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Targeting the epidermal growth factor receptor: prognostic and clinical implications

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Abstract

The epidermal growth factor receptor (EGFR) has been implicated in many tumorigenic processes and is therefore a promising target for novel anticancer agents such as the EGFR tyrosine kinase inhibitor gefitinib ('Iressa', ZD1839). Clinical trials in non-small-cell lung cancer have shown gefitinib to have encouraging antitumour activity; however, not all patients exhibit the same response. This review explores whether there are potential prognostic indicators – such as biological markers, disease characteristics, or surrogate markers – that might predict the response to agents such as gefitinib. Interestingly, although expression levels of the EGFR itself do not appear to predict response to gefitinib, a number of candidate genes associated with clinical response or resistance have been identified. Various demographic factors, including performance status, gender and histology, appear to have prognostic value in some settings; however, the skin toxicity associated with EGFR-targeted agents does not appear to be predictive of response to gefitinib.

Keywords: Gefitinib; Iressa; ZD1839; Prognostic factors; Epidermal growth factor receptor (EGFR)

1. Introduction

The epidermal growth factor receptor (EGFR) is a promising target for novel anticancer agents, as it has been implicated in a number of tumorigenic processes, including cell proliferation, angiogenesis and metastasis, and decreased apoptosis (Fig. 1) [1,2]. The EGFR is expressed in all normal epithelial cells and has a wide range of functions, including organ morphogenesis, maintenance and repair, depending on the origin of the tissue and its state of differentiation. This ubiquity means that the EGFR has the potential to be a contributory factor in the pathogenesis of a wide range of solid tumours. Indeed, increased levels of EGFR expression have been observed in lung, prostate, breast, gastric and ovarian cancers, amongst others [2], and high levels of EGFR signalling are associated with late-stage disease and a high degree of invasiveness, metastasis and resistance to chemical or hormonal therapy [3]. Furthermore, the association between elevated EGFR expression and poor prognosis is particularly strong in head and neck, ovarian, bladder and esophageal cancer [4]. In addition, mutations in the EGFR have been identified in some tumours; in particular a truncated mutant, EGFRvIII, which has constitutive kinase activity, is found in brain, ovarian, breast and prostate cancers [5,6]. This evidence of a role for EGFR in the pathogenesis of various cancers has led to the rational design and development of agents that target this receptor.

One such agent is the orally active EGFR tyrosine kinase inhibitor (EGFR-TKI) gefitinib ('Iressa', ZD1839), which has been shown not only to reduce cell proliferation in a variety of human tumour cell lines and xenografts but also to induce cell cycle arrest, increase apoptosis and decrease angiogenesis, cell migration and invasiveness [7–11]. These preclinical data provided a rationale for assessing the antitumour activity of gefitinib in clinical trials.

The potential clinical benefits of gefitinib were first demonstrated in four phase I studies when evidence of antitumour activity was observed in patients with

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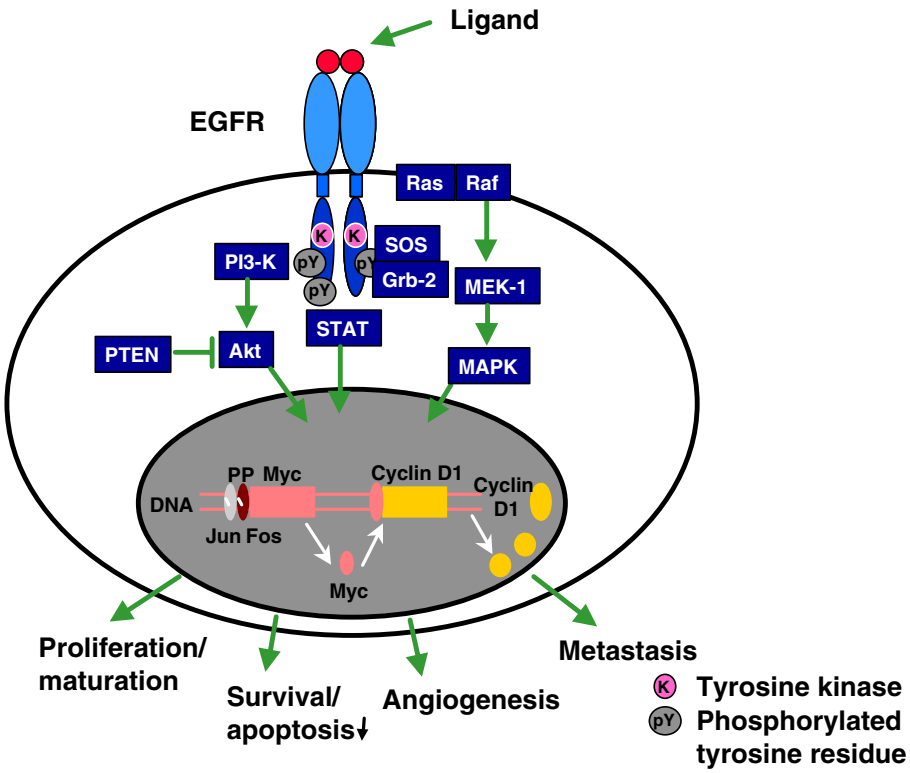


Fig. 1. EGFR signal transduction. Reproduced with permission from: Baselga J. New technologies in epidermal growth factor receptor-targeted therapy. *Signal* 2000, **1**, 12–21.

advanced non-small-cell lung cancer (NSCLC) [12–15]. The antitumour activity and a favourable tolerability profile were confirmed by two large phase II studies – 'Iressa' Dose Evaluation in Advanced Lung cancer (IDEAL) 1 and 2 – in patients with pretreated advanced NSCLC [16,17] (clinical data are reviewed by Thomas J Lynch, this volume). However, since not all patients exhibit the same response, there is considerable interest in prognostic indicators that might predict the response to agents such as gefitinib. Such indicators may include biological markers, disease characteristics or surrogate markers.

2. Are there biological markers that predict response to EGFR-TKIs?

There are a number of potential biological markers that might predict response to agents targeted to the EGFR, including components of the EGFR signalling pathway and molecular markers of proliferation and apoptosis. Of course, the most obvious candidate is the EGFR itself, and there has been much speculation whether the expression levels of EGFR could be used to predict response to EGFR-TKIs. This question has been addressed for gefitinib by an exploratory analysis using tumour biopsies taken prior to treatment from patients enrolled in two phase II studies of gefitinib in advanced NSCLC [18]. The baseline EGFR membrane staining intensity of the biopsies was measured using a validated immunohistochemical assay [19] and its correlation with the probability of objective tumour response was investigated. This analysis did not reveal any evidence for a consistent, biologically plausible correlation between the levels of membrane EGFR expression as measured and tumour response in patients treated with gefitinib for advanced NSCLC [18]. Substantial numbers of patients who were EGFR negative using this assay showed a tumour response, while some with intense EGFR staining showed no response (Fig. 2). There did appear to be an association between EGFR membrane staining and probability of symptom improvement; however, the association is not strong enough to warrant selecting patients on this basis, as at least 27% of patients with symptom improvement would be excluded from treatment. These data suggest that this method of EGFR assessment is unable to predict with sufficient precision which patients with advanced NSCLC are likely to experience clinical benefit following treatment with gefitinib.

This result is not particularly surprising, as there are several mechanisms by which the activity of receptor tyrosine kinases such as EGFR may become deregulated, many of which are not related to expression levels. These include genomic rearrangements that result in the expression of oncogenic fusion proteins, gain-of-function mutations (such as the constitutively active EGFRvIII) or small deletions, loss of inhibition by downregulation of inhibitory phosphatases and development of an autocrine

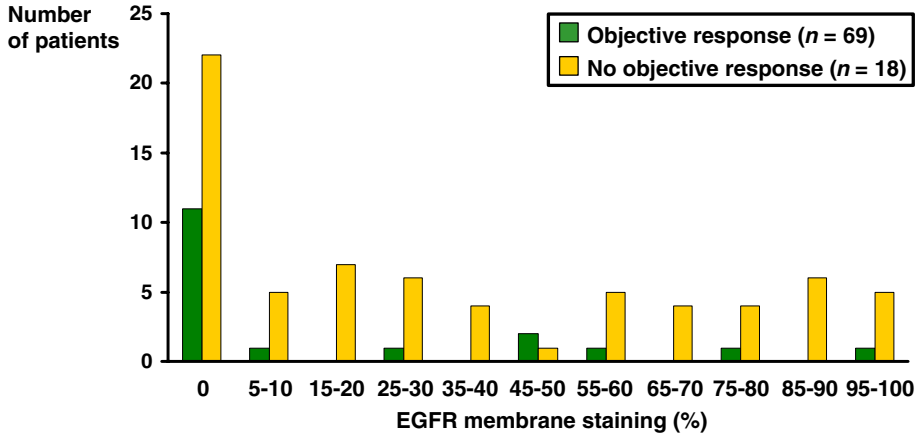


Fig. 2. Distribution of EGFR membrane staining intensity for patients with or without objective tumour response with gefitinib in IDEAL 1.

loop via overexpression of a growth factor or other ligand [20,21]. It is possible that assessing levels of activated EGFR may prove to have greater prognostic value than measuring expression levels alone.

An alternative approach to determining molecular predictors of response to EGFR-TKIs is to explore the correlation between quantitative gene expression in tumours and clinical response to these agents. A small study presented at ASCO 2003 identified a short list of candidate genes that appear to be associated with response to gefitinib [22]; these include signal transducers and activators of transcription (STAT) 5A and STAT5B, downstream elements of the EGFR signalling pathway, and γ-catenin. In accordance with the EGFR analysis described earlier, the expression of EGFR was not associated with a positive response to gefitinib. Interestingly, neither were a number of genes associated with proliferation [22]. Other studies are also underway to identify genes associated with sensitivity to gefitinib. For example an analysis of the gene-expression profiles of 13 NSCLC tumour xenografts identified 114 genes whose expression levels correlated significantly with gefitinib sensitivity [23]. Further studies are required to characterise these candidate markers of response.

In addition to identifying markers that predict a positive response to EGFR-TKIs, the identification of molecules that confer resistance to these agents may provide clinically useful information and important therapeutic leads. When the expression levels of the EGFR family members and their common ligands were examined in wild-type xenografts and in xenografts that had been developed to be resistant to gefitinib, there was no difference in RNA levels between the gefitinib-resistant and -sensitive tumours [22]. Furthermore, there were no mutations in the kinase domains of EGFR or erbB2, suggesting that alterations in EGFR might not be responsible for the acquired gefitinib resistance. However, one novel gene was upregulated in the gefitinib-resistant tumours. This putative gefitinib-resistance gene (GRG1) has been found to code for a cell-surface protein that is closely associated with erbB2. Interestingly, it is found in normal tissues that are naturally 'resistant' to EGFR-TKIs, such as the oral and oesophageal mucosa and the liver (EGFR-TKIs are not associated with adverse events such as stomatitis and hepatic toxicity in these tissues), but not in tissues 'sensitive' to EGFR-TKIs, such as the skin and colonic mucosa (the sites of adverse events commonly associated with EGFR-TKIs). Furthermore, clinical data suggest that the probability of response to gefitinib is significantly associated with low expression of GRG1. These initial data suggest that a combination of existing EGFR-TKIs with new molecules that target GRG1 could be a potentially useful new treatment strategy for NSCLC and other EGFR-driven tumour types.

3. Are there baseline prognostic factors that predict response to gefitinib?

Certain baseline factors appear to be predictive of improved clinical outcome in patients with advanced NSCLC who are receiving chemotherapy. The most important of these include disease stage, hypercalcaemia, superior vena caval obstruction, weight loss, performance status, age and gender [24]. In order to identify factors that might help to predict response to gefitinib, 22 factors were evaluated independently during a phase II study of gefitinib in advanced NSCLC to assess their value in predicting response (Table 1). Only four factors showed a significant effect on response: patients with performance status 0 or 1 (versus 2), female gender, adenocarcinoma histology (versus other histologies) or prior immuno/hormonal therapy such as picibanil, minomycin, marimastat and tamoxifen (versus no prior therapy) were more likely to respond to gefitinib treatment [16]. Uni- and multivariate analyses from a similar large phase II trial confirmed female gender and adenocarcinoma as prognostic factors for improved response to gefitinib (AstraZeneca data on file). Female gender has been identified previously as a prognostic factor for improved response rate and survival following first-line chemotherapy in NSCLC, as has a good performance

Table 1
Baseline factors evaluated for prognostic value in a phase II trial of gefitinib

Gender	***Performance status***
Histology	***Previously received immuno/hormonal therapy***
Age group (<65 vs ≥65 years)	Previously received docetaxel
Baseline lung cancer subscale score	Previously received radiotherapy
Body mass index at entry	Previously received surgery
Duration of previous chemotherapy treatment	Progressed on a previous chemotherapy
History of lung disorder, chest pain, dyspnoea, increased cough or haemoptysis	Stage of disease (III vs IV)
Months from diagnosis to randomisation	Time from last dose of chemotherapy to randomisation
Number of evaluable lesions at entry	Tumour burden at entry
Number of measurable lesions at entry	Type of disease (measurable/non-measurable)
Number of previous chemotherapies	Visceral metastases at entry

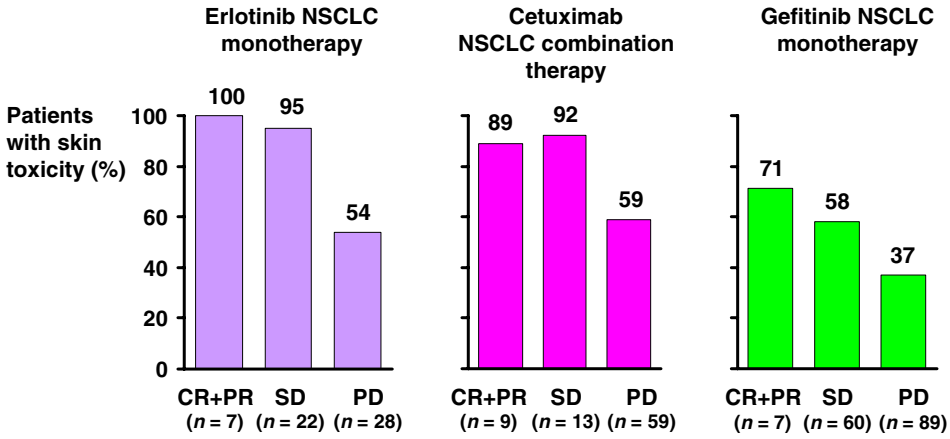
Factors that showed prognostic value are indicated in bold italics

status [25]. It is interesting that adenocarcinoma appears to be a prognostic factor, as these cells generally have low levels of EGFR compared with other histological types; it may be that there is an unknown factor at the protein level that determines sensitivity to gefitinib.

Survival following gefitinib treatment was explored using the data from two large, placebo-controlled, randomised studies combining gefitinib or placebo with chemotherapy (INTACT 1 = gemcitabine/cisplatin; INTACT 2 = carboplatin/paclitaxel). This multivariate analysis demonstrated that poor performance status, weight loss, and bone and liver metastases were significantly associated with a poor survival outcome in both trials, while male gender and brain metastases were identified as prognostic factors for poor survival in INTACT 2 only [26]. These analyses did not show any consistent effects of gefitinib combined with chemotherapy versus chemotherapy and placebo for any of the factors analysed.

4. Does skin toxicity predict response to EGFR-TKIs?

Recent reports have claimed that response to EGFR-TKIs might be predicted by the skin toxicity that is a common side effect of these agents. The bases of these claims are that most patients with a response, stable disease or long-term survival have a skin rash, that the incidence of rash amongst these patients is higher than in non-responders, and that survival endpoints are improved in patients with a rash. This phenomenon has been observed with the EGFR-TKIs gefitinib [27,28] and erlotinib [29], and also with the antibody cetuximab [30] (Fig. 3). However, as patients with an objective response are likely to remain on treatment for longer than those with disease progression, it might be expected that they would have a higher incidence of rash, suggesting that the correlation might not be predictive. It may be more appropriate, therefore, to determine whether the *early* development of skin toxicity could be used to predict objective response. To investigate this, a retrospective analysis examined the development of skin toxicity within 14 and 28 days in patients entered into a phase II study of gefitinib monotherapy in NSCLC who survived at least 28 days' therapy. A multivariate analysis indicated no statistically significant difference in the objective response rate between those with or without early-onset skin toxicity. This analysis was conducted independently in



NSCLC, non-small-cell lung cancer; CR, complete response; PR, partial response; SD, stable disease, PD, progressive disease

Fig. 3. Correlation between response and skin rash with EGFR-targeted agents.

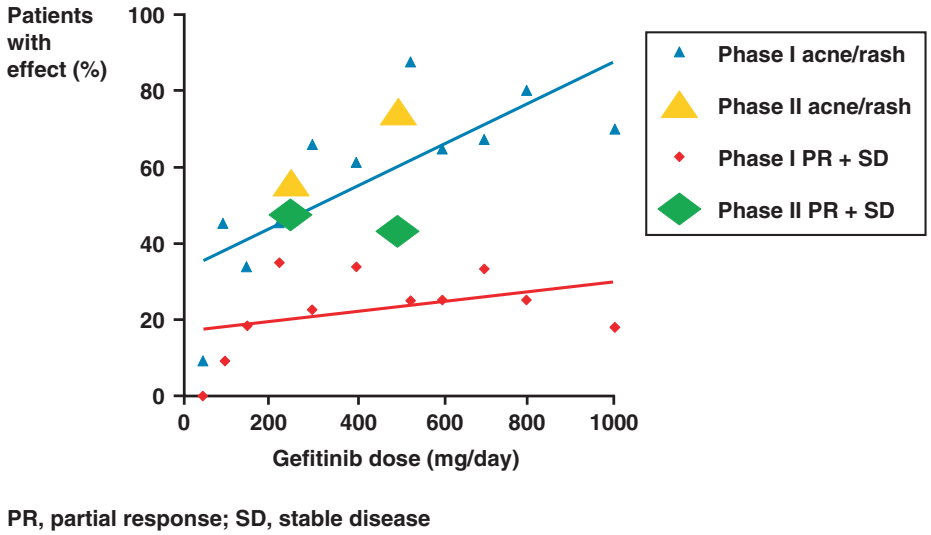


Fig. 4. Relationship between dose and incidence of acne or skin rash and response in gefitinib phase I and II trials.

each dose group (250 and 500 mg/day) and the results were the same for each dose. By day 14, 8/12 patients (67%) who ultimately achieved an objective response with gefitinib 250 mg/day had not developed any skin toxicity, while by day 28, 3 of these patients (25%) still had not developed skin toxicity (AstraZeneca data on file).

The clinical trial programme of gefitinib has shown that increasing the dose will increase the incidence of rash, but this does not translate into a higher response rate. In the phase I trials over the dose range 150–1000 mg/day, the incidence of acne or rash increased with increasing dose (Fig. 4) [12–15]. This was also seen in the large phase II IDEAL trials, in which the incidence of acne or rash was higher at 500 than at 250 mg/day, although this was not associated with higher objective response rates (Fig. 4), prolonged time to progression or survival [16,17]. Furthermore, analysis of one of these phase II studies showed that the incidence of rash was the same in responders and non-responders [31].

These data are also supported by a pharmacodynamic analysis in which paired skin biopsies were taken from patients receiving gefitinib (150–1000 mg/day) in a phase I study [32]. Gefitinib treatment significantly inhibited the activation of EGFR and mitogen-activated protein kinase, a downstream EGFR-inducible molecule, while increasing the expression of $p27^{KIP1}$, a marker of EGFR pathway inhibition and growth arrest (Fig. 5). Cell proliferation (evaluated using the proliferation marker Ki67) was reduced and apoptosis increased following gefitinib treatment [32]. These pharmacodynamic responses were not significantly associated with the development of adverse skin reactions. Furthermore, there was no noteworthy dose- or plasma concentration-response effect in this pharmacodynamic study, possibly because the starting dose of 150 mg/day resulted in significant receptor inhibition. This is supported by the fact that clinical benefit and tumour response were seen at all dose levels [32]. These data suggest that gefitinib has a wide therapeutic margin,

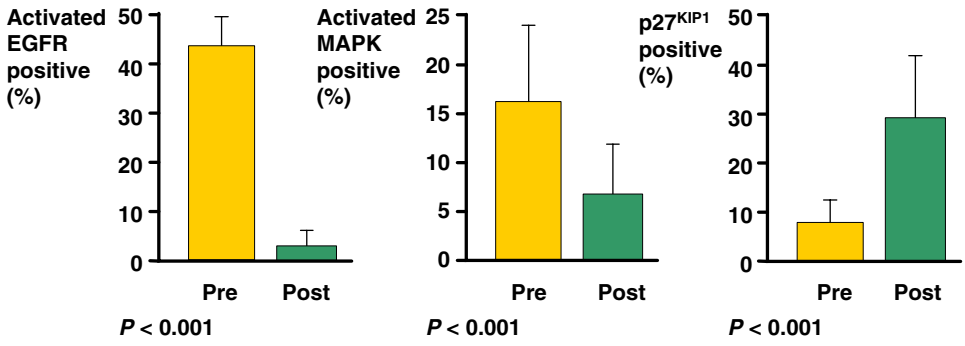


Fig. 5. Activation of EGFR and MAPK and expression of $p27^{KIP1}$ before and after gefitinib treatment. Reproduced with permission from: Albanell J, Rojo F, Averbuch S, *et al*. Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. *J Clin Oncol* 2002, **20**, 110–24. Reprinted with permission from the American Society of Clinical Oncology.

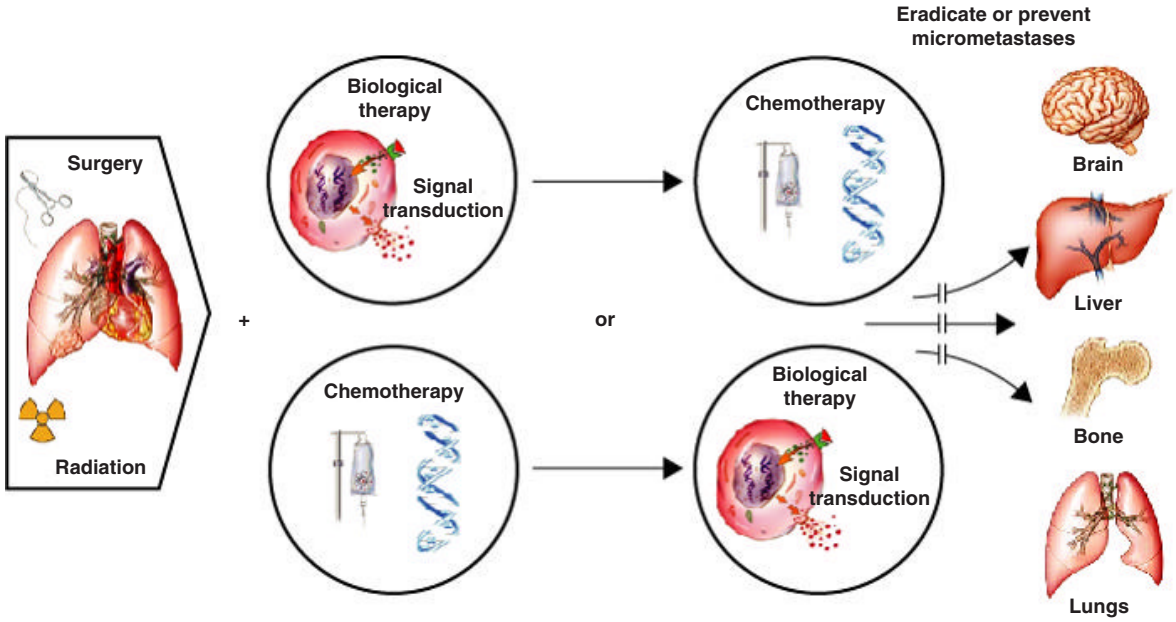


Fig. 6. New paradigm for the use of biologically targeted therapy with chemotherapy in advanced metastatic NSCLC.

and that the clinical dose should be selected based on the optimal balance of efficacy and tolerability, rather than having to dose at or close to the maximum tolerated dose for optimal efficacy.

5. Conclusions

The data presented here demonstrate that there are several factors that might have value for predicting clinical response to gefitinib and other EGFR-targeting agents. Although it appears that EGFR expression levels cannot predict response to gefitinib, a number of other candidate biological markers have been identified and are being studied in further detail. The identification of a gene associated with resistance to gefitinib (GRG1) is particularly exciting, as it offers the potential for a new therapeutic strategy, targeting both the EGFR and GRG1 simultaneously. It will be interesting to see whether GRG1 also confers resistance to other EGFR-targeted agents. There are a number of clinical baseline factors, notably performance status, gender and histology, that appear to predict a greater probability of response to gefitinib, some of which also predict a good response to other NSCLC treatments. Despite the publication of data demonstrating an association between skin toxicity and response, there does not appear to be a correlation between the early onset of skin toxicity and response, thus lessening any potential prognostic value. In the IDEAL 1 and 2 studies, symptom improvement was associated with tumour response and with increased overall survival and therefore has the potential to be used to predict the response to gefitinib. This association is discussed fully by Peter Harper (this volume).

Further studies exploring other potential prognostic factors are warranted, and it is hoped that the new data will drive the rational use of novel agents in different patient populations, alone and in combination with traditional chemotherapeutic agents (Fig. 6).

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