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;
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.

### 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 1).

Eligible patients were Caucasian, aged ≥18 years, had a life expectancy of ≥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 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 ≥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 ≥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 1 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 ≥1 activating, sensitising EGFR mutation with no ineligible mutations; positive-ineligible – samples that were positive for ≥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 ≥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 ≥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 population) using Kaplan–Meier methods and Greenwood, 1926 (PFS rates) and Brookmeyer and Crowley (median PFS). Overall survival was summarised as for ORR. Progression-free at 12 months (Figure 2A). Median OS was 19.2 months (95% CI: 17.0–not calculable; 27% mortality) (Figure 2B).

Safety and tolerability. Safety and tolerability data (FAS 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 FAS) experienced ≥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 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 (≤65 vs >65 years) and WHO PS (0–1 vs ≥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 Clogger–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 38.3% of patients progression-free at 12 months (Figure 2A). Median OS was 19.2 months (95% CI: 17.0–not calculable; 27% mortality) (Figure 2B).

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...
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 2.99; 95% CI: 1.26–7.1% ; P = 0.0001) (Mitsudomi et al, 2010). Finally, the First-SIGNAL study reported higher ORR (84.6%)}
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

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*                   | 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/missingab              | 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.

aCaucasians were considered to be patients of European, North African or Middle Eastern descent only for the purpose of this study.
bOther 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) | 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-adenocarcinoma                    | 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.
post-hoc (70%) and central-review (50%) ORRs was investigated further. (Yang et al, 2012). Consistent with the mechanism of action of EGFR TKIs, rash and diarrhoea were the most commonly observed 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 retro-

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 = 61.5%) with tumour tissue (13.7% in evaluable samples) than in plasma (10.6%). 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 RQ 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

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

| MedDRA preferred term | All AEs | 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: AEs = adverse events; CTC = Common Toxicity Criteria; EFS = evaluable for safety; MedDRA = Medical Dictionary for Regulatory Activities.

*A includes AEs with an onset data between the date of first dose and 30 days following the date of last dose of study medication.

*MedDRA version 15.0 and CTC AE version 4.0.

**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.
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.

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