Detection of *EGFR* mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial

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Summary

Background Detection of *EGFR* mutations in tumour tissue is the gold-standard approach to ascertain if a patient will benefit from treatment with an *EGFR* tyrosine kinase inhibitor. However, if tissue is scant, another strategy is to use circulating tumour DNA (ctDNA), but this method needs validation in clinical trials. We did a prospective clinical trial to assess ctDNA-based *EGFR* mutation detection as a selection criterion for patients with lung adenocarcinoma receiving gefitinib as first-line treatment.

Methods BENEFIT is a multicentre, single-arm, phase 2 clinical trial at 15 centres in China. Patients aged 18–75 years with stage IV metastatic lung adenocarcinoma and *EGFR* mutations detected in ctDNA were given oral gefitinib 250 mg once daily as first-line treatment. The primary endpoint was the proportion achieving an objective response. Secondary endpoints included median progression-free survival and safety. Next-generation sequencing (NGS) of a 168-gene panel was used for genetic analysis of baseline blood samples. The primary efficacy analysis was done by intention to treat in patients who had at least one post-baseline tumour assessment. The safety analysis was done in all patients who received at least one dose of study treatment. This trial is registered with ClinicalTrials.gov, number NCT02282267.

Findings Between Dec 25, 2014, and Jan 16, 2016, 426 patients were screened for the trial, of whom 188 with *EGFR* mutations in ctDNA were enrolled and received gefitinib. 183 patients had one or more post-baseline tumour assessment and were included in the primary efficacy analysis. Median follow-up was 14·5 months (IQR 12·2–16·5). At the time of data cutoff (Jan 31, 2017), 152 patients had progressive disease or had died. The proportion achieving an objective response was 72·1% (95% CI 65·0–78·5). Median progression-free survival was 9·5 months (95% CI 9·07–11·04). Of 167 patients with available blood samples, 147 (88%) showed clearance of *EGFR* mutations in ctDNA at week 8, and median progression-free survival was longer for these patients than for the 20 patients whose *EGFR* mutations persisted at week 8 (11·0 months [95% CI 9·43–12·85] *vs* 2·1 months [1·81–3·65]; hazard ratio [HR] 0·14, 95% CI 0·08–0·23; p<0·0001). From baseline NGS data in 179 patients, we identified three subgroups of patients: those with *EGFR* mutations only (n=58), those with mutations in *EGFR* and tumour-suppressor genes (n=97), and those with mutations in *EGFR* and oncogenes (n=24). Corresponding median progression-free survival in these subgroups was 13·2 months (95% CI 11·5–15·0), 9·3 months (7·6–11·0), and 4·7 months (1·9–9·3), respectively (*EGFR* mutations only *vs* mutations in *EGFR* and tumour-suppressor genes, HR 1·78, 95% CI 1·23–2·58; p=0·002; *EGFR* mutations only *vs* mutations in *EGFR* and oncogenes, 2·66, 1·58–4·49; p=0·0003). The most common grade 3 or 4 adverse events were hepatic function abnormalities (n=24). Serious adverse events were reported in 17 (9%) patients. No unexpected safety events for gefitinib were recorded.

Interpretation Detection of *EGFR* mutations in ctDNA is an effective method to identify patients who might benefit from first-line gefitinib treatment. Further analyses of dynamic alterations of *EGFR* mutations and accompanying gene aberrances could predict resistance to gefitinib.

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Introduction

The presence of *EGFR*-sensitising mutations in patients with advanced lung adenocarcinoma is the gold-standard biomarker for prediction of suitability for first-line *EGFR* tyrosine kinase inhibitor (EGFR-TKI) therapy.[1–5] *EGFR* mutations are detected usually in tumour tissue; however, in clinical practice, use of biopsy specimens might not always be possible because of suboptimum quantity, inadequate tissue quality, or intratumoral heterogeneity.

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Research in context

Evidence before this study

We searched PubMed from Jan 1, 2006, to July 31, 2017, and proceedings of international meetings (eg, American Society of Clinical Oncology annual meeting), with the keywords "cell-free DNA", "circulating tumor DNA", and "*EGFR* mutation". We restricted our search to the English language. Several prospective studies have validated use of circulating tumour DNA (ctDNA) for detection of *EGFR* mutations; however, these studies did not use ctDNA-based *EGFR* mutation detection as a selection criterion to guide EGFR tyrosine kinase inhibitor (EGFR-TKI) treatment. Our search yielded no prospective clinical trials of EGFR-TKIs as first-line therapy in patients with advanced non-small-cell lung cancer who underwent ctDNA-based *EGFR* mutation analysis.

Added value of this study

To the best of our knowledge, our prospective clinical trial (BENEFIT) is the first to report the efficacy of first-line gefitinib with ctDNA-based *EGFR* mutation status as a screening criterion, and provides clinical evidence for use of ctDNA-based *EGFR* mutation status to ascertain eligibility for EGFR-TKI treatment. We investigated the diagnostic use of plasma ctDNA-based *EGFR* mutation detection using droplet digital PCR in patients with advanced lung adenocarcinoma receiving the EGFR-TKI gefitinib as first-line treatment. We also analysed dynamically *EGFR*-sensitising and *EGFR*-resistance mutation status in relation to clinical outcomes through to disease progression.

Implications of all the available evidence

The proportion of patients achieving an objective response and the duration of progression-free survival recorded in our trial are similar to those reported in previous studies using tissue-based *EGFR* mutation detection. Thus, *EGFR* mutation detection in patients with *EGFR*-positive plasma ctDNA could be used prospectively to select patients with advanced lung adenocarcinoma for first-line EGFR-TKI therapy when an insufficient tumour specimen is available for tissue-based *EGFR* mutation detection. Dynamic alterations in *EGFR*-sensitising and *EGFR*-resistance mutations could be used to predict disease progression, ahead of radiological results.

Circulating tumour DNA (ctDNA) in blood provides an alternative to tumour samples for *EGFR* mutation analysis. In Europe and the USA, cell-free DNA-based *EGFR* mutation analysis is approved by the European Medicines Agency and the US Food and Drug Administration (FDA), respectively, for detection of *EGFR* mutations if tumour tissue is scant, as a selection criterion for first-line EGFR-TKI therapy. In many studies, *EGFR* mutation status has been investigated in matched peripheral blood and tumour tissue in patients with advanced non-small-cell lung cancer (NSCLC), and researchers have reported retrospectively that patients with ctDNA-based *EGFR* mutation status had superior clinical outcomes with EGFR-TKIs to patients without *EGFR* mutations.[6–13] Furthermore, in several studies, the high specificity (92–100%) and positive predictive value (94·0–98·6%) of ctDNA-based *EGFR* mutation detection was validated prospectively, with tissue as reference.[7,10,14,15]

In 2018, Ramalingam and colleagues[16] showed that ctDNA genotyping for the *EGFR* Thr790Met mutation in plasma samples was an ideal predictor to guide third-generation EGFR-TKI treatment. However, *EGFR*-sensitising and *EGFR*-resistance mutations in ctDNA had diverse sensitivity (43–80%) when using tumour genotyping status as reference.[16] Quantitative PCR techniques with potentially increased sensitivity—eg, droplet digital PCR (ddPCR) and BEAM (beads, emulsion, amplification, magnetics) digital PCR—have been used to genotype *EGFR* mutations in ctDNA and monitor dynamic gene alterations during EGFR-TKI therapy.[12,17,18] Yung and colleagues[12] reported that dynamic alterations of *EGFR* mutation status detected by ddPCR in ctDNA from patients with NSCLC could predict treatment response and monitor progressive disease. Also, Oxnard and colleagues[19] reported that emergence of the *EGFR* mutation Thr790Met up to 16 weeks before radiographic progression could be used to guide subsequent treatment. Intratumoral heterogeneity is recognised to be one of the molecular mechanisms of resistance to EGFR-TKI therapy, and substantial alterations of a patient's genetic makeup can take place during treatment and at progression. Thus, dynamic monitoring of gene aberrances in ctDNA, and generation of an integrated genomic profile from high-throughput next-generation sequencing (NGS), could help to tailor targeted treatment for individual patients in clinical practice.

Despite study findings showing the feasibility of detection and monitoring of *EGFR* mutations in ctDNA, the application of blood-based mutation analysis into routine clinical practice has several limitations. First, previous results came from retrospective biomarker analyses, which need to be verified in prospective trials using ctDNA-based genotyping to ascertain targeted therapy.[20] Second, in some prospective studies, dynamic changes of ctDNA *EGFR* mutations during EGFR-TKI treatment were assessed. However, most studies did subgroup analyses. To undertake a prospective study of dynamic monitoring of plasma *EGFR* mutations is difficult.[14,15] Finally, previous studies typically only focused on detection of one driver gene, rather than multiple sensitive or resistant alterations, which might affect clinical outcomes.

The third-generation EGFR-TKI osimertinib has been approved by the FDA as one of the standard options for

first-line therapy based on findings of the FLAURA study.[21] However, this first-line indication has not been approved in most Asian countries, which is why we chose to assess gefitinib in our study. The strategy of use of osimertinib in patients with the *EGFR* Thr790Met mutation after resistance to first-generation EGFR-TKIs is widely acceptable in China.[22] Here, we report the results of Blood Detection of *EGFR* Mutation For Iressa Treatment (BENEFIT), in which we aimed to assess prospectively the diagnostic and clinical use of ctDNA-based *EGFR* mutation detection by ddPCR in patients with lung adenocarcinoma receiving gefitinib as first-line treatment. We also analysed dynamic alterations of *EGFR*-sensitising and *EGFR*-resistance mutations in relation to clinical outcomes.

Methods

Study design and participants

BENEFIT is an open-label, single-arm, prospective, multicentre, phase 2 clinical trial at 15 centres across China. We judged patients eligible for the trial if they were aged 18–75 years with histologically confirmed, systemic treatment-naive (no previous chemotherapy, immunotherapy, or other systemic anticancer treatment), stage IV lung adenocarcinoma (according to the 7th American Joint Committee on Cancer Staging System), *EGFR*-sensitising mutations (exon 19 deletion or exon 21 Leu858Arg) detected by ddPCR in pre-treatment plasma, and a WHO performance status of 0–2. Radical surgery, radiotherapy, and adjuvant chemotherapy had to be completed at least 6 months before the start of the trial. At baseline, patients had to have at least one lesion (10 mm in the longest diameter in non-lymph-node lesions, or short axis >15 mm in lymph nodes), not previously irradiated, that could be measured by CT or MRI, and suitable for repeated measurement. The main exclusion criteria were: presence of histologically confirmed squamous and adenosquamous carcinoma or other co-existing malignant disease, or malignant disease diagnosed within the previous 5 years; newly diagnosed CNS metastasis or spinal-cord compression, unless treated with surgery or radiation and stable without steroids for at least 2 weeks; uncontrolled pleural or pericardial effusion; evidence of severe uncontrolled systemic disease; known allergy to gefitinib or any product excipient; and pregnancy or lactation.

All patients provided written informed consent. We obtained study approval from independent ethics committees at every study centre. The study was undertaken in accordance with local legal and regulatory requirements and the general principles of the International Ethical Guidelines for Biomedical Research Involving Human Subjects, the International Conference on Harmonisation guidelines on Good Clinical Practice, and the Declaration of Helsinki. An independent steering committee interpreted results.

Procedures

We gave eligible patients 250 mg gefitinib (AstraZeneca, Macclesfield, UK) once daily, to be taken orally in tablet form, as first-line treatment until disease progression (defined according to Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1),[23] death, or treatment cessation for other reasons (including intolerable toxic effects or withdrawal from the study). After disease progression, we followed up patients every 12 weeks until death, loss to follow-up, or withdrawal. Follow-up entailed obtaining survival information by telephone or at an outpatient clinic. We encouraged collection of imaging data but it was not mandatory.

We obtained samples of blood and tumour tissue for biomarker analysis and transported them to a designated central laboratory for *EGFR* mutation testing (Amoy Diagnostics, Xiamen, China). We gathered blood for plasma isolation at baseline (within 7 days before first study dose), every 8 weeks after the first study dose, and at the time of disease progression. We obtained whole blood samples in Streck cell-free DNA blood collection tubes (Streck, Omaha, NE, USA) and transported them at room temperature from every medical centre to the central laboratory. We obtained tumour tissue either at the initial diagnosis, resection, or biopsy 14 days before first study dose and stored it as formalin-fixed, paraffin-embedded (FFPE) samples. If gefitinib treatment failed, we recommended optional rebiopsy for exploratory analyses.

We ascertained *EGFR* mutation status (either *EGFR*-sensitising [exon 19 deletion, exon 21 Leu858Arg] or *EGFR*-resistance [exon 20 Thr790Met]) of plasma-derived ctDNA by ddPCR assay, as described previously.[24] We detected *EGFR* mutations in FFPE tissue specimens by ADx-ARMS (Amplified Refractory Mutation System; Amoy Diagnostics, Xiamen, China), as described previously.[9] We did NGS in plasma-derived ctDNA to detect additional mutations in oncogenic drivers and tumour-suppressor genes using an ultra-deep (20 000×) 168-gene panel named LungPlasma (Burning Rock Biotech, Guangzhou, China) in a subset of patients who provided sufficient blood at baseline (appendix).

See Online for appendix

Outcomes

The primary outcome measure was objective response, defined as the percentage of patients with a confirmed complete response (CR) or partial response (PR) according to RECIST version 1.1.[23] Secondary outcomes were progression-free survival, disease control, overall survival, and safety and tolerability of gefitinib. We defined progression-free survival as the time from start of study drug until either objective disease progression (assessed by an investigator using RECIST version 1.1) or death from any cause. We defined disease control as the percentage of patients who achieved disease control (ie, CR, PR, or stable disease according to RECIST version 1.1) at 8 weeks or more after screening. We defined overall survival as the time from start of

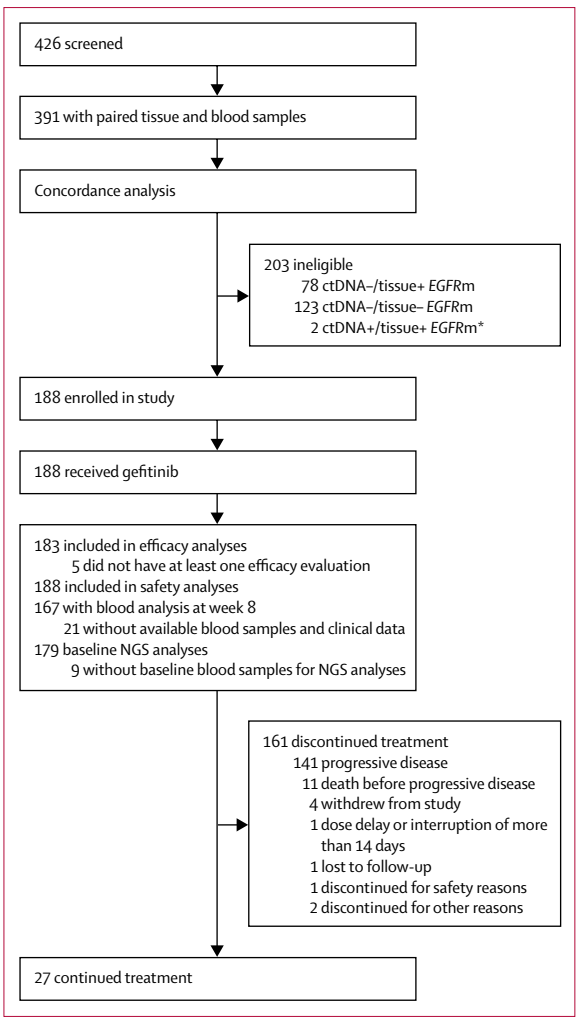

Figure 1: **Flow of participants in the study**
ctDNA=circulating tumour DNA. *EGFR*m=epidermal growth factor receptor mutation. NGS=next-generation sequencing. *Were not recruited for other reasons: one opted to receive erlotinib, the other refused to participate because of the relatively intense follow-up in hospital.

study drug until death from any cause. We censored patients who had not progressed or who had died at the time of statistical analysis, or who were lost to follow-up before death, at the time of their last evaluation. We assessed safety and tolerability according to Common Terminology Criteria for Adverse Events version 4.0.

We also assessed the concordance, sensitivity, specificity, positive predictive value, and negative predictive value of detection of *EGFR* mutations in ctDNA (assessed by ddPCR) versus tumour tissue (assessed by ARMS). We could not calculate the concordance between ddPCR and NGS for detection of *EGFR*-sensitising mutations because patients without *EGFR* mutations in blood samples by ddPCR were not enrolled for gefitinib treatment and, thus, were not tested by NGS, per protocol. Preplanned exploratory outcomes included the correlation of EGFR-TKI efficacy with dynamic changes in two *EGFR*-sensitising mutations (exon 19 deletion and exon 21 Leu858Arg) and one *EGFR*-resistance mutation (exon 20 Thr790Met) in plasma ctDNA, and the correlation of EGFR-TKI efficacy with qualitative detection of other biomarkers in plasma ctDNA or tumour tissue using NGS.

Statistical analysis

We calculated the target sample size with respect to the primary outcome measure. We estimated that 72% of patients would achieve an objective response after 10 months of follow-up of the last enrolled patient, with 159 patients providing a precision of +7% or –7% for this estimate. Therefore, the lower and upper limits of the 95% CI were 65% and 79%, respectively (Wilson score method). Based on 10% dropouts, we planned to enrol 177 patients in the trial.

We calculated concurrently in the full analysis set (ie, all patients who received at least one dose of gefitinib and had at least one post-baseline efficacy measurement) the proportion achieving an objective response and disease control every 8 weeks, progression-free survival, and overall survival. We assessed safety and tolerability in all patients who received at least one dose of gefitinib (safety population). We analysed sensitivity, specificity, concordance, positive predictive value, and negative predictive value of *EGFR* mutation detection in plasma versus tissue in the screening population set (ie, patients with evaluable samples) after the last patient received the first dose of study drug. For exploratory endpoints, we compared the proportion of patients achieving an objective response between subgroups using the χ^2 test or Fisher's exact test. We estimated progression-free survival using the Kaplan-Meier method and compared between subgroups using a Cox proportional hazards model and Wald 95% CIs, where available. Ties were approached using the method of approximate likelihood of Breslow in Cox models. We did all analyses with SAS software (version 9.4).

This trial is registered with ClinicalTrials.gov, number NCT02282267.

Role of the funding source

The funder provided scientific support for study design, biomarker testing, and data interpretation. The corresponding authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

From Dec 25, 2014, to Jan 16, 2016, 426 patients were screened for *EGFR* mutations in plasma-derived ctDNA by ddPCR. 391 patients had paired blood and tissue samples and underwent concordance analysis (figure 1). 188 patients with *EGFR* mutations in ctDNA were enrolled and received gefitinib treatment, of whom 180 had *EGFR* mutations in both tissue and ctDNA and eight had *EGFR* mutations only in ctDNA. 183 patients were included in the efficacy analysis (full analysis set);

five patients were excluded because they did not have at least one post-baseline efficacy assessment. At the time of data cutoff (Jan 31, 2017), 152 patients had progressive disease or had died; overall survival data are not yet mature. Median follow-up was 14·5 months (IQR 12·2–16·5). Demographic and baseline characteristics of the 183 patients in the full analysis set are summarised in table 1.

The *EGFR* mutation status of 391 patients with paired plasma and tissue samples from the screening population set is summarised in table 2. Plasma versus tissue showed a high specificity and positive predictive value for *EGFR* mutation status, with a sensitivity of 70·0% (95% CI 64·0–75·5), specificity of 93·9% (88·3–97·3), positive predictive value of 95·8% (91·9–98·2), negative predictive value of 61·2% (54·1–68·0), a concordance of 78·0% (73·6–82·0), and a positive likelihood ratio of 11·46 (3·69–19·24). High concordance was also obtained for de novo Thr790Met between baseline plasma and tissue samples (appendix).

Of 183 patients in the full analysis set, 132 had at least one confirmed PR; no CRs were reported. Thus, the proportion achieving an objective response in the full analysis set was 72·1% (95% CI 65·0–78·5). Median progression-free survival was 9·5 months (95% CI 9·07–11·04; figure 2A). At week 8, disease control was reported in 169 patients (PR, n=108; stable disease, n=61); 14 patients had progressive disease. Thus, the proportion achieving disease control at week 8 was 92·3% (95% CI 87·5–95·8). Notably, among the eight patients with *EGFR* mutations detected in ctDNA but not in tissue, median progression-free survival was 6·0 months (95% CI 2·0–not estimated).

Analyses of objective response and progression-free survival by subgroups of *EGFR* mutation status in ctDNA at baseline (exon 19 deletion *vs* exon 21 Leu858Arg; de novo Thr790Met-positive *vs* Thr790Met-negative) are summarised in the appendix. Compared with patients without the Thr790Met mutation at baseline (n=174), the proportion achieving an objective response with a de novo Thr790Met mutation (n=9) was lower (74·1% [95% CI 67·0–80·5] *vs* 33·3% [7·5–70·1]), and median progression-free survival was shorter (9·6 months [95% CI 9·17–11·10] *vs* 5·6 months [1·25–11·37]; hazard ratio [HR] 2·60, 95% CI 1·32–5·12; p=0·004; figure 2B). After excluding the nine patients with de novo Thr790Met, the proportion achieving an objective response was increased in patients with ctDNA *EGFR* exon 19 deletion (n=89; 83·1%, 95% CI 73·7–90·2) compared with those with ctDNA exon 21 Leu858Arg (n=85; 64·7%, 53·6–74·8); however, although median progression-free survival was extended, the difference between subgroups was not significant (11·0 months [95% CI 9·20–12·68] *vs* 9·2 months [7·06–11·14]; HR 0·8, 95% CI 0·57–1·11; p=0·18; figure 2C).

Dynamic alterations of *EGFR* mutations were investigated further. At week 8, in 167 patients on treatment and with available plasma samples, 147 (88%) had *EGFR* mutation clearance in ctDNA and 20 (12%) had persisting *EGFR* mutations. Median progression-free survival was prolonged significantly in patients with *EGFR* mutation clearance at week 8 compared with those with persisting *EGFR* mutations (11·0 months [95% CI 9·43–12·85] *vs* 2·1 months [1·81–3·65]; HR 0·14, 95% CI 0·08–0·23; p<0·0001; figure 2D). 90% of *EGFR* mutations had been cleared at week 16, and this percentage rose with time after week 16, similar to the increase seen with the Thr790Met mutation (appendix). The *EGFR* exon 19 deletion was cleared in more patients at week 8 than was the *EGFR* exon 21 Leu858Arg mutation (94·1% *vs* 81·7%; p=0·017). In 123 patients with available plasma samples at progressive disease, an *EGFR* mutation reappeared in ctDNA in 56 (46%). The abundance of mutant DNA at baseline differed significantly in patients with clearance at week 8 of *EGFR* exon 19 deletion versus those without clearance at week 8 (mean 172·9 copies per mL [SD 7·1] *vs* 1787·2 copies per mL [9·9]; p=0·012); no difference was noted in abundance of mutant DNA at baseline for

	Participants (n=183)
Age (years)	57 (32–74)
Men	81 (44%)
Women	102 (56%)
ECOG performance status	
0	41 (22%)
1	131 (72%)
2	11 (6%)
Smoking history	
Never smoker	135 (74%)
Former smoker	32 (17%)
Current smoker	16 (9%)
EGFR mutation status*	
Exon 19 deletion	93 (51%)
Exon 21 Leu858Arg	90 (49%)

Data are median (range) or number (%). ECOG=Eastern Cooperative Oncology Group. **EGFR* mutation status was ctDNA-based.

Table 1: **Demographic and baseline characteristics (full analysis set)**

	Tissue-positive	Tissue-negative	Total tissue samples
ctDNA-positive	182*	8	190
ctDNA-negative	78	123	201
Total ctDNA samples	260	131	391

Data are number of patients. Tumour tissue was assessed for *EGFR* mutations by ARMS and ctDNA was analysed by ddPCR. ARMS=Amplified Refractory Mutation System. ctDNA=circulating free tumour-derived DNA. ddPCR=droplet digital PCR. *Includes 180 patients who enrolled in the trial and received gefitinib treatment and two patients who failed to be recruited (one opted to receive erlotinib, one refused to participate because of the relatively intense follow-up in hospital).

Table 2: ***EGFR*** **mutation status at baseline (screening population)**

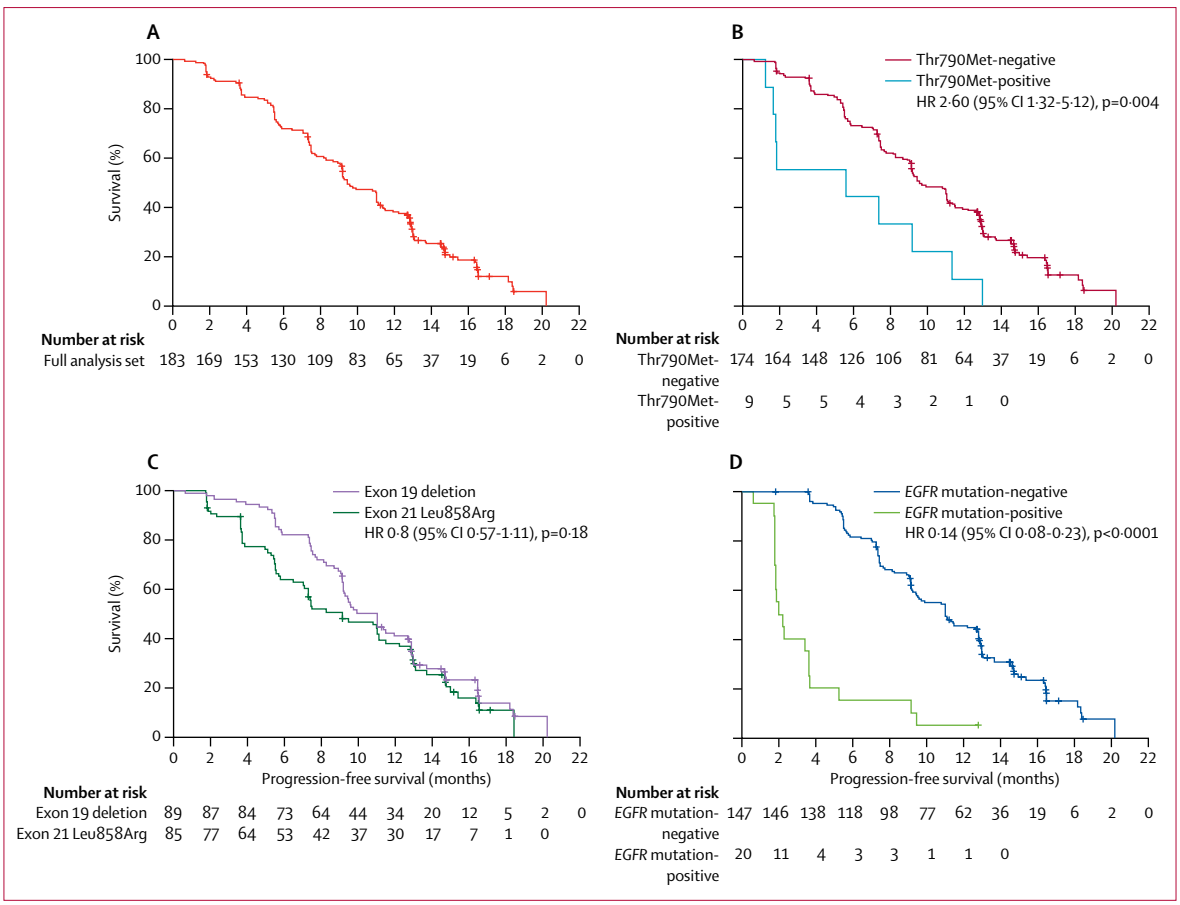

Figure 2: **Kaplan-Meier curves for progression-free survival**
(A) Progression-free survival in the full analysis set (n=183). (B) Progression-free survival according to baseline de novo Thr790Met status in plasma ctDNA (n=183). (C) Progression-free survival according to baseline *EGFR* exon 19 deletion-positive status versus exon 21 Leu858Arg-positive status in plasma ctDNA in patients without de novo Thr790Met (n=174). (D) Progression-free survival according to *EGFR* mutant status in plasma ctDNA at week 8, in patients on-treatment with gefitinib (n=167). ctDNA=circulating free tumour-derived DNA. HR=hazard ratio.

patients with clearance or not at week 8 of *EGFR* exon 21 Leu858Arg (mean 173·2 copies per mL [SD 6·6] *vs* 508·5 copies per mL [10·6]; p=0·060). Patients with *EGFR* mutation clearance at week 8 had significantly decreased tumour burden versus non-clearance at week 8 (% change of the mean sum of maximum diameters of target lesions, 39·2% [SD 21·8] *vs* 9·9% [33·7]; p=0·002).

Among 69 patients with an acquired Thr790Met mutation identified by ddPCR during gefitinib treatment, the median time from baseline negativity to Thr790Met positivity was 7·6 months (95% CI 6·0–10·0). The median time from Thr790Met positivity to progressive disease was 2·0 months (95% CI 2·0–4·9). At data cutoff, 123 patients without de novo Thr790Met provided blood samples at the timepoint of progressive disease, when acquired Thr790Met mutations were detected in 40 (33%) patients by ddPCR and 43 (35%) patients by NGS. Dynamic testing showed that the rate of Thr790Met positivity rose gradually over time, along with the corresponding increase in progressive disease (appendix).

Of 179 patients with baseline NGS data, all had *EGFR*-sensitising mutations by both NGS and ddPCR. No patient was identified with the *EGFR* exon 20 insertion mutation. 118 (66%) patients had concurrent *EGFR*-sensitising mutations and tumour-suppressor gene mutations, including 21 patients who also harbored driver oncogenic mutations. The 179 patients were separated into three subgroups according to the presence or absence of additional gene aberrations (figure 3). The first subgroup (n=58) harboured only *EGFR*-sensitising mutations; the second subgroup (n=97) had concurrent *EGFR*-sensitising mutations and mutations in tumour-suppressor genes (*TP53*, *RB1*, or *PTEN*); and the third subgroup (n=24) had multiple alterations in oncogenic drivers (*MET*, *ERBB2*, *KRAS*, *BRAF*, *RET*, or *ROS1*) besides *EGFR*-sensitising mutations, irrespective of tumour-suppressor gene aberrances. Median progression-free survival was 13·2 months (95% CI 11·5–15·0), 9·3 months (7·6–11·0), and 4·7 months (1·9–9·3) for the three subgroups, respectively (*EGFR*-sensitising mutations *vs* *EGFR*-sensitising and tumour-suppressor

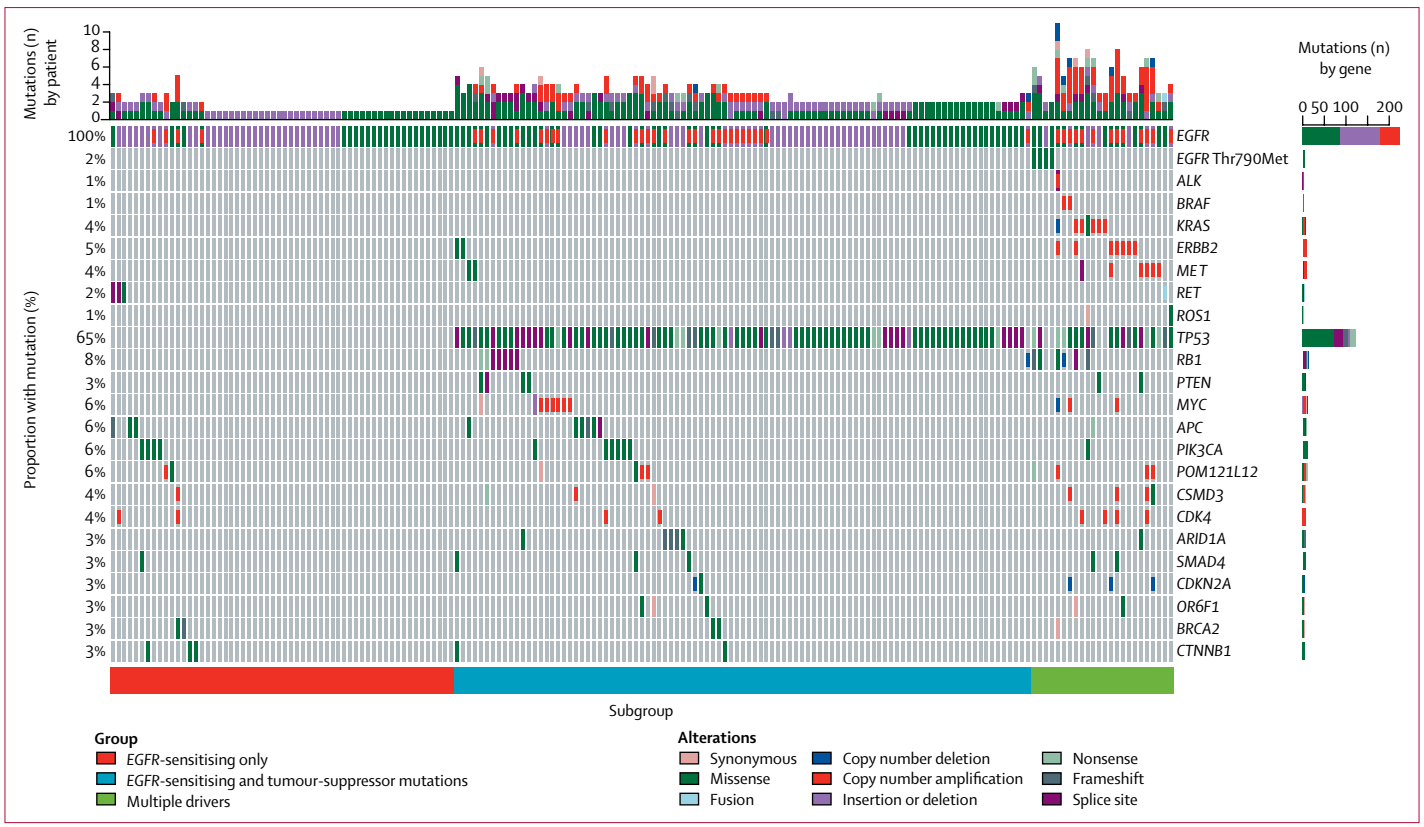

***Figure 3*: Distribution of gene aberrances, stratified by subgroups**

Subgroups were defined as patients with either *EGFR*-sensitising mutations (red block, n=58), *EGFR*-sensitising mutations and concomitant mutations in the tumour-suppressor genes *TP53*, *RB1*, or *PTEN* (blue block, n=97), or multiple alterations in oncogenic drivers *MET*, *ERBB2*, *KRAS*, *BRAF*, *RET*, or *ROS1* besides *EGFR*-sensitising mutations, regardless of tumour-suppressor gene aberrances in baseline ctDNA (green block, n=24). Some patients in the red block had alterations beyond *EGFR*-sensitising mutations; these alterations have not been identified for certain as functional driver gene mutations.

mutations, HR 1·78, 95% CI 1·23–2·58; p=0·002; *EGFR*-sensitising mutations *vs* *EGFR*-sensitising and multiple driver mutations, HR 2·66, 95% CI 1·58–4·49; p=0·0003; figure 4). After further analysis of baseline gene status in the 20 patients who had persisting *EGFR* mutations at week 8, 90% had coexisting tumour-suppressor genes, oncogenic drivers, or both.

176 (94%) of 188 patients had at least one adverse event (appendix). No unexpected safety events for gefitinib were recorded.

Discussion

To our knowledge, BENEFIT is the first clinical trial to ascertain prospectively the feasibility of first-line EGFR-TKI treatment using plasma ctDNA-based *EGFR* mutation analysis. Our results showed that patients selected for gefitinib treatment by ctDNA-based *EGFR* mutation analysis had a satisfactory clinical outcome, with results in line with tissue detection-based clinical trials such as IPASS (progression-free survival 9·5 months) and WJTOG 3405 (9·2 months).[2,3]

In previous studies, researchers gathered blood samples prospectively, but they did not use ctDNA-based *EGFR* mutation analysis as an inclusion criterion to guide EGFR-TKI treatment.[14,15,25] The correlation of blood-based *EGFR* mutation detection and efficacy of EGFR-TKIs has been investigated in several retrospective studies;[6–13] therefore, for the first time, we ascertained prospectively the predictive value of ctDNA-based *EGFR* mutation detection in guiding EGFR-TKI therapy. Using ctDNA-based *EGFR* mutation detection to predict the efficacy of first-line gefitinib had similar power to tumour tissue-based *EGFR* mutation analysis.[1–3] Therefore, ctDNA can be used for *EGFR* mutation detection to guide EGFR-TKI treatment, particularly in cases of insufficient tissue samples.

The low sensitivity of ctDNA *EGFR* mutation genotyping has been a bottleneck that has limited this technique's routine clinical application.[6,9,10] Using ddPCR, we obtained a satisfactory specificity of 93·9%; however, the sensitivity of 70·0% seemed not to be superior compared with those reported in retrospective studies.[25,26] Two explanations should be considered. First, to ensure the specificity of ddPCR assays, observation of at least two positive droplets was defined as mutant-positive in our study. This very rigorous cutoff might exclude some true-positive patients with a low abundancy of mutant DNA. Indeed, after rechecking primary

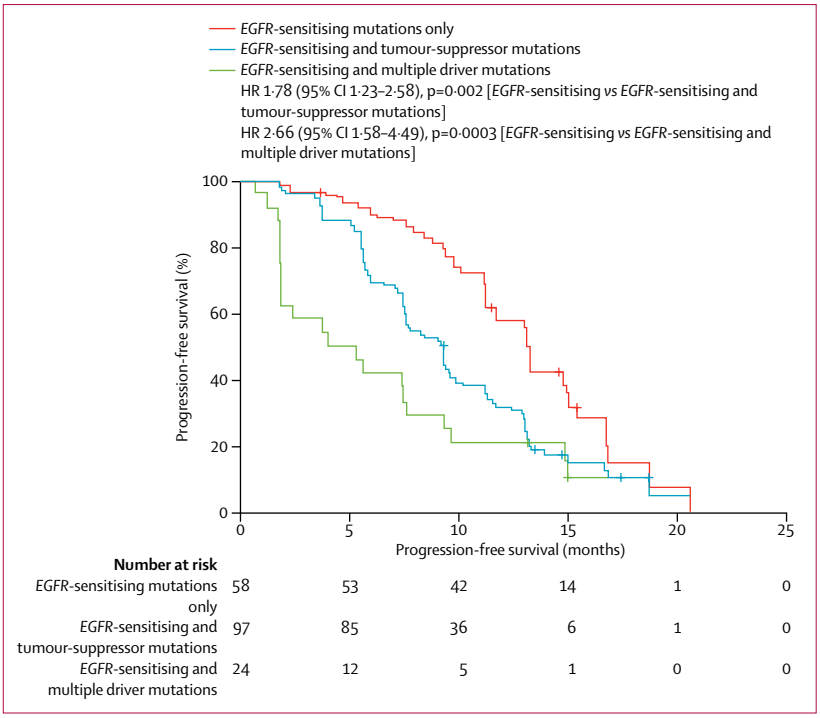

Figure 4: **Kaplan-Meier curves for progression-free survival in patients with baseline NGS data (n=179)**
HR=hazard ratio. NGS=next-generation sequencing.

ddPCR data, 18 samples were interpreted as negative because only one emulsion droplet with a positive *EGFR* mutation was detected. If these patients were added, the sensitivity of ddPCR would rise to almost 80%, similar to that reported in previous studies.[26,27] Second, transportation of samples could be linked to the low sensitivity. To facilitate and standardise ctDNA extraction, we obtained whole blood samples in Streck cell-free DNA blood collection tubes and transported them at room temperature from every study centre to the central laboratory. This type of Streck tube is designed specifically for stabilisation of cell-free DNA and nucleated blood cells at room temperature for up to 7 days. The procedure we used differs from that done in other studies, which comprises on-site plasma separation and frozen plasma transportation to central laboratories for ctDNA extraction, and might increase the chance of mild degradation of ctDNA, resulting in false-negative results for *EGFR* mutations.

Increasing evidence shows that dynamic changes occur in the genetic makeup of lung cancers. Biopsy tissues can only provide limited information on heterogeneous tumours;[28,29] however, ctDNA-based analysis could both assess the general genetic status and monitor change over time.[30] Retrospective studies have shown the predictive value of dynamic ctDNA *EGFR* mutation changes to response of EGFR-TKI treatment before radiographic progression.[10,31] BENEFIT is the first study to show prospectively, in a large patient population, that ctDNA *EGFR* mutation clearance after 8 weeks of gefitinib treatment significantly prolongs progression-free survival compared with persisting *EGFR* mutations. Our data suggest that dynamic monitoring of ctDNA-derived *EGFR* mutation status at week 8 could identify patients who might not benefit much from EGFR-TKI therapy. Furthermore, in our study, persistent ctDNA *EGFR* mutations at week 8 were predictive of poor clinical outcomes. Indeed, NGS analysis confirmed the complex genetic background in this subgroup, with roughly 90% of these patients having aberrances in multiple oncogenic driver genes and tumour suppressor genes. Therefore, analysis of baseline genotype and subsequent dynamic alterations in driver genes could allow identification of alternative therapeutic strategies for patients with *EGFR* mutations who have a poor response to EGFR-TKI monotherapy.

Acquired resistance to EGFR-TKIs is a common clinical problem, emerging at a median progression-free survival of 10–14 months, and is 50–60% mediated by the emergence of the *EGFR*-resistance mutation on exon 20, Thr790Met.[18,21,22] The current standard of care for Thr790Met-mediated resistance is treatment with third-generation EGFR-TKIs, such as osimertinib, after radiographic or clinical progression. However, Thr790Met can be detected in ctDNA about 2–4 months before clinical progression.[17] In our study, the median time to emergence of the acquired Thr790Met mutation in ctDNA was 7·6 months, and 2·0 months from first detection of progressive disease. Thus, dynamic monitoring of ctDNA for the Thr790Met mutation during EGFR-TKI treatment could identify molecular progression before clinical progression, which might prompt more intensive follow-up and potential treatment adjustment. Furthermore, intervention with a third-generation EGFR-TKI might be a better strategy for these patients at this timepoint, but this idea needs further verification in prospective clinical studies.

In this study, at baseline, a de novo Thr790Met mutation was detected in 5% of patients (n=9). The higher sensitivity of detection of Thr790Met in plasma than in tissue might be attributable to our use of the highly sensitive ddPCR method.[12,17] However, considering that patients with baseline Thr790Met were rare, these results could be subject to bias. Larger cohorts with Thr790Met-positive patients detected by ddPCR or another platform should be studied further, with comparison of plasma and tissue samples. Furthermore, patients with de novo Thr790met had significantly shorter median progression-free survival than did those without this mutation; therefore third-generation EGFR-TKIs given as first-line treatment are an ideal option for these patients. In 179 patients with baseline ctDNA NGS data, wide intratumoral and intertumoral gene mutation variations were noted.[32] Patients with *EGFR*-sensitising mutations alone had better outcomes compared with those with additional oncogenic drivers, tumour suppressors, or both; these data could account

partly for why 20–35% of patients with *EGFR*-sensitising mutations had progression-free survival of 6 months or less on first-line EGFR-TKI therapy.[3,4] Indeed, previous studies have shown mutations in *TP53* and *PTEN* contribute to EGFR-TKI resistance in *EGFR*-mutant lung cancer.[33,34] Alternative therapeutic strategies should, therefore, be considered for patients with mutations in multiple oncogenes and tumour suppressor genes, and ctDNA-based NGS analysis at baseline should be done to identify patients who could benefit from EGFR-TKI monotherapy or combination approaches.

Our study has several limitations. First, the sample size of some subgroups was small and, therefore, the results analysis should be interpreted with caution. Second, subgroup data for objective response and progression-free survival might be biased because we did not adjust for potential confounding factors—eg, the distribution of clinicopathological characteristics and accompanying genetic aberrances. Third, the frequency of *EGFR* mutations in our screening population was more than 50%, which could be accounted for by the high proportion (74%) of never-smokers in our study, similar to a previous report in the Chinese population.[35] Finally, only patients carrying *EGFR*-sensitising mutations (exon 19 deletion and exon 21 Leu858Arg) were enrolled for gefitinib therapy. Because the efficacy of first-generation EGFR-TKIs such as gefitinib was uncertain in patients with uncommon *EGFR* mutations at the time the study was designed, and it is still not well established now, we screened for patients with the two most common *EGFR*-sensitising mutations, which account for roughly 85–90% of lung adenocarcinomas with *EGFR* mutations and represent the main population, thus reducing the potential interference of uncommon mutations. In the future, we would be in a better position to investigate the efficacy of EGFR-TKIs in prospective trials based on ctDNA-derived uncommon *EGFR* mutations detected by other methods, such as NGS.

In conclusion, use of ctDNA-based *EGFR* mutation detection to ascertain suitability for first-line EGFR-TKI therapy is feasible. Dynamic assessment of *EGFR* mutation status at week 8 and comprehensive analysis of accompanying gene aberrances could predict resistance to gefitinib, ahead of radiographic information.

Contributors

JW designed the trial. JW, ZW, and Y-lW contributed to data interpretation and preparation of the report. JH and SC were responsible for statistical planning and data analysis. XY, MZ, and GZ were involved in biomarker testing. YC, TA, HG, KW, QZ, YaH, YoS, CD, FP, LL, YiH, CH, CZ, YuS, and LZ were involved in data collection and preparation and finalisation of the report.

Declaration of interests

JW declares speaker fees from AstraZeneca, Roche, Pfizer, and Eli Lilly. Y-lW declares speaker fees from AstraZeneca, Eli Lilly, Pfizer, Roche, and Sanofi. LZ declares research grants from Bristol-Myers Squibb, Eli Lilly, and Pfizer. ZW declares speaker fees from AstraZeneca and Roche. All other authors declare no competing interests.

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