First-line Afatinib plus Cetuximab for *EGFR*-Mutant Non–Small Cell Lung Cancer: Results from the Randomized Phase II IFCT-1503 ACE-Lung Study

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ABSTRACT

Background: Double inhibition of epidermal growth factor receptor (EGFR) using a tyrosine kinase inhibitor plus a monoclonal antibody may be a novel treatment strategy for non–small cell lung cancer (NSCLC). We assessed the efficacy and toxicity of afatinib + cetuximab versus afatinib alone in the first-line treatment of advanced *EGFR*-mutant NSCLC.

Methods: In this phase II, randomized, open-label study, patients with stage III/IV *EGFR*-positive NSCLC were randomly assigned (1:1) to receive afatinib (group A) or afatinib + cetuximab (group A + C). Oral afatinib 40 mg was given once daily; cetuximab 250 mg/m^2 was administered intravenously on day 15 of cycle 1, then every 2 weeks at 500 mg/m^2 for 6 months. The primary endpoint was time to treatment failure (TTF) rate at 9 months. Exploratory analysis of *EGFR* circulating tumor DNA in plasma was performed.

Results: Between June 2016 and November 2018, 59 patients were included in group A and 58 in group A + C. The study was ended early after a futility analysis was performed. The percentage of patients without treatment failure at 9 months was similar for both groups (59.3% for group A vs. 64.9% for group A + C), and median TTF was 11.1 (95% CI, 8.5–14.1) and 12.9 (9.2–14.5) months, respectively. Other endpoints, including progression-free survival and overall survival, also showed no improvement with the combination versus afatinib alone. There was a slight numerical increase in grade ≥3 adverse events in group A + C. Allele frequency of the *EGFR* gene mutation in circulating tumor DNA at baseline was associated with shorter PFS, regardless of the treatment received.

Conclusions: These results suggest that addition of cetuximab to afatinib does not warrant further investigation in treatment-naïve advanced *EGFR*-mutant NSCLC.

Introduction

First-line treatment of epidermal growth factor receptor (*EGFR*) mutant non–small cell lung cancer (NSCLC) has been revolutionized in recent years by the development of EGFR tyrosine kinase inhibitors (TKI). Multiple phase III trials with first-generation agents such as gefitinib and erlotinib, both reversible EGFR inhibitors, have demonstrated the superiority of TKIs over platinum-based chemotherapy (1–4). Due to the development of acquired resistance, however, almost all patients with an initial response to a first-generation agent experience disease progression, which occurs at a median time of 10–12 months after starting TKI therapy (1–4). Although acquired *EGFR* T790M mutation is the most common resistance mechanism occurring in approximately 50% to 60% of cases, other mechanisms have been identified, including activation of alternative signaling pathways such as MET and HER2, and histologic transformations (5, 6).

Second-generation EGFR TKIs were developed to overcome acquired therapeutic resistance to first-generation molecules. These agents, which irreversibly inhibit EGFR and include afatinib and dacomitinib, showed enhanced activity versus first-line agents in cell lines and preclinical models (7, 8). In the clinic, while second-

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

First-line therapy with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) is the standard of care for advanced *EGFR*-mutant non–small cell lung cancer. Although osimertinib has recently shown improved efficacy in comparison with first-generation TKIs, tumor progression occurs systematically because of the occurrence of secondary molecular resistances. Thus, strategies aiming at sparing osimertinib (which is also very effective on the *EGFR*-T790M secondary resistance mutation) for the second-line setting are still under active consideration. Such strategies mostly rely on combinations involving first- or second-generation EGFR TKIs. Here we report the results of a randomized phase II study showing that double inhibition of EGFR using a second-generation TKI, afatinib, and an EGFR monoclonal antibody, cetuximab, does not yield supplementary efficacy and does not seem to change the pattern of mechanisms of resistance. Moreover, we show that the baseline allele frequency of activating EGFR mutations was associated with shorter PFS upon EGFR inhibition.

generation TKIs have failed to demonstrate the effectiveness in the event of failure of first-generation agents, they have demonstrated superiority over first-generation TKIs as first-line treatment. For example, afatinib showed a benefit in progression-free survival (PFS) and time to treatment failure (TTF) versus gefitinib in the LUX LUNG 7 trial (9), and in the ARCHER trial dacomitinib was associated with improved PFS and overall survival (OS) compared with gefitinib (10, 11).

In order to delay tumor progression while limiting the heterogeneity of resistance mechanisms, strategies based on therapeutic combinations are of great interest, even with the availability of new-generation TKIs. To this end, dual targeting of EGFR using a TKI combined with a monoclonal antibody is a novel therapeutic approach that has been supported by both preclinical and clinical data. Notably, dual EGFR inhibition with afatinib combined with cetuximab, an anti-EGFR antibody, was able to overcome the resistance associated with the T790M mutation in preclinical models by inducing a degradation of EGFR (12). Furthermore, time to progression with afatinib plus cetuximab was also doubled in comparison with afatinib alone or erlotinib in TKI-naïve mouse models (13). In a phase I/II trial of 126 patients, the afatinib–cetuximab combination showed significant anti-tumor activity in patients who were heavily pretreated and had progressed during treatment with an EGFR TKI, independent of the T790M mutation (objective response rate, ORR, 32% in T790M-positive patients, and 25% in T790M-negative patients; ref. 14). Despite double inhibition of EGFR, the tolerance profile was acceptable in this study, as well as in other phase I and II trials evaluating the same drug combination (15, 16).

Considering these encouraging preclinical and clinical results, we initiated a phase II study to assess the efficacy and toxicity of the afatinib and cetuximab combination or afatinib alone in the first-line treatment of advanced *EGFR*-mutant NSCLC.

Materials and Methods

Study design and participants

This was a phase II, randomized, noncomparative, open-label study conducted at 27 centers in France (clinicaltrials.gov: NCT NCT02716311).

Eligible patients were ≥18 years of age with histologically or cytologically confirmed non-squamous NSCLC (stage III/IV), inaccessible to local treatment (surgery/radiotherapy), and with an *EGFR* mutation detected by a French NCI molecular genetics platforms (exon 19 deletions, L858R mutation, G719X, L861Q, and S768I mutations, or exon 19 insertions; T790M mutations or exon 20 insertions were not allowed). In addition, patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, with an estimated life expectancy >3 months, and a measurable disease according to RECIST1.1. Patients with a history of central nervous system metastases or spinal cord compression could be included if they had been treated definitively (surgery and/or radiotherapy) and were clinically stable for at least 1 month before the start of treatment. Patients were excluded if they had received prior systemic anti-neoplastic therapy for NSCLC (including EGFR inhibitor therapy), radiotherapy within 2 weeks of study treatment. Other exclusion criteria included the presence of diffuse underlying interstitial lung disease or another neoplastic disease requiring treatment, or symptomatic central nervous system metastases requiring immediate brain radiotherapy.

The study protocol was approved by a French national ethics committee, and written informed consent was obtained from all patients prior to performing study-related procedures. The study was conducted in accordance with the declaration of Helsinki.

Randomization and study procedures

Eligible patients were randomly assigned (1:1) to receive either afatinib (group A) or afatinib plus cetuximab (group A + C), with randomization stratified by site, *EGFR* mutation (exon 19 deletions vs. L858R mutation of exon 21 vs. other mutations), and smoking status (nonsmoker vs. smoker). Individuals directly involved in the conduct and analysis of the trial did not have access to the randomization schedule.

Patients in group A received afatinib 40 mg orally once daily in continuous 28-day cycles until disease progression or dose-limiting toxicity. Patients in group A + C received afatinib according to the same schedule, and cetuximab intravenously on day 15 of cycle 1 at a dose of 250 mg/m^2, then every 2 weeks at 500 mg/m^2, for 6 months. Treatment beyond progression was not allowed.

The dose of afatinib (40 mg/day) corresponds to the usual dose for this indication and was used in the two major trials assessing afatinib as first-line treatment [LUX LUNG 3 (17) and LUX LUNG 6 (18)]. The dose and administration schedule used for the combination of afatinib (40 mg/day) and cetuximab (500 mg/m^2 on days 1 and 15) matches those used in the phase I/II trial (14). The dosage and administration of cetuximab at cycle 1 (250 mg/m^2 at day 15) were adapted to better distinguish between the toxicity linked to afatinib and to the afatinib plus cetuximab combination, and to enable the correction of adverse events (AE) occurring on afatinib. Cetuximab was discontinued after 6 months of treatment to limit the cumulative toxicity of the combination, while preserving the principle of rapid and profound reduction in tumor load at treatment initiation.

In both groups, if patients had any grade 3 or higher treatment-AE, or grade 2 diarrhea lasting 2 days or more, grade 2 rash lasting for longer than 1 week, or an increase in serum creatinine of grade 2 or more, then the study drug was paused until recovery to grade 1 or less. Afatinib was reduced by 10 mg decrements to a minimum dose of 20 mg/day, and cetuximab dose was reduced to 300 mg/m^2; 3 individual occurrences of any of the above events with either treatment resulted in permanent treatment discontinuation. Afatinib or cetuximab treatment was also permanently discontinued in patients who did

not recover to grade 1 or less within 21 days (if afatinib related) or 14 days (if cetuximab related). A review of tolerability data (grade 3/4 toxicities and toxicities leading to modification of treatment) was performed after 20 patients had received 2 cycles of afatinib–cetuximab treatment to ensure proper tolerance of the study regimen and to allow continuation of recruitment.

Chest and supramesocolic CT scans as well as brain CT or MRI scans were performed systematically at enrolment. During the study, tumors were assessed via chest and supramesocolic CT scans and, if metastasis was present or suspected, brain CT or MRI scans and/or bone scintigraphy or PET scans. Assessments were made at baseline and every 8 weeks up to 12 months, then every 12 weeks according to RECIST criteria (version 1.1; ref. 19). Safety was evaluated via recording of AEs, physical examination (including vital signs), World Health Organization (WHO) PS, and laboratory tests. AEs were assessed by investigators from the start of treatment according to seriousness, severity (NCI Common Terminology Criteria for Adverse Events v4.0; ref. 20), and causal relationship to study treatment.

Endpoints

The primary endpoint was treatment failure-free survival (TTF) at 9 months, according to the RECIST 1.1 criteria (19). Treatment failure was defined as treatment discontinuation for any reason (including disease progression, death, or toxicity). Of note, TTF (rather than PFS) was chosen as the primary endpoint as it considers both effectiveness and toxicity and the risk of premature treatment discontinuation. Key secondary endpoints included PFS (time between randomization and tumor progression or death by any cause), OS (time between enrolment and death by any cause), ORR, disease control rate, and safety (AEs).

Exploratory biological analyses

As previously described, plasma samples were collected for each patient before treatment initiation, after 2 weeks, 4 weeks, at each tumor assessment, and at RECIST progression. The samples were collected in cell-free DNA BCT tubes (Streck) and sent to a centralized laboratory. Upon receipt, tubes were centrifuged at 2,000 × *g* for 10 minutes. The supernatant was then collected and centrifuged at 16,000 × *g* for 3 minutes. Plasma was prepared and frozen at −80°C until use.

Circulating tumor DNA (ctDNA) was extracted from 3 mL of plasma using the Maxwell RSC LV (large volume) Circulating Cell-Free Plasma Kit (Promega) and eluted in 50 μL of elution buffer as recommended by the supplier. DNA extracts were frozen at −20°C until analysis. We quantified the ctDNA for each patient using digital PCR (QuantStudio 3D Digital PCR System; ThermoFischer). For each sample, a reaction mixture was prepared with 7.6 μL of DNA extract, 8 μL of a PCR mix comprising Taq polymerase, dNTPs and ROX reference dye, and 0.4 μL of PCR primers and hydrolysis fluorescent probes. When the *EGFR* mutation was detailed in the patient file, the corresponding specific probe was used (Thermo Fisher). The following mutations were tested: p.L858R (c.2573T>G), p.G719A (c.2156G>C), p.L861Q (c.2582T>A), and different exon 19 deletions: p.E746_A750del (c.2235_2249del), p.E746_A750del (c.2236_2250del), and p.L747_T751del (c.2240_2254del). If the sequence of the exon 19 deletion was not available, we used a drop-off digital PCR assay that we previously described (21). This mixture was then partitioned onto a 20,000 well-chip by diffusion, using a semiautomatic device to standardize this step. After sealing the chips, the amplification reaction was carried out using a suitable thermal cycler, according to the following program: hold 10 minutes at 96°C and then 39 cycles alternating for 2 minutes at 60°C and 30 seconds at 98°C. At the end of the amplification reaction, the fluorescence emitted by each well was read using a dedicated reader. These fluorescence data were then analyzed using a software of our design (unpublished), which provides the proportion of mutation-positive wells. This proportion of mutation-positive wells is an estimator of the probability that a well contains mutated copies. Given the number of wells filled with PCR reaction mix (ROX positive), it is possible to calculate the number of mutated copies of the assay and its 95% confidence intervals (CI), using the Poisson law. The measurement variability was calculated from this CI, and the number of mutated copies per mL of plasma was then deduced, considering the parameters of ctDNA extraction and analysis (22). A sample was considered positive if it contained at least 2 mutated copies per assay, i.e., 8 mutated copies/mL of plasma under our conditions of extraction and analysis.

For clearance analysis, plasma samples collected after 2 weeks of treatment were tested as described above. The proportion of dPCR mutation-positive wells between this point and the baseline was compared using a one-sided Z-test as previously described 21). The biological response (bR) was thus defined as a decrease in ctDNA at week 2 compared with the baseline level that was greater than the variability of the dPCR measurement.

Statistical considerations

We originally planned to enroll 172 patients (86 per treatment group) in this noncomparative study to show a difference in the survival rate without treatment failure at 9 months of 15% (one-sided test, power = 90%, alpha = 5%; % of patients without treatment failure of 50% in group A and 67% in group A + C). A planned futility analysis was performed after inclusion of 36 patients per treatment group (72 patients in total); futility was not demonstrated and therefore patient recruitment continued as planned. However, following preliminary publication of the results of the SWOG S1403 study (23), which suggested no additional benefit of adding cetuximab to afatinib for first-line treatment of EGFR-mutated NSCLC, we conducted an unplanned interim analysis in September 2018, after inclusion of 117 patients. Based on this analysis, the steering committee recommended to halt the study in November 2018. Thus, these results correspond to the final analysis and are presented in this article.

Demographic/baseline characteristics were described for the intention-to-treat (ITT) population, which comprised all included/randomized patients. All included patients without major eligibility criteria deviations were evaluable for efficacy (the evaluable population), and all patients who received at least one dose of study treatment were evaluable for safety. Median duration and 95% CIs for TTF, PFS, and OS were analyzed using the Kaplan–Meier method; a log-rank test was used to test for differences between treatment groups. All data were analyzed with SAS version 9.4 (Statistical Analysis System, RRID:SCR_008567).

Results

Patient disposition and baseline characteristics

As of the analysis cutoff date, a total of 117 of 172 (68%) patients initially planned had been included in the study between June 2016 and November 2018 and randomly assigned to group A ($n = 59$) or group A + C ($n = 58$; **Fig. 1**); these 117 patients comprised the ITT population. Of included/randomized patients, one patient originally assigned to group A + C was deemed noneligible following randomization (PS of 2) and was therefore excluded from the evaluable

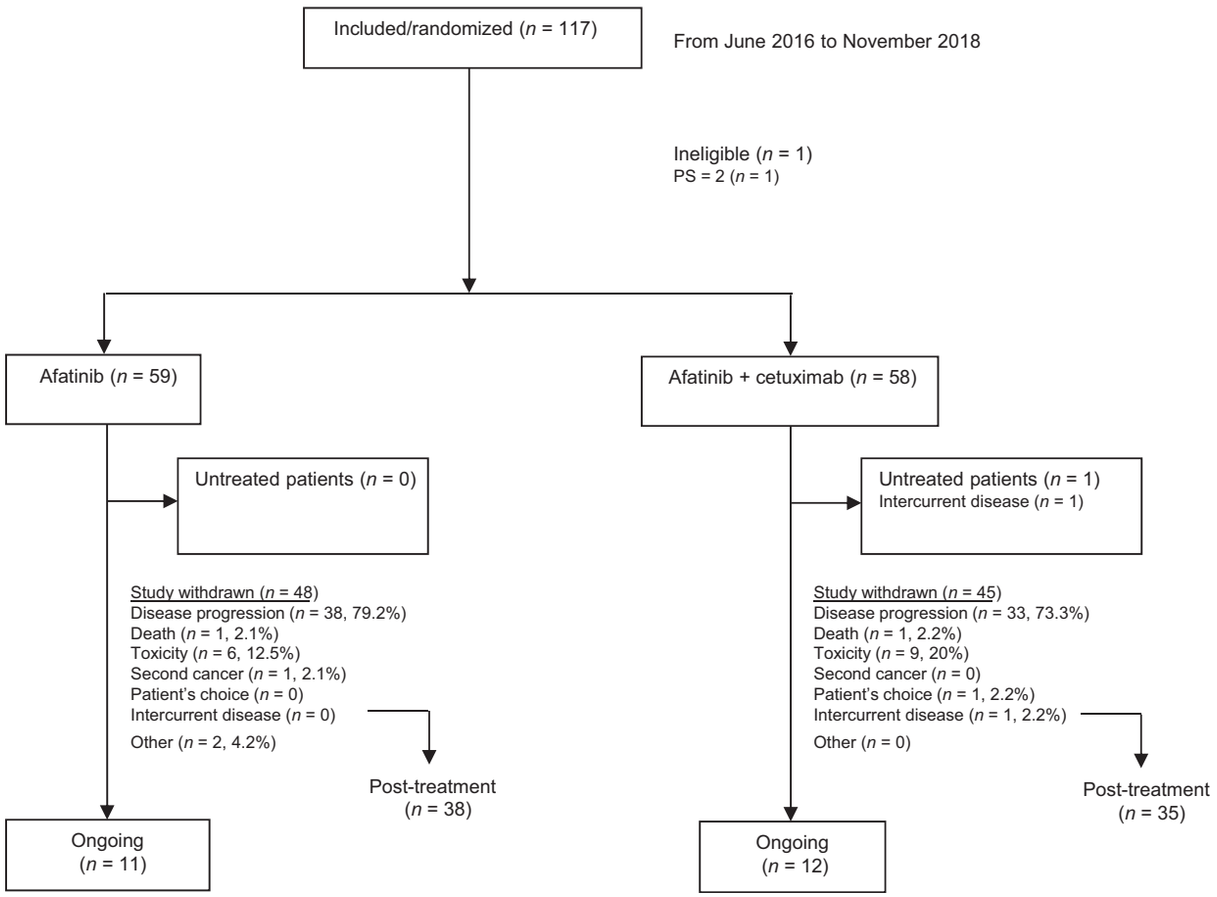

Figure 1.

CONSORT flow diagram.

population. Only one patient (group A + C) did not receive any study treatment due to the presence of intercurrent disease.

Patient demographic and baseline clinical characteristics were well balanced between the two treatment groups (**Table 1**). The majority of patients (71.8%) were women, over half (57.3%) were never-smokers, and the mean (±SD) age overall was 65 (±11) years. Almost all patients had lung adenocarcinomas (96.6%), and *EGFR* mutations were mainly deletions in exon 19 (55.6%) and L858R mutations (40.2%), with a similar distribution between the two groups.

In terms of treatment exposure, 31 (52.5%) patients in group A and 29 (50.9%) in group A + C had a dose modification of afatinib, and 2 patients in group A + C did not receive cetuximab due to afatinib toxicity. The median number of cetuximab injections in patients who received at least one dose of cetuximab was 10.5 (range, 1–13). At the time of the analysis, there were 11 patients (18.6%) ongoing in group A and 12 patients (20.7%) ongoing in group A + C.

Efficacy

During a median follow-up time of 21.7 months (interquartile range, 16.79–26.59), 38 patients (79.2%) and 33 patients (73.3%) in group A and group A + C, respectively, were discontinued from the study for disease progression, 6 patients (12.5%) and 9 (20%) were discontinued for toxicity, and 2 patients (1 in each group) died.

The number (%) of patients without treatment failure at 9 months was 35 (59.3%) in group A and 37 (64.9%) in group A + C, and median TTF was 11.1 months (95% CI, 8.5–14.1) and 12.9 months (9.2–14.5), respectively (**Fig. 2A**). Accordingly, the median PFS was similar in both groups: 11.9 months (95% CI, 9.1–14.7) in group A and 13.4 months (9.7–13.8) in group A + C (**Fig. 2B**). The objective response rate was 76.3% in group A and 77.2% in group A + C, and the disease control rate was 98.3% and 93.0%, respectively (**Table 2**). Finally, the 12-month survival rate was 87.9% (95% CI, 76.3–94.0) in group A and 89.4% (77.9–95.1) in group A + C. Median OS was 26.6 months (20.6–33.6) in group A + C, while OS was not reached in group A (**Fig. 2C**). Considering these results, which showed no benefit of addition of cetuximab to afatinib, the study steering committee recommended that patient inclusion be stopped.

Safety and tolerability

Treatment-related AEs (see **Table 3**) were observed in 59 patients (100%) and 56 patients (98.2%) in group A and group A + C, respectively, with grade 3 events reported in 22 patients (37.3%) and 30 patients (52.6%), respectively, and grade 4 events in 3 patients (5.1%) in group A (only). No grade 5 events occurred.

As shown in Supplementary Table S1, treatment-related AEs were mainly digestive and skin disorders, in accordance with the known safety profile of EGFR inhibitors. Diarrhea (any grade) was reported in

Q5 **Table 1.** Patient baseline and demographic characteristics (ITT population[a]).

Characteristic	Afatinib (*N* = 59)	Afatinib + cetuximab (*N* = 58)	Total (*N* = 117)
Age, years			
Median	68.1	63.8	64.7
Range	(34; 86.2)	(41.7; 84.3)	(34; 86.2)
Gender, *n* (%)			
Female	43 (72.9)	41 (70.7)	84 (71.8)
Male	16 (27.1)	17 (29.3)	33 (38.2)
Smoking history			
No	35 (59.3)	32 (55.2)	67 (57.3)
Yes	24 (40.7)	26 (44.8)	50 (42.7)
Median (range) (pack.years)	20 (2–112)	16 (1–60)	18 (1–112)
EGFR mutation type, *n* (%)			
Deletion exon 19	33 (55.9)	32 (55.2)	65 (55.6)
Mutation G719X exon 18	2 (3.4)	0	2 (1.7)
Mutation L858R exon 21	23 (39)	24 (41.4)	47 (40.2)
Mutation L861Q	1 (1.7)	2 (3.4)	3 (2.6)
ECOG performance status, *n* (%)			
0	21 (35.6)	21 (36.2)	42 (35.9)
1	38 (64.4)	36 (62.1)	74 (63.2)
2	0	1 (1.7)	1 (0.9)
TNM stage, *n* (%)			
IIIa	1 (1.7)	0	1 (0.9)
IIIb	0	3 (5.2)	3 (2.6)
IVa	17 (28.8)	13 (22.4)	30 (25.6)
IVb	41 (69.5)	42 (72.4)	83 (70.9)
Brain metastases, *n* (%)			
No	44 (74.6)	46 (79.3)	90 (76.9)
Yes	15 (25.4)	12 (20.7)	27 (23.1)
Histologic type, *n* (%)			
Adenocarcinoma (unspecified)	57 (96.6)	56 (96.6)	113 (96.6)
Non–small cell non-squamous cancer	1 (1.7)	1 (1.7)	2 (1.7)
Mixed carcinoma	1 (1.7)	1 (1.7)	2 (1.7)

[a]ITT population comprised all included and randomized patients.

93.2% of patients in group A and 89.5% of patients in group A + C, and grade 3–4 diarrhea was reported in 18.7% and 12.3%, respectively. We observed a higher incidence of skin rash in group A + C than group A (any grade, 94.7% vs. 79.7%, respectively), including grade 3–4 events (21.1% vs. 10.2%, respectively). Skin dryness, paronychia, and stomatitis were also more common in group A + C, and mainly grade <3 in severity. Among the 15 patients who discontinued the study for treatment-related AEs, 2 patients discontinued for grade 4 events (vomiting in one patient and diarrhea in another, both in group A).

Analysis of baseline ctDNA

To better understand the biological impact of the afatinib–cetuximab combination, we analyzed the *EGFR* mutations in the ctDNA of patients included in the study.

At baseline, blood samples were available for 104 patients in total (54 in group A and 50 in group A + C); of these, ctDNA was detected for 81 (77.9%) patients (41 in group A and 40 in group A + C). *EGFR* mutations were consistent with those found in the tissue. Use of digital polymerase chain reaction (dPCR) made it possible to measure allele frequencies. The median allele frequency of the mutated allele compared with unmutated alleles was 4.3% (range, 0.05%–92.8%) overall, and similar in both groups [median (range) values: 4.5% (0.05%–52.8%) in group A; 3.7% (0.1%–92.8%)] in group A + C].

Multivariate analyses were performed using a Cox proportional hazard regression model, adjusted according to stratification factors. The presence of ctDNA at baseline was not predictive of objective response (Supplementary Table S2) or better PFS [HR, 1.86 (0.96–3.62); $P = 0.0671$] in the adjusted analysis. However, allele frequency greater than the median value (4.3%) was associated with shorter PFS compared with patients with allele frequency below the median value [HR, 1.95 (1.11–3.41), $P = 0.02$; see **Fig. 2D**]. Accordingly, for increasing values of allele frequency, PFS was poorer [HR 1.02 (1.00–1.03), $P = 0.018$]. This remained true whatever the treatment arm (Supplementary Table S3).

For 74 of the 81 patients who were ctDNA positive at baseline, we were able to analyze plasma collected after 2 weeks of afatinib in the two arms of treatment, as cetuximab was added at day 15. A bR was observed in 49 patients (66.2%): 22/35 (62.9%) in group A and 27/39 (69.2%) in group A + C. However, the bR was not associated with an improved PFS or OS.

Analysis of ctDNA at progression

At RECIST progression ($n = 76$), a blood sample was available for 48 patients (67.6%; 25 in group A, 23 in group A + C). Of these, ctDNA was detectable in 27 patients (56.3%): 12 in group A (48.0%) and 15 in group A + C (65.2%). A T790M mutation was detected in 9 of the 27 patients (33.3%) in whom the *EGFR*-activating mutation was detectable (6 of 12 patients in group A and 3 patients of 15 patients in group A + C). The presence of a T790M mutation was not associated with better PFS. For the 9 patients who were T790M

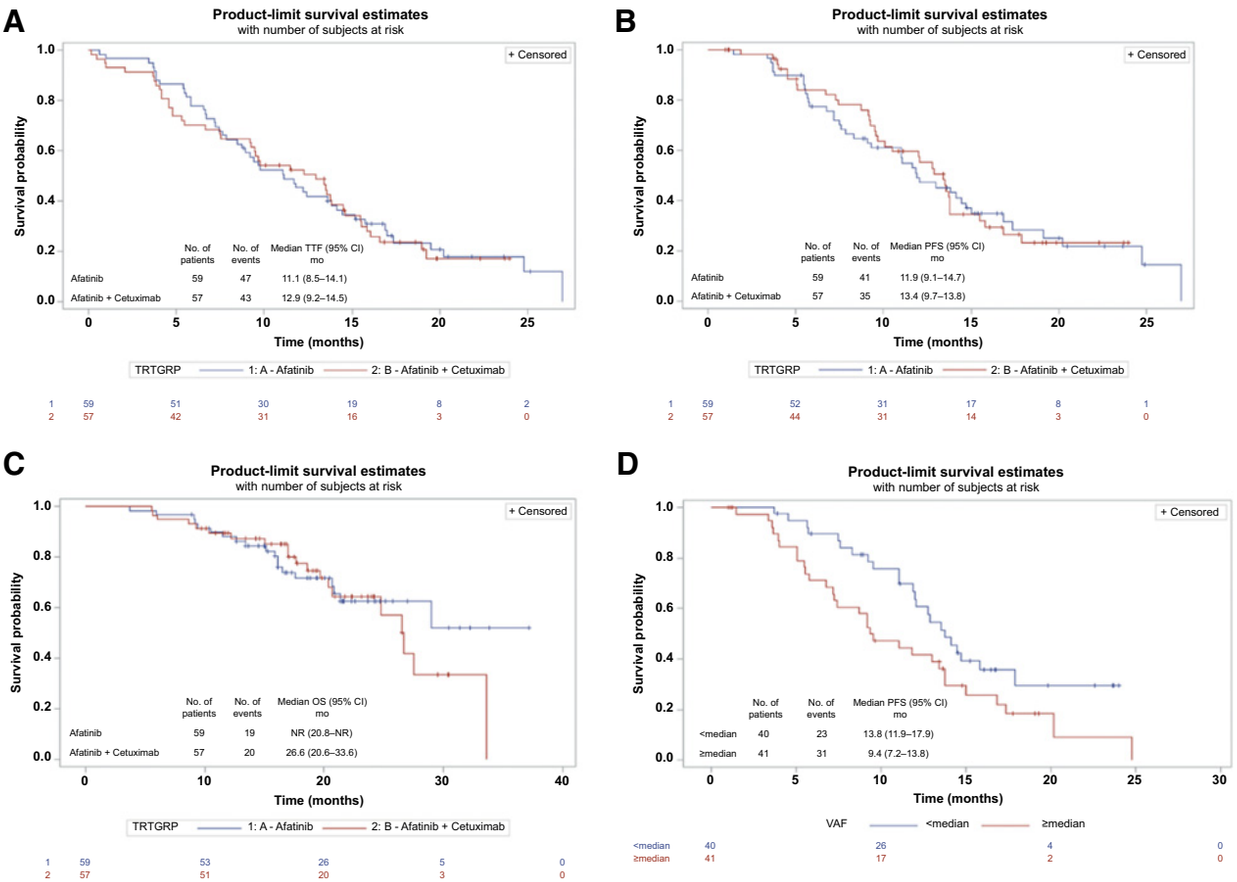

Figure 2.
Time to treatment failure (**A**), PFS (**B**), and OS (**C**) and PFS according to EGFR-mutant allele frequencies < or ≥ to the median value at baseline (**D**).

Table 2. Response rates and disease control (eligible population).

	Afatinib ($N = 59$)	Afatanib + cetuximab ($N = 57$)
Response after 2 treatment cycles, *n* (%)		
Complete response	2 (3.4)	–
Partial response	40 (67.8)	37 (64.9)
Stable disease	16 (27.1)	16 (28.1)
Progressive disease	–	1 (1.8)
Not done/evaluable	1 (1.7)	3 (5.3)
Objective response rate[a]	42 (71.2)	37 (64.9)
Disease control rate[b]	58 (98.3)	53 (93)
Best response, *n* (%)		
Complete response	3 (5.1)	2 (3.5)
Partial response	42 (71.2)	42 (73.7)
Stable disease	13 (22.0)	9 (15.8)
Progressive disease	–	1 (1.8)
Not done/evaluable	1 (1.7)	3 (5.3)
Objective response rate[a]	45 (76.3)	44 (77.2)
Disease control rate[b]	58 (98.3)	53 (93.0)

[a]Objective response rate = complete response + partial response.
[b]Disease control rate = complete response + partial response + stable disease.

positive, median PFS values were similar for the two treatment groups [11.0 months (95% CI, 5.4–24.7) in group A and 12 months (7.3–13.8) in group A + C].

Discussion

In this randomized phase II study (ACE-Lung study), we did not observe any benefit of adding cetuximab to afatinib for the first-line treatment of *EGFR*-mutated NSCLC. The safety profile was manageable and was consistent with that reported previously for double EGFR inhibition (14, 15).

Currently, first-line treatment of *EGFR*-mutated NSCLC is based on first, second, or third-generation EGFR TKIs. Both second- and third-generation TKIs have shown superiority to the first-generation agents, as demonstrated in the LUX LUNG 7 (9), ARCHER (9, 10), and FLAURA (24) trials. On the other hand, second-generation TKIs have never been compared with third-generation molecules. Regardless of the TKI used, tumor progression occurs almost systematically. The mechanisms behind the acquired resistance are mainly the T790M mutation in the case of first- or second-generation TKIs, which can be targeted by osimertinib, a third-generation, irreversible EGFR TKI that selectively inhibits both *EGFR* TKI-sensitizing and *EGFR* T790M resistance mutations. Resistance mechanisms to third-generation TKIs, however, are much more varied and difficult to target (25–27).

Table 3. Summary of treatment-related adverse events (safety population).

Treatment-related adverse events	Afatinib (*N* = 59)	Afatanib + cetuximab (*N* = 57)
All treatment-related AEs	59 (100)	56 (98.2)
Grade 3	22 (37.3)	30 (52.6)
Grade 4	3 (5.1)	—
Treatment-related serious AEs[a]	12 (20.3)	5 (8.8)
Related to afatinib only	12 (20.3)	1 (1.8)
Related to cetuximab only	—	1 (1.8)
Related to afatinib and cetuximab	—	3 (5.3)
Treatment-related AEs leading to study discontinuation	6 (10.2)	9 (15.8)
AEs leading to death	—	—

[a]Data presented are number of patients with AE (% of patients).

Thus, strategies to improve the effectiveness of first-line treatment while preserving the possibility of using third-generation TKIs are therefore under consideration. Such strategies are based mainly on therapeutic combinations, for example, with chemotherapy, antiangiogenics, other targeted therapies or combinations of TKIs and antibodies directed against the same target (28).

Based on preclinical studies, double EGFR inhibition by TKI and antibodies directed against EGFR is more effective than TKI inhibition alone, whether targeting initial mutations (13) or certain resistance mechanisms (29). The present study is the first publication to report the results of a therapeutic combination of afatinib with a fixed duration of cetuximab. Results from the SWOG S1403 study (23) showed a lack of benefit from the addition of cetuximab to afatinib, both maintained until disease progression or unacceptable toxicity, in the first-line treatment of EGFR-mutated NSCLC. One of the causes of failure was suspected to be the increased toxicity of the afatinib–cetuximab combination, which resulted in more grade 3 or higher AEs, and more dose reductions than afatinib alone. The ACE-Lung study was designed with particular attention to limit the risk of toxicity of the combination: cetuximab was introduced 2 weeks after starting afatinib, first at mid-dose and then at full dose, and appropriate dose reduction strategies were employed. Treatment with the combination was limited to a period of 6 months with the objective of reducing minimal residual disease. Interestingly, we did not observe more AEs in the combination group than in the afatinib group. Moreover, we chose to use TTF as the primary endpoint to take into account the potential toxicity of afatinib and cetuximab combination and found similar differences between the 2 groups regarding TTF and PFS. Altogether, these results suggest that increased toxicity is not the reason for the lack of efficacy of afatinib and cetuximab combination.

The reasons for the lack of additional efficacy of adding cetuximab to afatinib, whereas it was found active in pretreated patients and in animal models as first-line therapy, remain poorly understood. This is unlikely to be due to the limited duration of cetuximab treatment, because maintaining cetuximab until progression has also not demonstrated any benefit on PFS in the SWOG S1403 study (30). Consistent with our initial hypothesis, the proportion of T790M mutations was not significantly different between the two groups, suggesting that cetuximab did not alter the type of resistance mechanism. Research into other resistance mechanisms will be important to confirm this hypothesis and better understand the biological impact of the afatinib–cetuximab combination. Conceivably, the afatinib–cetuximab combination may not be active on residual disease. Different results between animal models and human patients may result from differences in the genetic background. Human EGFR-mutated tumors frequently harbor other mutations, usually seen as passenger mutations. However, these mutations may have an impact on response to EGFR TKIs and may have limited the antitumor activity of A + C (31, 32). Another hypothesis is that A + C combination may be more active in TKI-pretreated tumors than in TKI-naïve tumors. This could be due to a higher dependency on EGFR signaling following therapeutic pressure with prior EGFR TKI, as emphasized by the acquired T790M mutation, or a differential EGFR expression. Indeed, EGFR downregulation has been observed in TKI-resistant EGFR-mutant tumors (33). Because EGFR overexpression has been proposed as a mechanism of resistance to A + C, this could explain the higher sensitivity of TKI-pretreated tumors to this combination (34).

Our study also provides original data on the detection of *EGFR* mutations on ctDNA in the context of a prospective randomized study. We confirm the feasibility of detecting baseline *EGFR* mutations, with good sensitivity, in line with what has been reported in the literature (35, 36). Interestingly, the allele frequency of the *EGFR* mutation in ctDNA was associated with shorter PFS, regardless of the treatment received in this prospective trial. This could reflect a higher tumor burden. Although we did not find any association of allele frequency with tumor stage, the analysis was limited by the high proportion of patients with stage IVb disease. Whether this result may help to select which patients could benefit from more intensive strategies such as combination of EGFR TKI and chemotherapy remains uncertain.

On the other hand, the detection of ctDNA at progression was less sensitive. This is likely because in this prospective study, progression was defined by RECIST radiologic progression, which corresponds to an increase in the sum of the diameters of the target lesions by 20% or the appearance of new lesions. Thus, RECIST progression can be retained even if the tumor volume remains relatively low, which then decreases the chances of detection of ctDNA. Although trials are currently being conducted to assess the relevance of the use of ctDNA to determine tumor progression (37), our results suggest that detecting molecular progression earlier than radiologic progression will require different technical approaches.

In conclusion, our findings from the phase II ACE-Lung study suggest that addition of cetuximab to afatinib does not warrant further investigation in treatment-naïve patients with advanced *EGFR*-mutant NSCLC. Baseline ctDNA could help identify different patient profiles benefiting from EGFR inhibition.

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