, colorectal, head and neck, prostate, pancreatic and other cancers [5]. Mechanisms involved in the activation of EGFR include receptor overexpression [6], autocrine activation by overproduction of ligands [7], ligand-independent activation through other receptor systems [8] and mutant receptors resulting in ligand-independent activation. An example of the latter are class III mutations (EGFRvIII), which are recognised as the most important of seven variants characterised so far. EGFRvIII contains a deletion of exons 2–7 within the extracellular domain (ECD), resulting in an in-frame deletion of 801 base pairs of the coding sequence and the generation of a novel glycine residue at the fusion junction [9]. This mutant form is the most frequently detected genomic variant in brain tumours and other cancers [10, 11], and is another specific antigen for targeted therapies. Both wildtype EGFR and EGFRvIII overexpression have been correlated with a poor prognosis in some cases (for review see [12]).

This minireview is intended to update strategies for targeting the EGFR receptor, including the role of somatic mutations in the tyrosine-kinase domain of EGFR recently found in a subgroup of patients with non-small-cell lung cancers who responded impressively to treatment with tyrosine-kinase inhibitors (TKI).

Various strategies targeting the EGF receptor

Preclinical and clinical studies have shown that targeting EGFR is a valid strategy for anticancer therapy. Currently four treatment strategies for targeting EGFR and blocking its downstream signalling pathways have been developed, including 1) monoclonal antibodies directed against the extracellular domain of EGFR, 2) small molecules blocking tyrosine-kinase activation intracellularly (tyrosine-kinase inhibitors; TKIs), 3) antisense oligonucleotides inhibiting EGFR synthesis and 4) antibody-based immunoconjugates such as immunotoxins or immunoliposomes for specific and efficient delivery of anticancer agents to EGFR-overexpressing tumours.

These strategies are at various stages of clinical development; so far only monoclonal antibodies and TKIs have entered clinical phase III trials or been approved for first indications in countries such as the US and several European states (summary in table 1).

Table 1

Clinical applications of cetuximab, gefitinib und erlotinib.

Substrate	Study	Design	Results
Cetuximab (Erbitux™)	Colorectal	Phase II/III study; progressive disease on irinotecan; ± cetuximab	RR = 23 vs. 11% TTP = 4.1 vs. 1.5 months
	NSCLC	Randomized phase II study; 1st line; vinorelbine/cisplatin ± cetuximab	RR = 50% vs. 29%
	Pancreatic cancer	Phase II study in combination with gemcitabine; 1st line	RR = 51% TTP = 12 months
	H&N	Randomised phase II/III study; progressive disease on cisplatin; ± cetuximab	RR = advantage, no survival benefit
Gefitinib (Iressa™)	NSCLC (Ideal 1)	Randomised phase II study; 2nd and 3rd line; 250 vs. 500 mg/day	RR = 18%
	NSCLC (Ideal 2)	Randomised phase II study, 3rd line; 250 vs. 500 mg/die	RR = 8–11%
	NSCLC (Intact 1)	Randomised phase III study; gemcitabin/cisplatin ± gefitinib	No advantage
	NSCLC (Intact 2)	Randomised phase III study; carboplatin/paclitaxel ± gefitinib	No advantage
	Colorectal	Phase II study; 2nd line; 750 mg/day	No response
	Prostate cancer	Randomised phase II study; hormone-refractory; 250 vs. 500 mg/day	No response
	H&N	Phase II study; monotherapy	RR = 11%
Erlotinib (Tarceva™)	NSCLC (BR.21)	Phase II/III study; 2nd and 3rd line	RR = 12%; 1y survival = 40%
	HCC	Phase II study; 1st line	RR = 50%
	Ovarian	Phase II study; 2nd and 3rd line	RR = 6%
	H&N	Phase II study; 2nd and more lines	RR = 5%

NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; TTP, time to progression; RR, response rate

Monoclonal antibodies

Monoclonal antibodies (MAbs) target the extracellular ligand-binding domain of the EGF receptor and are able to compete with EGF and TGF-α, thereby inhibiting subsequent receptor activation and initiation of downstream signalling cascades [13]. In addition, binding of MAbs to EGFR induces receptor dimerisation, internalisation and receptor downregulation [14]. These processes consequently lead to several effects such as cell-cycle arrest via upregulation of cyclin-dependent kinase inhibitor $p27^{KIP1}$ [15] and potentiation of apoptosis, which correlates with induction of Bax and activation of caspase 8 [16]. The clinically most advanced members of this drug class are cetuximab (IMC-C225; Erbitux™; ImClone Systems Inc., New York, NY, USA resp. Merck KGaA, Darmstadt, Germany), matuzumab (EMD 72000; Merck KGaA, Darmstadt, Germany) and panitumumab (ABX-EGF; Abgenics/Amgen, San Francisco, CA, USA).

Cetuximab

Pharmacokinetic studies revealed half-lives of this MAb of 106–111 hours [17]. Subsequently, clinical trials evaluating cetuximab employed a loading dose of 400 mg/m^2 followed by weekly maintenance doses of 250 mg/m^2 body surface area (BSA).

Two preliminary phase II trials by Saltz et al. [18] demonstrated the activity of cetuximab in EGFR-positive irinotecan refractory metastatic colorectal cancer (CRC) as a single agent and in combination with irinotecan. The response rate in the first study of cetuximab as monotherapy in heavily pretreated patients reached 11%; in combination with irinotecan a RR of 17% was achieved as demonstrated in the second study. These two regimens were then compared in a multicentre randomised phase III trial (BOND Study) [19]. In this study, 329 patients with irinotecan-resistant CRC were randomised to cetuximab alone or in combination with irinotecan. The combination arm showed statistically significant improvements in RR (23% versus 11%), stable disease (55% versus 32%) and time to progression (TTP; 4.1 versus 1.5 months). Although a trend was seen, no statistically significant difference in overall survival between the two treatments was detected (8.6 versus 6.9 months). On the basis of these results cetuximab was approved in Switzerland, Europe and the US for the treatment of metastatic CRC in 2nd or 3rd line chemotherapy. In the meantime, first data have also become available from phase II studies investigating cetuximab in combination with 5-FU/leucovorin/irinotecan (FOLFIRI) and 5-FU/leucovorin/oxaliplatin (FOLFOX-4) as first-line treatment; and phase III studies in second-line chemotherapy settings are ongoing (e.g. EPIC and EXPLORE trials).

Cetuximab has also been evaluated in other cancers, in particular in head and neck and non-small-cell lung cancer (NSCLC). For example, in a phase III study 424 patients with squamous cancer of the head and neck were randomised to cetuximab in combination with high-dose radiotherapy or to high-dose radiotherapy alone. Patients who received the combination treatment had significanctly improved survival at 2 years (62% versus 55%) and 3 years (57% versus 44%) [20]. In patients with NSCLC, activity of cetuximab as single agent and in combination with standard chemotherapy regimens was seen; however, further phase III clinical trials will be required to confirm whether cetuximab improves the efficacy and outcome of chemotherapy in patients with NSCLC.

Matuzumab

Matuzumab (EMD 72000) is a humanised anti-EGFR monoclonal antibody which also prevents ligand-induced receptor activation and is currently in phase I and II studies [21]. This antibody has a prolonged half-life which may allow less frequent administration than the other antibodies, which are given on a weekly basis. In an ongoing trial, preliminary efficacy and pharmacodynamic data indicate that a more convenient administration schedule of every 2–3 weeks may actually be feasible with EMD72000 [22].

Panitumumab

Panitumumab (ABX-EGF) (Abgenics/Amgen; San Francisco, CA) is a fully human anti-EGFR MAb which binds with high affinity, inhibits ligand-dependent receptor activation and effectively inhibits the growth of human tumour xenografts. In a phase II study of ABX-EGF in advanced renal cell carcinoma, 88 patients whose treatment had failed or who were unable to receive interleukin-2/interferon-alfa completed one 8-week cycle of ABX-EGF and were assessable for response [23]. Objective responses were seen in five patients and stable disease was attained in 44/88 patients.

Side effects

The main side effects of all MAbs directed against EGFR are an acneiform rash (overall 70–86%, grade 3 or 4 in 5–16%), allergic reactions, diarrhoea and lethargy. The acneiform rash commonly develops within the first 3 weeks of treatment; dose reduction or omitting doses of cetuximab may produce improvement.

Apart from the fact that the "epidermal" growth factor receptor is to some extent expressed in the skin, little is known about the aetiology of this rash, and there are no clear evidence-based management recommendations. Histological data indicate that the rash may be caused by HER1/EGFR inhibition in skin, although this has not been confirmed. Findings suggest that there is

a relationship between the development of rash and response and/or survival, making rash a potential surrogate marker of activity. Data from multiple studies with cetuximab and also tyrosine-kinase inhibitors show a consistent relationship between rash and response, as well as between rash and survival [18, 19]. However, the cause of the possible relationship between rash and clinical benefit remains unclear at present, and additional studies are needed to determine the clinical utility of this observation (for review, see [24]).

Tyrosine-kinase inhibitors (TKIs)

The small molecule inhibitors compete with ATP for binding to the tyrosine kinase portion of the receptor and thereby abrogate the receptor's catalytic activity. Some of these small molecules may induce formation of inactive EGFR homodimers and EGFR/HER2 (ErbB1/ErbB2) heterodimers [25], which impair EGFR-mediated transactivation of the potent ErbB2 tyrosine kinase. In contrast to the monoclonal antibodies, this class of agents does not downregulate EGFR. In addition, because of the over 80% homology in the kinase domain between the EGFR (ErbB1) and HER2 (ErbB2), some ATP-competitive small molecule inhibitors can block the catalytic activity of both receptors. Also, binding to the ATP site can be either reversible or irreversible. Based on these differences, four different groups of TKIs can be identified: 1) reversible EGFR inhibitors (e.g. gefitinib, erlotinib or PKI-166), 2) irreversible EGFR inhibitors (e.g. EKB-569), 3) reversible pan-ErbB inhibitors (GW-2016) and 4) irreversible pan-ErbB inhibitors (CI-1033) (table 2). In contrast to monoclonal antibodies, which must be administered intravenously, tyrosine-kinase inhibitors can be taken orally. Gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; Roche) have already been approved in Europe and the USA for use in patients with NSCLC.

Gefitinib

Preclinical studies with gefinitib have shown antitumour activity in a variety of cultured tumour-cell lines and in human tumour xenografts, both as a single agent and in combination with chemotherapy and radiotherapy. Initial phase I clinical trials were performed to determine the maximum tolerated dose (MTD), which was found to be 700 mg/day [26]. Terminal elimination half-life was approximately 28 h (range 12–51 h), which is one reason for the commonly used daily dosing schedule of tyrosine-kinase inhibitors. Dose-limiting toxicities were diarrhoea and acneiform rash. Biologically relevant plasma concentrations were maintained at doses >150 mg/day, and skin biopsies demonstrated EGFR inhibition at the same dose as well as inhibition of the downstream signalling pathways involving MAPK, for example. On the basis of these phase I clinical trials, dose levels of 250 or 500 mg/day were selected for further investigation. In the large, randomised, double-blind, multicentre IDEAL trials (Iressa Dose Evaluation in Advanced Lung Cancer) I and II, gefitinib at a dose of either 250 or 500 mg/day was tested in patients with advanced NSCLC in whom prior chemotherapy had failed [27, 28]. While no difference in efficacy (response rate, overall survival and symptom improvement) was seen between the 250 and 500 mg/day dose, fewer and less severe side effects were seen in the patients treated with the lower dose. Response rates ranged from 9–19% and temporary disease control was achieved in another 40%.

Interestingly, subgroup analyses of these studies have shown that patients with the best chance of benefiting from treatment with gefitinib are female, non-smokers, of Asian origin and with adenocarcinoma. If all these criteria are fulfilled the probability of responding is as high as 56%, compared to 3% if none of these features is present [29].

As discussed earlier, gefitinib has shown promising activity in preclinical models, in particular in combination therapies. It seemed logical to test gefitinib in combination with standard chemo-

Table 2

Comparison of EGFR tyrosine-kinase inhibitors (TKI's) – so-called small molecules.

	Substrate	EGFR IC_{50} (nM)	HER2 IC_{50} (nM)	Clinical studies
Reversible EGFR-TKI	Gefitinib (Iressa)	23	3700	NSCLC, H&N and prostate (Phase III)
	Erlotinib (Tarceva)	20	3500	NSCLC, H&N and ovarian (Phase III)
	PKI-166	7	Unknown	Solid tumours (Phase I)
Irreversible EGFR-TKI	EKI-569	38	1200	Solid tumours (Phase I)
Reversible Pan-HER-TKI	GW2016 (Lapatinib)	9	9	Solid tumours (Phase I)
Irreversible Pan-HER-TKI	CI-1033	0.8	19	Solid tumours (Phase I)

IC_{50}, concentration necessary to result in cell death of 50%

therapy in a first line setting, as was done in two randomised clinical trials (INTACT 1 and 2; Iressa NSCLC Trial Assessing Combination Treatment). In the first trial, 1250 patients were randomised in Europe to receive cisplatin plus gemcitabine plus/minus gefitinib [30]; in the second trial, 1037 patients were randomised in the US to receive carboplatin plus paclitaxel plus/minus gefinitib [31]. In neither trial was any advantage observed by combining gefitinib with standard chemotherapy with regard to progression-free survival, survival or symptom control. Why gefitinib failed to exhibit an activity when associated with chemotherapy in chemo-naive patients is unknown, but several explanations and hypotheses have been proposed [32]. The first and one of the most frequently proposed hypotheses is antagonism or a competitive effect with chemotherapy. The results of preclinical studies and clinical trials with EGFR-targeted monoclonal antibodies suggested a synergistic or additive effect between gefitinib and chemotherapy. However, a trend towards better survival in the gefitinib arm after the completion of chemotherapy has shown that sequential administration after chemotherapy may be of interest. Secondly, an inadequate design for clinical trials evaluating targeted agents has been suggested: a targeted therapy would logically deploy its best anti-tumour activity in tumours expressing the target. In contrast to other targeted approaches, NSCLC patients included in these clinical trials were not selected strictly biologically with regard to a precise definition of EGFR expression when these trials were designed. As a result, the molecular heterogeneity of lung cancer led to a dilution of patients sensitive and resistant to gefitinib, thus might explaining the lack of benefit in these clinical trials.

Also disappointingly, another large randomised clinical phase III study (Iressa Survival Evaluation in Lung Cancer [ISEL STUDY]) comparing gefitinib to placebo in second- and third-line therapy in 1692 patients failed to show significant survival benefit from gefitinib in either the overall population (5.6 versus 5.1 months) or in patients with adenocarcinoma (6.3 versus 5.4 months). There was, however, a statistically significant improvement in tumour shrinkage (objective response rate), which did not translate into a statistically significant survival benefit. Again, prospective subgroup analyses suggested survival benefits in patients of Asian origin and in patients who had never smoked.

Currently gefitinib is also being studied in phase I and II clinical studies in head and neck, gastric and prostate cancer.

Erlotinib

Similarly to gefitinib, erlotinib (Tarceva) was first studied in classical phase I clinical trials and its MTD was found to be 150 mg/day. However, one possibly important difference in further development of this tyrosine-kinase inhibitor was the fact that consecutive studies were carried out at its MTD of 150 mg/day and not at its biologically relevant plasma concentration, as was done in the case of gefitinib. Erlotinib was evaluated in a phase II clinical trial in patients with advanced NSCLC (n = 57) in whom prior chemotherapy had failed [33]. The overall response rate was 12% and 1-year survival was 40%, both parameters very similar to gefitinib. Three larger phase II/III clinical trials in patients with NSCLC have recently been completed involving erlotinib in combination with standard chemotherapeutic regimens (TALENT and TRIBUTE trials) or as monotherapy (BR.21 study). When used in combination with carboplatin/paclitaxel (TALENT trial) or cisplatin/gemcitabine (TRIBUTE trial), erlotinib was not found to improve survival. These results contrast with what would be predicted from preclinical trial outcomes, but confirm the phase III reports involving similar chemotherapy regimens combined with gefitinib (INTACT 1 and 2 trials) described above.

Conversely to gefitinib, erlotinib has resulted in overall survival benefits when used as monotherapy: in the BR.21 study 731 patients with advanced NSCLC and one or two previous chemotherapies were treated with either erlotinib 150 mg/day or placebo, randomly assigned in a ratio of 2:1. The 1-year survival for patients treated with erlotinib (n = 488) was 31% versus 21% for the control group (n = 243). Median survival increased by 2 months from 4.7 to 6.7 in patients treated with the tyrosine-kinase inhibitor ($p < 0.001$). Even when the comparison was made after eliminating data from patients with partial and complete responses, there was a statistically significant difference in overall survival (median survival in the erlotinib group of 7.4 versus 6.7 months, p-value = 0.037). Interestingly, and again contrary to experience with gefitinib, patients with squamous-cell carcinoma of the lung also benefited from treatment with erlotinib with regard to survival (HR = 0.67, CI 0.50–0.90) [34].

Currently erlotinib is also being evaluated in phase I/II clinical trials in ovarian, head and neck, hepatocellular and pancreatic cancer.

At present it is unclear whether these two agents, which are members of the same class of reversible EGFR TKIs, mediate the same disease-specific activity. Both agents were found not to improve survival in combination with standard chemotherapy in patients with advanced NSCLC. As single agents, again, in patients with NSCLC, erlotinib statistically improved survival in one large study while in the corresponding gefitinib trial this drug failed to show the same benefit, although a trend in favour of the active treatment arm was found. Clearly, although they belong to the same class of agents, gefitinib and erlotinib possess different properties and characteristics, such as pharmacokinetic and binding affinities, which may explain the converse results reported. Or is it just a chance finding?

The ISEL study in particular has taught investigators that the development process for targeted drugs should be strengthened. It is important to fully understand the target pharmacodynamics before the drug is developed clinically. In the case of gefitinib, the negative results of the ISEL trial resulted in a new labelling and distribution programme on the part of the Food and Drug Administration (FDA). This programme limits the administration of gifitinib in the US to patients in the following situations: patients currently receiving and benefiting from the drug, patients who have previously received and benefited from the drug, and previously enrolled patients or new patients in non-Investigational New Drug clinical trials approved by an Investigational Review Board prior to June 17, 2005. For the moment erlotinib remains the only EGFR inhibitor approved by the FDA for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen.

Side effects

Since the target of the previously described monoclonal antibodies and TKIs is EGFR, it is not surprising that side effects such as acneiform rash and diarrhoea are similarly common for both classes of agents. However, gastrointestinal toxicity has been associated more frequently with TKIs [35] and constitutes their main dose-limiting toxicity.

Receptor mutations

The EGF receptor triggers cancer via at least three major mechanisms: overexpression of EGFR ligands, amplification of EGFR and mutational activation of EGFR. When gefitinib and erlotinib were introduced to the clinical setting the specific targets of these drugs in human tumours were unidentified, and little was known regarding their detailed mechanisms of action. Meanwhile, three groups focused on different approaches leading to the identification of EGFR kinase domain mutations in NSCLC. All these groups were motivated by the observation that tumours may be dependent on signalling from aberrant tyrosine-kinases and other oncogenes [36]. Human examples include BCR-ABL-dependent chronic myelogenous and acute lymphoblastic leukaemias [37], and KIT-dependent gastrointestinal stromal tumours [38].

One group first looked for rearrangements within the extracellular domain of EGFR which are characteristic of gliomas [39]. When no rearrangements were found, they subsequently sequenced the entire coding region of the gene and analysed tumours from patients who did or did not respond to treatment with gefitinib. The second group performed a genome-wide screen of tyrosine-kinases to determine whether mutations in specific kinases play a causal role in NSCLC [40]. As an initial screen, the investigators amplified and sequenced the exons encoding the activation loops of 47/58 human receptor tyrosine-kinase genes from a cohort of NSCLC samples. Mutations were detected only in EGFR, prompting a more detailed analysis of the entire EGFR gene in this cohort. Interestingly, EGFR mutations were found more frequently in patients with adenocarcinoma, in women, in patients of Asian origin and in never-smokers – the same patient group reported to have the best response to treatment with gefitinib [28]. The third group [41] extended the studies and found that similar EGFR mutations are also associated with responses to erlotinib.

To date 13 studies have reported the EGFR mutation status of a total of 499 patients treated with either gefitinib or erlotinib (ASCO 2005). Taking these studies together, 104 out of 143 tumours (73%) from patients with NSCLC experiencing at least a partial response to treatment with gefitinib or erlotinib have been shown to contain a mutation in the exons encoding the EGFR tyrosine-kinase domain. In contrast, only 24 of the 356 patients (7%) not responding to a treatment with these TKIs had a mutation of this kind.

Thus far, 192 different EGFR tyrosine-kinase domain mutations have been reported [42]. Nearly 86% of these mutations occur in two hot spots: 56% are in-frame deletions which eliminate four highly conserved amino acids encoded by exon 19; the remaining 44% are point mutations in exon 21 which result in a specific amino acid substitution at position 858. Importantly, only some of the various EGFR mutations have been associated with responses to gefitinib or erlotinib, such as those on exon 18, some of the common exon 19 deletions and some of the exon 21 point mutations. All the EGFR mutations found in NSCLC are clustered within the tyrosine-kinase domain of the protein. Hypothetically, all of the relevant mutations result in conformational changes leading to increased activity as well as TKIs sensitivity [39, 40]. The effect of mutations in the tyrosine-kinase domain on EGFR function is a major focus of ongoing studies. It has been reported that EGFR mutations lead to abnormally sustained responses to EGF [39] and activation of different downstream signalling pathways, such as Akt and STAT [43]. Remarkably, and in contrast to NSCLC cells without mutations in the EGF receptor, NSCLC cell lines which contain EGFR mutations remain dependent on the activity of the mutant EGFR for survival. This may partly explain why mutations of EGFR are associated with response to TKIs. In addition, the ability of EGFR to activate specific tyrosine-kinases

appears to be inhibited by TKIs at lower doses of drug than those required for wild type EGFR. For example, the L858R mutant is approximately 10 times more sensitive to gefitinib or erlotinib. However, among patients with NSCLC receiving erlotinib the presence of an EGFR mutation increases responsiveness to the agent but is not indicative of a survival benefit [44].

The aetiology of EGFR mutations remains unclear. Since mutations are more frequent in non-smokers (51% versus 9% in former/current smokers), they do not seem to be directly related to tobacco exposure. In addition, mutations are also more frequent in females (38%) than in males (13%), in adenocarcinoma (31%) as opposed to other histologies (2.3%), and in patients of Asian origin (29%) versus non-Asian patients (8%) [45]. Overall, mutations are expected to be present in 10–20% of patients with NSCLC in Europe.

The EGFR mutations associated with drug responses to gefitinib or erlotinib overlap, but, again, whether these two TKIs target exactly the same or common subsets of NSCLC patients is unknown.

In addition to mutations, high EGFR gene copy numbers were identified as an effective molecular predictor for efficacy of tyrosine-kinase inhibitors in advanced NSCLC [46]. In this study, amplification or high polysomy of the EGFR gene (33 of 102 patients) and high protein expression (58 of 98 patients) were significantly associated with better response (36% versus 3%), disease control rate (67% versus 26%), time to progression (9.0 versus 2.5 months), and survival (18.7 versus 7.0 months).

Other strategies

A different approach using the EGF receptor as a target for anticancer therapies is represented by the so-called immunoconjugates, such as immunotoxins, immunoradionucleotides or immunoliposomes. In theory, specific binding of the immunocomponent (MAb or its fragment) to EGFR, followed by internalisation, renders possible specific transport of the attached cytotoxic agent to target cells. One example are immunotoxins, generated by fusion of Pseudomonas or diphtheria toxin to MAbs or TGF-α. Preclinical studies have demonstrated promising anti-tumour activity in vitro and in vivo [47]. However, these constructs are highly immunogenic, resulting in rapid clearance and high accumulation in the liver and other organs [48].

Another promising strategy is the use of immunoliposomes, which are antibody-targeted liposomes (ILs) combining monoclonal antibody (MAb) and liposome technologies [49]. ILs are constructed to create agents capable of targeting drug carriers to tumour cells, providing a specific and efficient transport of encapsulated drugs to target cells while sparing normal tissue. We have developed immunoliposomes for specific recognition of EGFR, followed by receptor-mediated endocytosis [50]. We have demonstrated that EGFR-targeted immunoliposomes show extensive internalisation in the cytoplasm of EGFR-overexpressing cells (up to 30,000 ILs/cell) but not in non-overexpressing cells. EGFR-targeted ILs showed marked cytotoxicity after encapsulation of any of several chemotherapy drugs. Additionally, therapeutic studies in a series of tumour models have demonstrated superior efficacy for immunoliposome delivery. Anti-EGFR ILs containing different drugs (doxorubicin, epirubicin or vinorelbine) showed marked antitumour effects against moderate sized to large established tumours, including tumour regressions and cures in many mice. For each compound, the efficacy of the immunoliposome agent was significantly superior to all other treatments tested, including free drug, non-targeted liposomal drug and chemotherapy combined with free MAb [51]. By penetrating the plasma membrane and providing drug release within the cytoplasm, drug delivery is not only targeted but also very efficient, and has the potential to bypass membrane-bound efflux mechanisms ([52], and preliminary data). A first clinical phase I trial using doxorubicin-loaded anti-EGFR immunoliposomes has been designed and is scheduled to start in early 2006.

Outlook

One of the main focuses of ongoing work is the search for predictive factors for targeted therapies, including mutation, overexpression and amplification of EGFR. Apart from these molecular alterations, alternative approaches to predicting response to tyrosine kinase inhibitors have been studied. For example, it was found that patients with activated (phosphorylated) p-Akt had a better response rate, disease control and time to progression compared to patients with no p-Akt activation [53].

Another open question is why EGFR expression does not necessarily correspond to responsiveness to EGFR inhibition via TKIs or mono-

clonal antibodies [54], as was observed in the case of inhibition of HER2 through trastuzumab (Herceptin™). Future research must therefore define the exact mechanisms and pathways involved which are responsible for antitumour efficacy.

Conclusion

Inhibitors of the EGF receptor have already displayed activity in various types of advanced human cancers, and their role in the treatment of earlier stages and other cancer types is currently being evaluated.

Apart from all the open questions it should be kept in mind that these targeted therapies generate significant costs for moderate benefits in only a small subgroup of patients. For example, treatment with gefitinib or erlotinib costs approximately € 2000 per month, and treatment with cetuximab twice as much (table 3). However, patients who are sensitive to these kinds of therapy may respond impressively and clearly benefit from such treatment. Future preclinical investigations and, more importantly, clinical trials are necessary to help us to understand better and predict responsiveness to EGFR inhibition.

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Table 3
Therapy costs (only medication without administration).

Substrate	Schedule	costs per month (in euros)*
Gefitinib (Iressa)	250 mg p.o., daily	1975
Erlotinib (Tarceva)	150 mg p.o., daily	2250
Cetuximab (Erbitux)	250 mg/m^2 i.v., weekly (starting dose 400 mg/m^2)	1st month = 4550, followed by 3950

* Based on prices published in Switzerland (May 2005) and a median BSA of 1.85 m^2 (175 cm/70 kg)

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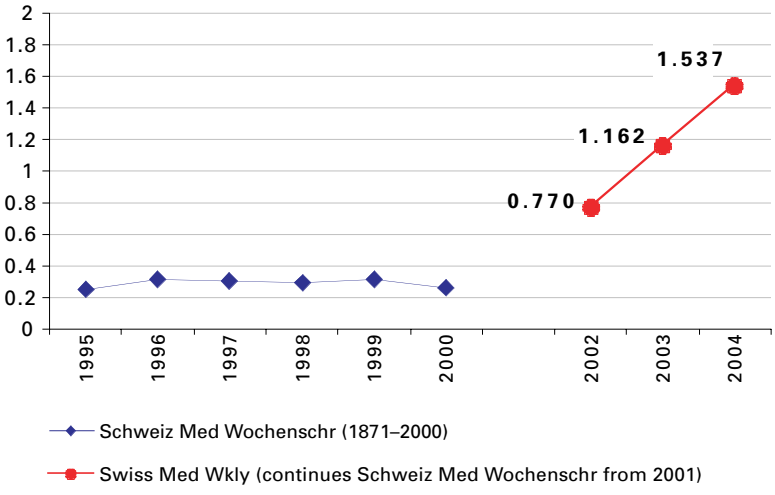

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