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First-line gefitinib in Caucasian *EGFR* mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study

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Background: Phase-IV, open-label, single-arm study (NCT01203917) to assess efficacy and safety/tolerability of first-line gefitinib in Caucasian patients with stage IIIA/B/IV, epidermal growth factor receptor (*EGFR*) mutation-positive non-small-cell lung cancer (NSCLC).

Methods: Treatment: gefitinib 250 mg day^{-1} until progression. Primary endpoint: objective response rate (ORR). Secondary endpoints: disease control rate (DCR), progression-free survival (PFS), overall survival (OS) and safety/tolerability. Pre-planned exploratory objective: *EGFR* mutation analysis in matched tumour and plasma samples.

Results: Of 1060 screened patients with NSCLC (859 known mutation status; 118 positive, mutation frequency 14%), 106 with *EGFR* sensitising mutations were enrolled (female 70.8%; adenocarcinoma 97.2%; never-smoker 64.2%). At data cutoff: ORR 69.8% (95% confidence interval (CI) 60.5–77.7), DCR 90.6% (95% CI 83.5–94.8), median PFS 9.7 months (95% CI 8.5–11.0), median OS 19.2 months (95% CI 17.0–NC; 27% maturity). Most common adverse events (AEs; any grade): rash (44.9%), diarrhoea (30.8%); CTC (Common Toxicity Criteria) grade 3/4 AEs: 15%; SAEs: 19%. Baseline plasma 1 samples were available in 803 patients (784 known mutation status; 82 positive; mutation frequency 10%). Plasma 1 *EGFR* mutation test sensitivity: 65.7% (95% CI 55.8–74.7).

Conclusion: First-line gefitinib was effective and well tolerated in Caucasian patients with *EGFR* mutation-positive NSCLC. Plasma samples could be considered for mutation analysis if tumour tissue is unavailable.

The aims of personalised health care and optimal, targeted treatment for patients with advanced non-small-cell lung cancer (NSCLC) are steadily becoming a reality for many patients with the disease (Vallieres *et al*, 2012). Investigations into the molecular basis of increased response seen in some patients with advanced NSCLC when treated with the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) gefitinib led to the identification of activating mutations of the *EGFR* gene (Lynch *et al*, 2004; Paez *et al*, 2004).

The EGFR TKI gefitinib (Iressa) has been shown to prolong progression-free survival (PFS) compared with first-line chemotherapy in patients with advanced NSCLC with activating mutations of the *EGFR* gene, and has been associated with improved tolerability and quality of life compared with chemotherapy (Mok *et al*, 2009; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010; Han *et al*, 2012). Objective response rates (ORRs) in patients with *EGFR* mutation-positive tumours treated with gefitinib have been reported between 62% and 85% (Mok *et al*, 2009; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010; Han *et al*, 2012). Patients with *EGFR* mutation-positive advanced NSCLC have also experienced longer PFS with the EGFR TKI erlotinib compared with first-line chemotherapy (Zhou *et al*, 2011;

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Rosell *et al*, 2012). Objective response rates of 58% and 83% were reported in these studies, including a randomised study in a non-Asian (Caucasian) *EGFR* mutation-positive NSCLC population (Rosell *et al*, 2012).

In 2009, the European Medicines Agency approved gefitinib for the treatment of adult patients with locally advanced or metastatic NSCLC with activating mutations of the *EGFR* TK (European Medicines Agency, 2009), based partly on the results of the IRESSA Pan-ASia Study (IPASS) (Mok *et al*, 2009). As part of this approval, a single-arm, follow-up measure study was required to address the fact that a relatively low number of non-Asian patients with *EGFR* mutation-positive tumours had been treated with gefitinib in the first-line setting at that time. Here we report the efficacy and tolerability results from this open-label, phase-IV, follow-up study of efficacy, safety and tolerability of gefitinib in Caucasian patients with *EGFR* mutation-positive NSCLC. Exploratory biomarker analyses were also pre-planned objectives of the study. The aim of these biomarker analyses was to investigate the utility of surrogate samples (plasma) for *EGFR* mutation analysis and assess whether these samples containing circulating-free tumour DNA (cfDNA) could be used to reliably determine *EGFR* mutation status, thus enabling those patients who do not have tumour tissue samples available to be offered an optimised, molecular-based therapy. The results of the pre-planned Exploratory Biomarker Objective comparing baseline tumour and plasma *EGFR* mutation status in all screened patients with evaluable results are reported here. Further pre-planned Exploratory Biomarker Objectives will be reported separately.

MATERIALS AND METHODS

Study design and patients. The gefitinib follow-up measure study (NCT01203917) was a prospective, open-label, multicentre, single-arm study to characterise the efficacy, safety and tolerability of gefitinib ($250\,mg\,day^{-1}$) as a first-line treatment of Caucasian patients with *EGFR* mutation-positive, locally advanced or metastatic NSCLC. The primary end point was ORR (investigator assessment). Secondary end points included PFS, disease control rate (DCR), overall survival (OS), safety and tolerability, and correlation between clinical characteristics and baseline tumour *EGFR* mutation status. Pre-planned exploratory objectives included the comparison of baseline tumour and plasma *EGFR* mutation status in all screened patients with evaluable results (Exploratory Biomarker Objective I).

Eligible patients were Caucasian, aged $\geqslant$18 years, had a life expectancy of $\geqslant$12 weeks, histologically confirmed stage-IIIA/B/IV NSCLC (stage IIIA/B eligible only if considered by the investigator unsuitable for therapy of curative intent) with activating, sensitising *EGFR* mutations, irrespective of histological type or smoking status, a World Health Organization (WHO) performance status (PS) of 0–2 and were eligible for standard first-line treatment (including patients who had received previous adjuvant chemotherapy or had completed prior surgery or radiotherapy $>$6 months prior to the start of study treatment and patients who had received palliative radiotherapy $\geqslant$4 weeks prior to the start of the study treatment). Provision of tumour samples and duplicate plasma samples for *EGFR* mutation testing at baseline was mandatory. Patients whose tumours harboured an *EGFR* mutation reported to confer resistance to EGFR TKIs (exon 20 point mutations T790M or S768I; exon 20 insertions, either alone or in combination with activating, sensitising mutations) were excluded from the study.

All patients provided written, informed consent, including for the provision of tumour and plasma samples for biomarker analyses. Study approval was obtained from independent ethics committees at each institution. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice, applicable regulatory requirements and AstraZeneca's policy on bioethics.

Treatment. Patients received gefitinib ($250\,mg\,day^{-1}$ orally) administered continuously until objective disease progression, intolerable toxicity or discontinuation from the study for another reason. Upon disease progression, patients were offered subsequent anti-cancer treatment at their physician's discretion.

Assessments. Tumour assessment by computed tomography scan was performed every 6 weeks. The ORR (complete response (CR) plus partial response (PR)) was determined by the Response Evaluation Criteria In Solid Tumors (RECIST) (Eisenhauer *et al*, 2009) version 1.1. A secondary, supportive ORR was also calculated from a central, independent review of scans. Progression-free survival (time from start of the study treatment to date of objective tumour progression (excluding clinical deterioration without evidence of objective progression)) and DCR (CR plus PR plus stable disease $\geqslant$6 weeks) were also determined by RECIST 1.1. Overall survival was assessed from the start of study treatment to death from any cause. Safety and tolerability were assessed by adverse events (AEs) and clinical laboratory results, classified according to Common Toxicity Criteria (CTC) version 4.0.

EGFR mutation analysis. One tumour sample and two plasma samples (plasma 1 and 2) were collected from each patient at baseline (screening) for DNA extraction and *EGFR* mutation analysis. Optional plasma samples were collected at disease progression. The pre-planned Exploratory Biomarker Objective I included the comparison of baseline tumour and plasma 1 *EGFR* mutation status in all screened patients with evaluable results.

A central laboratory (LabCorp, Durham, NC, USA) performed DNA extraction and mutation analysis. Tumour DNA was extracted using the Qiagen QIAamp DNA Mini Kit, whereas cfDNA was extracted from plasma using the Qiagen QIAamp Circulating Nucleic Acid Kit.

EGFR mutation status of all samples was assessed using a Scorpion Amplification Refractory Mutation System (ARMS)-based *EGFR* mutation detection kit (Therascreen *EGFR* RGQ PCR kit, Qiagen, Crawley, UK), which detects 29 mutations across the *EGFR* gene. For tumour samples, all mutations in the kit were analysed.

EGFR mutation status was assigned to baseline tumour samples according to the agreed eligibility criteria (Supplementary Appendix Table 1): positive – samples that were positive for $\geqslant$1 activating, sensitising *EGFR* mutation with no ineligible mutations; positive-ineligible – samples that were positive for $\geqslant$1 ineligible mutation (exon 20 point mutations T790M or S768I; exon 20 insertions, either alone or in combination); negative – samples with no mutations detected; and unknown – samples for which no mutation results were available (exhaustion of samples, poor quality or low DNA yield).

For plasma samples, only the exon 19 deletions, L858R point mutation and T790M point mutation were analysed. The following *EGFR* mutation status was assigned to plasma 1 samples: positive – samples that were positive for $\geqslant$1 mutation (L858R, exon 19 deletions [19 different mutations] and T790M); negative – samples for which no mutations were detected; and unknown – samples for which no mutation results were available (no sample, poor quality or low DNA yield).

Correlation between clinical characteristics (including gender, age, race, tumour histology, WHO PS and smoking history) and baseline tumour *EGFR* mutation status was assessed in the subset of all screened patients with tumours evaluable for mutation status (-positive or -negative); tumour samples of unknown status or with ineligible mutations were excluded from this analysis.

Data for Exploratory Biomarker Objective I is reported here and in the Supplementary Appendix.

Statistical analysis. Data were analysed using a data cutoff at 6 months after the last patient had started study treatment (15 February 2012; data cutoff: 15 August 2012). It was estimated that 1250 Caucasian patients with advanced NSCLC would have to be screened to obtain 100 patients with eligible *EGFR* mutation-positive tumours for gefitinib treatment. A total of 100 patients would ensure precise ORR estimation, with the ORR 95% CI no more than 10% above or 10% below the observed ORR (e.g. if the observed ORR was 50%, the CI would be within 40% to 60%).

The ORR (primary end point) was calculated from investigator data and summarised in the full analysis set (FAS; all screened patients with an eligible, positive *EGFR* mutation status who received $\geqslant 1$ dose of gefitinib), with 95% CIs (Wilson score intervals). The secondary, supportive, central ORR review was also calculated and summarised. The DCR was summarised in the FAS population as for ORR. Progression-free survival was estimated with 95% CIs (FAS) using Kaplan–Meier methods and Greenwood's formula (Greenwood, 1926) (PFS rates) and Brookmeyer and Crowley (median PFS). Overall survival was summarised as for PFS. Safety was analysed in the evaluable-for-safety (EFS) population (all patients who received $\geqslant 1$ dose of gefitinib) with AEs summarised according to system organ class and Medical Dictionary for Regulatory Activities (MedDRA) version 15.0 preferred term. Safety analysis included incidence of AEs, interruption in study treatment, actual data/changes in laboratory safety data, vital signs, electrocardiogram results and physical examination.

Correlation between clinical characteristics and baseline tumour *EGFR* mutation status was calculated using a multivariate logistic regression model (*EGFR* mutation-positive *vs* -negative) with covariates: histology (adenocarcinoma *vs* non-adenocarcinoma), smoking status (never- *vs* ever-smoker), gender (female *vs* male), age ($\leqslant 65$ *vs* >65 years) and WHO PS (0–1 *vs* $\geqslant 2$). Clinical characteristics of patients with unknown mutation status were only summarised.

Baseline tumour and plasma 1 *EGFR* mutation status in patients evaluable for both samples was compared by cross-tabulation of the adjusted mutation status and mutation subtype from tumour at baseline *vs* the mutation status from plasma 1 at baseline. The following were presented with rates (percentages) using the Clopper–Pearson method to derive 95% CIs: concordance (either positive or both negative), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

The trial is registered with ClinicalTrials.gov (NCT01203917).

Role of the funding source. This study was funded by AstraZeneca. AstraZeneca employees participated in the conception and design of the study, collection and assembly of data, data analysis and interpretation. The corresponding author had full access to all the study data and final responsibility for the decision to submit for publication.

RESULTS

Patients and treatment. Of 1060 patients screened from 13 countries, 118 (11.1%) harbouring an *EGFR* mutation were enrolled from 8 September 2010 to 15 February 2012. Twelve of the enrolled patients did not start treatment and 106 constituted the FAS population (Figure 1). Patient baseline demographics/characteristics (FAS population) are presented in Table 1. Briefly, 70.8% were female, 97.2% had tumours of adenocarcinoma histology and 64.2% were never-smokers. The detection rate of *EGFR* mutation-positive status among patients with evaluable tumour samples was 13.7% (118 out of 859); 65.1% of patients with a positive *EGFR* mutation status had tumours with exon 19 deletions and 31.1% with L858R point mutations. Gefitinib treatment was started in 107 patients, of whom 106 had activating, sensitising *EGFR* mutations and one had a resistant *EGFR* mutation. Post-discontinuation therapy is reported in the Supplementary Appendix.

Efficacy. Median duration of follow-up was 13.0 months. As of data cutoff (15 August 2012), a response was seen in 74 patients, with an ORR of 69.8% (95% CI: 60.5–77.7%) based on investigator assessment ($n=2$ with CR; $n=72$ with PR). Objective response rates were consistent across clinical subgroups (Table 2). The ORR by secondary, supportive, central review was 50.0% (95% CI: 40.6–59.4%). The ORR from a *post-hoc* analysis of the patients with measurable disease at baseline according to the central review (i.e. excluding 17 patients assessed as having measurable disease by the investigator, but not the central, review) was 59.6%.

The DCR was 90.6% (95% CI: 83.5–94.8%). Median PFS was 9.7 months (95% CI: 8.5–11.0%), with 38.5% of patients progression-free at 12 months (Figure 2A). Median OS was 19.2 months (95% CI: 17.0–not calculable; 27% maturity) (Figure 2B).

Safety and tolerability. Safety and tolerability data (EFS population) are summarised in Table 3. Median duration of exposure was 245 days (8.0 months). A total of 100 out of 107 patients (93.5% of EFS) experienced $\geqslant 1$ AE during the study (onset between first dose and 30 days following last dose of gefitinib). Most common AEs were rash (44.9%), diarrhoea (30.8%), vomiting (13.1%), asthenia, cough and dry skin (all 11.2%), and nausea (10.3%). Only two patients (1.9%) experienced a serious AE that was considered by the investigator to be related to treatment with gefitinib. In total, 8 out of 107 patients (7.5%) experienced AEs that led to treatment discontinuation. A total of five patients (4.7%) died due to AEs (cardiac failure $n=2$, pneumonia $n=2$, Alzheimer's-type dementia $n=1$); for two of these patients, disease progression was a secondary cause of death. None of the fatal AEs were considered by the investigator to be related to gefitinib. One patient (0.9%) experienced an AE of interstitial lung disease (CTC grade 3) which recovered and was considered by the investigator to be causally related to gefitinib.

EGFR mutation status and clinical characteristics. Calculation of correlation between clinical characteristics and baseline tumour *EGFR* mutation status in the screened population ($N=1060$) included 850 patients: 118 with *EGFR* mutation-positive and 732 with *EGFR* mutation-negative tumours. Nine patients had tumours with ineligible mutations and *EGFR* mutation status was unknown in 201 patients due to technical reasons (e.g. low tumour content, poor sample quality, insufficient quantity, poor/inappropriate fixation, no DNA). Clinical characteristics that significantly predicted tumour *EGFR* mutation-positive status were histology (adenocarcinoma *vs* non-adenocarcinoma; odds ratio [(OR] 6.8); smoking status (never-smoker *vs* ever-smoker; OR 5.5); gender (female *vs* male; OR 2.8) (all $P<0.0001$). Age ($\leqslant 65$ *vs* >65 years; OR 1.2; $P=0.4226$) and PS (0–1 *vs* $\geqslant 2$; OR 0.8; $P=0.5563$) were not predictive of *EGFR* mutation status. Clinical characteristics of the overall screened population ($N=1060$) are shown in Supplementary Appendix Table 2, and for the overall screened population ($N=1060$) for patients with baseline tumour samples and patients with baseline plasma 1 samples in Supplementary Appendix Table 3.

Exploratory Biomarker Objective I. A total of 652 patients provided matched baseline tumour and plasma 1 samples, for which both samples were evaluable for EGFR mutation status (Table 4). Fewer patients with *EGFR* mutation-positive status were identified with plasma-derived cfDNA (*EGFR* mutation detection rate 10.6%; 69 out of 652 patients) than with tumour tissue. The false-negative and false-positive rates for plasma-derived cfDNA

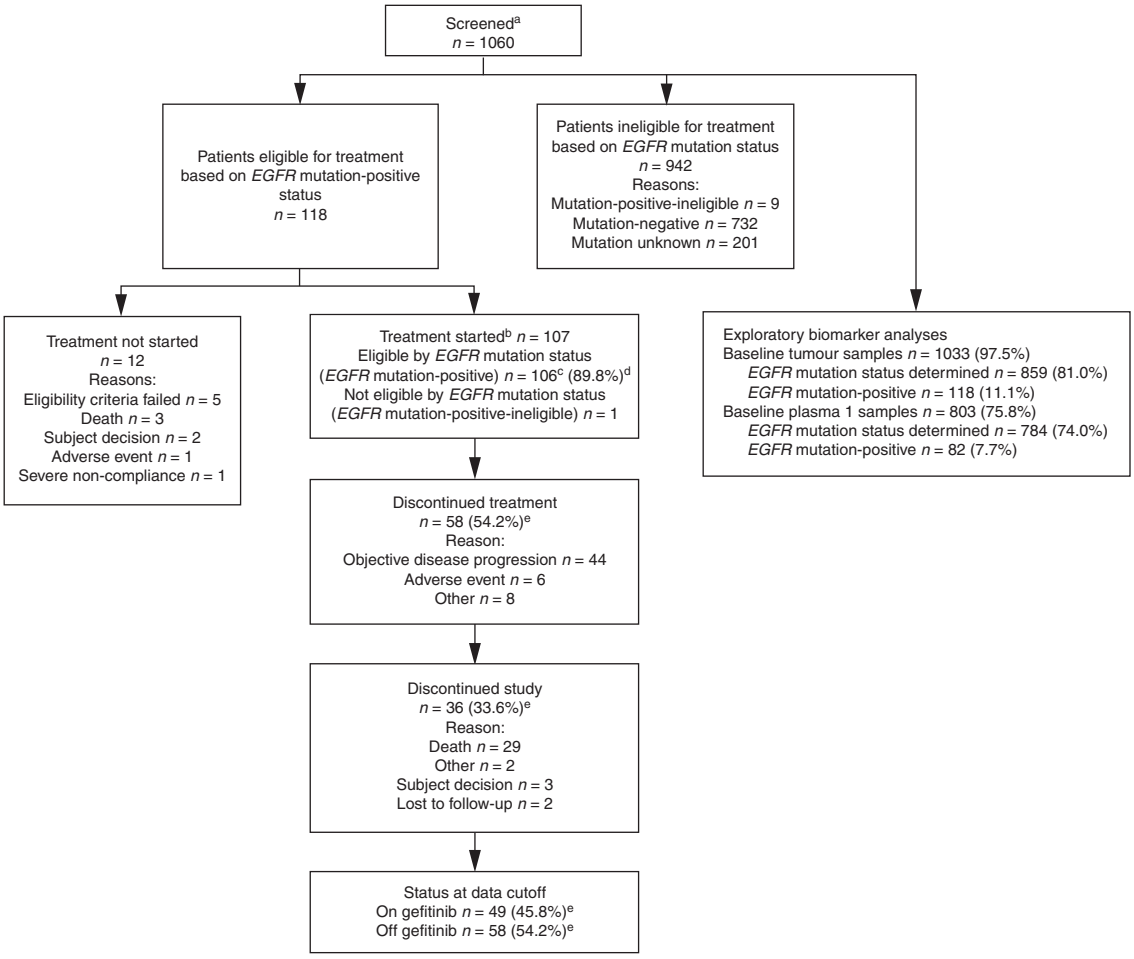

Figure 1. Patient disposition flow diagram. [a]All screened patients. Used to calculate the correlation between clinical characteristics and tumour *EGFR* mutation status and the comparison of *EGFR* mutation status between tumour DNA and plasma-derived circulating free DNA. [b]One patient of *EGFR* mutation-positive-ineligible status was treated in error and included in the evaluable-for-safety population. A total of 107 patients therefore started study treatment. [c]Full analysis set population. Used to summarise efficacy data, and for the comparison of *EGFR* mutation status in plasma and tumour samples. [d]Number of patients with *EGFR* mutation-positive tumours ($n = 118$) used as the denominator for the percentage calculation. [e]Number of patients started on treatment ($n = 107$) used as the denominator for the percentage calculation. Abbreviation: *EGFR* = epidermal growth factor receptor.

were 34.3% (36 out of 105 patients) and 0.2% (1 out of 547 patients), respectively. In 201 patients with an unknown *EGFR* mutation status due to technical problems with tumour samples (see above), 12 mutations were identified in the corresponding plasma samples.

The sensitivity, specificity PPV and NPV of mutation-status detection between baseline tumour and plasma samples for patients evaluable for both samples are presented in Table 4.

DISCUSSION

The gefitinib follow-up measure study reported here is, to our knowledge, the first prospective, large-scale study of first-line gefitinib to be conducted in Caucasian patients with *EGFR* mutation-positive, advanced NSCLC. Our study demonstrates that first-line gefitinib is effective in this population, as assessed by ORR (70%) (supported by DCR (91%), median PFS (9.7 months) and median OS (19.2 months)). First-line gefitinib was well tolerated, with AEs consistent with the characterised tolerability/safety profile for gefitinib and previous studies (Fukuoka *et al*, 2003; Kris *et al*, 2003; Kim *et al*, 2008; Mok *et al*, 2009). Female gender, adenocarcinoma histology and never-smoker status correlated with the presence of *EGFR* mutations, consistent with observations in Asian patients (Mok *et al*, 2009). The *EGFR* mutation rate in the Caucasian patients in our study was 13.7%.

The ORR in our *EGFR* mutation-positive Caucasian population is similar to that observed in the IPASS *EGFR* mutation-positive Asian population; IPASS reported an ORR of 71.2% with first-line gefitinib ($n = 132$) *vs* 47.3% with carboplatin/paclitaxel ($n = 129$) (OR 2.75; 95% CI: 1.65–4.60%) (Mok *et al*, 2009). The ORR in our study is also similar to that reported in the gefitinib arm of three further randomised, phase III studies comparing first-line gefitinib with doublet chemotherapy in Asian NSCLC populations (Mitsudomi *et al*, 2010; Han *et al*, 2012; Inoue *et al*, 2013). In NEJ002, ORR was 73.7% (84 out of 114) with gefitinib *vs* 30.7% (35 out of 114) ($P < 0.001$) with carboplatin/paclitaxel in 198 prospectively randomised patients with *EGFR* mutation-positive tumours (Inoue *et al*, 2013). The similarly designed WJTOG3405 study reported ORRs of 62.1% with gefitinib ($n = 36$ out of 58) and 32.2% with cisplatin/docetaxel ($n = 19$ out of 59) (OR 29.9; 95% CI: 12.6–47.1%; $P < 0.0001$) (Mitsudomi *et al*, 2010). Finally, the First-SIGNAL study reported higher ORR (84.6%;

Table 1. Key demographic and baseline characteristics for patients in the FAS population

Characteristic	FAS (*N* = 106)
Median age, years (range)	65 (32–82)
Age group, years, *n* (%)	
⩾18 to <65	52 (49.1)
⩾65 to <75	28 (26.4)
⩾75	26 (24.5)
Gender, *n* (%)	
Male	31 (29.2)
Female	75 (70.8)
Race, *n* (%)	
Caucasian[a]	106 (100.0)
Black/African American	0 (0.0)
Histology, *n* (%)	
Adenocarcinoma (NOS)	92 (86.8)
Adenocarcinoma bronchiolo-alveolar	10 (9.4)
Adenosquamous carcinoma	2 (1.9)
Large-cell carcinoma (NOS)	1 (0.9)
Other/missing[b]	1 (0.9)
Disease stage at screening, *n* (%)	
IIIA	2 (1.9)
IIIB	6 (5.7)
IV	98 (92.5)
Other/missing	0 (0.0)
Time from original diagnosis, *n* (%)	
<6 months	55 (51.9)
⩾6 months	34 (32.1)
Unknown	17 (16.0)
Performance status, *n* (%)	
0	48 (45.3)
1	51 (48.1)
2	7 (6.6)
Other/missing	0 (0.0)
Smoking status, *n* (%)	
Never	68 (64.2)
Current	6 (5.7)
Former	32 (30.2)
Missing	0 (0.0)
Prior treatment, *n* (%)	
Radiotherapy	14 (13.2)
Chemotherapy	10 (9.4)
***EGFR* mutation subtype, *n* (%)**	
Exon 19 deletions	69 (65.1)
L858R	33 (31.1)
L861Q	2 (1.9)
G719X (G719S/A/C)	2 (1.9)

Abbreviations: *EGFR* = epidermal growth factor receptor; FAS = full analysis set; NOS = not otherwise specified.
[a]Caucasians were considered to be patients of European, North African or Middle Eastern descent only for the purpose of this study.
[b]Other histologies included squamous cell carcinoma, undifferentiated carcinoma and adenocarcinoma tubulopapillary.

Table 2. ORR by investigator assessment (overall and by subgroups; FAS population)

Category	FAS (*N* = 106) (*n*)	Objective responders (*n*)	ORR (%)	95% CI
Total response	106	74	69.8	60.5–77.7
CR	2	–	1.9	–
PR	72	–	67.9	–
Age				
⩽65 years	55	36	65.5	52.3–76.6
>65 years	51	38	74.5	61.1–84.5
Sex				
Male	31	22	71.0	53.4–83.9
Female	75	52	69.3	58.2–78.6
Performance status				
0–1	99	69	69.7	60.0–77.9
⩾2	7	5	71.4	29.0–96.3
Smoking status				
Never	68	50	73.5	62.0–82.6
Ever	38	24	63.2	47.3–76.6
***EGFR* mutation type**				
Exon 19 deletions	69	50	72.5	61.0–81.6
L858R	33	21	63.6	46.6–77.8
L861Q	2	1	NC	NC–NC
G719X (G719S/A/C)	2	2	NC	NC–NC
Histology				
Adenocarcinoma	103	72	69.9	60.5–77.9
Non-adencocarcinoma	3	2	NC	NC–NC

Abbreviations: CI = confidence interval; CR = complete response; *EGFR* = epidermal growth factor receptor; FAS = full analysis set; NC = not calculable; ORR = objective response rate; PR = partial response.

n = 22 out of 26) with gefitinib in the *EGFR* mutation-positive subgroup, *vs* gemcitabine/cisplatin (ORR 37.5%; *n* = 6 out of 16) (Han *et al*, 2012). One can, therefore, conclude that gefitinib appears to be consistent in efficacy in patients with *EGFR* mutation-positive tumours, irrespective of their ethnicity.

Response data from our study are also encouraging when put into context with previously published studies of first-line EGFR TKIs in Caucasian patients with *EGFR* mutation-positive advanced NSCLC (Sequist *et al*, 2008; Rosell *et al*, 2012). The phase II, iTARGET study reported an ORR of 55% (95% CI: 33–70%) in 34 patients treated with gefitinib, of whom only two were of Asian ethnicity (Sequist *et al*, 2008). Similarly, the phase III EURTAC study reported an ORR of 58% (50 out of 86 patients) with first-line erlotinib *vs* 15% (13 out of 87 patients) with chemotherapy in European patients (Rosell *et al*, 2012). As in our study, iTARGET and EURTAC reported generally mild to moderate AEs (rash and diarrhoea), although 13% of patients (11 out of 84) in EURTAC did experience grade-3/4 rash with erlotinib (there were no grade-3/4 rash events with gefitinib reported in our study). Mutation subtype analysis results in iTARGET and EURTAC were also similar to our study, with exon 19 deletions and L858R the most

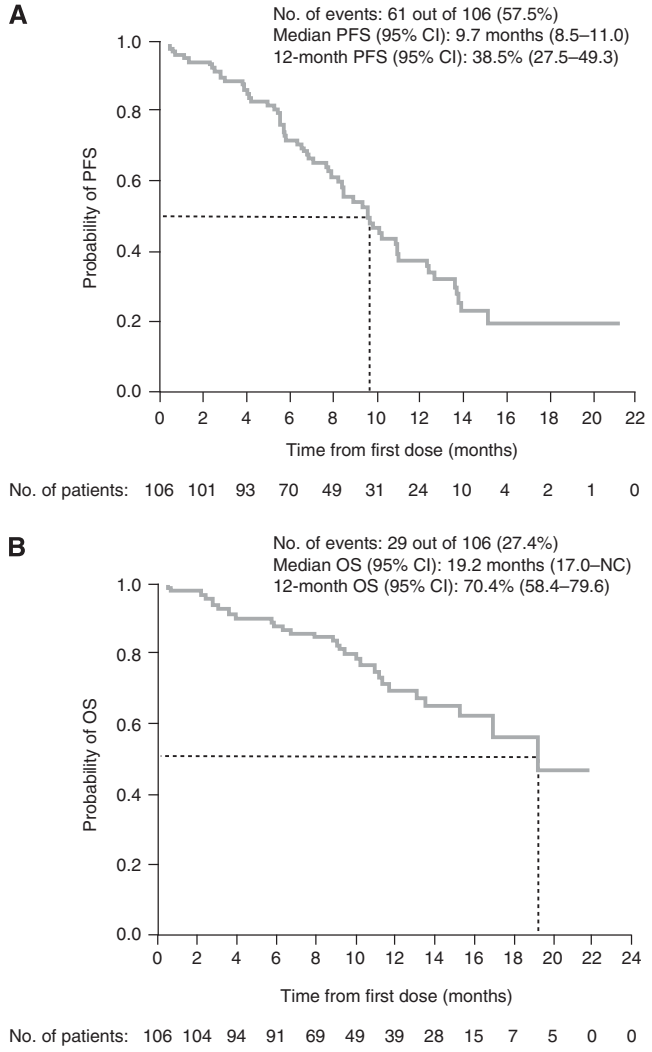

Figure 2. Kaplan–Meier curves for (A) PFS and (B) OS (FAS population). Patients without a PFS event at the time of the primary analysis were censored at the date of their last objective tumour assessment. Abbreviations: CI = confidence interval; NC = not calculable; OS = overall survival; PFS = progression-free survival.

Table 3. Adverse events (frequency of ⩾3%) by MedDRA preferred term and AEs of CTC grade ⩾3 (EFS population)

	EFS (*N* = 107) (*n*) (%)[a]	
MedDRA preferred term[b]	**All AEs[c]**	**AEs CTC grade ⩾3**
Total	100 (93.5)	16 (15.0)
Rash	48 (44.9)	0 (0.0)
Diarrhoea	33 (30.8)	4 (3.7)
Vomiting	14 (13.1)	0 (0.0)
Asthenia	12 (11.2)	0 (0.0)
Cough	12 (11.2)	0 (0.0)
Dry skin	12 (11.2)	0 (0.0)
Nausea	11 (10.3)	0 (0.0)
Decreased appetite	10 (9.3)	0 (0.0)
Alanine aminotransferase increased	9 (8.4)	1 (0.9)
Hypertension	8 (7.5)	0 (0.0)
Dermatitis acneiform	7 (6.5)	0 (0.0)
Urinary tract infection	7 (6.5)	0 (0.0)
Aspartate aminotransferase increased	6 (5.6)	0 (0.0)
Pneumonia	4 (3.7)	3 (2.8)
Cardiac failure	3 (2.8)	2 (1.9)

Abbreviations: AE = adverse event; CTC = Common Toxicity Criteria; EFS = evaluable for safety; MedDRA = Medical Dictionary for Regulatory Activities.
[a]Includes AEs with an onset date between the date of first dose and 30 days following the date of last dose of study medication.
[b]MedDRA version 15.0 and CTC AE version 4.0.
[c]Adverse events sorted in decreasing frequency order of MedDRA preferred term.
For the majority of patients (81 out of 107; 75.7%), the investigator considered the AEs to be causally related to gefitinib. In total, 16 out of 107 patients (15.0%) experienced an AE of CTC grade ⩾3. A total of 20 out of 107 patients (18.7%) experienced serious AEs, two (1.9%) of which were considered by the investigator to be related to treatment with gefitinib.

common mutation subtypes detected. Additionally, an ORR of 56% was reported with first-line afatinib ($n = 230$) *vs* 23% with pemetrexed/cisplatin ($n = 115$) ($P < 0.0001$) in the phase III LUX-Lung 3 study's multinational (72% Asian) NSCLC population (Yang *et al*, 2012). Consistent with the mechanism of action of EGFR TKIs, rash and diarrhoea were the most commonly reported AEs.

Although investigator and central-review variability is not uncommon and has been observed previously in oncology studies (Tang *et al*, 2010), in our study the difference between investigator (70%) and central-review (50%) ORRs was investigated further. When the ORR data from our study were analysed *post-hoc*, 17 patients were assessed as having no measurable disease at baseline by central review. If these 17 patients are excluded from the ORR analysis, the central-review ORR is 60%. This retrospective analysis may, therefore, help to explain differences between investigator and central-review results. Since the presence of measurable disease was an inclusion criterion for this study, this result may be considered to represent the likely outcome had central review been the principal designation of ORR.

In an assessment of the utility of cfDNA from plasma, we demonstrated that *EGFR* mutation detection rates were higher with tumour tissue (13.7% in evaluable samples) than in plasma (10.6%) in patients with both samples; concordance was very high at 94.3%, with assay specificity of 99.8% and sensitivity of 65.7%. Our results compare very favourably with several studies, including IPASS, which reported a mutation rate of 23.7% with cfDNA ($n = 194$) *vs* 61.5% with tumour tissue ($n = 91$) in the Japanese subset, although the rate of false negatives was high, (56.9%) (Goto *et al*, 2012). The high rate of false negatives reported in IPASS and the differences seen in assay sensitivity between IPASS and the study reported here may be attributed to differences in sample type, DNA extraction kit and mutation analysis methodology. In IPASS, cfDNA samples were prepared from serum using the QIAamp DNA minikit. Whereas in the study reported here, cfDNA samples were obtained from plasma using the QIAamp circulating nucleic acid kit, which has been optimised for cfDNA preparation and isolation of small fragments of DNA. Additionally, two different versions of the ARMS-based *EGFR* mutation detection kit were used in the studies: IPASS used the DxS *EGFR* mutation test kit (DxS, Manchester, UK) and the study reported here used the Therascreen *EGFR* RGQ PCR kit (Qiagen, Crawley, UK). The high concordance, specificity and sensitivity reported here demonstrate that *EGFR* mutation status can be accurately assessed using cfDNA and can be considered appropriate when tumour tissue is unavailable or the sample is exhausted. From the authors' clinical experience, ~10–15% of patients with advanced NSCLC attending clinic do not have tumour tissue samples available, thus making molecular-based treatment decisions difficult. Although this result

Table 4. Exploratory Biomarker Objective I data

Tumour and plasma 1 – screened patients evaluable for both samples (*N* = 652)					
	Plasma 1 *EGFR* mutation status (*n*)				
	Positive		Negative		Total
Adjusted baseline tumour *EGFR* mutation status, *n*					
Positive	69		36		105
Negative	1		546		547
Total	70		582		652
	Exon 19 deletions	**L858R**	**L858R and T790M**	**Negative**	**Total**
Exon 19 deletions	48	0	0	23	71
L858R	0	21	0	12	33
L858R and T790M	0	0	0	1	1
Negative	0	1	0	546	547
Total	48	22	0	582	652
	N		**Rate (%)**		**95% CI**
Concordance	652		94.3		92.3–96.0
Sensitivity	105		65.7		55.8–74.7
Specificity	547		99.8		99.0–100.0
PPV	70		98.6		92.3–100.0
NPV	582		93.8		91.5–95.6

For the comparison of tumour and plasma data, the tumour DNA mutation status was adjusted for the mutations analysed in cfDNA from plasma (i.e. for exon 19 deletions, L858R point mutations and T790M point mutations only). Abbreviations: cfDNA = circulating free tumour DNA; CI, confidence interval; *EGFR* = epidermal growth factor receptor; PPV = positive predictive value; NPV = negative predictive value.

is very encouraging and suggests that plasma is a suitable substitute for mutation analysis when tumour tissue is unavailable, tumour tissue should be considered the preferred sample type when available.

In summary, the results of this follow-up measure study confirm that first-line gefitinib is effective and well tolerated in Caucasian patients with *EGFR* mutation-positive NSCLC, supporting the use of tumour molecular characteristics to help define a patient's treatment regime, irrespective of ethnicity or clinical characteristics.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Professor Jean-Yves Douillard contributed towards provision of study patients, study design, data analysis and data interpretation. Dr Gyula Ostoros contributed towards provision of study patients, study design, data collection and data interpretation. Dr Manuel Cobo contributed towards provision of study patients. Dr Tudor Ciuleanu contributed towards provision of study patients, data collection and data interpretation. Dr Rose McCormack contributed towards study design (including protocol development), study implementation with a focus on biomarker analysis (delivery of prospective tumour testing and plasma testing) and data interpretation. Mr Alan Webster contributed towards data analysis and data interpretation. Dr Tsveta Milenkova contributed towards study design, protocol writing, consultation on patient eligibility/toxicity, data cleaning, data interpretation and writing of the clinical study report. All authors contributed to the writing and critical review of the manuscript.

REFERENCES

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* **45**: 228–247.

European Medicines Agency (2009) European Public Assessment Report Iressa Summary of Product Characteristics. Available at http://www.ema.europa.eu/. Accessed 13 August 2013.

Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard J-Y, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong R-P, Baselga J (2003) Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. *J Clin Oncol* **21**: 2237–2246.

Goto K, Ichinose Y, Ohe Y, Yamamoto N, Negoro S, Nishio K, Itoh Y, Jiang H, Duffield E, McCormack R, Saijo N, Mok T, Fukuoka M (2012) Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. *J Thorac Oncol* **7**: 115–121.

Greenwood M (1926) The natural duration of cancer. Reports on public health and medical subjects. *Her Majesty's Stationery Office* **33**: 1–26.

Han JY, Park K, Kim SW, Lee DH, Kim HY, Kim HT, Ahn MJ, Yun T, Ahn JS, Suh C, Lee JS, Yoon SJ, Han JH, Lee JW, Jo SJ, Lee JS (2012) First-SIGNAL: First-Line Single-Agent Iressa Versus Gemcitabine and Cisplatin Trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol* **30**: 1122–1128.

Inoue A, Kobayashi K, Maemondo M, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Saijo Y, Hagiwara K, Morita S, Nukiwa T. for the North-East Japan Study Group (2013) Updated overall survival results from a randomized phase III trial comparing gefitinib with carboplatin-paclitaxel for chemo-naïve non-small cell lung cancer with sensitive EGFR gene mutations (NEJ002). *Ann Oncol* **24**: 54–59.

Kim ES, Hirsch V, Mok T, Socinski MA, Gervais R, Wu Y-L, Li L-Y, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao M-L, Østerlind K, Reck M, Armour AA, Shepherd FA, Lippman SM, Douillard J-Y (2008) Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* **372**: 1809–1818.

Kris MG, Natale RB, Herbst RS, Lynch Jr TJ, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC (2003) Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer. A randomized trial. *JAMA* **290**: 2149–2158.

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **350**: 2129–2139.

Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S. Nukiwa T for the North-East Japan Study Group (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **362**: 2380–2388.

Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M. West Japan Oncology Group (2010) Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* **11**: 121–128.

Mok TS, Wu Y-L, Thongprasert S, Yang C-H, Chu D-T, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang J-J, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* **361**: 947–957.

Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**: 1497–1500.

Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P, Longo F, Moran T, Insa A, De Marinis F, Corre R, Bover I, Illiano A, Dansin E, de Castro J, Milella M, Reguart N, Altavilla G, Jimenez U, Provencio M, Moreno MA, Terrasa J, Muñoz-Langa J, Valdivia J, Isla D, Domine M, Molinier O, Mazieres J, Baize N, Garcia-Campelo R, Robinet G, Rodriguez-Abreu D, Lopez-Vivanco G, Gebbia V, Ferrera-Delgado L, Bombaron P, Bernabe R, Bearz A, Artal A, Cortesi E, Rolfo C, Sanchez-Ronco M, Drozdowskyj A, Queralt C, de Aguirre I, Ramirez JL, Sanchez JJ, Molina MA, Taron M, Paz-Ares L. Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica (2012) Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* **13**: 239–246.

Sequist LV, Martins RG, Spigel D, Grunberg SM, Spira A, Jänne PA, Joshi VA, McCollum D, Evans TL, Muzikansky A, Kuhlmann GL, Han M, Goldberg JS, Settleman J, Iafrate AJ, Engelman JA, Haber DA, Johnson BE, Lynch TJ (2008) First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic *EGFR* mutations. *J Clin Oncol* **26**: 2442–2449.

Tang PA, Pond GR, Chen EX (2010) Influence of an independent review committee on assessment of response rate and progression-free survival in phase III clinical trials. *Ann Oncol* **21**: 19–26.

Vallieres E, Peters S, Van Houtte P, Dalal P, Lim E (2012) Therapeutic advances in non-small cell lung cancer. *Thorax* **67**: 1097–1101.

Yang JCH, Schuler M, Yamamoto N, O'Byrne KJ, Hirsch V, Mok TS, Geater SL, Orlov SV, Tsai C-M, Boyer MJ, Su WC, Bennouna J, Kato T, Gorbunova VA, Lee KH, Shah RNH, Massey D, Lorence RM, Shahidi M, Sequist LV (2012) LUX-Lung 3: a randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. *J Clin Oncol* **30**(suppl): LBA7500.

Zhou C, Wu Y-L, Chen G, Feng J, Liu X-Q, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang Y, Xiu Q, Ma J, Zhang L, You C (2011) Erlotinib versus chemotherapy as first-line treatment for patients with advanced *EGFR* mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* **12**: 735–742.

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