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REVIEW

The challenge of targeting EGFR: experience with gefitinib in nonsmall cell lung cancer

A.A. Armour and C.L. Watkins

ABSTRACT: As the first approved epidermal growth factor receptor (EGFR)-targeted therapy for nonsmall cell lung cancer (NSCLC), the clinical development of gefitinib was complex. Advances in scientific understanding of the target biology during its clinical development enabled the identification of a biomarker to define patients most likely to derive benefit from gefitinib. Initial phase II trials showed clinically meaningful anti-tumour activity in 12–18% of unselected pretreated patients with advanced NSCLC at the optimum biological dose (250 mg). Subgroup analyses of these and subsequent phase III trials in unselected patients suggested that *EGFR* mutation and some clinical characteristics associated with a higher incidence of *EGFR* mutation (Asian ethnicity, adenocarcinoma histology, never-smoking and female sex) were linked with increased response to gefitinib. Consequently, the IRESSA Pan-Asia Study (IPASS) was conducted in never-smokers or former light-smokers in East Asia who had adenocarcinoma of the lung. IPASS showed that *EGFR* mutation was the strongest predictor of improved progression-free survival (mutation-positive subgroup hazard ratio (HR) 0.48, 95% CI 0.36–0.64 ($p<0.001$, $n=261$); mutation-negative subgroup HR 2.85, 95% CI 2.05–3.98 ($p<0.001$, $n=176$); interaction test $p<0.001$) with gefitinib *versus* carboplatin/paclitaxel as first-line therapy for advanced NSCLC. Important lessons for the development of future personalised medicines are discussed.

KEYWORDS: Epidermal growth factor receptor, gefitinib, nonsmall cell lung cancer, personalised medicine

Lung cancer is the most common cause of cancer deaths worldwide [1]. Many cases are not diagnosed until the disease is at an advanced stage, and the prognosis is poor with a 5-yr survival rate of ~15% [2]. Nonsmall cell lung cancer (NSCLC) accounts for ~80% of all lung cancers and comprises three main types: adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Advances have been made in the control of local disease with the addition of systemic therapy; however, for patients with metastatic disease, chemotherapy has remained the established treatment since the mid-1990s [3]. Platinum-based doublet chemotherapy has been the mainstay of first-line treatment for advanced NSCLC in patients with a good performance status; however, despite the development of new chemotherapy regimens, the prognosis remains poor and the toxicity remains significant [4]. Targeted cancer therapies focusing on molecular changes specific to cancer may be more effective, and give rise to predictable and more favourable tolerability, than traditional chemotherapy that interferes with all rapidly dividing cells.

Gefitinib (IRESSA™) is an orally active epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) and was the first EGFR-targeted therapy to be approved for the treatment of NSCLC. The understanding of the biological role of the target/receptor was limited during the early stages of gefitinib development. The clinical programme needed to be adapted in response to advances in scientific understanding of the target biology.

Phase II clinical studies demonstrated anti-tumour activity of gefitinib against pretreated advanced NSCLC, with response rates of 12–18% in an unselected population [5, 6]. Gefitinib was subsequently approved in Japan in 2002 and in the USA in 2003. The results of the phase III

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IRESSA Survival Evaluation in Lung cancer (ISEL) study available in 2004 failed to show a statistically significant benefit in overall survival for gefitinib compared with best supportive care in the overall unselected, predominantly refractory study population [7]. This led to the use of gefitinib being restricted in June 2005 by the US Food and Drug Administration (FDA) and to AstraZeneca withdrawing the marketing authorisation application under review in Europe. Greater benefit for gefitinib *versus* placebo observed in subgroups such as never-smokers and patients of Asian origin suggested the potential for improved patient selection based on clinical characteristics, which was explored in further work in conjunction with tumour biology studies. In 2008, the phase III IRESSA Pan-Asia Study (IPASS) showed that the presence of an *EGFR* mutation was the strongest predictor of a more favourable outcome with gefitinib compared with carboplatin/paclitaxel as first-line treatment. In June 2009, the European Medicines Agency (EMA) approved gefitinib for use in adult patients with locally advanced or metastatic NSCLC with activating mutations of EGFR-tyrosine kinase.

This article discusses the complex journey of discovery and clinical development of gefitinib and the many challenges that were successfully resolved. Advances in the scientific understanding of the target biology, which resulted in the ability to biologically define those patients who were most likely to derive the greatest benefit from treatment, are also reviewed.

TARGETING THE EGFR WITH GEFITINIB

EGFR belongs to a family of four related transmembrane receptors: EGFR (HER1), HER2, HER3 and HER4 [8, 9]. EGFR is activated by binding of one of its specific ligands, such as epidermal growth factor (EGF) or transforming growth factor-α, to its extracellular domain, resulting in dimerisation, and receptor autophosphorylation and transphosphorylation through intrinsic tyrosine kinase activity. This triggers intracellular pathways that can result in cell proliferation, inhibition of apoptosis, invasion and metastasis, and tumour-induced angiogenesis [10]. Many common solid tumours of epithelial origin express high levels of EGFR and it has been associated with advanced disease and poor prognosis [8]; it is, therefore, an attractive target for anti-tumour therapies.

Gefitinib is a low molecular weight, synthetic anilinoquinazoline that was designed to inhibit the EGFR pathway. Gefitinib inhibits the tyrosine kinase activity of EGFR, blocking its autophosphorylation and subsequent downstream signalling (fig. 1) [12]. In preclinical studies, gefitinib inhibited EGF-stimulated cell growth and tumour growth in nude mice bearing a range of human tumour xenografts, illustrating its potential for cancer therapy [12].

DETERMINING THE OPTIMUM BIOLOGICAL DOSE

The tolerability, pharmacokinetics and anti-tumour activity of oral gefitinib at doses up to 1,000 mg·day^{-1} were investigated in four phase I dose-escalation multicentre studies in patients with a range of solid tumours known to express EGFR, including 100 patients with advanced NSCLC [13–16]. In two initial phase I studies, gefitinib was administered once daily for 14 days followed by 14 days of observation in 28-day cycles [15, 16]. The tolerability profile was acceptable and the two subsequent studies were conducted using 28-day cycles of once daily oral dosing at 150–1,000 mg·day^{-1} gefitinib [13, 14]. The maximum tolerated dose (MTD) was 800 mg·day^{-1} and 1,000 mg·day^{-1} with once daily oral dosing, and the predominant dose-limiting toxicity was diarrhoea [13, 14]. The most common adverse events were rash/acne, diarrhoea, nausea, vomiting and asthenia, and were dose-related: the majority were mild or moderate (common toxicity criteria (CTC) grade 1 or 2), with grade 3 or 4 events generally occurring at doses

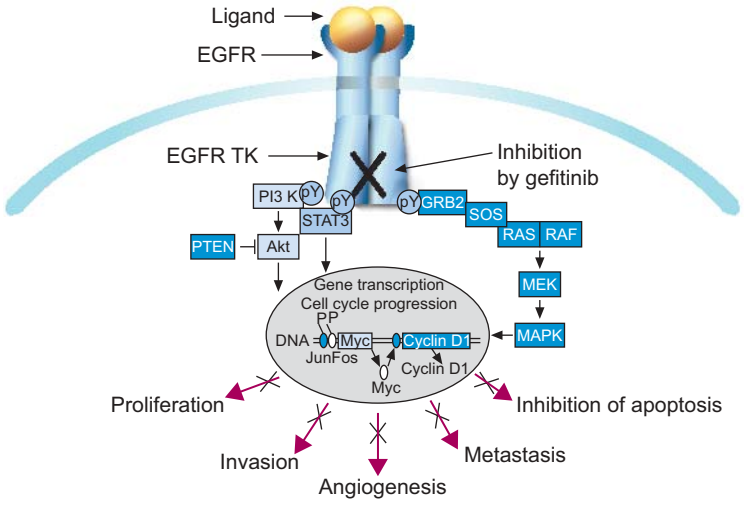

FIGURE 1. Schematic representation of the role of epidermal growth factor receptor (EGFR) in cancer and its inhibition by gefitinib. Activation of the EGFR by ligand binding causes receptor dimerisation and the autophosphorylation of specific tyrosine residues of the intracellular tyrosine kinase (TK) domain. This leads to the stimulation of downstream signalling pathways, including the phosphatidylinositol 3-kinase (PI3K)–Akt and RAS–mitogen-activated protein kinase (MAPK) pathways, which promote the processes of cell proliferation, angiogenesis, invasion/metastasis and the inhibition of apoptosis. Gefitinib inhibits the TK activity of the EGFR TK domain, blocking the signalling pathways important in the survival and proliferation of tumour cells. PTEN: phosphatase and tensin homologue; STAT3: signal transducer and activator of transcription 3; GRB2: growth factor receptor-bound protein 2. Reproduced from [11] with permission from the publisher.

>600 mg·day^{-1} [13, 14]. Radiographic responses were observed across the whole dose range, with all of the 10 partial responses across the trials occurring in patients with NSCLC [13–16].

Dose selection for gefitinib was based on the optimum biological dose approach with the aim of achieving maximum inhibition of the EGFR target at a dose level below the MTD [17, 18]. In the phase I studies, biologically relevant plasma concentrations of >100 ng·mL^{-1} (above the 90% maximal inhibitory concentration for inhibition of growth of KB oral carcinoma cells [9]) were generally maintained at doses >100 mg·day^{-1} across the 24-h dosing period [15–17]. Pharmacodynamic studies in skin biopsies from patients taking ⩾150 mg·day^{-1} gefitinib revealed changes indicative of inhibition of the EGFR signalling pathway at every dose level evaluated [13, 19]. Based on these results, the activity observed across the dose range and the more favourable tolerability at doses ⩽600 mg·day^{-1}, two doses of gefitinib (250 and 500 mg·day^{-1}) were selected for phase II evaluation in patients with advanced pretreated NSCLC.

Towards the end of 2000, two uncontrolled, dose-randomised, double-blind, multicentre phase II studies were initiated to evaluate 250 and 500 mg·day^{-1} doses of gefitinib in patients with locally advanced or metastatic NSCLC who had previously received platinum-based chemotherapy [5, 6]: the IRESSA Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 (n=210) and IDEAL 2 (n=221) studies [5, 6]. Both studies showed that once daily oral treatment with gefitinib resulted in clinically meaningful anti-tumour activity, with objective response rates (ORRs) of 18% and 19% in IDEAL 1 and 12% and 9% in IDEAL 2 at 250 and 500 mg·day^{-1}, respectively. Disease-related symptom improvement rates were 40% and 37% among evaluable patients in IDEAL 1 and 43% and 35% in IDEAL 2, respectively. Median overall survival times were 7.6 and 8.0 months in IDEAL 1 and 7 and 6 months in IDEAL 2, respectively. The most common gefitinib-related adverse events were consistent with those observed in the phase I trials. Improved tolerability was noted at 250 mg·day^{-1}, with fewer patients requiring dose interruptions or dose reductions, withdrawing from treatment and experiencing grade 3/4 events than at 500 mg·day^{-1} (16% and 28%, 0% and 10%, 2% and 9%, and 2% and 5% in IDEAL 1, respectively). Thus, 250 mg·day^{-1} was identified as the optimum biological dose for gefitinib and taken forward into the phase III clinical programme.

The concept of using optimal biological dose (rather than MTD dosing) for gefitinib is further supported by more recent clinical data, which have shown that, due to its pharmacokinetic properties, gefitinib concentrates more in tumour tissue (*i.e.* at the target) relative to plasma, reaching concentrations greater than 40-fold higher in breast tumour [20] and 60-fold higher in NSCLC tumour [21] than in coincident plasma samples.

Based on the phase II study results, the first marketing authorisation for gefitinib was granted in July 2002 in Japan for the treatment of inoperable or recurrent NSCLC.

IDENTIFYING POTENTIAL PREDICTORS OF RESPONSE TO GEFITINIB

The first clues as to which clinical subgroups of patients were most sensitive to gefitinib were provided by the phase II studies, in which higher ORRs were observed in patients with female sex and adenocarcinoma histology in IDEAL 1 and 2 [5, 6], and with Japanese ethnicity in IDEAL 1 [5]. A number of retrospective studies showed that never-smoking status was also associated with sensitivity to gefitinib [22–24]. However, as there were no control arms, it was not clear if similar results would be seen for any treatment or whether these were predictive factors specific to gefitinib.

Initial exploratory biomarker studies assessing EGFR protein expression using immunohistochemistry reported mixed results on the link between EGFR expression and outcome with gefitinib. While no relationship was found between EGFR protein expression and response for the IDEAL studies [25], other studies were inconclusive [26] or showed that EGFR protein expression was related to clinical outcome [27, 28]. Another potential biomarker investigated was *EGFR* gene copy number. An increase in *EGFR* gene copy number may occur as a result of polysomy (extra copies of chromosomes) or gene amplification (the presence of multiple copies of a gene) and can be investigated using fluorescence *in situ* hybridisation (FISH) [26]. Initial retrospective analyses showed an association between high *EGFR* gene copy number and outcome [27–29].

In 2004, sensitising mutations of *EGFR* were described in patients with advanced NSCLC [30–32]. In a retrospective analysis, 25 (81%) of 31 patients who had experienced partial responses or marked clinical improvement on gefitinib or erlotinib (an EGFR-TKI) were found to have tumours with mutations in the *EGFR* tyrosine kinase domain [31]. Conversely, none of the 29 patients who did not respond to gefitinib or erlotinib was found to harbour *EGFR* mutations. These and subsequent studies showed that *EGFR* mutations were more prevalent in females, never-smokers, patients with adenocarcinoma histology and those of Asian ethnic origin [30–34], *i.e.* those groups in whom increased responsiveness to gefitinib had been observed.

In the early studies, *EGFR* mutational analyses of tumour samples were performed by the sequencing and analyses of PCR fragments in both sense and antisense directions for all exons of the *EGFR* tyrosine kinase domain. Two mutations in the *EGFR* tyrosine kinase domain (either multinucleotide in-frame deletions of amino acids in exon 19 or point mutations resulting in a specific amino acid substitution at position 858 (L858R) in exon 21) were found to account for 49 (88%) of 56 of the sensitising NSCLC-associated *EGFR* mutations [31]. As a small number of mutation types were shown to account for the vast majority of *EGFR* mutations, more sensitive targeted techniques have subsequently been developed that detect only the most common mutation types.

These initial results for EGFR biomarkers required further investigation in large scale, randomised, controlled, prospective studies.

THE GEFITINIB MONOTHERAPY EXPERIENCE: FROM AN UNSELECTED PRETREATED POPULATION TO FIRST-LINE USE IN PATIENTS WITH EGFR MUTATION-POSITIVE NSCLC

Initial phase III studies in unselected first-line NSCLC patients showed that gefitinib combined with platinum-based doublet chemotherapy gave no additional efficacy benefit over the

TABLE 1 Summary of efficacy end-points for gefitinib from the IPASS, INTEREST and ISEL studies

Study	Population	Subjects n	Objective response rates	Progression-free survival[#]	Overall survival[#,¶]
IPASS[+]					
Gefitinib *versus* carboplatin/ paclitaxel in chemo-naïve, never- or former light-smokers with adenocarcinoma in East Asia	Overall	1217	43.0% *versus* 32.2% OR 1.59 95% CI 1.25–2.01 p<0.001	HR 0.74 95% CI 0.65–0.85 5.7 *versus* 5.8 months p<0.001	HR 0.91 95% CI 0.76–1.10 18.6 *versus* 17.3 months
	EGFR mutation-positive	261	71.2% *versus* 47.3% OR 2.75 95% CI 1.65–4.60 p=0.0001	HR 0.48 95% CI 0.36–0.64 9.5 *versus* 6.3 months p<0.001	HR 0.78 95% CI 0.50–1.20 NR *versus* 19.5 months
	EGFR mutation-negative	176	1.1% *versus* 23.5% OR 0.04 95% CI 0.01–0.27 p=0.0013	HR 2.85 95% CI 2.05–3.98 1.5 *versus* 5.5 months p<0.001	HR 1.38 95% CI 0.92–2.09 12.1 *versus* 12.6 months
INTEREST[§]					
Gefitinib *versus* docetaxel in previously treated patients	Overall	1466	9.1% *versus* 7.6% OR 1.22 95% CI 0.82–1.84 p=0.33	HR 1.04 95% CI 0.93–1.18 2.2 *versus* 2.7 months p=0.47	HR 1.020 96% CI 0.905–1.150[##] 7.6 *versus* 8.0 months p=0.7332
	EGFR mutation-positive	44	42.1% *versus* 21.1% OR 25.22 95% CI 1.23–515.53 p=0.0361	HR 0.16 95% CI 0.05–0.49 7.0 *versus* 4.1 months p=0.001	HR 0.83 95% CI 0.41–1.67 14.2 *versus* 16.6 months p=0.60
	EGFR mutation-negative	253	6.6% *versus* 9.8% OR 0.63 95% CI 0.23–1.73 p=0.3720	HR 1.24 95% CI 0.94–1.64 1.7 *versus* 2.6 months p=0.14	HR 1.02 95% CI 0.78–1.33 6.4 *versus* 6.0 months p=0.91
ISEL[f]					
Gefitinib *versus* best supportive care in previously treated patients	Overall	1692	8.0% *versus* 1.3% OR 7.28 95% CI 3.1–16.9 p<0.0001	TTF HR 0.82 95% CI 0.73–0.92 3.0 *versus* 2.6 months p=0.0006	HR 0.89 95% CI 0.77–1.02 5.6 *versus* 5.1 months p=0.087
	EGFR mutation-positive	26	37.5% *versus* 0% NC	NC	NC
	EGFR mutation-negative	189	2.6% *versus* 0% NC	NC	HR 1.16 95% CI 0.79–1.72 3.7 *versus* 5.9 months p=0.4449

Odds ratios >1 favour gefitinib. Hazard ratios <1 favour gefitinib. IPASS: IRESSA Pan-Asia Study; INTEREST: IRESSA NSCLC Trial Evaluating Response and Survival *versus* Taxotere; ISEL: IRESSA Survival Evaluation in Lung cancer; EGFR: epidermal growth factor receptor; NR: not reached; NC: not calculated; TTF: time to treatment failure. [#]: median month values are presented; [¶]: IPASS overall survival follow-up is ongoing; [+]: values presented for IPASS are for gefitinib *versus* carboplatin/paclitaxel; [§]: INTEREST values are for gefitinib *versus* docetaxel; [f]: ISEL values are for gefitinib *versus* placebo; [##]: confidence interval entirely below non-inferiority margin of 1.154. Data are taken from [7, 28, 37, 38, 39] and previously unpublished studies.

chemotherapy regimen alone [35, 36], and therefore the phase III programme progressed evaluating gefitinib as monotherapy.

Gefitinib monotherapy versus *placebo or docetaxel in pretreated patients*

Two phase III studies, ISEL and IRESSA NSCLC Trial Evaluating Response and Survival *versus* Taxotere (INTEREST), designed as phase IV commitments with the FDA to support the approval, evaluated the role of gefitinib monotherapy in pretreated patients. These studies were initiated in 2003 and early 2004, respectively, before the discovery of sensitising *EGFR* mutations, and recruited an unselected population. However, these two large studies provided the opportunity to evaluate the relationship between EGFR biomarkers and clinical outcome with gefitinib.

The placebo-controlled phase III ISEL study investigated the effect of gefitinib on survival for patients (n=1,692) with locally advanced or metastatic NSCLC who had received one or two previous chemotherapy regimens and were refractory to or intolerant of their latest chemotherapy regimen [7]. Treatment with gefitinib was associated with a numerical

improvement in survival in the overall unselected population, but this failed to reach statistical significance in the primary analysis (hazard ratio (HR) 0.89, 95% CI 0.77–1.02; p=0.087; table 1). The high proportion of chemotherapy refractory patients (90%) in ISEL may account in part for this outcome in the overall population, as these patients represent a very difficult to treat population with a poor prognosis. However, pre-planned subgroup analyses showed statistically significant increases in survival with gefitinib compared with placebo for never-smokers (n=375; HR 0.67, 95% CI 0.49–0.92; p=0.012) and patients of Asian origin (n=342; HR 0.66, 95% CI 0.48–0.91; p=0.01). The ORRs in the overall population were 8.0% *versus* 1.3% (p<0.0001) for gefitinib and placebo, respectively, and pre-planned subgroup analyses showed that the highest response rates with gefitinib were among never-smokers (18.1%), females (14.7%), patients of Asian origin (12.4%) and patients with adenocarcinomas (11.9%); the characteristics that are now known to be typically associated with increased incidence of *EGFR* mutation. Gefitinib was well tolerated, with the most common adverse events being rash (37% *versus* 10%) and diarrhoea (27% *versus* 9%); mostly CTC grade 1 or 2 in severity.

Based on the ISEL results, in June 2005, the FDA limited the use of gefitinib to patients who have previously taken gefitinib and are benefiting or have benefited from gefitinib, and AstraZeneca withdrew its marketing authorisation application under review in Europe. Given the more favourable results observed for patients of Asian ethnicity, the drug remained available in many Asian countries.

A panel of EGFR-related biomarkers was subsequently investigated in 460 tumour samples from patients in the ISEL study [28]. High *EGFR* gene copy number measured by FISH was found to be a predictor of a survival benefit with gefitinib compared with placebo (HR 0.61 and 1.16 for high and low copy number, respectively; interaction test (comparison of high *versus* low copy number HR) p=0.045). An association was also observed between EGFR protein expression and survival (HR 0.77 and 1.57 for positive and negative expression, respectively; interaction test p=0.049). There were insufficient *EGFR* mutation-positive samples for survival analysis by *EGFR* mutation status, although gefitinib-treated patients with mutations had higher ORRs than those without (table 1). Of all the clinical and biomarker subgroups assessed, gefitinib ORR was highest in the *EGFR* mutation-positive subgroup [7, 28].

In the INTEREST study, first reported in late 2007 [40], patients with locally advanced or metastatic NSCLC that had progressed or recurred after one or two previous platinum-based chemotherapy regimens were randomised to treatment with either gefitinib (n=733) or docetaxel (n=733) [37]. Non-inferior survival of gefitinib compared with docetaxel was demonstrated in the overall unselected population (HR 1.020, 96% CI 0.905–1.150; predefined non-inferiority margin 1.154) (table 1). Progression-free survival (PFS) and ORR were similar in both treatment groups (table 1). Significantly more patients had improvements in quality of life (QoL) with gefitinib compared with docetaxel as assessed by Functional Assessment of Cancer Therapy-Lung (FACT-L) total score (OR 1.99, 95% CI 1.42–2.79; p<0.0001) and the FACT-L Trial Outcome Index (TOI) (OR 1.82, 95% CI 1.23–2.69; p=0.0026), and there was no significant difference between the two arms in the proportions of patients who had improvements in lung cancer symptoms (p=0.13). Some clinical factors (never-smoking, Asian origin, female sex and adenocarcinoma histology) were associated with long survival, although similar effects were seen with both gefitinib and docetaxel and so no significant differences between treatments were observed (fig. 2). However, there was more heterogeneity in the treatment differences for the secondary end-points of PFS and ORR (fig. 2). Gefitinib had a more favourable tolerability profile than docetaxel. The most common adverse events were rash/acne (49% *versus* 10%) and diarrhoea (35% *versus* 25%) in the gefitinib group, and neutropenia (5% *versus* 74%), asthenic disorders (25% *versus* 47%) and alopecia (3% *versus* 36%) in the docetaxel group. Gefitinib was associated with lower rates of grade 3 or 4 adverse events (9% *versus* 41%), particularly grade 3 or 4 neutropenia (2% *versus* 58%). Other studies have consistently shown that the efficacy of gefitinib is similar to that of docetaxel in unselected patient populations in the pretreated setting, but with an improved tolerability and QoL profile [41–43].

The biomarker findings from ISEL prompted the introduction of a co-primary analysis of superior overall survival in patients with high *EGFR* gene copy number in the INTEREST study, *via* protocol amendment in 2006. Superior overall survival of gefitinib *versus* docetaxel in patients with high *EGFR* gene copy number was not proven (HR 1.09, 95% CI 0.78–1.51; p=0.62). One possible explanation for the difference in biomarker results between ISEL and INTEREST is that gefitinib and docetaxel have similar activity in patients with high or low *EGFR* gene copy number and that high *EGFR* gene copy number is predictive of a greater survival benefit over placebo for both treatments [38]. Another is that crossover to the alternative therapy at disease progression made it more difficult to detect an overall survival difference in INTEREST as many patients received both treatments. In contrast to overall survival, PFS and ORR advantages for gefitinib tended to be larger in patients with high *EGFR* gene copy number compared with those with low copy number [38]. There was no evidence of a difference in overall survival, PFS or ORR between treatments according to EGFR protein expression status. Of 297 patients with samples evaluable for *EGFR* mutations, 44 (15%) were *EGFR* mutation positive. Among patients with EGFR mutation-positive tumours, PFS and ORR were higher for gefitinib compared with docetaxel (table 1 and fig. 2). However, overall survival was similarly long with both gefitinib and docetaxel in patients with *EGFR* mutation-positive tumours and, hence, there was no statistically significant difference in overall survival between treatments in the small *EGFR* mutation-positive subgroup (HR 0.83, 95% CI 0.41–1.67; p=0.60) (table 1). A prospective trial with a higher number of patients with *EGFR* mutation-positive status was needed to make any definitive conclusions regarding *EGFR* mutation status and outcome.

Gefitinib versus *doublet chemotherapy as first-line treatment in clinically selected patients*

Although *EGFR* mutations had been identified at the time of designing the IPASS trial in 2005, the importance of these and other biomarkers relative to clinical characteristics was not clear at that point. Therefore, patients were selected for IPASS on the basis of clinical characteristics known to be associated

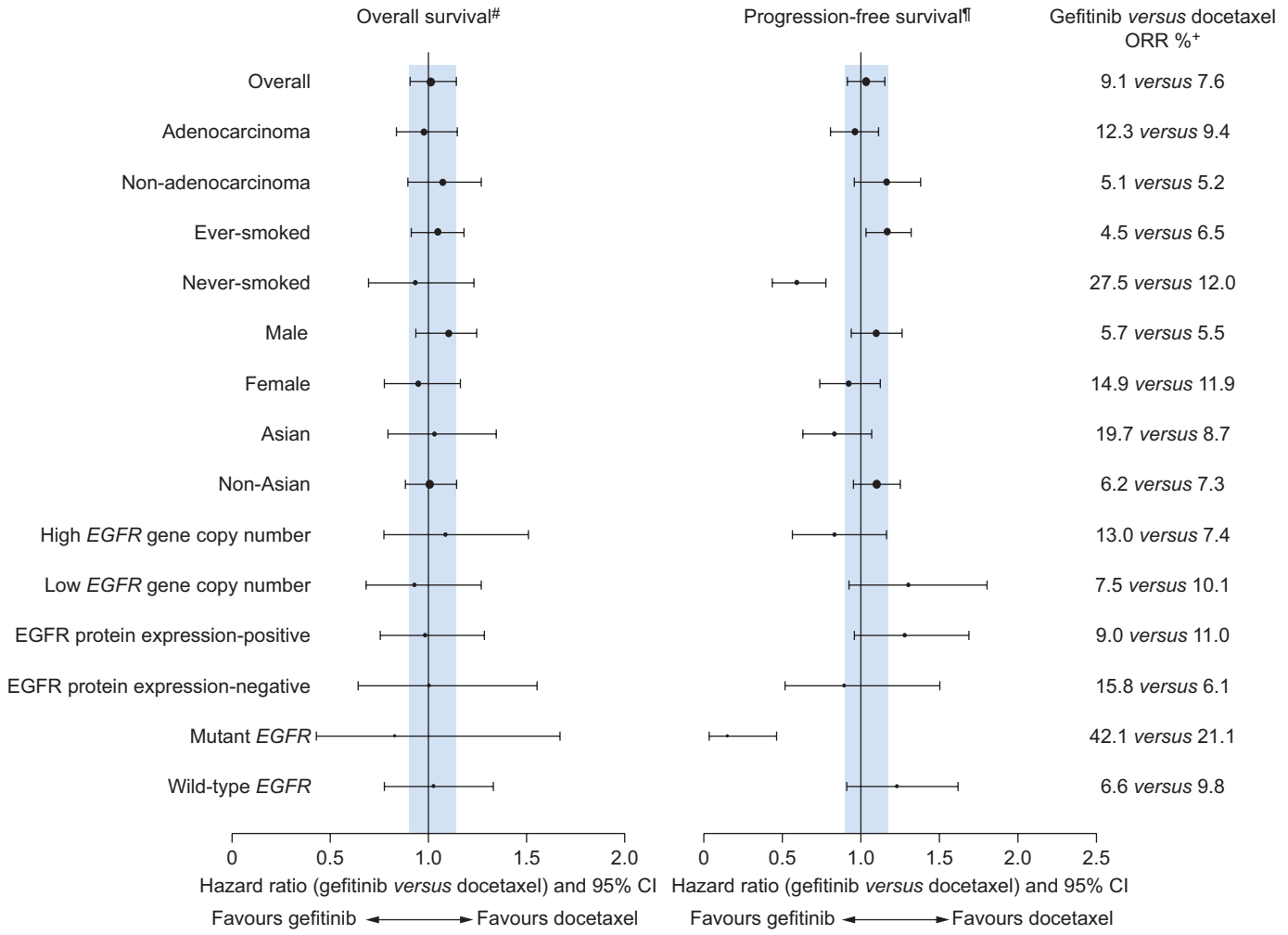

FIGURE 2. Forest plot of (left) overall survival and (right) progression-free survival for gefitinib *versus* docetaxel in the INTEREST (IRESSA NSCLC Trial Evaluating Response and Survival *versus* Taxotere) study (pretreated setting) by clinical characteristics and epidermal growth factor receptor (EGFR) biomarkers [37, 38]. ORR: objective response rate. #: unadjusted analysis – per-protocol population for clinical factors and intent-to-treat population for biomarker factors; ¶: adjusted analysis – evaluable for response (EFR) population; +: EFR population.

with increased response to gefitinib. Never-smokers or former light-smokers in East Asia who had adenocarcinoma of the lung were randomised, between March 2006 and October 2007, to receive either gefitinib (n=609) or carboplatin/paclitaxel (n=608) as first-line treatment [39]. The study met its primary objective of demonstrating non-inferiority and additionally showed the superiority of gefitinib compared with carboplatin/paclitaxel for PFS in the overall clinically selected population (HR 0.74, 95% CI 0.65–0.85; p<0.001) (table 1). The effect was not constant over time, with the probability of being progression-free in favour of carboplatin/paclitaxel in the first 6 months, and in favour of gefitinib in the following 16 months, likely driven by the differing effects in subgroups (see later). The ORR was significantly higher with gefitinib compared with carboplatin/paclitaxel (table 1) and significantly more patients had improvements in QoL with gefitinib compared with carboplatin/paclitaxel as assessed by FACT-L total score (OR 1.34, 95% CI 1.06–1.69; p=0.01) and the FACT-L TOI (OR 1.78, 95% CI 1.40–2.26; p<0.001). Similar proportions of patients had improvements in symptoms as assessed by FACT-L Lung Cancer Subscale (LCS) score (OR 1.13, 95% CI 0.90–1.42; p=0.30). As in previous studies, gefitinib had a more favourable tolerability profile than chemotherapy, with a lower rate of grade 3 or 4 adverse events (29% *versus* 61%). The most common adverse events in the gefitinib group were rash or acne (66% *versus* 22%) and diarrhoea (47% *versus* 22%), whereas in the carboplatin/paclitaxel group, the most common adverse events were neurotoxic effects (11% *versus* 70%), neutropenia (4% *versus* 67%) and alopecia (11% *versus* 58%).

Analysis of efficacy according to baseline biomarker status was a preplanned objective of IPASS. Samples were tested for the presence of *EGFR* mutations using the amplification refractory mutation system (the DxS EGFR29 mutation-detection kit; Qiagen, formerly DxS, Manchester, UK) (437 were evaluable for *EGFR* mutation status). The presence of *EGFR* mutation was a very strong predictor of improved PFS and ORR with gefitinib compared with carboplatin/paclitaxel. Among the subgroup of 261 patients who had *EGFR* mutation-positive tumours, PFS was significantly longer with gefitinib compared

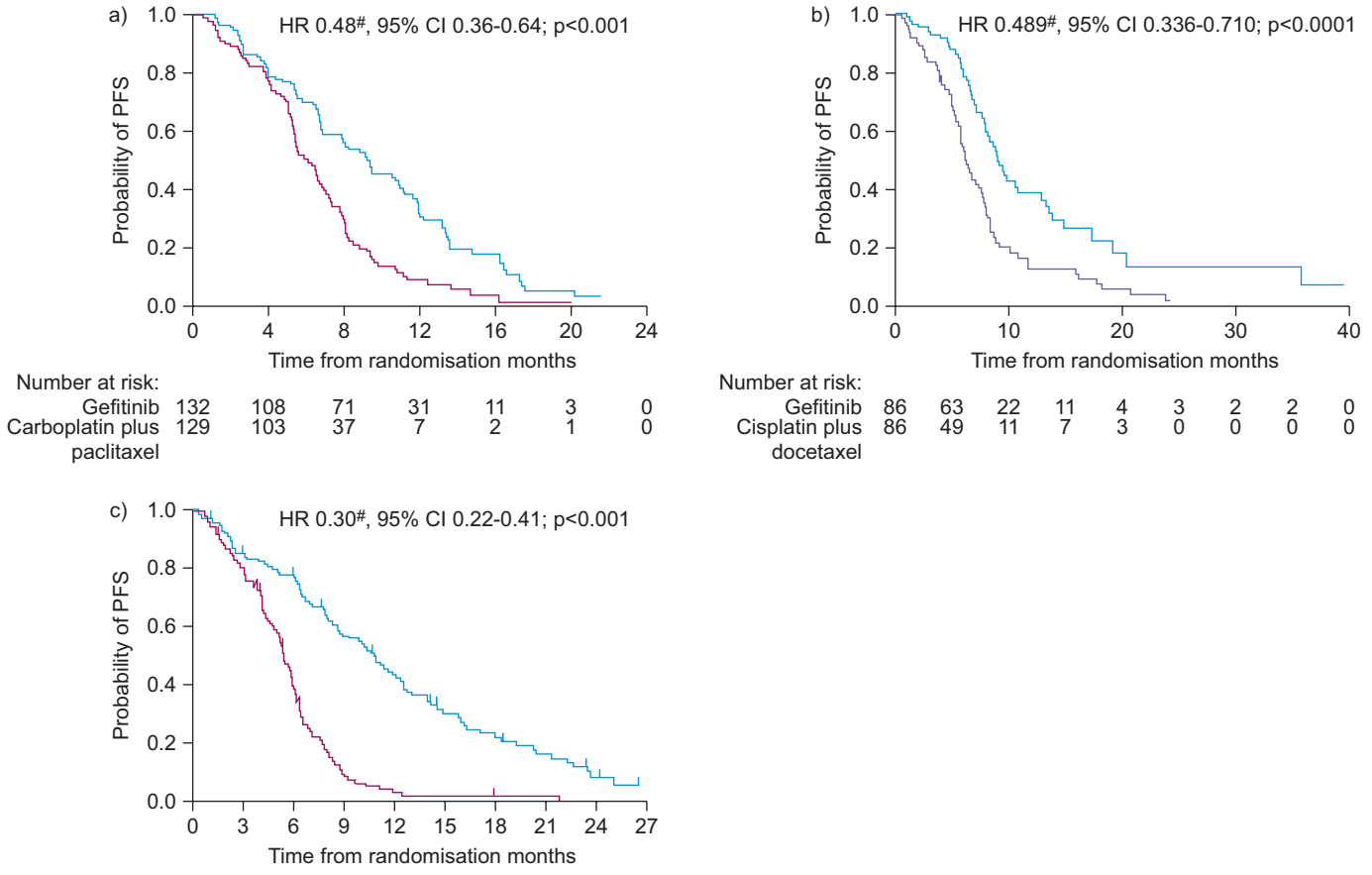

FIGURE 3. Kaplan–Meier curve for progression-free survival for gefitinib *versus* doublet chemotherapy in three phase III trials in first-line nonsmall cell lung cancer harbouring an activating epidermal growth factor receptor (*EGFR*) mutation: a) patients with *EGFR* mutation-positive status in the IRESSA Pan-Asia Study (IPASS; first-line setting) (——: gefitinib; ——: carboplatin plus paclitaxel) (reproduced from [39] with permission from the publisher; © 2009 Massachusetts Medical Society. All rights reserved), b) the overall population (all *EGFR* mutation-positive) in the WJTOG3405 study (first-line setting) (——: gefitinib; ——: cisplatin plus docetaxel) (reproduced from [44] with permission from the publisher; © 2010, with permission from Elsevier) and c) the overall population (all *EGFR* mutation-positive) in the NEJ002 study (first-line setting) (——: gefitinib (n=114); ——: carboplatin plus paclitaxel (n=110)) (reproduced from [45] with permission from the publisher; © 2009 Massachusetts Medical Society. All rights reserved). PFS: progression-free survival; HR: hazard ratio. #: HR <1.00 favours gefitinib.

with carboplatin/paclitaxel (HR 0.48, 95% CI 0.36–0.64; p<0.001) (table 1 and fig. 3a). Conversely, in the subgroup of 176 patients with *EGFR* mutation-negative tumours, PFS was significantly longer with carboplatin/paclitaxel compared with gefitinib (HR 2.85, 95% CI 2.05–3.98; p<0.001) (table 1). The treatment by mutation status interaction test p-value showed p<0.001. The benefit seen with gefitinib in the overall study population was driven primarily by the subgroup of patients with *EGFR* mutations, with ORRs of 71.2% *versus* 1.1% in mutation-positive *versus* mutation-negative subgroups (table 1). The ORRs with carboplatin/paclitaxel were 47.3% *versus* 23.5%, respectively (table 1).

Unplanned analysis of the relatively small amount of early overall survival data available at the time of the primary PFS analysis by mutation status showed a HR numerically in favour of gefitinib in the *EGFR* mutation-positive subgroup (HR 0.78, 95% CI 0.50–1.20; based on 81 events) (table 1) and numerically in favour of carboplatin/paclitaxel (HR 1.38, 95% CI 0.92–2.09; based on 94 events) (table 1) in the mutation-negative subgroup. This preliminary analysis was based on a relatively small number of events. Further follow-up for mature overall survival data is ongoing. However, even when the data are mature, analysis of the overall survival data is likely to be confounded by crossover to the comparator treatment; for this reason PFS was chosen as the primary outcome of this study. Additionally, as shown by BROGLIO and BERRY [46], even when overall survival is improved it can be difficult to demonstrate statistical significance if survival post-progression is long, such as longer than 12 months; this was the case for the IPASS mutation-positive subgroup, in which median PFS on the chemotherapy arm was 6.3 months but median overall survival was 19.5 months.

Significantly more patients in the *EGFR* mutation-positive subgroup had improvements in QoL with gefitinib compared with carboplatin/paclitaxel as assessed by FACT-L total score (OR 3.01, 95% CI 1.79–5.07; p<0.0001) and FACT-L TOI (OR 3.96, 95% CI 2.33–6.71; p<0.0001), and in symptoms as assessed by FACT-L LCS score (OR 2.70, 95% CI 1.58–4.62; p=0.0002). Conversely, in the mutation-negative subgroup, significantly more patients had improvements in QoL and symptoms with carboplatin/paclitaxel compared with gefitinib.

Analysis of PFS by *EGFR* gene copy number (measured by FISH) produced similar but less marked trends to those observed in the *EGFR* mutation analysis [47]. The treatment by gene copy number interaction test showed p=0.0437. PFS was significantly longer with gefitinib than with carboplatin/paclitaxel in the subgroup of 249 patients with high *EGFR* gene copy number (HR 0.66, 95% CI 0.50–0.88; p=0.0050) and numerically longer with carboplatin/paclitaxel than with gefitinib in the subgroup of 157 patients with low *EGFR* gene copy number (HR 1.24, 95% CI 0.87–1.76; p=0.2368). However, there was a high degree of overlap between *EGFR* mutation-positivity and high *EGFR* gene copy number: of 245 patients with high *EGFR* gene copy number whose *EGFR* mutation status was also known, 190 (78%) were also *EGFR* mutation positive. This suggests that the improved outcome in high *EGFR* gene copy number patients is being driven by the *EGFR* mutation-positive overlap. There was no relationship evident between EGFR protein expression status and PFS outcome.

In summary, patients selected by clinical characteristics for first-line treatment in IPASS had prolonged PFS and increased ORR with gefitinib. The finding that PFS treatment effect changed over time (favouring carboplatin/paclitaxel for the first 6 months and gefitinib thereafter) is likely due to the mixed population with regard to *EGFR* mutation status, the initial advantage for carboplatin/paclitaxel being attributed to the benefit of chemotherapy over gefitinib in the *EGFR* mutation-negative subgroup and the subsequent advantage for gefitinib attributed to the prolonged PFS in the *EGFR* mutation-positive subgroup with gefitinib. The presence of *EGFR* mutation was the strongest and most reliable predictor of improved PFS and ORR with gefitinib compared with carboplatin/paclitaxel as first-line therapy of advanced NSCLC.

The results in the *EGFR* mutation-positive subgroup from the IPASS trial are now supported by those of two other phase III randomised controlled trials conducted in Japan of gefitinib *versus* doublet chemotherapy as first-line treatment of advanced NSCLC harbouring *EGFR* sensitising mutations [44, 45]. In these two studies, gefitinib significantly prolonged PFS compared with doublet chemotherapy (n=172; HR 0.489, 95% CI 0.336–0.710; p<0.0001 *versus* cisplatin/docetaxel (fig. 3b); and n=224; HR 0.30, 95% CI 0.22–0.41; p<0.001 *versus* carboplatin/paclitaxel (fig. 3c)) [44, 45].

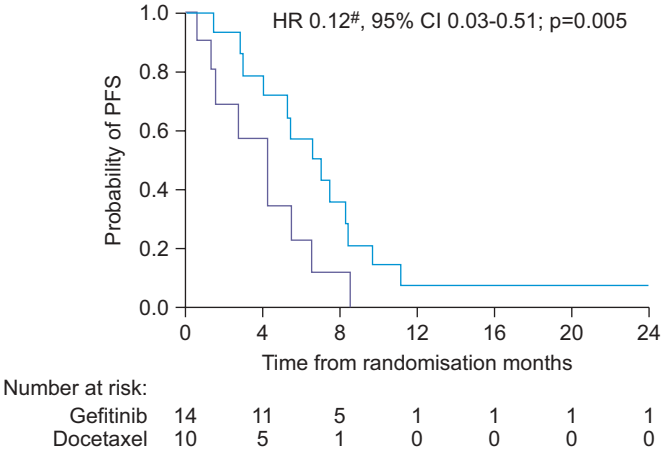

FIGURE 4. Kaplan–Meier curve for progression-free survival for non-Asian patients with epidermal growth factor receptor (*EGFR*) mutation-positive status in the INTEREST (IRESSA NSCLC Trial Evaluating Response and Survival *versus* Taxotere) study (pretreated setting). ——: gefitinib; ——: docetaxel. PFS: progression-free survival; HR: hazard ratio. #: HR <1.00 favours gefitinib. Reproduced from [53] with permission from the publisher.

Evidence suggests that patients with sensitising *EGFR* mutations have high response rates to gefitinib irrespective of ethnicity [48], although the proportion of patients with *EGFR* mutations is lower for non-Asian (~10–15%) [49, 50] compared with Asian patients (~30–40%) [51, 52]. A *post hoc* subgroup analysis of the INTEREST study (pretreated patients) showed that PFS was significantly longer with gefitinib than docetaxel in non-Asian patients with *EGFR* mutation-positive disease, although the patient numbers were low (fig. 4).

The IPASS result presents a paradigm shift in the treatment of lung cancer: a first-line oral treatment option for patients with *EGFR* mutation-positive tumours that is more effective than doublet chemotherapy, the standard of care as first-line treatment for advanced NSCLC since the 1980s. By targeting the EGFR, a receptor preferentially expressed by tumour cells, gefitinib is associated with predictable and more favourable tolerability compared with traditional chemotherapy that interferes with all rapidly dividing cells. In June 2009, the EMA granted marketing authorisation for gefitinib for adults with locally advanced or metastatic NSCLC with activating mutations of EGFR-tyrosine kinase, based on a submission package including the IPASS and INTEREST studies.

LESSONS LEARNED

The sooner a predictive biomarker for the effect of a new treatment is identified, the more focused and efficient the clinical development programme can become. One of the major challenges in the development of gefitinib was that scientific understanding of the drug's target biology, and potential biomarkers of outcome, advanced in conjunction with its clinical development. Consequently, the identification of clinical characteristics and biomarkers of response to gefitinib, and the refinement of these, occurred simultaneously to a large degree. Trials in unselected NSCLC populations first revealed clinical characteristics and then biomarkers associated with high response rates, ultimately leading to prospective studies of gefitinib in patients with *EGFR* mutation-positive disease. We now know that it is *EGFR* mutation rather than protein expression or gene copy number that is linked to the dependency on the EGFR pathway and, therefore, the mechanism of action of gefitinib. Sensitising mutations of the *EGFR* increase the activation and duration of receptor signalling after ligand binding compared with the wild-type receptor [32]. The mutated *EGFR* exhibits preferential signalling down the AKT pathway and this gives the cell a survival advantage that must be maintained. Over time, the tumour becomes heavily dependent on this target and oncogenic addiction occurs [54].

It is hoped that, in the future, technical advances will result in preclinical models that are better able to identify optimal biomarkers for response to new targeted anti-cancer therapies, enabling more efficient clinical development. In the absence of a targeted biomarker in early clinical development, a surrogate for the biomarker may help to identify those patients most

TABLE 2 Summary of multivariate logistic regression analysis to identify factors that independently predict for the presence of epidermal growth factor receptor (*EGFR*) mutations in non-Asian and Asian patients

Factors that predict for presence of *EGFR* mutation[#]	Non-Asian patients[¶]		Asian patients[+]	
	Odds (95% CI) of *EGFR* mutation	p-value	Odds (95% CI) of *EGFR* mutation	p-value
Smoking status	6.6 (3.9–11.1) times higher in never-smokers than ever-smokers	<0.0001	2.4 (0.9–5.9) times higher in never-smokers than ever-smokers	0.0702
Histology	3.8 (2.1–6.8) times higher in adenocarcinoma than non-adenocarcinoma	<0.0001	4.7 (1.7–12.8) times higher in adenocarcinoma than non-adenocarcinoma	0.0022
Sex	1.9 (1.2–3.1) times higher in females than males	0.0103	2.2 (0.9–5.5) times higher in females than males	0.1007
WHO PS	1.9 (1.0–3.6) times higher in PS 0–1 than PS 2	0.0594		

These data are previously unpublished. [#]: age (<65 years *versus* ⩾65 years) and WHO PS (0–1 *versus* ⩾2; Asians only) were not found to be significant predictors using p<0.2 selection criteria; [¶]: analysis based on INTEREST, INVITE, ISEL, INSTEP, INTACT 1 and 2, and IDEAL 1 and 2 baseline data in non-Asian patients combined (n=920); overall mutation-positive rate in non-Asian patients was 10%; [+]: analysis based on INTEREST, V-15-32, INTACT 1 and 2, and IDEAL 1 and 2 baseline data in Asian patients combined (n=140); overall mutation-positive rate in Asian patients was 39%. WHO: World Health Organization; PS: performance status; INTEREST: IRESSA NSCLC Trial Evaluating Response and Survival *versus* Taxotere; INVITE: IRESSA in NSCLC versus Vinorelbine Investigation in the Elderly; ISEL: IRESSA Survival Evaluation in Lung cancer; INSTEP: IRESSA NSCLC Trial Evaluating Poor Performance Status Patients; INTACT: IRESSA NSCLC Trial Assessing Combination Treatment; IDEAL: IRESSA Dose Evaluation in Advanced Lung Cancer.

likely to respond to treatment. Current evidence suggests that the early identified potential markers of outcome to gefitinib, including clinical characteristics and *EGFR* gene copy number, were probably surrogates for *EGFR* mutation status [47]. Indeed, a multivariate logistic regression analysis (to identify factors that independently predicted for the presence of *EGFR* mutations in a total of 1,060 patients included in clinical trials of gefitinib) confirmed that the clinical characteristics of never-smoker, adenocarcinoma histology and female sex are independent predictors of positive *EGFR* mutation status (table 2).

In the IPASS trial, patients were selected on clinical characteristics (never- or former light-smoker, adenocarcinoma histology and living in Asia), and 60% of those patients with evaluable samples were found to be *EGFR* mutation positive [39]. While the study showed a significant PFS benefit with gefitinib compared with carboplatin/paclitaxel overall and in those patients who harboured an *EGFR* mutation, patients without the mutation did better on chemotherapy. Therefore, clinical characteristics cannot be considered to be appropriate surrogates for *EGFR* mutation status when making treatment decisions regarding use of gefitinib *versus* doublet chemotherapy in the first-line setting. Nevertheless, clinical surrogates may be useful in other settings, for other drugs and biomarkers.

If biomarkers could potentially affect treatment outcome, then ideally, high quality tissue samples should be collected from all patients in the study. This maximises the ability to detect predictive biomarkers if they exist. However, the gefitinib development programme highlights the practical challenges in conducting biomarker research. These are mainly related to collection of adequate numbers of tissue samples that are of sufficiently high quality and quantity, which is particularly problematic in the case of lung cancer, where the tumour is relatively inaccessible, some patients have inoperable disease and where routine clinical practice does not necessarily generate the samples required for biomarker analysis. Other challenges include the informed consent process for biomarker evaluation, problems with incomplete pathology tracking consent and recovery of sufficient DNA from the samples for *EGFR* mutation analysis. In the case of multinational phase III trials, sample quality can be variable and the process of obtaining informed consent presents different challenges in different countries. For example, in the IPASS trial, despite 1,038 (85%) of the 1,217 trial participants providing consent for biomarker analyses, tissue samples were provided for only 683 of these, of which 437 were evaluable for *EGFR* mutations and 261 were found to have *EGFR* mutation-positive tumours [39]. In non-Asian populations, the difficulties are further compounded by the lower prevalence of *EGFR* mutations (~10–15%) [49, 50]. In the ISEL and INTEREST studies, insufficient *EGFR* mutation-positive tissue samples were identified for the definitive determination of whether *EGFR* mutation status was a predictive factor for the efficacy of gefitinib. If biomarkers might be important factors determining response, then maximising the number of high-quality tissue samples should be considered high priority in the design of the clinical programme, and studies should be sufficiently powered to detect clinically important differences in biomarker subgroups. Protocols and consents also need to allow for future developments in tests and technologies, as the science can evolve rapidly while trials are ongoing.

Another important requisite is a readily available and accurate diagnostic test for the biomarker that determines response, and the use of a consistent definition of positivity (cut-off). Three methods of measuring EGFR and determining outcome were assessed during the development of gefitinib: EGFR protein expression, *EGFR* gene copy number and *EGFR* mutation. It took some time to demonstrate that *EGFR* mutation was the strongest predictive factor in identifying patients that would benefit from gefitinib. A number of techniques for detecting *EGFR* mutation have been evaluated with gefitinib and new less invasive approaches for the patient, such as detection in cytology and serum samples, continue to be assessed.

It is possible that randomised controlled phase II trials would have enabled more rapid identification of the subgroups on which to focus gefitinib development, as they are more informative than uncontrolled trials. A predictive factor can only be identified from a controlled trial, and not from a single-arm study.

Finally, early engagement with regulatory agencies is important to ensure that the planned development programme will meet their requirements in this rapidly evolving area. Both the regulatory agencies and pharmaceutical companies are learning about the development of biomarker-targeted agents together and early discussions may facilitate opportunities for collaboration.

SUMMARY

The development of a new molecularly targeted agent represents a significant challenge, as the knowledge about the target is often limited at the time of designing the clinical programme. In fact, the drug under development may serve as a tool to further explore and improve understanding of the target biology. Gefitinib is now approved in Europe for the treatment of adults with locally advanced or metastatic NSCLC with activating mutations of *EGFR* tyrosine kinase. In various other countries, particularly those in Asia, gefitinib is indicated in an unselected pretreated NSCLC population and in some countries, the addition of a first-line indication in patients with activating mutations of *EGFR* tyrosine kinase has recently been granted.

For the first time, an oral treatment offers superior efficacy, in terms of PFS and ORR, and better tolerability and QoL compared with doublet chemotherapy (carboplatin/paclitaxel) as first-line treatment for patients with advanced NSCLC harbouring *EGFR* mutations. Physicians now have a test that will give a good indication that a treatment for lung cancer will work, which is in contrast to the usual position of administering chemotherapy and hoping that their patient will respond well to the chosen treatment.

However, it took several years and many large studies before the target patient population for gefitinib treatment in advanced NSCLC became clear. There are several useful lessons for future biomarker-targeted products from the gefitinib experience: understanding the science is fundamental in determining which biomarker the tumour is truly dependent on and how best to measure that biomarker; the number of tissue samples collected in trials needs to be maximised so that the significance of the biomarker can be fully evaluated; and the diagnostic test needs to be available together with the drug. Pharmaceutical companies and regulatory agencies will need to engage early on and work collaboratively to ensure that efficacious and safe personalised medicines are available to patients in a timely manner, without the requirement for excessively long and complex development programmes.

STATEMENT OF INTEREST

A.A. Armour and C.L. Watkins are employed by and own shares in AstraZeneca, UK.

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