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Resistance mechanisms to osimertinib in EGFR-mutated advanced non-small-cell lung cancer: A multicentric retrospective French study

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ABSTRACT

Objectives: The understanding of histo-molecular mechanisms associated with resistance to osimertinib is a critical step to define the optimal treatment strategy in advanced EGFR-mutated Non-Small-Cell-Lung-Cancer (NSCLC).

Materials and methods: We performed a multicentric retrospective analysis on a cohort of consecutive patients treated with osimertinib for an advanced EGFR-mutated NSCLC and collected histo-molecular data from plasma and tumor samples at the time of progression. Next-generation sequencing (NGS) was performed for all samples. Best Overall Response Rate (ORR), Progression Free Survival (PFS), Overall Survival (OS) and data on treatment post-progression efficacy were also collected.

Results: Two-hundred and twenty-six patients were included from 9 Academic French Hospitals between April 2015–October 2018. Osimertinib was given in second-line or more in 219 patients (97%). Best ORR was 52% and best central nervous system ORR was 56%. Median PFS and OS were 9.5 months (IQR 4.0-17.2) and 24 months (IQR 12.4-NR) respectively. At the time of analysis, 150 patients (66%) had tumor progression. Among them, 73 contributive samples (56 tumor biopsies) were available. The most frequent molecular alterations were C797S mutation (n = 9 (13%)) and MET amplification (n = 8 (11%)). Histologic transformation occurred in 5 patients (9% of tumor biopsies). In T790M + NSCLC, loss of T790 M occurred in 68% of cases. Median PFS and OS with treatment beyond progression were 6.0 months (IQR 2.0-10.4) and 15.1 months (IQR 6.7-NR) respectively and longer in case of osimertinib continuation beyond progression.

Conclusion: We confirmed the efficacy of osimertinib in patients with advanced *EGFR* mutation positive NSCLC. At progression, the most frequent molecular alterations were *MET* amplification and C797S mutation.

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1. Introduction

Around 15% of advanced non-small cell lung cancers (NSCLC) harbor a mutation of *Epidermal Growth Factor Receptor* (*EGFR*) gene in the Caucasian population [1]. Osimertinib is an irreversible third generation EGFR tyrosine kinase inhibitor (TKI). Beyond its activity on exon 19 and exon 21 *EGFR* mutations, osimertinib is also effective in case of T790 M *EGFR* mutation that is responsible of 50% of acquired resistance with first- and second-generation EGFR TKIs [2]. In NSCLC with T790 M mutation (T790M +), the AURA3 phase 3 trial has shown the superiority of osimertinib compared to chemotherapy after failure of first-generation EGFR TKI, with a median of Progression-Free-Survival (PFS) with osimertinib of 10.1 months vs. 4.4 months with chemotherapy (hazard ratio (HR) 0.30; 95% confidence interval [CI], 0.23 to 0.41; P < 0.001) [3]. More recently, a phase 3 trial (FLAURA trial) compared osimertinib with erlotinib or gefitinib in untreated patients with advanced *EGFR* mutated NSCLC, with improvement of PFS with osimertinib (median 18.9 months vs. 10.2 months with other TKI; HR = 0.46; 95% CI, 0.37 to 0.57; P < 0.001) [4]. Osimertinib is now approved in US and Europe for patients with advanced *EGFR*-mutated NSCLC in first-line treatment, or in any line in case of a T790 M mutation.

However, whereas resistance mechanisms are now well described with first- and second-generation EGFR TKIs, there is still few data available on histo-molecular resistance mechanisms with osimertinib. Small series, using plasma and/or tumor samples, showed that C797S *EGFR* resistance mutation and copy number alteration, mostly *Mesenchymal Epithelial Transition Factor (MET)* amplification, were the most frequent mechanisms found at the time of progression with osimertinib [5–8]. Lately, subset analyses from AURA and FLAURA trials, with a larger number of samples analyzed, confirmed these data [9–16]. However, these two studies only used plasma samples. There is therefore a need for new studies combining tissue and liquid biopsies to better characterize histological and molecular mechanisms associated with resistance to osimertinib.

We performed a multicentric retrospective analysis on a large cohort of patients treated with osimertinib for advanced *EGFR*-mutated NSCLC and collected histo-molecular data from plasma and tumor samples at the time of progression.

2. Materials and methods

2.1. Patients

All consecutive patients with advanced *EGFR*-mutated NSCLC from 9 Academic French Hospitals treated with osimertinib between April 2015 and October 2018 were included (hospital pharmacy dispensing).

Demographic and histo-molecular data were retrospectively collected. Tumor response was assessed locally in multi-disciplinary board, including radiologist specialized in thoracic oncology. Best Overall Response Rate (ORR), Time to Treatment Discontinuation (TTD), PFS and Overall Survival (OS) were collected, with a cut-off point on 02/06/2019. Toxicities during osimertinib treatment were recorded using CTCAE 4.0 classification.

2.2. Histo-molecular data at progression

Data from available circulating tumor DNA (ctDNA) (from plasma or other fluids as pleural effusion of cerebrospinal fluid) analyses and tumor biopsies collected at progression were recorded.

Molecular analyses were performed as part of clinical care, at the time of progression by Next Generation Sequencing (NGS). The description of the different NGS panels is presented in Suppl. Table 1. *MET* amplification was confirmed by Fluorescence In Situ Hybridization (FISH) on tumor biopsy, if available. Loss of T790 M was defined as the absence of T790 M mutation with presence of other molecular abnormality at the time of progression, in tumor harboring a T790 M mutation at the beginning of osimertinib treatment.

2.3. Ethical considerations

This retrospective study was approved by the Institutional Review Board (CEPRO) of the Société de Pneumologie de Langue Française (SPLF) on the 03/01/2019.

2.4. Statistical analysis

OS was defined as the time from treatment introduction to death. Patients were censored if they were alive at the time of last follow-up. OS-1 was defined as the time from osimertinib introduction to death. OS-2 was the time from new systemic treatment introduction (in association or not with osimertinib) after osimertinib to death. For patients who continued osimertinib beyond progression (minimum 3 months of treatment beyond progression, including dose increase of osimertinib, and/or association with local treatment like radiotherapy or therapeutic lumbar punctions or surgery), OS-2 was defined as the time from progression with osimertinib to death.

PFS was defined as the time from treatment introduction to progression or death. Patients alive who had not experienced progression as of the analysis cutoff date were censored at the last disease assessment. PFS-1 was defined as the time from osimertinib introduction to progression or death. PFS-2 was defined as the time from new systemic treatment introduction after osimertinib to progression or death. For patients who continued osimertinib beyond progression, PFS-2 was defined as the time from the first progression with osimertinib, to the second.

ORR was defined as the proportion of patients who achieve a complete or partial response. CNS-ORR was defined as the proportion of patients who achieve a complete or partial response on central nervous system (CNS) (evaluated by the referring physician and reviewed by multidisciplinary board) by MRI or CT scan (depends on local habits). For patients with meningeal involvement, response was defined as a diminution or a disappearance of tumoral cells in cerebrospinal fluid (CSF), if available, or as a significant clinical and/or radiological amelioration with treatment.

TTD was defined as the time until the end of therapy for any reason. Patients were censored at the date that they were last known to have received therapy.

TTD, PFS and OS were analyzed on osimertinib treatment, and also for post-osimertinib treatment. TTD, PFS and OS were calculated using Kaplan-Meier method. Log-rank test was used for survival comparisons according to specific endpoints (loss of T790 M, osimertinib continuation after progression).

Statistical analyses were performed using Xlstat software version 21.1.3.58422 (Addinsoft, FRANCE) software.

3. Results

3.1. Clinical and histo-molecular characteristics at osimertinib introduction

Two-hundred and twenty-six consecutive patients were included. Median of follow-up was 11.6 months (Interquartile range IQR 4.6-18.2 months). Characteristics of the patients are presented in Table 1. Median age was 66 years old (range 31–92), with a predominance of women (n = 157; 69%) and non-smokers (n = 144; 63%). One hundred and twenty-one patients (53%) had tumor involvement on central nervous system (CNS) at osimertinib introduction.

Main histologic subtype was adenocarcinoma (n = 218, 95%), mainly with exon 19 deletion (n = 134, 59%) or L858R mutation (n = 76, 33%). At introduction of osimertinib, one patient (0.4%) had *de novo* HER2 amplification; 5 patients (2%) presented *de novo* MET amplification. Molecular characteristics of the tumors at osimertinib

Table 1
Demographic and disease characteristics of patients at osimertinib introduction.

Characteristics	Global population (n = 226)	Patients treated in ≥2nd line with T790 M (n = 184)	Patients treated in ≥2nd line without T790 M at (n = 35)	Patients treated in first line (n = 7)
Age (median, range)	66 (31–92)	68 (33–92)	65 (31–87)	59 (46–80)
Female sex	157 (69)	126 (68)	28 (80)	3 (43)
Ethnic group				
Asian	32 (14)	29 (16)	3 (9)	0 (0)
Caucasian	151 (67)	120 (65)	29 (83)	2 (29)
African	43 (19)	35 (19)	3 (9)	5 (71)
Other				
Smoking status				
Never	145 (64)	118 (64)	23 (66)	4 (57)
Current	17 (8)	13 (7)	4 (11)	0 (0)
Former	62 (27)	51 (28)	8 (23)	3 (43)
unknown	2 (< 1)	2 (1)	0 (0)	0 (0)
PS				
≤2	144 (63)	118 (64)	21 (60)	3 (43)
> 2	8 (4)	4 (2)	4 (11)	0 (0)
unknown	74 (33)	60 (33)	10 (29)	4 (57)
Histology				
Non squamous	223 (99)	183 (99)	33 (95)	7 (100)
Squamous	3 (1)	1 (1)	2 (6)	0 (0)
CNS involvement	121 (54)	92 (50)	26 (74)	3 (43)
Previous systemic therapies				
TKI	214 (95)	184 (100)	30 (86)	–
Chemotherapy	95 (42)	72 (39)	23 (66)	–
Immunotherapy	10 (4)	7 (4)	3 (9)	–
Number of linesbefore OSI (median, IQR)	1 (1–2)	2 (2–3)	3 (2–4)	–
OSI given in 2nd line of treatment after 1 st/2nd G EGFR KI	117 (52)	105 (57)	12 (34)	–

Data are presented as n (%) unlike otherwise specified; IQR: interquartile range; PS: performance status; CNS: central nervous system; OSI: osimertinib; TKI: tyrosine kinase inhibitors; 1 st/2nd G: 1 st/2nd generation.

introduction are presented in Table 2.

Osimertinib was given in second-line or more in 219 patients (97%). One hundred and seventeen patients (52%) received osimertinib in second line after a first- or second-generation EGFR TKI, because of a T790 M mutation at progression (T790M + NSCLC). Median duration of first/second generation EGFR TKI treatment was 14.3months (IQR 1.7-21.7 months). Sixty-seven patients (30%) received osimertinib in further treatment line, because of a T790 M acquisition (T790M + NSCLC). Thirty-five patients (15%) received osimertinib in second line or more, without T790 M mutation at introduction of osimertinib. Most of these 35 patients without T790 M were treated in third line or more (n = 18) and among these 35 patients, 18 presented a CNS involvement and 8 a meningeal involvement at introduction of osimertinib; 6 patients had an *EGFR* exon 20 insertion at diagnostic.

Osimertinib was given in first-line treatment in 7 patients (3%). Of these 7 patients, two presented a *de novo* T790 M mutation.

3.2. Osimertinib efficacy

In the global population (n = 226), best ORR was 52% (n = 118; complete responses, n = 4 (2%); partial response: n = 114 (50%)). Fifty-three patients (23%) had tumor stabilization and 44 patients (19%) had tumor progression as best response. Best CNS ORR was 56% (n = 68/121). Median PFS-1 was 9.5 months (IQR 4.0-17.2 months) and median OS-1 was 24 months (IQR 12.4 months-not reached NR). Median TTD was 12.7 months (IQR 5.1–24 months) (Fig. 1).

In patients treated with osimertinib in 1st line (n = 7), best ORR was 71% (n = 5, all with partial response). Two patients (29%) had tumor stabilization. Median PFS-1 was 9.6 months (IQR 8.7-10.5 months) and median OS-1 was not reached. Median TTD was 24.0 months (IQR 15.4-32.5 months). Best CNS ORR was 100% (n = 3/3). In T790M + NSCLC patients treated with osimertinib in 2nd line or more (n = 184), best ORR was 54% (n = 99/184) (n = 99; complete responses: n = 4 (2%); partial response: n = 95 (52%)). Forty-three patients (23%) had tumor stabilization and 34 patients (18%) had tumor progression as best response. Best CNS ORR was 58% (n = 52/89). Median PFS-1 was 11.5 months (IQR 4.6-17.2 months) and median OS-1 was 27.0 months (IQR 14.1-37.1 months). Median TTD was 13.5 months (IQR 5.7-24.3 months) (Fig. 1). In T790M- NSCLC patients treated with osimertinib in 2nd line or more (n = 35), best ORR was 40% (n = 14/35) (all with partial response). Eight patients (23%) had tumor stabilization and 10 patients (29%) had tumor progression as best response. Best CNS ORR was 57% (n = 13/23). Median PFS was 6.0 months (IQR 1.7-11.5 months) and median OS 14.2 months (IQR 5.9 months-NR). Median TTD was 5.7 months (IQR 1.8-20.2 months) (Supplemental Fig. 1).

3.3. Histo-molecular profiles at progression

At the time of analysis, 150 patients (66%) had tumor progression. Consort diagram of patients with contributive samples at progression is presented in Supplemental Fig. 2.

Among these 150 patients, 62 patients (41%) had a new tumor biopsy at progression (45 patients with both new biopsy and plasma sample). Six biopsies over 62 at progression (10%) were not contributive (no tumor tissue). All patients (n = 6) with non-contributive biopsy also had a plasma sample: in 4 patients the plasma sample was contributive (either the initial EGFR mutation and/or a new resistance mutation found). Eighteen patients (12% of patients with progression) had both contributive biopsy and contributive plasma sample. All contributive biopsies (n = 56) were analyzed by NGS for the detection of molecular alterations.

Twenty-two patients (15%) had only a ctDNA analysis (plasma only: n = 19; plasma and other: n = 3; other only: n = 1) without tissue biopsy at progression. Other ctDNA analyses were from cerebrospinal fluid (n = 3) or pleural fluid (n = 1). Among these 22 patients, 17 had contributive analyses (plasma only: n = 14; plasma + other: n = 1; other only: n = 2).

Among all patients with any contributive sample (tissue and/or

Table 2
Molecular alterations at osimertinib introduction.

Type of alteration	Global population (n = 226)	Patients treated in ≥2nd line with T790 M (n = 184)	Patients treated in ≥2nd without T790 M (total = 35)	Patients treated in first line (n = 7)
***EGFR* Exon 19 deletion**	134 (59)	113(61)	16 (46)	5 (71)
***EGFR* L858R mutation**	76 (34)	63 (34)	11 (31)	2 (29)
Other*EGFR*mutation	20 (9)	12 (7)	8 (23)	0 (0)
Exon 20 insertion	7 (3)	1 (1)	6 (17)	–
pL861Q exon 21	3 (< 1)	2 (1)	1 (3)	–
other	11 (5)	9 (5)	2 (6)	–
***EGFR* T790 M mutation**	186 (82)	184 (100)	0 (0)	2 (29)
***BRAF*mutation**	2 (< 1)	0 (0)	2 (6)	0
***HER2*Amplification**	1 (< 1)	1 (1)	0 (0)	0
***MET*Amplification**	5 (2)	4 (2)	0 (0)	1 (14)
PIK3CA	5 (2)	5 (3)	0 (0)	0
RAS				
KRAS	5 (2)	3 (2)	1(3)	1 (14)
NRAS	1 (< 1)	0 (0)	1(3)	0
TP53	9 (4)	8 (4)	1(3)	0

Data are presented as n (%) unlike otherwise specified.

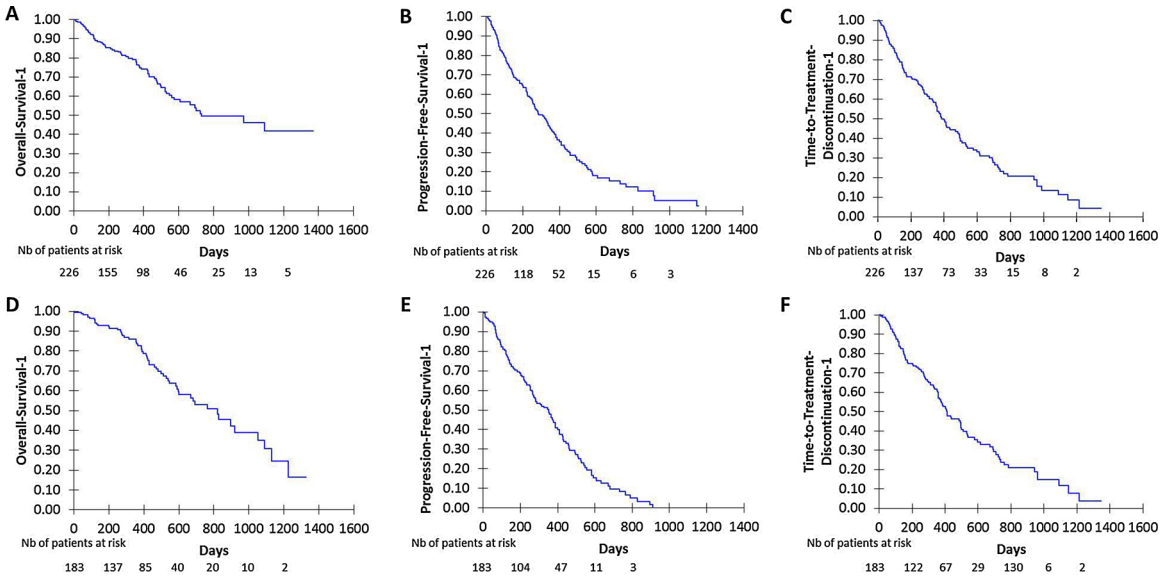

Fig. 1. Osimertinib efficacy. A: Overall-Survival-1 in the overall population; B: Progression-Free-Survival-1 in the overall population; C: Time-to-Treatment-Discontinuation-1 in the overall population; D: Overall-Survival-1 in the T790M + population; E: Progression-Free-Survival-1 in the T790M + population; F: Time-to-Treatment-Discontinuation-1 in the T790M + population.

ctDNA) at progression (n = 73), there was no evidence of any mechanism of resistance in 33 patients (46%).

Histo-molecular alterations at progression, in global population and according to the line of treatment, are summarized in Table 3. The description of resistance mechanisms according to the type of samples (tissue/ctDNA) for global population and for T790M + NSCLC patients is presented in Fig. 2, and for patients treated in first-line (n = 3) in Supplemental Table 2. The description of resistance mechanisms in patients with matched tissue and liquid biopsies (n = 18) is presented in Supplemental Table 3.

Acquisition of C797S mutation occurred in 9 patients (13%): 6 found on tumor biopsy; 2 on ctDNA; 1 case of 2 different C797S mutations (c.2389 T > A clone in ctDNA on ddPCR and c.2390 G > C clone in lymph node on NGS). In T790M + NSCLC, C797S occurred in all cases in *cis* position with T790 M mutation.

Eight patients (11%) had an acquisition of a *MET* amplification (found on tissue by NGS, and confirmed by FISH), 2 patients (3%) a *HER2* amplification and one patient (1%) a *BRAF* (V 600E) mutation, all found on tumor biopsy. Co-histo-molecular alterations were found in 11 patients (15%).

Histologic transformation was found in 5 patients (9% of patients who had a tumor biopsy at progression) (small-cell lung cancer: n = 4; squamous cell carcinoma: n = 1).

In T790M + NSCLC, loss of T790 M at progression occurred in 68%. There was no difference between patients treated by osimertinib with or without loss of T790 M mutation in term of PFS (median 7.0 vs 9.0 months, respectively, p = 0.570) or OS (median 23.7 months vs. NR, respectively, p = 0.48) (Supplemental Fig. 3).

3.4. Efficacy data on post-osimertinib treatment

Of the 150 patients with progression, 110 (77%) patients received a subsequent treatment. Median PFS-2 was 6.0 months (IQR 2.0-10.4 months) and median OS-2 was 15.1 months (IQR 6.7months-NR) (Fig. 2). Among these 110 patients, 62 patients (42%) received a new system treatment just beyond progression (mainly cytotoxic chemotherapy) and 48 patients (35%) continued osimertinib beyond progression (mainly with local ablative treatment such as surgery or

Table 3
Molecular alterations at progression after osimertinib in patients with contributive sample at progression.

Type of histo-molecular alteration	Global population	Patients treated in ≥2nd line with T790 M	Patients treated in ≥2nd line without T790 M	Patients treated in first line
Number of patients with contributive samples	**73**	**61**	**9**	**3**
EGFR C797S mutation	10[a] (13)	8[a] (13)	0 (0)	2 (67)
MET amplification	8 (11)	8 (13)	0 (0)	0 (0)
HER2 amplification	2 (3)	1 (2)	1 (11)	0 (0)
BRAF V600E mutation	1 (1)	1 (2)	0 (0)	0 (0)
Histologic transformation				
small cell lung cancer	4 (6)	4 (7)	0 (0)	0 (0)
squamous cell carcinoma	1 (1)	1 (2)	0 (0)	0 (0)
TP53 mutation	3 (4)	3 (5)	0 (0)	0 (0)
PTEN mutation	2 (3)	2 (3)	0 (0)	0 (0)
PIK3CA mutation	2 (3)	2 (3)	0 (0)	0 (0)
RAS mutation	2 (3)	2 (3)	0 (0)	0 (0)
pL718Q exon 18 *EGFR* mutation	2 (3)	2 (3)	0 (0)	0 (0)
CTNNB1 mutation	1 (1)	1 (2)	0 (0)	0 (0)
SMAD4 mutation	1 (1)	1 (2)	0 (0)	0 (0)
No evidence of molecular alteration	33 (46)	26 (43)	7 (78)	1 (33)

Data are presented as n (%) unlike otherwise specified.
[a] 1 patient had 2 different C797S mutations (c.2389 T > A clone in ctDNA on ddPCR and c.2390 G > C clone in lymph node on NGS).

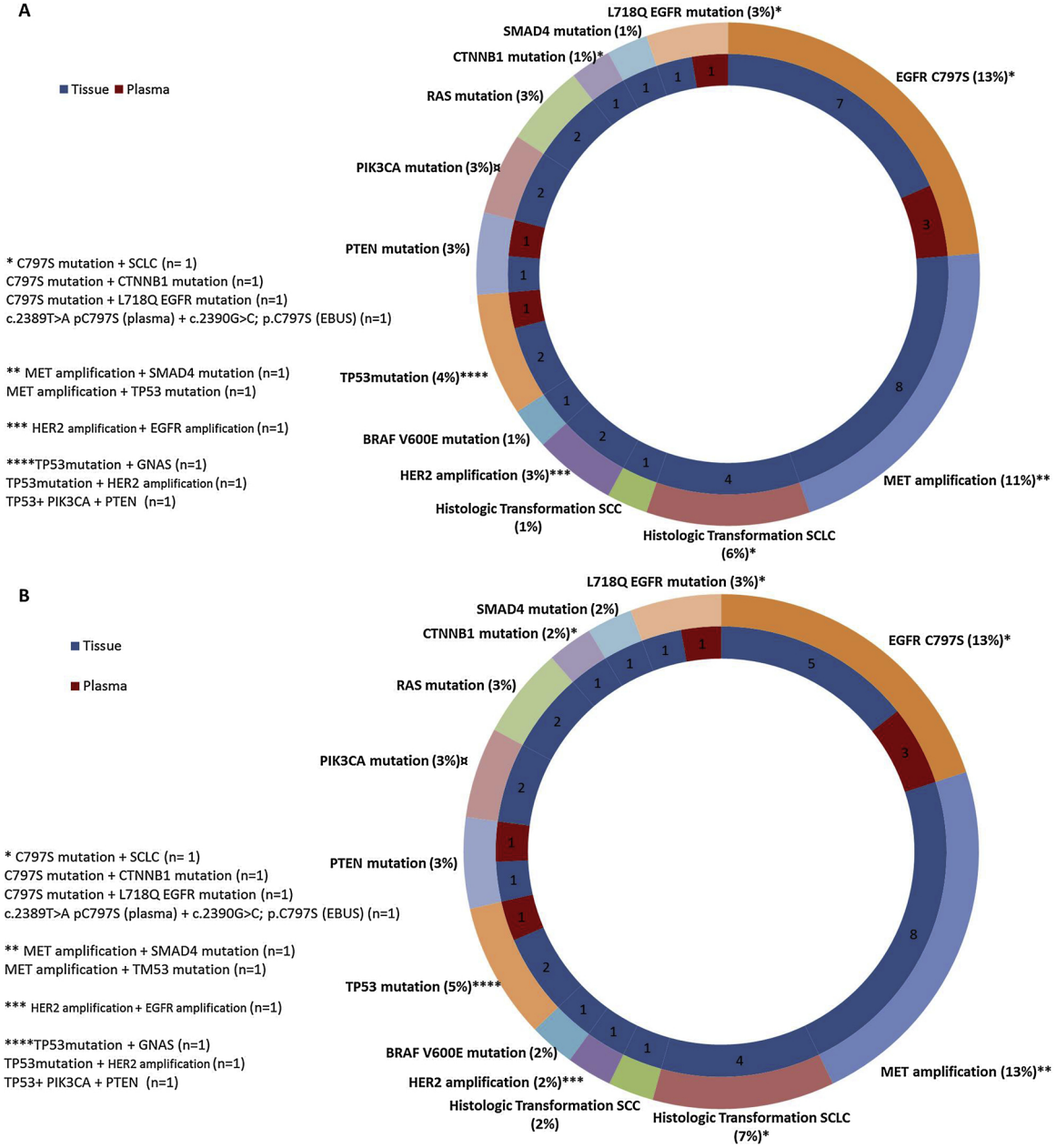

Fig. 2. Histo-molecular profiles at progression with osimertinib on contributive samples. A. global population (n = 73); B: patients treated in ≥2nd line with T790 M mutation at osimertinib introduction (n = 61). Numbers presented for tissue and plasma are numbers of positive samples for each histo-molecular profile. EBUS: endobronchial ultrasound.

radiotherapy, Supplemental Table 4). For patients who continued osimertinib beyond progression, PFS-2 was 10.4 months (IQR 8.7-20.6 months) and OS-2 22.6 months (IQR 9.9months-NR), versus 3.2 months (IQR 1.3-5.4 months) and 102 months (IQR 3,8-18,1) for patients who received a new systemic treatment ($p < 0.0001$ and $p = 0.002$ respectively) (Fig. 3). Patients who continued osimertinib beyond progression had a longer PFS-1 than patients who began a new systemic treatment beyond progression: 8.8 months (IQR 14.1-5.1) versus 4.7 months (2.3–10.1) ($p = 0.05$).

In T790M + NSCLC treated by osimertinib in second line or more, 95 patients (81%) received a treatment beyond progression (osimertinib continuation, n = 45; new systemic treatment, n = 50). In this population, PFS-2 and OS-2 were 6.0 months (IQR 2.1-10.4 months) and 22.6 months (IQR 7.5 months-NR) respectively (Supplemental Fig. 4). There was no difference between patients with or without loss of T790 M mutation treated in second line or more with osimertinib in term of PFS-2 (6.6 vs 8.6 months, respectively, $p = 0.97$) or OS-2 (13.2 months vs 17.1months, respectively ($p = 0.44$) (Supplemental Fig. 5).

In the global population, median OS from the diagnostic of metastatic disease to death was 72.2 months (IQR 33.1-125.6 months).

3.5. Tolerance of treatment

Most reported adverse events were of low severity (grade I or II). The most frequent adverse events were cutaneous rash (17%) or diarrhea (17%) (Supplemental Table 5). Three grade 5 adverse events were reported. One patient died by sepsis associated with aplasia, 3 days after the beginning of the treatment; one by sepsis 3 months after the introduction, and the last patient by acute respiratory distress complicating an interstitial pneumonia diagnosed one month after beginning of the treatment.

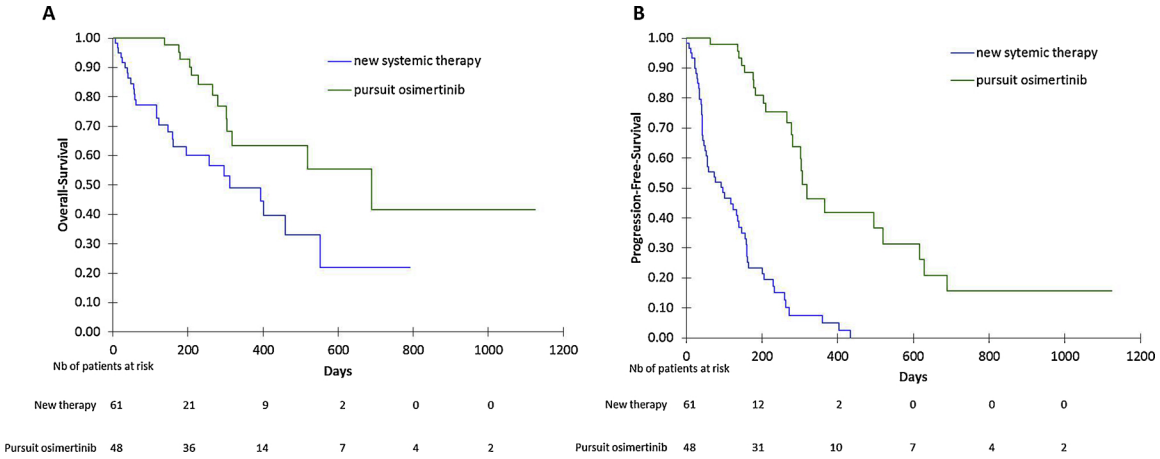

Fig. 3. Survivals according to post-progression treatment strategies. A: Overall-Survival-2 B: Progression-Free-Survival-2.

4. Discussion

In this work, we confirmed the efficacy of osimertinib in patients with advanced *EGFR* mutation positive NSCLC. In this large cohort of patients with progression with osimertinib, the majority of the patients had a new histo-molecular analysis at the time of progression, mainly with tissue rebiopsy (+ /- ctDNA). Contributive samples were obtained in almost half of the cases and the most frequent molecular alterations were *MET* amplification and *EGFR* C797S mutation.

The detection of specific mechanisms resistance is one of the most important challenges with the development of new targeted therapies in non-squamous cell lung cancer. Among these new therapies, osimertinib has shown its superiority over chemotherapy in second line in T790M + NSCLC [2,3], and over other EGFR TKIs in first line, regardless of the T790 M status [4]. However, all patients will have a tumor progression with osimertinib. The resistance profile is therefore a key point to select the appropriate drug after osimertinib failure.

First, our data confirmed the efficacy of osimertinib in patients with advanced *EGFR* mutation positive NSCLC. PFS and ORR were similar with data from large clinical trials; in our study, median PFS for the global population was 9.6 months, and 11.6 months for the specific population treated in second line with identified T790 M mutation. ORR was 52%, and 53%, respectively. In the AURA3 phase 3 trial, median PFS was 10.1 months and ORR was 71% in the group of patients with T790M + NSCLC treated in second line with osimertinib. Moreover, efficacy of osimertinib in CNS metastases has been confirmed, with specific ORR of 56% [3]. Few studies have reported osimertinib efficacy in real life setting. In the EXPLORE T790 M GFPC study on 205 patients [17], median PFS was similar to our study, at 12.4 months for patients with T790M + NSCLC treated by osimertinib in second line or more.

Second, our data showed that a large number of centers performed NGS in new tissue biopsies after progression: of all patients who progressed, 49% (73/150) had contributive sample after progression and in 70% of cases, at least a tissue biopsy was performed (n = 56). Comparatively, Oxnard et al. presented data from 95 patients with progression with osimertinib, with NGS analyses performed on tissue biopsy in 41 patients (43%) [9]. Our study is to date the largest study that included both tumor biopsies and plasma samples at progression with NGS analyses. We found that *MET* amplification and C797S mutation were the most frequent resistance mechanisms after progression with osimertinib (13% and 11% of cases respectively). This result is consistent with other studies. Oxnard *et al.* found the presence of a *MET* amplification or C797S mutation at progression in 10% and 22% of the cases, respectively. Another analysis on a cohort of 42 samples at progression with osimertinib showed respective proportions of 14% and 26% [12], whereas in the AURA1 trial [15], *MET* amplification was found in 23% and C797S mutation in 10% [9,15]. Moreover, the presence of multiple histo-molecular resistance mechanisms at progression was observed in up to 15% in our work, and unlike EGFR 1st or 2nd generation EGFR TKIs, most of these molecular alterations are EGFR independent. This highlights the need for a large molecular screening at the time of progression to have a better panorama of the underlying resistance mechanisms. Given the high incidence of gene amplifications in this setting, screening of resistance mechanisms at the time of progression with osimertinib may be based preferentially on tumor biopsy, when feasible.

In our study, loss of T790 M mutation at progression occurred in 68%, that is consistent with previous studies [9,11,12]. It was associated with slightly, although not significantly, shorter median survivals and TTD. Molecular analyses from the AURA phase III trial showed that loss of T790 M was associated with EGFR-independent resistance mechanisms and seemed to occur shortly after the beginning of osimertinib. However, Oxnard *et al.* did not found statistical difference in term of PFS or TTD according to loss/persistence of T790 M at progression [9,14].

Patients who received osimertinib had prolonged survival after progression. We found PFS-2 and OS-2 (evaluated from the beginning of post-progression treatment) at 6.0 months and 15.1 months, respectively. Especially, in the T790M + NSCLC group, with patients sometimes heavily pre-treated, PFS-2 and OS-2 were 6.0 months and up to 22.6 months, respectively. In comparison, preliminary data from FLAURA trial showed that PFS-2 after osimertinib failure in first line treatment was at least of 24 months [18]. Mature survival analyses from FLAURA and AURA-3 trials are awaited to better define the optimal treatment strategies with osimertinib in *EGFR*-mutated NSCLC.

Finally, after progression, osimertinib was continued in one third of cases, and survivals and TTD were longer in the group of patients who continued osimertinib beyond progression. The interpretation of such results is subject to caution, as this is not a randomized trial, with selection biases for the patients who continued osimertinib after progression. It is likely that these two patients' populations are very different. Interestingly, we found a longer PFS-1 in patients who continued osimertinib than patients who began a new systemic treatment beyond progression. This suggests a more indolent evolution of the tumor in this specific population. A recent study showed that 73% of patients with T790M + NSCLC had oligo-progression with osimertinib [13]. Such patients, amenable to local treatment, could therefore take benefit from osimertinib continuation after progression. Similar results were found with 1st line EGFR TKI [19]. In T790M + NSCLC, Le et al. observed also a significant longer median overall survival in the group of patients who continued osimertinib beyond progression (n = 47) (11.2 vs. 6.1 months, P = 0.02) [12].

The principal limitation of our study is the retrospective design of

this work. Moreover, some patients received osimertinib in second line or more without T790 M mutation, most of them because of meningeal involvement, and/or after multiple lines of therapies. However, the description of histo-molecular resistance mechanisms in these patients is still interesting, as it represents 15% of the patients treated with osimertinib in a real-life setting, as highlighted in our cohort. Another limitation of our study is the absence of CTs centralized rereading. Moreover, gene fusions were not screened for all patients, so the presence of such molecular feature could have been underestimated. We also only had 7 patients treated with osimertinib in first-line treatment (due to the period of inclusion), and from them 3 samples at progression (1 plasma and 2 tumor biopsies), limiting the interpretation of these results in this setting. However, there is currently only published data on plasma at progression with osimertinib given in first-line [13]. We present here for the first-time preliminary results on tumor rebiopsy at progression with first line osimertinib. Besides, our study has several strengths: this multicentric work is the largest study to date using both plasma and tissue at progression with osimertinib, dedicated to the description of resistance mechanisms to osimertinib, with large use of NGS analyses, and confirming the efficacy of osimertinib in a real-life setting.

In conclusion, this study confirmed the efficacy of osimertinib in *EGFR*-mutated NSCLC. Based on tissue and plasma analyses at progression, C797S and *MET* amplification were the most frequent resistance mechanisms but the diversity of molecular alterations must be emphasized. However, in almost half of the cases, no mechanism was found. Specific clinical trials dedicated to treatment strategies after osimertinib failure, are needed to determine the best therapeutics according to the different resistance profiles.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.09.019.

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