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# UNIVERSITÀ DEGLI STUDI DI TORINO

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# Activity of the EGFR-HER2 Dual Inhibitor Afatinib in EGFR-Mutant Lung Cancer Patients With Acquired Resistance to Reversible EGFR Tyrosine Kinase Inhibitors

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## Abstract

### Background

The purpose of this study was to evaluate the efficacy of afatinib in EGFR-mutant metastatic NSCLC patients with acquired resistance to erlotinib or gefitinib.

### Materials and Methods

We retrospectively analyzed the outcome of patients with EGFR-mutant advanced NSCLC treated with afatinib after failure of chemotherapy and EGFR TKIs.

### Results

A total of 96 individuals were included in the study. According to EGFR status, most patients ( $n = 63$ ; 65.6%) harbored a deletion in exon 19, and de novo T790M mutation was detected in 2 cases (T790M and exon 19). Twenty-four (25%) patients underwent repeated biopsy immediately before starting afatinib and secondary T790M was detected in 8 (33%) samples. Among the 86 patients evaluable for efficacy, response rate was 11.6%, with a median progression free-survival (PFS) and overall survival (OS) of 3.9 and 7.3 months, respectively. No significant difference in PFS and OS was observed according to type of last therapy received before afatinib, type of EGFR mutation or adherence to Jackman criteria, and patients benefiting from afatinib therapy had longer PFS and OS ( $P < .001$ ). Outcome results for repeated biopsy patients were similar to the whole population, with no evidence of response in T790M-positive patients. All patients were evaluable for toxicity, and 81% experienced an AE of any grade, with grade 3 to 4 AEs, mainly diarrhea and skin toxicity, occurring in 19 (20%) patients.

### Conclusion

Our results showed that afatinib has only modest efficacy in a real life population of EGFR mutant NSCLC patients with acquired resistance to erlotinib or gefitinib.

### Keywords

- Acquired resistance;
- Afatinib;
- Erlotinib;
- Gefitinib;
- NSCLC

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## Introduction

Epidermal growth factor receptor (EGFR)-activating mutations, mainly represented by deletion in exon 19 or the L858R substitution in exon 21, identified a distinct subgroup of non-small-cell lung cancer (NSCLC) with different prognosis and sensitivity to anti-tumor strategies.<sup>1, 2 and 3</sup> Eight large randomized studies have clearly demonstrated the superiority of EGFR tyrosine kinase inhibitors (TKIs) in terms of response rate (RR), progression free-survival (PFS) and tolerability compared with conventional first-line platinum-doublet chemotherapy.<sup>4, 5, 6, 7, 8, 9, 10 and 11</sup> Although no formal advantage in overall survival (OS) has emerged from the aforementioned studies, in all trials median survival was up to 2 to 3 years, indicating that EGFR-TKIs have changed natural history of EGFR-mutated NSCLC. Nevertheless, despite an initial dramatic tumor regression, after a median time of 9 to 12 months, all patients have disease progression due to the occurrence of resistance and the possibility of further control tumor growth inevitably decreases.

From a practical point of view, the widespread use of EGFR-TKIs as first-line therapy translates to an undoubted clinical benefit for EGFR-mutant patients, but it also leads to the emergence of a novel clinical entity. Indeed, EGFR-mutant patients with acquired resistance to EGFR TKIs represent a subgroup of individuals for whom approved treatment options are only modestly active and for whom there is an urgent need for novel targeted agents. So far, several mechanisms have been recognized as responsible for acquired resistance, with the secondary T790M mutation—a characteristic point mutation in the exon 20 of the *EGFR* gene—representing the most prominent, being detectable in more than 50% of patients exposed to gefitinib or erlotinib.<sup>12, 13 and 14</sup>

Afatinib (Giotrif) is an irreversible HER-family inhibitor and preclinical experience has demonstrated its activity in cell lines harboring EGFR mutations, including the T790M, thus suggesting a potential role in overcoming acquired resistance.<sup>15 and 16</sup> In 2 trials, the LUX-Lung 1 and LUX-Lung 4,<sup>17 and 18</sup> the role of afatinib was investigated at the daily dose of 50 mg in NSCLC patients resistant to EGFR TKIs defined according to the Jackman criteria,<sup>19</sup> demonstrating similar results. RR ranged from 8% to 10%, with a PFS of nearly 4 months, in the whole population and in subgroup analyses. Nevertheless, in both studies there was no molecular restriction for patient selection, thus precluding the possibility to derive definitive conclusions on the role of afatinib in the EGFR-mutant and resistant population.

The aim of the present study was to investigate the efficacy of afatinib in a real-life population of pretreated *EGFR*-mutant NSCLC patients with acquired resistance to reversible EGFR TKIs.

## Materials and Methods

### Patient Selection

In the present study we retrospectively analyzed the outcome of patients with *EGFR*-mutant advanced NSCLC treated with afatinib after failure of chemotherapy and reversible EGFR TKIs in 11 Italian institutions. Eligibility criteria included: availability of clinical information, such as demographic characteristics, presence of *EGFR* mutation, toxicity, and efficacy data of afatinib therapy. *EGFR* mutational status was assessed independently at each institution, according to the Società Italiana di Anatomia Patologica e Citopatologica Diagnostica guidelines and using direct

sequencing (Sanger method), pyrosequencing, or real-time polymerase chain reaction (Therascreen EGFR29 RGQ PCR mutation kit, Qiagen, Venlo, Netherlands).<sup>20</sup> Afatinib was provided by Boehringer Ingelheim Inc as a compassionate use and self-administered at a 50-mg dose orally once daily continuously until disease progression, unacceptable toxicity, or patient refusal to continue. Dose reductions to 40 mg per day and then to 30 mg per day were considered on the basis of individual tolerability. Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 every 4 weeks. In all patients, tumor assessment was performed every 2 months according to the Response Evaluation Criteria In Solid Tumors (RECIST) criteria<sup>21</sup> and drug resupply was subordinated to tumor reassessment. Each center received the approval of the local ethics committee for each patient included in the study. All patients provided informed consent.

## Statistical Analysis

A descriptive analysis on baseline characteristics was performed on the cohort of 96 EGFR-mutant lung cancer patients. RR was computed on 86 patients evaluable for efficacy. PFS was calculated from the time of starting therapy with afatinib to date of progression or last radiological assessment, and OS was calculated from the time of starting therapy with afatinib to death or last follow-up, with 95% confidence intervals using the Kaplan–Meier method. Differences in PFS or OS according to type of EGFR mutations, type of previous therapy, adherence to Jackman criteria, or response to afatinib therapy were evaluated using the Log rank test. The significance level for all analyses was set at  $P < .05$ . Statistical analysis was performed using SPSS version 20.

## Results

### Patient Characteristics

A total of 96 consecutive subjects treated with afatinib between 2011 and 2013 were included in the study. Patient demographic and baseline characteristics are listed in Table 1.<sup>19</sup> Most patients were female ( $n = 62$ ; 64.4%), never smokers ( $n = 62$ ; 64.4%), with an Eastern Cooperative Oncology Group performance status of 0 to 1 ( $n = 89$ ; 92.8%) and treated with at least 3 or more therapy lines ( $n = 68$ ; 70.8%). All patients had received previous EGFR TKI treatment such as gefitinib ( $n = 46$ ; 47.9%), erlotinib ( $n = 46$ ; 47.9%), or both ( $n = 4$ ; 4.2%), as their first ( $n = 27$ ; 28.1%), second ( $n = 57$ ; 59.3%), or subsequent ( $n = 19$ ; 19.7%) line of therapy. More than two-thirds of patients ( $n = 70$ ; 72.9%) responded (complete response [CR] or partial response [PR]) to previous EGFR TKI therapy. Regarding therapy received before starting afatinib, 53.1% of patients ( $n = 51$ ) received chemotherapy, and 41 (41.7%) fulfilled the Jackman criteria for acquired resistance. Seventeen patients (17.7%) received 1 or more subsequent lines of therapy after afatinib progression, including gefitinib in 35%. According to *EGFR* mutational status, 63 (65.6 %) patients harbored a mutation in exon 19, 25 (26.0%) in exon 21, 4 (4.2%) in exon 18, and 2 (2.1%) patients had an activating mutation not otherwise specified. *De novo* T790M mutation was detected in 2 (2.1%) patients and in both cases it was associated with mutation in exon 19. In 24 patients it was possible to perform a second biopsy immediately before starting afatinib treatment. Secondary T790M was detected in 8 (33%) samples, in 2 (8%) samples a novel *EGFR* mutation in exon 18 was detected, and 2 other (8%) resulted as wild type, and the remaining 12 (50%) samples displayed the same *EGFR* mutation detected at baseline. No additional biomarker was investigated.

Table 1.

Patient Characteristics (n = 96)

Characteristic	n	%
<b>Median Age (Range)</b>	62.0 (29.6-84.7)	
<b>Men/Women</b>	34/62	35.4/64.6
<b>Smoking History</b>		
Never/former	62/31	64.6/32.3
Current	3	3.1
<b>ECOG Performance Status</b>		
0/1/2/3	59/30/6/1	61.5/31.3/6.3/1.0
<b>Previous Chemotherapy Lines</b>		
1/2/3/>3	2/26/34/34	2.1/27.1/35.4/35.4
<b>Best Response to Previous Reversible EGFR-TKI</b>		
CR/PR/SD/PD	5/65/22/4	5.2/67.7/22.9/4.2
<b>Previous EGFR-TKI</b>		
Gefitinib/erlotinib/both	43/45/8	44.8/46.9/8.3
<b>Type of <i>EGFR</i> Mutation at Baseline</b>		
Exon 19	63	65.6
Exon 21	25	26.0
Exon 20 (T790M)	2	2.1
Exon 18	4	4.2
Not specified <sup>a</sup>	2	2.1
<b>Repeated Biopsy Immediately Before Starting Afatinib</b>		
Yes/No	24/72	25/75

Characteristic	n	%
<b>EGFR Status in Repeated Biopsy</b>	24	100
Same mutation of at baseline	12	50.0
EGFR wild type	2	8.3
EGFR mutated + T790M	8	33.4
Other EGFR mutation <sup>b</sup>	2	8.3
<b>Last Therapy Before Afatinib</b>		
EGFR-TKI	45	46.9
Chemotherapy	51	53.1
<b>Fulfilled the Jackman Criteria<sup>19</sup></b>		
Yes/No	40/56	41.7/58.3

Abbreviations: ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; TKI = tyrosine kinase inhibitor.

A Unknown means EGFR mutation not otherwise specified.

B Only in exon 18.

## Efficacy

Eighty-six patients were evaluable for response according to RECIST criteria (Table 2). Overall, 10 (11.6%) patients achieved confirmed response, including 1 CR and 9 PRs, and 38 (44.2%) obtained disease stabilization (SD) as their best response, with a disease control rate (CR + PR + SD) of 55.8%; furthermore, an additional 38 (44.2%) patients' disease progressed within the first 2 months of therapy. All responders had previously responded to reversible EGFR-TKIs and for 70% of them chemotherapy was the last therapy received before afatinib. No difference in RR was detected in patients fulfilling Jackman criteria versus patients not fulfilling the same criteria (5% in both groups).

Table 2.

Response Rate in the Overall Population

	Response Rate	
	n	%
<b>Evaluable Patients</b>	86	100
<b>CR</b>	1	1.1
<b>PR</b>	9	10.5
<b>SD</b>	38	44.2
<b>PD</b>	38	44.2
<b>CR + PR</b>	10	11.6
<b>CR + PR + SD</b>	48	55.8

All patients were assessable for PFS and OS. In the whole population, median PFS and OS were 3.9 (95% confidence interval [CI], 3.26-4.62) and 7.3 (95% CI, 4.03-10.69) months, respectively (Figure 1). No difference in PFS and OS was observed according to type of last received therapy (EGFR TKIs vs. chemotherapy), type of EGFR mutation (exon 19 vs. exon 21 vs. other) or adherence to Jackman criteria (Supplemental Table 1, Supplemental Figure 1, Supplemental Figure 2 and Supplemental Figure 3). As expected, a significant difference in PFS (7.1 months vs. 1.9 months;  $P < .001$ ) and OS (13.4 months vs. 4.7 months;  $P < .001$ ) was observed for patients benefiting from afatinib therapy (Figure 2).

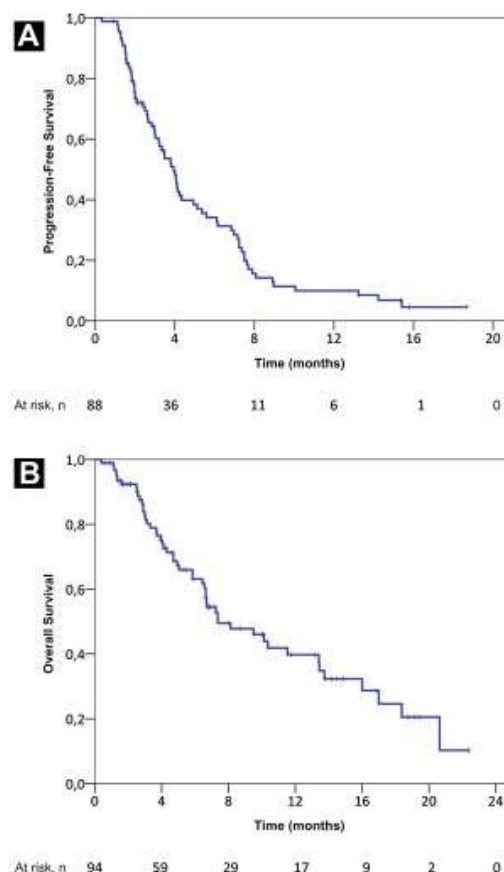




Figure 1.

(A) Kaplan–Meier Curve of Time to Progression-Free Survival and (B) Overall Survival in the Whole Population

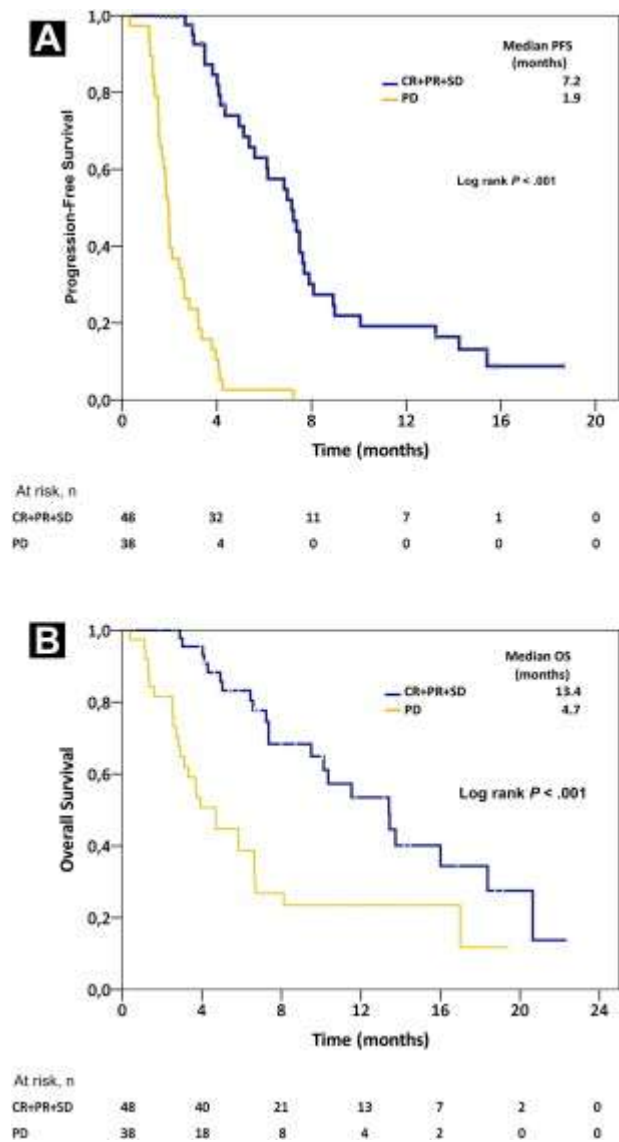


Figure 2.

(A) Kaplan–Meier Curve of Time to Progression-Free Survival and (B) Overall Survival According to Response to Afatinib

Abbreviations: OS = Overall Survival; PFS = Progression-Free Survival.

We further analyzed outcome in the subgroup of patients who received a repeated biopsy. This subgroup was representative of the whole population, with no difference in PFS (3.8 vs. 3.9 months;  $P = .5$ ) or OS (10.3 vs. 7.3 months;  $P = .2$ ), as shown in Table 3. Among the 22 evaluable patients who receive a repeated biopsy, overall RR was 4.5% (1 patient with PR), with SD of 45.4% and a progressive disease (PD) rate of 50%. Notably, none of the 6 individuals harboring the T790M mutation responded, and the only responder had the less common mutation in exon 18 in both tumor samples.

Table 3.

## Outcome in Patients With Repeated Biopsy

Pt ID	EGFR Status At Baseline	EGFR Status Before Afatinib	Best Response to Afatinib	PFS, Months	OS, Months
2	Exon 19	Exon 19	PD	2.00	14.85
3	Exon 19	Exon 19 + T790M	PD	1.54	16.99
4	Exon 19	Exon 19 + T790M	SD	15.41	19.16
5	Exon 21	EGFR Wild type	SD	7.49	18.76
9	Exon 19	Exon 19	SD	3.48	6.44
10	Exon 19	Exon 19	PD	1.68	6.67
11	Exon 18	Exon 18	PR	7.89	13.74
15	Exon 19	Exon 19 + T790M	PD	1.97	2.14
31	Exon 21	Exon 21	PD	7.23	19.39
35	Exon 19	Exon 19 + T790M	SD	2.27	5.32
42	Exon 19	Exon 19	SD	8.97	13.14
43	Exon 19	Exon 19	PD	1.74	6.64
45	Exon 19	Exon 19	PD	1.97	2.89
47	Exon 19	Exon 19 + T790M	SD	8.08	9.50
50	Exon 19	EGFR Wild type	PD	1.84	6.70
55	Exon 19	Exon 19 + T790M	SD	3.81	3.91
67	Exon 21	Exon 21 + T790M	SD	5.13	10.35
70	Exon 19	Exon 19 + T790M	PD	1.18	2.50
73	Exon 19	Exon 18	SD	1.61	1.91
79	Exon 19	Exon 19	PD	3.78	3.94

Pt ID	EGFR Status At Baseline	EGFR Status Before Afatinib	Best Response to Afatinib	PFS, Months	OS, Months
80	Exon 19	Exon 19	PD	1.28	1.31
83	Exon 19	Exon 18	SD	4.07	14.16
Overall			RR, 4.5%	PFS, 3.8	OS, 10.3

Abbreviations: EGFR = epidermal growth factor receptor; OS = overall survival; PFS = progression-free survival; RR = response rate.

### Toxicity

Ninety-five patients were assessable for toxicity and 77 (81.0%) subjects experienced a drug-related adverse event (AE) of any grade, including diarrhea and skin toxicity, this latter defined as skin rash/acneiform dermatitis, palmar-plantar erythrodysesthesia syndrome, pruritus, xerosis, nail changes, and paronychia (Table 4). Grade 3 to 4 AEs occurred in 19 patients (20%), with diarrhea and skin toxicity as the most frequent events (10.6% and 11.6%, respectively); however, the occurrence of both types of adverse reactions was 5%. Other Grade 3 to 4 AEs included stomatitis in 1 patient and respiratory distress without clinical features of interstitial lung disease in another patient. Overall, 29 patients (30%) required a dose reduction to 40 mg (22%) and to 30 mg (8%) because of persistent Grade 2 or 3 skin rash (24%), diarrhea (31%), or both (41%). Thirty patients (31.6%) had treatment delays because of toxicity, with only 3.2% of patients discontinuing afatinib because of unresolved AEs.

Table 4.

Most Common Treatment-Related AEs

Toxicity	All Grade		Grade 3/4	
	n	%	n	%
Evaluable Patients	95	100	95	100
Total With Any Grade AEs	77	81.0	19	20.0
Diarrhea	47	49.5	10	10.6
Cutaneous Toxicity <sup>a</sup>	52	59.7	11	11.6
Stomatitis	1	1.0	1	1.0

Toxicity	All Grade		Grade 3/4	
	n	%	n	%
Fatigue	2	2.1	0	0
Respiratory Distress	1	1.0	1	1.0

Abbreviation: AEs = adverse events.

a Cutaneous toxicity included: skin rash/acneiform dermatitis, palmar-plantar erythrodysesthesia syndrome, pruritus, xerosis, nail changes, and paronychia.

## Discussion

The present study, specifically conducted in *EGFR*-mutated NSCLCs with acquired resistance to erlotinib or gefitinib, showed that afatinib is effective only in a small fraction of NSCLC patients pretreated with reversible *EGFR*-TKIs.

*Epidermal growth factor receptor*-mutant NSCLC represents a growing clinical entity for which efficacious therapeutic options are still lacking. In clinical practice, rechallenge with *EGFR* TKIs has been considered as a reasonable choice and clinical trials are currently under way to investigate such a strategy. In addition, several studies to evaluate retreatment with reversible *EGFR*-TKIs<sup>22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33</sup> or investigate the efficacy of irreversible *EGFR* TKIs<sup>17 and 18</sup> in the setting of acquired resistance to reversible inhibitors showed that there is a constant proportion of patients ranging up to 10% who continued to benefit from such an agent.<sup>17, 18, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33</sup> Nevertheless, it is not possible to accurately predict which patients will further belong to this small subgroup.

In our study, we reported a RR of 11%, quite similar to historical data<sup>17, 18, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33</sup>; more interestingly, we noted that in most cases, chemotherapy was the last therapy received before afatinib. Moreover, responding patients progressed later and lived longer than those who did not. Although this result seems too obvious, it reinforces the conviction that irrespective of mechanism responsible for erlotinib or gefitinib failure, reexposure to *EGFR*-TKIs after a break period could restore the sensitivity to driver inhibition probably because of the reexpansion of the initially sensitive clones. However, for the remaining 90% of our population, afatinib did not seem to produce any benefit, even when splitting results according to type of *EGFR* mutation or adherence to Jackman criteria.

From a biological point of view, acquired resistance is a more complex phenomenon than a simple radiologic progression during treatment and a molecular definition should be mandatory, to allocate the correct patient to the correct treatment. Prolonged exposure to erlotinib or gefitinib provides selective pressure for the development of tumor clones able to grow irrespective of the drug inhibition. Some of the mechanisms underlying the phenomenon of secondary resistance are so far elucidated,<sup>12, 13, 14, 34, 35, 36 and 37</sup> including the upregulation of the downstream signal by mesenchymal-epidermal transition amplification, *EGFR* amplification, *phosphatidylinositol-3 kinase catalytic subunit alpha (PI3KCA)* mutations, transition from epithelial to mesenchymal differentiation, and for a small percentage of

resistant tumors, transformation into small-cell lung cancer. Furthermore, several studies recognized the emergence of the T790M *EGFR* gatekeeper mutation as most prominent, explaining approximately half of gefitinib/erlotinib treatment failures.<sup>12, 13, 14, 36 and 37</sup>

Because of its ability to arrest tumor growth in in vitro models of *EGFR* mutant clones resistant to gefitinib and harboring the T790M mutation, afatinib has emerged as the good candidate to test in the clinical setting of acquired resistance with a specific focus on T790M-mediated resistance.<sup>15, 16 and 37</sup> The combination of afatinib and cetuximab, a monoclonal antibody against *EGFR*, showed promising efficacy in patients with acquired resistance to *EGFR* TKIs with an increased risk of toxicity.<sup>38</sup> Anecdotal series reported a potential efficacy of afatinib even in the presence of the T790M mutation.<sup>39 and 40</sup> Nevertheless, additional studies showed the lack of efficacy of the drug in patients with *EGFR* TKI acquired resistance.<sup>17 and 18</sup> The LUX-Lung 1 was a large phase III trial specifically designed to demonstrate the superiority of afatinib versus best supportive care in heavily pretreated NSCLC patients with secondary resistance to reversible *EGFR* TKIs. Although the study failed to meet its primary end point of OS, a modest but significant improvement in RR and PFS was observed for patients allocated in the active arm than in the placebo arm.<sup>17</sup> Similarly, in the LUX-Lung 4, a phase II single-arm Japanese trial, RR and PFS were 8.2% and 4.4 months, respectively.<sup>18</sup> Notably, in both trials there was not a molecular restriction for patient selection, even if the requirement for at least 12 weeks of previous *EGFR* TKI treatment was adopted as an enrichment strategy to increase the number of *EGFR*-mutated patients. As a consequence, archival tissues for *EGFR* assessment was available in a small percentage of patients, with only 6 cases (4 and 2 patients, in LUX-Lung 1 and LUX-Lung 4, respectively) carrying the T790M; in addition, tumor samples were collected at the time of initial diagnosis rather than after erlotinib or gefitinib progression, thus precluding the possibility to postulate any hypothesis on the role of afatinib in presence of such a mutation.<sup>17 and 18</sup> Although repeating tumor biopsy is not often feasible in NSCLC, in our cohort, a not negligible number of patients underwent repeated biopsy and we identified secondary T790M in 33% of cases, with no evidence of tumor response. It is interesting to note that 3 of these patients had a relative longer PFS; nevertheless, in such cases we cannot rule out a potential effect of the drug, even if the presence of T790M could be predictive for an indolent outcome.<sup>36</sup>

These unmet expectations could be probably explained by the afatinib ability to inhibit not only the mutated *EGFR* but also the wild type protein, limiting the use of the optimal dose.<sup>15 and 41</sup> Therefore, a new potentially effective strategy consists of the use of a new class of covalent irreversible *EGFR* inhibitors, sparing the *EGFR* wild type and effective only against the mutated form, including T790M. CO-1686 and AZD6162 are new third-generation *EGFR* TKIs and preliminary results of 2 recently presented phase I studies showed promising activity in a resistant setting with the absence of typical class-related AEs.<sup>42 and 43</sup>

Taking into account all of these data suggested that the resistant setting is not the correct place to use afatinib. Furthermore, as shown in 2 recently published phase III trials conducted in more than 700 patients, the best performance is obtained when afatinib is used early in the course of disease.<sup>10 and 11</sup> In LUX-Lung 3, the first trial to use the most fit comparator arm of cisplatin-pemetrexed, patients treated with afatinib had a 42% relative reduction in risk of progression compared with those who received standard chemotherapy.<sup>10</sup> Again, treatment with the *EGFR* TKI was also associated with greater RR and a better toxicity profile than chemotherapy, although Grade 3 diarrhea and skin rash occurred in 14% and 16% of cases receiving the experimental drug, respectively. The second trial, the LUX-Lung 6, in which afatinib was compared with standard doublet of cisplatin-gemcitabine in an Asian population,

replicated these findings. Treatment with afatinib doubled PFS, tripled RR, and it was responsible for a 35.6% of Grade 3 to 4 drug-related AEs, mainly diarrhea and skin rash.<sup>11</sup>

At this proposal, it is a general opinion that afatinib is more toxic than the first-generation TKIs, erlotinib and gefitinib. In the metastatic setting, the preservation of quality of life still remains one of the goals of therapy, mainly in second and subsequent lines of treatment. Moreover, regarding safety profile, our findings were consistent with the well known toxicity profile of afatinib.<sup>10, 11, 17 and 18</sup> In our series we reported an overall incidence of any grade AEs of 81%, quite similar to those described in all afatinib trials.<sup>10, 11, 17 and 18</sup> Furthermore, Grade 3 to 4 AEs, mainly diarrhea and skin rash, occurred in 20% of subjects. This percentage was not unexpected, probably because we used as a starting dose 50 mg, instead of the recommended 40 mg dose.<sup>10 and 11</sup> Nevertheless, only 3% of patients discontinued afatinib because of unresolved toxicity, thus suggesting that, with appropriate dose reduction and adequate supportive care, afatinib was manageable also in a cohort of heavily pretreated patients.

## Conclusion

Our results showed that afatinib has only modest efficacy in a real-life population of EGFR-mutant NSCLCs with acquired resistance to erlotinib or gefitinib and its use in *EGFR*-mutant patients should be reserved for EGFR TKI naive individuals. Third-generation irreversible EGFR TKIs seem to offer important advantages over older compounds, especially in the management of resistant tumors, and confirmatory trials are urgently awaited.

## Clinical Practice Points

- In *EGFR*-mutant NSCLC patients, 8 randomized studies have clearly demonstrated the superiority of EGFR-TKIs in terms of outcome and tolerability compared with standard first-line platinum-doublet chemotherapy.
- Currently, the approved treatment options in *EGFR*-mutant patients with acquired resistance to first-generation EGFR TKIs are only modestly active and there is an urgent need for novel targeted agents.
- Afatinib is a second-generation irreversible HER-family inhibitor and preclinical models suggest a potential role in overcoming acquired resistance, including secondary T790M mutation.
- In patients with acquired resistance to EGFR TKIs, no study has been specifically focused on individuals with *EGFR* mutations, precluding the possibility to derive definitive conclusions on the role of this drug in resistant cases.
- In our study, we retrospectively evaluated the outcome of 96 *EGFR* mutant NSCLC patients treated with afatinib after failure of chemotherapy and EGFR TKI treatments. Our data showed that afatinib was effective only in a small fraction of NSCLC patients with acquired resistance to EGFR TKIs.
- Afatinib treatment should be reserved only for EGFR-TKI-naive *EGFR*-mutant NSCLC patients.

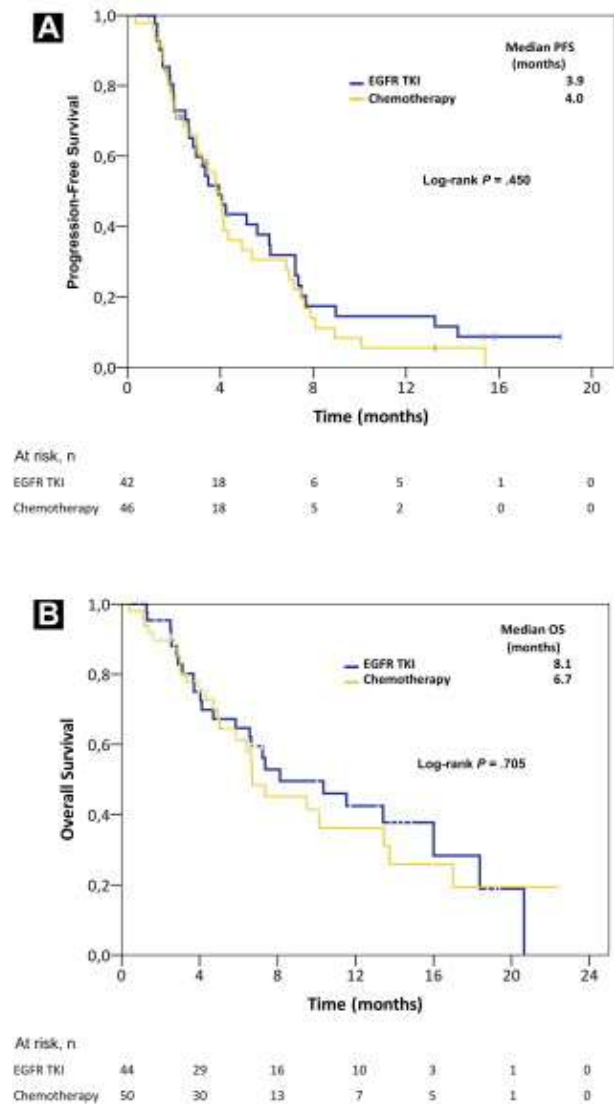
## Disclosure

F.C. and L.C. have received honoraria from AstraZeneca e Roche. F.d.M. has received honoraria from AstraZeneca, Roche, and Boehringer Ingelheim. S.N. has received honoraria from AstraZeneca, Roche, and Eli Lilly. The remaining authors have stated that they have no conflicts of interest.

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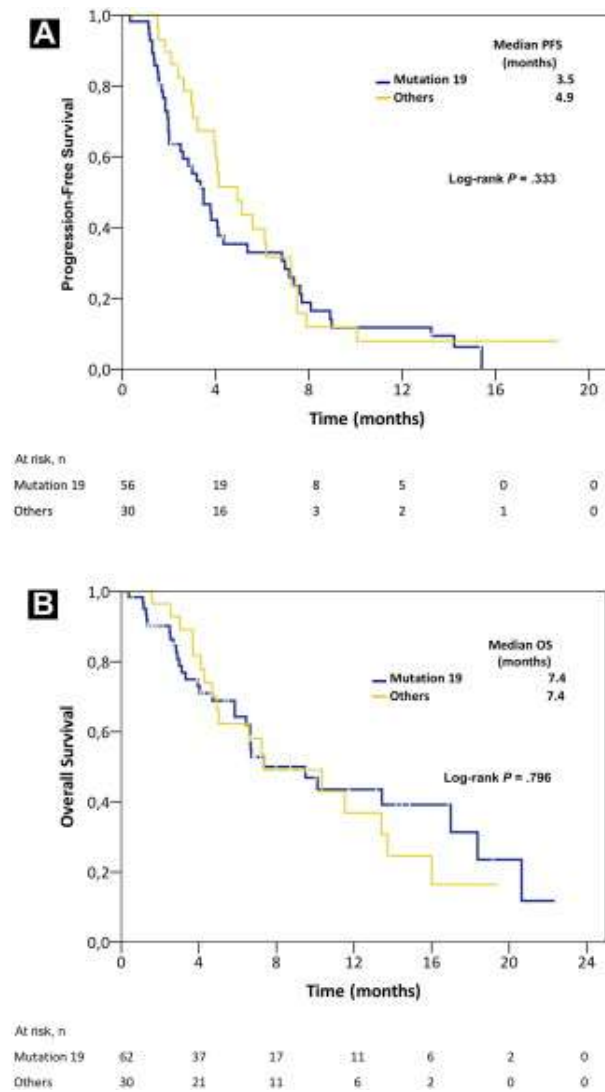
Supplemental Data



Supplemental Figure 1.

(A) Kaplan–Meier Curve of Time to Progression-Free Survival (PFS) and (B) Overall Survival (OS) According to Therapy Received Before Afatinib

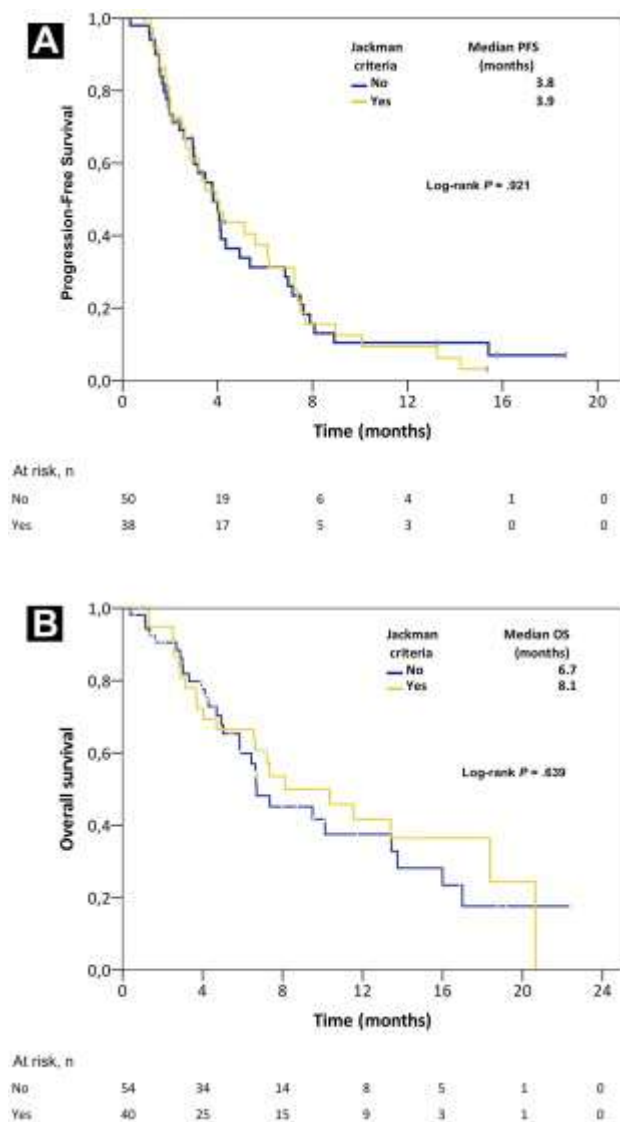
Abbreviations: EGFR = Epidermal Growth Factor Receptor; TKI = Tyrosine Kinase Inhibitor.



Supplemental Figure 2.

**(A)** Kaplan–Meier Curve of Time to Progression-Free Survival (PFS) and **(B)** Overall Survival (OS) According to Type of EGFR Mutation





Supplemental Figure 3.

(A) Kaplan–Meier Curve of Time to Progression-Free Survival (PFS) and (B) Overall Survival (OS) According to Jackman Criteria

Supplemental Table 1.

## Outcome Results in Subgroup Analyses

	Last Therapy Before Afatinib		Type of Mutation		Fulfilled Jackman Criteria	
	CT	EGFR-TKI	Exon 19	Exon 21 (and Other)	Yes	No
<b>Patients, n</b>	51	45	63	33	40	56
<b>PFS, Months</b>	4.0	3.9	3.4	4.9	3.9	3.8
95% CI	3.18-4.83	2.75-5.12	2.48-4.47	3.22-6.63	2.67-5.20	2.80-4.81
<i>P</i>	.4		.3		.9	
<b>OS, Months</b>	6.7	8.1	7.3	7.3	8.1	6.7
95% CI	3.67-9.72	2.96-13.27	3.06-11.66	2.42-12.30	3.32-12.91	3.57-9.83
<i>P</i>	.7		.8		.6	

Abbreviations: CT = chemotherapy; EGFR = epidermal growth factor receptor; OS = overall survival; PFS = progression-free survival; TKI = tyrosine kinase inhibitor.

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