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ORIGINAL RESEARCH

Activity of osimeRTInib in non-small-cell lung Cancer with UNcommon epidermal growth factor receptor mutations: retrospective Observational multicenter study (ARTICUNO)

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Background: Osimertinib represents the standard of care for the treatment of advanced non-small-cell lung cancer (NSCLC) harboring classical epidermal growth factor receptor (*EGFR*) mutations, constituting 80%-90% of all *EGFR* alterations. In the remaining cases, an assorted group of uncommon alterations of *EGFR* (uEGFR) can be detected, which confer variable sensitivity to previous generations of EGFR inhibitors, overall with lower therapeutic activity. Data on osimertinib in this setting are limited and strongly warranted.

Patients and methods: The ARTICUNO study retrospectively evaluated data on osimertinib activity from patients with advanced NSCLC harboring uEGFR treated in 21 clinical centers between August 2017 and March 2023. Data analysis was carried out with a descriptive aim. Investigators collected response data according to RECIST version 1.1 criteria. The median duration of response, progression-free survival (mPFS), and overall survival were estimated by the Kaplan—Meier method.

Results: Eighty-six patients harboring uEGFR and treated with osimertinib were identified. Patients with 'major' uEGFR, that is, G719X, L861X, and S768I mutations ($n = 51$), had an overall response rate (ORR) and mPFS of 50% and 9 months, respectively. Variable outcomes were registered in cases with rarer 'minor' mutations ($n = 27$), with ORR and mPFS of 31% and 4 months, respectively. Among seven patients with exon 20 insertions, ORR was 14%, while the best outcome was registered among patients with compound mutations including at least one classical *EGFR* mutation ($n = 13$). Thirty patients presented brain metastases (BMs) and intracranial ORR and mPFS were 58% and 9 months, respectively. Amplification of *EGFR* or *MET*, *TP53* mutations, and *EGFR* E709K emerged after osimertinib failure in a dataset of 18 patients with available rebiopsy.

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Conclusion: The ARTICUNO study confirms the activity of osimertinib in patients with uEGFR, especially in those with compound uncommon—common mutations, or major uEGFR, even in the presence of BMs. Alterations at the E709 residue of EGFR are associated with resistance to osimertinib.

Key words: atypical EGFR, compound mutations, NSCLC, osimertinib, uncommon EGFR mutations

INTRODUCTION

Identification of epidermal growth factor receptor (*EGFR*) gene-activating mutations has been the first step toward the age of targeted therapy in lung cancer, the most commonly diagnosed cancer and the leading cause of cancer-related mortality worldwide.[1] *EGFR* mutations can be detected in ∼10%-20% of non-small-cell lung cancer (NSCLC) cases in Europe and North America, and in up to one-half of NSCLC diagnosed in patients with no history of tobacco smoking.[2] In 80%-90% of *EGFR*-mutated NSCLC cases, tumor analyses exhibit exon 19 deletions (del19) and exon 21 L858R (common *EGFR* mutations) and the use of orally available tyrosine kinase EGFR inhibitors (TKIs) can allow achieving high rates of durable tumor responses.[3] Osimertinib, a third-generation EGFR TKI, represents the standard of care for the treatment of patients with metastatic NSCLC harboring common *EGFR* mutations.[4] Indeed, osimertinib demonstrated improved overall survival (OS) compared with first-generation TKIs,[5] with the capability to overcome the acquired T790M mutation in exon 20 of *EGFR* (commonly seen after failure of first- and second-generation TKIs),[6] better toxicity profile, and greater intracranial activity, the latter being a critical feature considering the high frequency of brain metastases (BMs) in patients with NSCLCs.

In the remaining 10%-20% of *EGFR*-mutated NSCLC cases, uncommon alterations of *EGFR* (uEGFR) comprise G719X in exon 18, L861Q in exon 21, and S768I in exon 20, which are the most frequent (major uEGFR), and a variety of rarer alterations (minor uEGFR) such as gene fusions, duplications of the EGFR-kinase domain (EGFR-KDD), and mutations in exons 2-15.[7-9] Such mutations may occur as single alterations or together with other common or uncommon *EGFR* mutations (compound or complex mutations).[10]

For patients harboring uEGFR, data regarding the clinical benefit of TKIs are limited, as they have been usually excluded from clinical trials because of their high molecular heterogeneity, low prevalence, and uncertain biological function and drug sensitivity. However, the likelihood of detecting them is increasing with the spread of next-generation sequencing (NGS). Overall, data from limited series in NSCLC with uncommon mutations indicate that treatment with first-generation TKIs has achieved a lower overall response rate (ORR) and progression-free survival (PFS) compared with NSCLC with common mutations.[11] Higher activity has been described with afatinib, a second-generation TKI, although with variable outcomes, especially in NSCLC with minor uEGFR.[12] Afatinib is the only Food and Drug Administration (FDA)-approved TKI for the treatment of metastatic NSCLC harboring major uEGFR based on the pooled analysis of the LUX-Lung 2, 3, and 6 trials, with median PFS (mPFS) between 8.2 and 14.7 months.[13] When ARTICUNO was designed, the only available clinical data with osimertinib in this setting came from a single Korean phase II trial, with 37 patients enrolled. The ORR was 50% and the disease control rate (DCR) was 89%. The mPFS ranged between 8.2 and 15.2 months in major uEGFR.[14] An additional peculiar class of uncommon *EGFR* alterations is the insertions of exon 20 (ins20). In this regard, no generation of TKIs demonstrated, overall, any substantial activity, except rare helical specific types of exon 20 insertions, considering that >100 different forms of ins20 are reported.[15]

The scarcity of available data motivated the present ARTICUNO study to document the activity of osimertinib in this very heterogeneous and poorly studied NSCLC subpopulation.

PATIENTS AND METHODS

Patients

ARTICUNO was an academic-initiated, retrospective, multicenter study that included patients with advanced NSCLC treated with osimertinib in 21 Italian cancer centers between August 2017 and March 2023.

The main inclusion criteria were as follows: age ≥18 years, histologically or cytologically confirmed advanced NSCLC, presence of any uEGFR (single or complex mutations, fusions, EGFR-KDD), and at least 4 weeks of treatment with osimertinib. Cases with *de novo* T790M were also included. Patients with common *EGFR* mutations, that is, del19 (comprising molecular variants of del19, with or without insertions between codons E746 and A750) and L858R, occurring alone or with acquired T790M, were excluded.

Data collection

Information relating to patient demographics, case history, and survival was collected and extracted by local investigators in each treating center through retrospective chart review. The study size was estimated with preliminary projections obtained from agreeing centers.

Complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) were locally estimated using RECIST version 1.1. ORR was defined as the sum of CR and PR; and DCR as the sum of CR, PR, and SD. PFS and OS were defined as the time between the start of osimertinib and disease progression or death, whichever occurred first, and as the treatment start and death, respectively.

Study data were collected and managed using REDCap (Vanderbilt University, Nashville, TN) electronic data capture

tools, according to strict privacy standards. This study was conducted in accordance with the Declaration of Helsinki and approval was obtained by each local ethics committee.

Statistical analyses

Data analysis was carried out with a descriptive aim. The median values (with range) and frequencies (percentages) were provided for descriptions of continuous and categorical variables, respectively. PFS, OS, and duration of response were estimated using the Kaplan—Meier method and described using median values with 95% confidence intervals (95% CIs). Follow-up was calculated using the reverse Kaplan—Meier method.

All statistical analyses were carried out with R studio version 4.2.2 (R Foundation, Vienna, Austria). *P*-values <0.05 were considered statistically significant, and all tests were two-sided.

RESULTS

Patients' characteristics

Eighty-six patients were registered in the ARTICUNO study. The main demographic and characteristics of patients are summarized in Table 1.

Of the included patients, 7 had ins20 and identified as group C (who are expected to be less responsive to osimertinib) and 79 had other single or compound uEGFR. Among them, 13 had compound mutations, including a classic del19 (deletion between E746 and A750) or L858R, and were designated separately as group B (who are expected to be more responsive to osimertinib). The remaining patients ($n = 66$) with neither common mutations nor ins20 were described as group A.

Overall, the most prevalent mutations were G719X ($n = 28$ patients, 33%), L861X ($n = 20$, 23%), and S768I ($n = 13$, 15%). A variety of minor uncommon alterations were found in 27 patients (31%), either alone or in combination. Compound mutations were identified in 38 patients (44%), mostly including G719X (19/38), T790M (13/38, in 7 cases being acquired after previous treatment with TKIs), and S768I (12/38). Molecular analysis was carried out in 49 patients (57%) by NGS and at least one concomitant alteration in a different gene was described in 12 patients (25%; Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103592). Programmed death-ligand 1 (PD-L1) immunohistochemistry results were available for 73 patients: 47% had a tumor proportion score $<1\%$, 37% had a tumor proportion score of 1%-49%, and 16% had high PD-L1 expression ($\geq 50\%$).

Sixty-six patients (77%) had distant metastases at the time of diagnosis. Among the remaining patients, the median time from first diagnosis of NSCLC to diagnosis of advanced disease was 6 months (range 1-46 months).

Overall, 70 patients (81%) received osimertinib as the first line of treatment, 76 (88%) were TKI-naive and 10 (12%) received osimertinib as the second EGFR-TKI (Table 1 and Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103592).

Outcomes

The best response and treatment duration for each patient are represented in Figure 1. Response evaluation, as assessed by local investigators, was available for 83 patients (97%). Among them, CR was reported in 3 patients (4%; 95% CI 0.8-10.2), PR in 31 patients (37%; 95% CI 27.0-48.7), SD in 29 patients (35%; 95% CI 24.8-46.2), and PD in 20 patients (24%; 95% CI 15.4-34.7). The median duration of response was 13 months (95% CI 7 months-not reached). With a median follow-up of 28 months (95% CI 24-30 months), 62 patients (72%) experienced disease progression. The mPFS was 8 months in the whole study cohort (95% CI 6-13 months). Osimertinib treatment was ongoing for 30 patients (35% overall, 29% in group A, 77% in group B, and 14% in group C). Among them, the treatment was continued beyond progression in 11 patients (group A: 8; group B: 3; and group C: 0). A total of 40 patients (47%) were alive at data cutoff [group A: 27 (41%); group B: 12 (92%); and group C: 1 (14%)]. The median OS was 20 months in the overall population (95% CI 15-35 months). Response and survival data for each group are reported in Table 2. The Kaplan—Meier curves of PFS and OS of the whole cohort and by groups are presented in Figure 2A and 2B, respectively.

Subgroup descriptive analyses

Looking at specific *EGFR* alterations, the ORR and mPFS were 50% and 9 months in patients with major uEGFR, respectively. The outcome for each major uEGFR (G719X, L861X, or S768I) is reported in Table 2. Patients with minor mutations responded to osimertinib with variable outcomes (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103592; Figure 1). Overall, the ORR and mPFS for minor uEGFR were 31% and 4 months, respectively. Of note, one patient with EGFR-KDD achieved a CR, maintained at the time of writing this paper, after 28 months of osimertinib therapy. Another patient with EGFR-RAD51 fusion had a PR with a PFS of 20 months.

Focusing on group A, 47 patients had at least one major uEGFR with ORR, DCR, and mPFS of 48% (95% CI 33% to 63%), 87% (95% CI 74% to 95%), and 8 months (95% CI 6-13 months), respectively. Among 25 patients with compound mutations, ORR, DCR, and mPFS were 42% (95% CI 22% to 63%), 88% (95% CI 86% to 97%), and 10 months (95% CI 7-13 months), respectively.

Outcome with brain metastasis

Overall, 30 patients (35%) presented with BMs at baseline and 9 patients received radiation therapy on BMs before the start of osimertinib therapy. In group A, among 21 patients presenting measurable cerebral disease, intracranial ORR was 67%, intracranial DCR 100%, and intracranial mPFS was 9 months (95% CI 5-13 months), according to RECIST version 1.1. Considering RT-naive patients in group A

Table 1. Patients' characteristics

Characteristics	All patients (*N* = 86)	Group A: uncommon only (*n* = 66)	Group B: common with uncommon (*n* = 13)	Group C: exon 20 insertions (*n* = 7)
Age (years), median (range)	68.5 (30-87)	65.5 (30-87)	68 (42-80)	62 (40-87)
Sex, *n* (%)				
Female	54 (63)	41 (62)	7 (54)	6 (86)
Male	32 (27)	25 (38)	6 (46)	1 (14)
ECOG PS at initiating of osimertinib, *n* (%)				
PS 0	30 (35)	22 (33)	6 (46)	2 (29)
PS 1	43 (50)	29 (44)	6 (46)	3 (42)
PS ≥2	13 (15)	15 (23)	1 (8)	2 (29)
Cigarette smoking history, *n* (%)				
Never	32 (37)	26 (39)	3 (23)	2 (29)
Former (stop > 5 years from diagnosis)	25 (29)	23 (35)	5 (38)	1 (14)
Current (or stop ≤ 5 years from diagnosis)	27 (31)	16 (24)	4 (31)	4 (57)
Unknown	2 (3)	1 (2)	1 (8)	0 (0)
Ethnicity, *n* (%)				
White	81 (94)	62 (94)	13 (100)	6 (86)
Other races	5 (6)	4 (6)	0 (0)	1 (14)
Histology, *n* (%)				
Adenocarcinoma	80 (93)	60 (91)	13 (100)	7 (100)
Other	6 (7)	6 (9)	0 (0)	0 (0)
Line of therapy, *n* (%)				
First line	70 (81)	53 (80)	11 (85)	6 (86)
Second line	12 (14)	9 (14)	2 (15)	1 (14)
Third line or further	4 (5)	4 (6)	0 (0)	0 (0)
Previous treatments for advanced disease, *n* (%)				
EGFR-TKI	10 (12)	8[a] (12)	1[a] (8)	1[a] (14)
Chemotherapy	8 (9)	7 (11)	1 (8)	0 (0)
Immunotherapy	3 (3)	2[b] (3)	0 (0)	0 (0)
Immunotherapy + chemotherapy	1 (1)	1[b] (2)	0 (0)	0 (0)
TNM stage at initiating of osimertinib, *n* (%)				
III	3 (3)	2 (3)	1 (8)	0 (0)
IV	83 (97)	64 (97)	12 (92)	7 (100)
Sites of metastasis at initiating of osimertinib, *n* (%)				
Brain	30 (35)	24 (36)	2 (15)	4 (57)
Bone	38 (44)	29 (44)	7 (54)	2 (29)
Lung	51 (59)	39 (59)	6 (46)	6 (86)
Nonregional lymph nodes	31 (36)	22 (33)	5 (38)	4 (57)
Liver	16 (19)	10 (15)	4 (31)	2 (29)
Adrenal glands	7 (8)	5 (8)	1 (8)	1 (14)
Pleura	19 (22)	15 (23)	3 (23)	1 (14)
Initial EGFR mutation detected on, *n* (%)				
Tissue biopsy	78 (91)	61 (93)	10 (77)	7 (100)
Liquid biopsy	2 (2)	1 (2)	1 (8)	0 (0)
Both	6 (7)	3 (5)	2 (15)	0 (0)
Method for EGFR analysis, *n* (%)				
Sanger/RT-PCR	37 (43)	28 (42)	4 (31)	2 (29)
NGS	49 (57)	38 (58)	9 (69)	5 (71)
Reasons for stopping osimertinib, *n* (%)				
PD or death	54 (63)	45 (68)	3 (23)	6 (86)
Toxicity	1 (1)	1 (2)	0 (0)	0 (0)
Other	1 (1)	1 (2)	0 (0)	0 (0)
Treatment after osimertinib, *n*				
Immunotherapy	4	4[c]	0	0
Chemotherapy	26	23	1	2
Immunotherapy + chemotherapy	1	1[d]	0	0

ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; PD, progressive disease; TKI, tyrosine kinase inhibitor; TNM, tumor—node—metastasis.
[a]Previous TKI group A: one afatinib; group B: four afatinib, three erlotinib, and one gefitinib and afatinib; and group C: one afatinib.
[b]One atezolizumab and two pembrolizumab.
[c]Two atezolizumab, two pembrolizumab, and one nivolumab.
[d]Chemotherapy + pembrolizumab.

(*n* = 17), 14 had measurable BMs and the intracranial ORR was 57% (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2024.103592).

Mechanisms of resistance

At the time of progression on osimertinib, a rebiopsy was carried out in 18/62 patients: 14 tissue biopsies and 8 liquid biopsies (both in 4 patients). NGS was carried out by the investigators in 14/18 cases with variable gene panels. Putative mechanisms of resistance were identified in five patients: *EGFR* amplification (*n* = 2), *MET* amplification (*n* = 1), *TP53* mutation (*n* = 1), and *EGFR* E709K (*n* = 1; Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2024.103592).

Outcome with immune checkpoint inhibitors

Eight patients received immune checkpoint inhibitors (ICIs), three before and five after the osimertinib course. An objective response with ICI-containing therapy was reported in two patients (25%; 95% CI 3-65), while the best response was PD in the remaining six patients. Of the six rapid progressors to ICIs, four achieved a benefit from osimertinib, with PFS >6 months (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2024.103592).

DISCUSSION

To our knowledge, the ARTICUNO study represents the largest real-world dataset of patients with advanced NSCLC harboring uEGFR and treated with the third-generation EGFR TKI osimertinib. In this multicenter retrospective

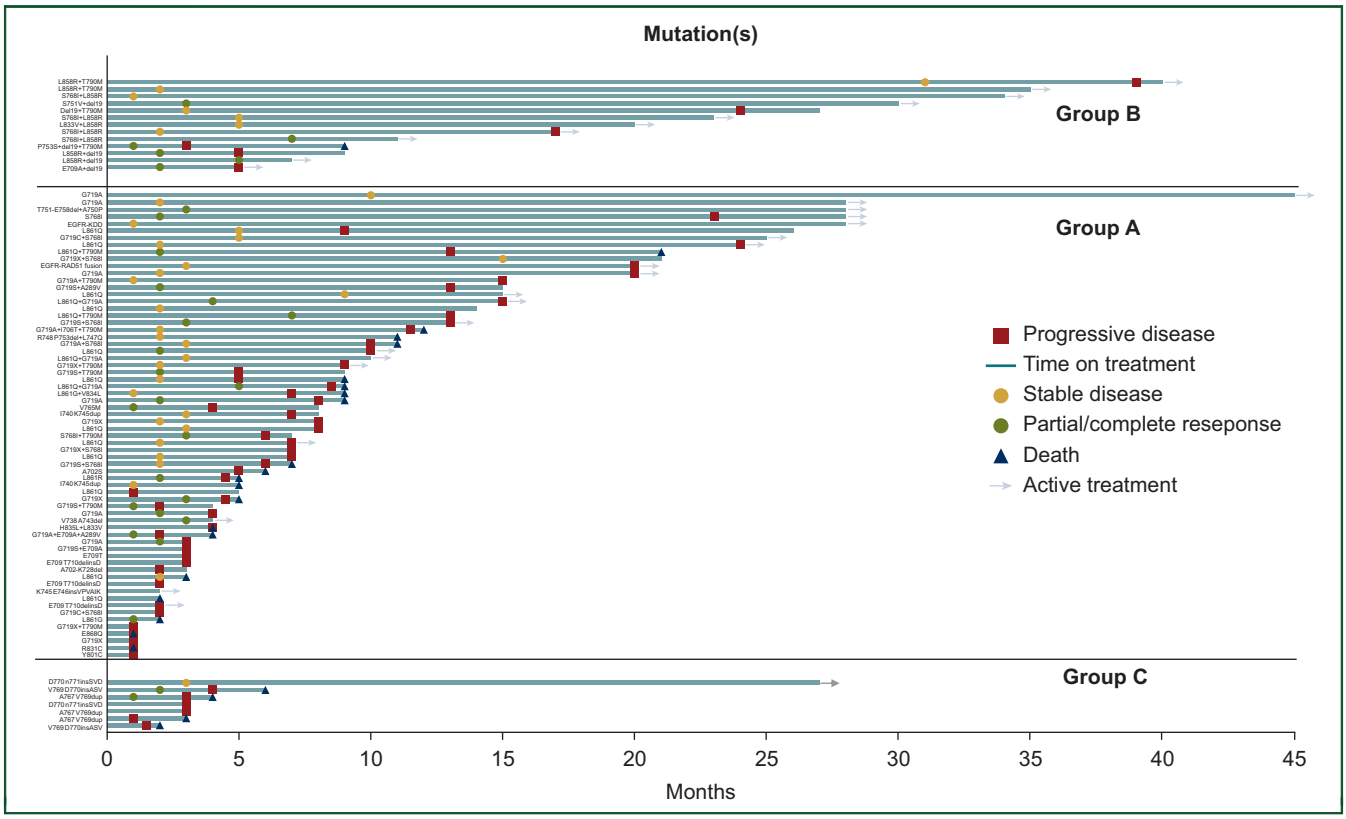

Figure 1. Swimmer's plot of patients classified in the ARTICUNO study as group A (with uncommon *EGFR* alterations, excluding those harboring compound mutations with a common mutation and those harboring ins20, $n = 66$), arranged by time on treatment.
EGFR, epidermal growth factor receptor.

analysis, ORR and mPFS were 43% and 8 months in group A and 42% and 8 months in the overall cohort ($n = 86$), respectively, with most patients being TKI-naive (88%). A major uEGFR (G719X, L861Q, or S768I) was found in 59% of cases, and in 44% there were compound mutations. In our study, 57% of the samples underwent NGS, while in the remaining cases, analyses were carried out by Sanger sequencing or also by real-time PCR that could potentially miss some alterations (i.e. some exon 20 insertions, gene fusions, exceedingly rare mutations). However, the distribution of uEGFR is in line with other large cohorts,[12,16] taking into account the underrepresentation of ins20 (8% in this cohort) because osimertinib is not considered an optimal treatment for these patients.

Table 2. The activity of osimertinib in the study cohort and in uEGFR subgroups

Group of patients	N. / N. evaluable	ORR (95% CI)	DCR (95% CI)	mPFS (95% CI), months	mDOR (95% CI), months	mOS (95% CI), months	mFU (95% CI), months
All patients	86/83	42% (31% to 54%)	77% (67% to 86%)	8 (6-13)	13 (7-NR)	20 (15-35)	28 (24-30)
Group A	66/63	43% (30% to 56%)	76% (64% to 86%)	8 (6-12)	9 (6-21)	17 (12-24)	27 (21-30)
Group A: TKI-naive	58/55	44% (30% to 58%)	75% (61% to 85%)	7 (5-10)	7 (5-NR)	17 (11-NR)	27 (21-30)
Group B	13/13	54% (25.1% to 80.7%)	100% (75% to 100%)	40 (17-NR)	20 (14-NR)	NR (NR-NR)	30 (20-NR)
Group C	7/7	14% (0.04% to 57.8%)	43% (10% to 81.6%)	3 (2-NR)	NR (NR-NR)	6 (3-NR)	27 (27-NR)
L861X single: $n = 14$ compound: $n = 6$	20/20	55% (32% to 77%)	90% (68% to 99%)	8.5 (7-15)	5 (4-NR)	28 (28-NR)	15 (9-NR)
G719X single: $n = 9$ compound: $n = 19$	28/27	44% (25% to 65%)	85% (66% to 96%)	8.5 (5-15)	13 (7-NR)	20 (12-NR)	25 (21-NR)
S768I single: $n = 1$ compound: $n = 12$	13/12	58% (28% to 85%)	92% (62% to 100%)	17 (7-NR)	NR (7-NR)	NR (NR-NR)	23 (15-NR)

CI, confidence interval; DCR, disease control rate; EGFR, epidermal growth factor receptor; mDOR, median duration of response; mFU, median follow-up; mOS, median overall survival; mPFS, median progression-free survival; NR, not reached; ORR, overall response rate; TKI, tyrosine kinase inhibitor; uEGFR, uncommon alterations of *EGFR*.

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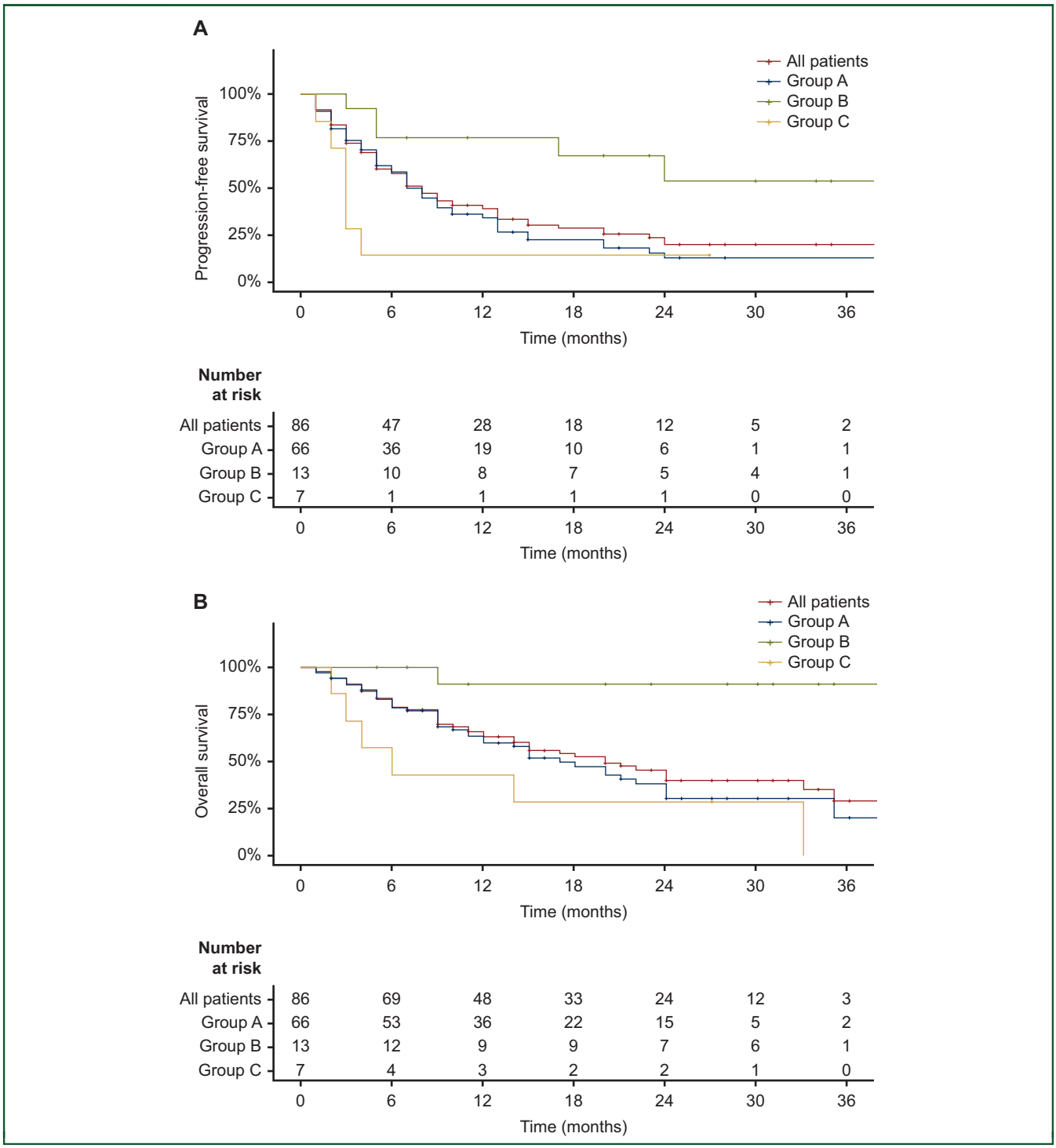

Figure 2. Kaplan–Meier curves of (A) progression-free survival (PFS) and (B) overall survival (OS). Data are represented for group A (uEGFR without common mutations), group B (uEGFR with common mutations), and group C (ins20). Vertical lines show censored events. Log-rank of group A versus B: PFS $P = 0.0072$; OS $P = 0.0017$; group B versus C: PFS $P = 0.0039$, OS $P = 0.00036$; group A versus C: PFS $P = 0.16$, OS $P = 0.2$.
EGFR, epidermal growth factor receptor; OS, overall survival; PFS, progression-free survival; uEGFR, uncommon alterations of *EGFR*.

As expected,[10,17] the best outcomes were observed in patients with compound mutations including either the classical L858R or del19. Still, the exceptional PFS of 40 months observed in group B could be biased by the small sample size ($n = 13$). Conversely, we found the lowest ORR in the ins20 cohort (group C), but a sustained PR in one out of two patients with D770_N771insSVD, one of the most prevalent ins20 and associated with sensitivity to osimertinib in the preclinical setting.[18] However, no response was registered in a Chinese cohort of patients with such specific ins20 ($n = 17$).[19] These results could suggest that more clinical data are needed in non-Asian patients to clarify if there is a role for osimertinib against D770_N771insSVD.

Table 3. Summary of available studies evaluating osimertinib in patients with advanced NSCLC harboring uEGFR

Study	Study type	Patients assessable (ins20 excluded), *n/n*	TKI-naive, *n*	Major/minor/classical[a], *n*	ORR (95% CI)	DOR (95% CI), months	DCR (95% CI)	mPFS (95% CI), months	ORR in major uEGFR (95% CI)	ORR in minor uEGFR (95% CI)
ARTICUNO	Retrospective	79/76	70	51/28/13	45% (33% to 57%)	13 (7-NR)	80% (70% to 89%)	9 (7-13)	50% (36% to 64%)	31% (14% to 52%)
Cho et al. *JCO* (2020) (KCSG-LU15-09)[14]	Phase II	35/35	35	32/3/0	51% (34% to 67%)	11.2 (7.7-14.7)	89% (73% to 97%)	8.2 (5.2-14.7)[b]	53% (35% to 71%)	33% (0% to 90%)
Bar et al. *JTO* (2022) (UNICORN)[16]	Retrospective	60/51	60	28/29/13	61% (47% to 73%)	17.4 (9.1-NR)	92% (81% to 98%)	9.5 (8.5-17.4)	57% (37% to 76%)	68% (43% to 87%)
Ji et al. *JTO CRR* (2023)[39]	Retrospective	43/36	23[c]	26/18/0	36.1% (20.8% to 53.8%)	NA	NA	NA	32.1% (15.9% to 52.4%)	54% (25% to 81%)
Villaruz et al. *ESMO Open* (2023)[40]	Phase II	17/17	17	17/2/0	47% (23% to 72%)	8.7 (1.9-13.9)	94% (71% to 100%)	10.5 (3.7-15.2)	47% (23% to 72%)	0%
Okuma et al. *JAMA Oncol* (2024) (UNICORN—Japan)[41]	Phase II	40/40	40	32/8/7	55% (38.5% to 70.7%)	22.7 (9.5-NR)	90% (76.3% to 97.2%)	9.4 months (3.7-15.2)	53.1% (36% to 69%)	75% (41% to 93%)

Major uEGFR: G719X, L861X, and S768I; minor uEGFR: any EGFR alteration other than major uEGFR, T790M, and classical EGFR mutations.
CI, confidence interval; DCR, disease control rate; DOR, duration of response; EGFR, epidermal growth factor receptor; mPFS, median progression-free survival; NA, not available; NR, not reached; NSCLC, non-small-cell lung cancer; ORR, overall response rate; TKI, tyrosine kinase inhibitor; uEGFR, uncommon alterations of *EGFR*.
[a]Number of patients with at least one major uEGFR alone or in combination/one minor uEGFR alone or in combination/one classical *EGFR* mutation in combination with uEGFR.
[b]In the whole cohort ($n = 36$, including 1 ins20).
[c]A total of 23 patients in the whole cohort ($n = 50$, including 7 with ins20) were TKI-naive.

In patients with major uEGFR, we registered an ORR of 50% and an mPFS of 9 months. Such mutations have been associated with reduced activity with first-generation TKIs, but available data are frequently inconsistent.[10,18] Afatinib, the second-generation TKI, presents the most robust data from the *post hoc* analysis of clinical trials, compassionate-use and expanded-access programs, case series, or observational studies.[12,13,20] High activity was observed with afatinib against compound mutations (ORR 73.5%) and against major uncommon mutations (ORR 59%; median duration of response 17.1 months, 95% CI 11.0-20.8 months).[12,13] Still, the width of CIs may reflect the heterogeneity of mutations clustered in these groups, with potential heterogeneous sensitivity. While the ARTICUNO study was being conducted, more clinical data became available with osimertinib in this setting and the ORR in patients with major uEGFR appears in line with our results (Table 3). The ORR was similar for each major mutation, ranging from 44% with G719X to 55% with L861X. We registered a numerically higher ORR among 12 patients with L861Q alone compared with other mutations in group A ($P = 0.12$), in line with previous clinical results.[14,16]

Looking at minor uEGFR, among 28 patients included in our study (33%), the ORR was 31% with 4 months of PFS. Such results compare negatively with those of a large international case series that described the activity of osimertinib among 60 TKI-naive patients with uEGFR (UNICORN). The authors reported an ORR of 61% and an mPFS of 9.5 months in the overall cohort.[16] The high ORR reported in UNICORN appears at least partially driven by the good performance of osimertinib registered in patients harboring minor uEGFR (48% of the study cohort, ORR 68%), reflecting the biological heterogeneity of this subgroup and the limitation of cross-study comparisons.

In detail, a number of very rare or so far unreported uEGFR cases were documented in the ARTICUNO study. Alterations at E709 in exon 18 are the most frequent among minor uEGFR.[21] Such residue is close to the phosphate-binding loop and may be susceptible to deletions and substitutions. Both have been associated with response to afatinib.[12,22] In ARTICUNO, none of the seven patients with alterations at E709 (E709T, E709_T710delinsD, E709A+G719S, E709A+del19, E709A+G719A+A289V) achieved a response with osimertinib. Thus alterations at the E709 codon may anticipate a lack of benefit from osimertinib, also in case of compound mutations. A702S was previously reported as first- and second-generation EGFR-TKIs resistant,[23] and a case in our study achieved a PD as the best response. I740_K745dup is a rare insertion of exon 19, associated with TKI response.[24] Both patients in ARTICUNO with such uEGFR achieved a PR. Both A750P and T751_E758del have been separately reported as targetable by TKIs,[25,26] and we found a persistent SD (>2 years) in a patient with a combination of these alterations. A patient with V765M, previously associated with response to both gefitinib and afatinib,[20] achieved an SD with 4 months of PFS. Notably, deep response and prolonged benefit were also observed with osimertinib in patients with both *EGFR*

fusion and EGFR-KDD, in line with previous reports.[9,27,28] Data on sensitivity to TKIs are missing for Y801C,[29] which was associated with no response in our study. E868Q, V738_A743del, A702_K728del, and L747Q have never been described in cancers and R831C has never been found in lung cancers.[30] No response to osimertinib was observed among these patients. However, a single patient with the L747Q mutation, detected alongside the R748_P753del deletion, a rare exon 19 deletion, exhibited no available TKI sensitivity data in the literature.[31] In this first clinical report, such complex *EGFR* alterations appeared targetable with osimertinib.

Robichaux et al.[32] proposed a different classification of *EGFR* mutations based on their impact on the structure and function of EGF receptors, with the potential to predict sensitivity to different TKIs. A subgroup of mutations occurring on the interior surface of the ATP-binding pocket or C-terminal end of the αC-helix, predicted to be P-loop and αC-helix compressing (PACC), appeared more sensitive to afatinib, while mutations not predicted to alter the drug-biding pocket were more sensitive to osimertinib. Accordingly, in our cohort, ORR was 37.5% (95% CI 22.7% to 54.2%) among uEGFR classifiable as PACC ($n = 40$) and 50% (95% CI 27.2% to 72.8%) among those classifiable as classical-like ($n = 20$) (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2024.103592).

A valuable advantage of osimertinib compared with previous TKIs is the high intracranial activity in patients with common *EGFR* mutations, with a central nervous system ORR of ∼90% reported in the FLAURA trial.[33] In this cohort, we registered an intracranial ORR of 58% according to RECIST version 1.1, and similar results were found in the subgroup of patients with untreated BMs. These data confirm the activity of osimertinib within the central nervous system even in the population with uEGFR.

In addition, molecular analysis after osimertinib failure was available for 18 patients, providing, to our knowledge, the widest set of data regarding the putative resistance mechanisms in this population. Analysis by NGS (with different panels) was carried out in 14/18 patients, with *EGFR* and *MET* amplification, *TP53* mutation, or *EGFR* E709K emerging at progression. *EGFR*, *MET*, and *TP53* alterations are already involved in the potential processes of resistance to osimertinib in classical *EGFR* mutations.[34,35] In our study, no patients with E709 alterations at baseline achieved any response from osimertinib; furthermore, E709K appeared at disease progression in a patient with G719A at baseline. This is consistent with data from UNICORN, where no benefit was derived in two patients with E709_T710delinsD at baseline, while E709K was found at progression in two patients with G719A.[16] These data, in accordance with preclinical results,[22] suggest that alterations at residue E709 may be associated with lower activity of osimertinib and, in particular, with resistance in cases with E709K. Conversely, afatinib exhibited robust activity against E709X mutations, suggesting it could be the optimal treatment choice for patients with either *de novo* or acquired E709 alterations.[12,22]

Finally, eight patients also received an ICI-based treatment. In general, ICIs hold a modest activity in cases of NSCLC with *EGFR* mutations, and very few data are available regarding uEGFR.[36,37] In our cohort, one out of six patients treated with ICI alone achieved a prolonged PR. Notably, this smoker patient experienced rapid PD to osimertinib and presented high PD-L1 expression. A patient carrying EGFR-RAD51 and no PD-L1 expression achieved a response to chemo-immunotherapy. In this case, a mutation of a DNA damage repair-related gene, *ATR,* was also identified, which is potentially associated with improved clinical outcomes with either platinum chemotherapy or PD-(L)1 blockade.[38]

There are some limitations of the ARTICUNO study beyond its retrospective design and its descriptive aim. First, local testing was used to detect *EGFR* mutations, without central revision. However, NGS was adopted in most cases, which is today the technique of reference. Second, even if this is the largest dataset available today, considering the heterogeneity of uEGFR, ARTICUNO does not allow firm comparisons regarding the activity of osimertinib in different molecular subgroups. Third, no central revision of radiologic findings was carried out. Anyhow, this is a multicenter study providing real-world data that could be more representative of both the epidemiology of uEGFR and osimertinib sensitivity in the European population.

In summary, findings from this large study confirm that osimertinib holds relevant activity against uEGFR (ins20 excluded), even in cases with intracranial disease. Better results could be obtained in cases with compound mutations, including either the classical del19 or L858R, and in cases with the major uncommon mutations. Alterations at E709 codon, the most common among minor uEGFR, are associated with a lack of benefit from osimertinib, and E709K may drive acquired resistance.

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