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ORIGINAL ARTICLE

Treatment outcome of atypical *EGFR* mutations in the German National Network Genomic Medicine Lung Cancer (nNGM)

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Background: Atypical *EGFR* mutations occur in 10%-30% of non-small-cell lung cancer (NSCLC) patients with *EGFR* mutations and their sensitivity to classical epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKI) is highly heterogeneous. Patients harboring one group of uncommon, recurrent *EGFR* mutations (G719X, S768I, L861Q) respond to EGFR-TKI. Exon 20 insertions are mostly insensitive to EGFR-TKI but display sensitivity to exon 20 inhibitors. Clinical outcome data of patients with very rare point and compound mutations upon systemic treatments are still sparse to date.

Patients and methods: In this retrospective, multicenter study of the national Network Genomic Medicine (nNGM) in Germany, 856 NSCLC cases with atypical *EGFR* mutations including co-occurring mutations were reported from 12 centers. Clinical follow-up data after treatment with different EGFR-TKIs, chemotherapy and immune checkpoint inhibitors were available from 260 patients. Response to treatment was analyzed in three major groups: (i) uncommon mutations (G719X, S7681, L861Q and combinations), (ii) exon 20 insertions and (iii) very rare *EGFR* mutations (very rare single point mutations, compound mutations, exon 18 deletions, exon 19 insertions).

Results: Our study comprises the largest thus far reported real-world cohort of very rare *EGFR* single point and compound mutations treated with different systemic treatments. We validated higher efficacy of EGFR-TKI in comparison to chemotherapy in group 1 (uncommon), while most exon 20 insertions (group 2) were not EGFR-TKI responsive. In addition, we found TKI sensitivity of very rare point mutations (group 3) and of complex *EGFR* mutations containing exon 19 deletions or L858R mutations independent of the combination partner. Notably,

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treatment responses in group 3 (very rare) were highly heterogeneous. Co-occurring *TP53* mutations exerted a non-significant trend for a detrimental effect on outcome in EGFR-TKI-treated patients in groups 2 and 3 but not in group 1.
Conclusions: Based on our findings, we propose a novel nNGM classification of atypical *EGFR* mutations.
Key words: non-small-cell lung cancer, *EGFR*, atypical *EGFR* mutations

INTRODUCTION

L858R mutations and exon 19 deletions represent the most common, classical *EGFR* mutations in patients with non-small-cell lung cancer (NSCLC) treated with epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitors (TKIs) in first line (1L).[1] Retrospective studies indicate that combinations of classical *EGFR* mutations with other co-mutations such as *TP53* can be detrimental for outcome.[2]

Atypical *EGFR* mutations comprise 10%-30% of *EGFR*-mutated NSCLC,[3-6] either alone or in combination with other *EGFR* mutations (atypical or classical). Only few prospective clinical trial data are available to assess the activity of EGFR-TKI in patients with atypical *EGFR* mutations. In a *post hoc* analysis of different prospective trials, data from 100 patients with rare *EGFR* mutations were analyzed.[3] Seventy-five patients were treated with afatinib and 25 with chemotherapy and rare *EGFR* mutations were categorized into 3 groups. Group 1 contained point mutations or duplications in exons 18-21, either alone or in combination, group 2 patients with *de novo* T790M mutation and group 3 patients with exon 20 insertions. This study showed clinical benefit of afatinib in patients assigned to group 1. G719X, L861Q and S768I either alone or in combination were the most common mutations in this group. Upon treatment with afatinib, progression-free survival (PFS) was 10.7 months [95% confidence interval (CI) 5.6-14.7 months] and overall survival (OS) was 19.4 months (16.4 months-not estimable). Patients with atypical *EGFR* mutations from group 2 (*de novo* T790M) and group 3 (exon 20 insertions) did not benefit from afatinib compared to chemotherapy.[3]

Despite the relatively low frequency of atypical *EGFR* mutations, real-world outcome data from patients with such *EGFR* mutations are crucial for clinical decision making. Today most data are available for G179X, L861Q, S768I and combinations of such and for treatment with afatinib or osimertinib. This group is often referred to as 'major uncommon' or 'uncommon, group 1' and is considered sensitive to EGFR-TKI. In current recommendations, afatinib and/or osimertinib are suggested for treatment of patients with these mutations, also because less data are available from other EGFR-TKIs.[3,5-8]

Exon 20 insertions are considered less sensitive toward classical EGFR-TKI. Recently, the Food and Drug Administration (FDA) approved two drugs specifically for treatment of NSCLC with exon 20 insertions[9,10]: (i) mobocertinib, an oral, irreversible TKI designed to selectively target in-frame exon 20 insertions and (ii) amivantamab, a bi-specific MET-EGFR monoclonal antibody.[11,12] Although this is a tremendous step forward for patients harboring exon 20 insertions, these still represent a very heterogeneous group of mutations.

In an attempt to better understand and categorize the very heterogeneous group of atypical *EGFR* mutations, Robichaux et al. recently suggested a structure- and *in vitro* drug sensitivity-based approach for improvement of prediction of drug sensitivity for atypical *EGFR* mutations.[4] They screened >16 000 *EGFR* mutations from five different databases and reported 7199 cases with atypical *EGFR* mutations. Furthermore, *in silico* prediction models were used to identify four potential subgroups based on structural information: (i) classical-like mutations, which were distant from the ATP-binding pocket and were predicted to have little effect on the drug-binding pocket, containing mutations such as L861Q, T725M and 763_764YinsFQEV besides classical *EGFR* mutations; (ii) mutations in the interior surface of the ATP-binding pocket or C-terminal end of the C-helix, which were predicted to be P-loop and C-helix compressing (PACC), thereby interfering with third-generation TKI binding. *In vitro* assays comparing sensitivity of different TKIs in cell lines expressing 76 different atypical *EGFR* mutations suggested that second-generation TKIs were indeed more effective in PACC mutations. Uncommon mutations including G719X, S786I and exon 18 deletions were considered PACC mutations; (iii) T790M-like mutations, with at least one mutation in the hydrophobic core, mostly consisting of complex mutations in combination with T790M mutations and (iv) exon 20 insertions. Here, they suggested that C-helix insertions were classical-like (i.e. 763_764YinsFQEV) and that insertions following the C-helix could be divided into near loop (NL) and far loop (FL) insertions based on *in vitro* sensitivities. In these *in vitro* assays, second-generation TKIs (especially to poziotinib) and exon 20-specific TKIs appeared more sensitive in NL insertions than in FL insertions. Interestingly, data from the phase II ZENITH20 study also suggested that poziotinib was more efficient in patients with NL insertions,[13] while this difference was not observed for mobocertinib or amivantamab.[11,12]

The authors also confirmed that patients with PACC mutations had a better response to afatinib than patients with non-PACC mutations and that patients with PACC mutations benefited more from second-generation EGFR-TKIs (mostly afatinib) than third-generation TKI osimertinib. However, most mutations in the second-generation EGFR-TKI-treated group were already known to respond well to afatinib.[3]

Taken together, there is still lack of functional data and a clinical knowledge gap for many atypical *EGFR* mutations. Here, we present a retrospective, multicenter study of the national Network Genomic Medicine (nNGM) in Germany analyzing the frequency of atypical *EGFR* mutations, co-mutations and response to EGFR-TKI, chemotherapy and immune checkpoint inhibitors (ICIs).

PATIENTS AND METHODS

Study design and participants

Twelve German comprehensive cancer centers participating in the nNGM contributed data to this retrospective study (Berlin, Dresden, Düsseldorf, Erlangen, Essen, Frankfurt, Hamburg, Köln/Bonn, Mainz, München, Tübingen/Stuttgart, Würzburg).

Inclusion criteria were non-resectable NSCLC with atypical *EGFR* mutations in exons 18-21 including compound mutations with atypical or classical *EGFR* mutations (nNGM cohort). Information about co-occurring mutations in other genes and follow-up (FUP) information including PFS and OS upon palliative systemic treatment with EGFR-TKI, chemotherapy and immune therapy were reported if available (nNGM clinical FUP cohort). We excluded exon 19 deletions and L858R mutations, and combinations of such with T790M, and *de novo* T790M mutations. Response to treatment was assessed locally without central review.

Sample preparation and sequencing were carried out at the centers according to local guidelines. Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.02.225, gives an overview of different techniques and next-generation sequencing (NGS) panels that were used in each cohort. Overall, in the nNGM cohort, customized NGS panels were used in 85.7% of the cases (nNGM cohort) and in the clinical FUP cohort in 78.5% of the cases. Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.02.225, lists the targeted genes of the customized NGS panels. In 0.8% ($n = 7$ cases) of the nNGM cohort and 1.6% ($n = 4$) of the clinical FUP cohort, *EGFR*-targeted monogene assays were used not including *EGFR* exon 20 alterations.

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). It received approval by the ethics committee II of Medical Faculty Mannheim at the University of Heidelberg (no. 2021-800). Informed consent was obtained when required by law.

Classification of mutations

Classification of atypical *EGFR* mutations into subgroups is shown in Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225, and explained in the Results section.

Statistical analysis

PFS was calculated from the day of initiation of systemic palliative treatment until disease progression or death for 1L treatment of each patient and additionally for each drug applied during the treatment history of the patients regardless of line of therapy (all lines). OS was calculated from the day of initiation of 1L palliative treatment to death. Survival was estimated by Kaplan—Meier (KM) plots. Hazard ratios (HRs) for EGFR-TKI versus chemotherapy were calculated using Cox regression models. SPSS version 27 (IBM, Armonk, NY) and R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) with packages survival 3.2-11, survminer 0.4.9, swimplot 1.2.0, ggplot2 3.3.5 and cowplot 1.1.1 were used.

We analyzed PFS and OS as main outcome parameters because objective response rates were available only for a minority of patients.

RESULTS

Frequency and distribution of different uncommon EGFR mutations

A total of 856 patients with 276 different atypical *EGFR* aberrations were reported (nNGM cohort; Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 1A). Atypical *EGFR* mutations were categorized into three major subgroups based on previous analyses[5,6]: (i) more frequent uncommon *EGFR* mutations including G719X, S768I, E709X and L861Q and combinations of those with classical *EGFR* mutations L858R and exon 19 deletions ('uncommon' based on group 1 from Yang et al.[3]); (ii) exon 20 insertions (group 3 based on Yang et al.[3]) and (iii) 'very rare' mutations including very rare point mutations, exon 18 deletions, exon 19 insertion and complex mutations in which very rare point mutations are at least one combination partner (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 1A). Throughout this manuscript, we use the term 'atypical' for all non-classical *EGFR* mutations and the terms 'uncommon' and 'very rare' for groups 1 and 3, respectively.

Exon 20 insertions represented the largest subgroup of atypical *EGFR* mutations (group 2, 227 cases, 26.5% of the nNGM cohort, $n = 57$ different exon 20 insertions), most of which were located in the loop following the C-helix ($n = 224$, 98.7% of exon 20 insertions, Figure 1A and Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225).[14] These loop insertions were mainly NL insertions (79%, AA767-772, Supplementary Figure S1A, available at https://doi.org/10.1016/j.annonc.2022.02.225).

A subgroup of comparable size comprised rare point mutations (223 cases, 26.1% of nNGM cohort, $n = 113$ different mutations). This subgroup was remarkably heterogeneous with approximately half of the mutations occurring only once ($n = 57$, 50.4% of very rare point mutations). However, some mutations such as P848X ($n = 16$, 7.3%), L747P ($n = 12$, 5.4%), K713X ($n = 11$, 4.9%), A840X ($n = 7$, 3.1%), R776X ($n = 6$, 2.6%) and N816X ($n = 6$, 2.6%) were recurrent (Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225).

Altogether, 244 aberrations were complex mutations (28.5% of nNGM cohort). While 51% of uncommon *EGFR* mutations (group 1) occurred as complex mutations, only 31% ($n = 117$) of complex mutations were reported in the group of very rare *EGFR* mutations (group 3). The largest subgroup of complex mutations in group 3 was combinations of very rare point mutations with classical *EGFR* mutations (L8585R or exon 19 deletions, 12.6%, $n = 48$,

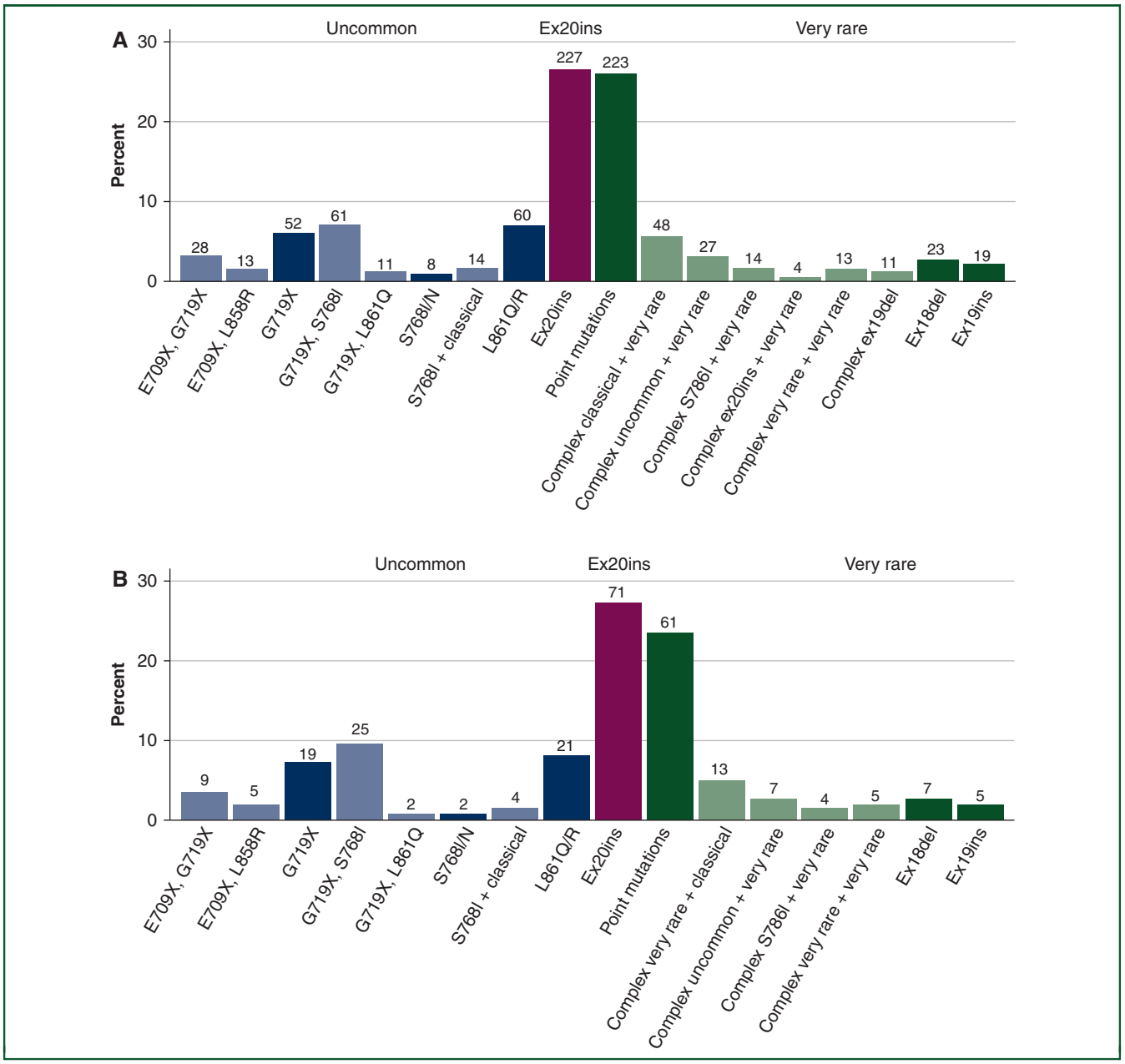


Figure 1. Frequency and distribution of rare *EGFR* mutations by subgroup.
(A) The nNGM cohort ($n = 856$ patients) and (B) the nNGM clinical FUP cohort ($n = 260$ patients). Lighter shades indicate complex mutations. Uncommon mutations with known EGFR-TKI sensitivity, based on Yang et al.[3]
EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; Ex18del, exon 18 deletions; Ex19ins, exon 19 insertions; Ex20ins, exon 20 insertions; FUP, follow-up; nNGM, national Network Genomic Medicine.

Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 1A).

The overall distribution of the different types of *EGFR* mutations in the nNGM cohort was similar to that in previous reports.[5,6]

Characteristics of the nNGM clinical FUP cohort

Clinical information about systemic treatment and outcome was available for 260 patients (nNGM clinical FUP cohort). This cohort comprised 135 different atypical *EGFR* aberrations. The distribution in groups 1-3 was similar to that in the nNGM cohort and comparable with previous reports (Figure 1A and B, Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2022.02.225).

The median age at diagnosis was 64.4 years. Overall, 90% ($n = 235$) of patients had adenocarcinomas, and the lowest frequency of this subtype was observed in patients with very rare *EGFR* mutations (group 3). Interestingly, this group was associated with a higher frequency of squamous cell carcinoma histology and co-occurring *KRAS* mutations (Table 1).

Two hundred and thirty-four patients were treated with either EGFR-TKI ($n = 95$), chemotherapy ($n = 123$) or mono-ICI treatment ($n = 16$) as 1L. Twenty-six of 260 patients were not assessable for 1L therapy or received a

Table 1. Patient's characteristics—nNGM clinical FUP cohort

Characteristics	Clinical FUP cohort	Group 1 Uncommon	Group 2 Exon 20 insertions	Group 3 Very rare	*P* value
Total, *n* (%)	260 (100)	87 (100)	71 (100)	102 (100)	
Age in years, mean (range)	64.4 (29-92)	67.5 (44-92)	64.7 (29-87)	63.4 (32-86)	0.059
Sex, *n* (%)					
Male	109 (42)	39 (45)	28 (39)	42 (41)	0.777
Female	151 (58)	48 (55)	43 (61)	60 (59)	
Smoking status, *n* (%)					
Current	55 (24)	20 (25)	9 (15)	26 (29)	0.096
Former	92 (40)	31 (39)	23 (37)	38 (43)	
Never	83 (36)	28 (35)	30 (48)	25 (28)	
Missing	30	8	9	13	
Histology, *n* (%)					
Adeno	235 (90)	83 (95)	67 (94)	85 (83)	0.008
Squamous	16 (6)	1 (1)	1(1)	14 (14)	
Others	9 (4)	3 (3)	3 (4)	3 (3)[a]	
TP53, *n* (%)					
Wild type	127 (63)	46 (68)	33 (52)	48 (69)	0.099
Mutated	74 (37)	22 (32)	33 (48)	22 (31)	
Missing	59	19	8	32	
KRAS, *n* (%)					
Wild type	192 (93)	65 (94)	63 (98)	64 (87)	0.022
Mutated	15 (7)	4 (6)	1 (2)	10 (14)	
Missing	53	18	7	28	
Treatment, *n* (%)					
1L	234	82	61	91	
EGFR-TKI	95 (41)	42 (51)	13 (21)	40 (44)	0.006
Chemo	123 (53)	37 (45)	42 (69)	44 (48)	
ICI	16 (7)	3 (4)	6 (10)	7 (8)	
2L	136	43	33	60	
EGFR-TKI	65 (48)	29 (67)	8 (24)	28 (47)	0.005
Chemo	43 (32)	10 (23)	14 (42)	19 (32)	
ICI	28 (21)	4 (9)	11 (33)	13 (22)	
3L	69	17	19	33	
EGFR-TKI	18 (26)	8 (47)	3 (16)	7 (21)	0.211
Chemo	37 (54)	6 (35)	11 (58)	20 (61)	
ICI	14 (20)	3 (18)	5 (26)	6 (18)	
4L	26	8	7	11	
EGFR-TKI	7 (27)	1 (13)	2 (29)	4 (37)	0.813
Chemo	13 (50)	5 (63)	3 (43)	5 (46)	
ICI	6 (23)	3 (25)	2 (29)	2 (18)	

1L, first line; 2L, second line; 3L, third line; 4L, fourth line; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; FUP, follow-up; ICI, immune checkpoint inhibitor; LCNEC, large cell neuroendocrine carcinoma; nNGM, national Network Genomic Medicine.
[a]Including two LCNEC; percent may not add to 100 due to rounding.

therapy not fitting into these categories. One hundred and thirty-nine of 260 patients were assessable for second line (2L) and 71 patients for third line (3L) therapy. Only 27 patients were reported who received ≥4 lines of treatment (Table 1). The choice of treatment differed between groups. While patients with exon 20 insertions were mostly treated with chemotherapy in 1L (69%), patients with uncommon mutations and very rare *EGFR* mutations were given EGFR-TKI or chemotherapy with similar frequency in 1L (uncommon mutations: 51% EGFR-TKI, 45% chemo; very rare *EGFR* mutations: 44% EGFR-TKI, 48% chemo). In 2L, however, EGFR-TKIs were administered more often in patients with uncommon (EGFR-TKI 67%, chemo 23%) and very rare mutations (EGFR-TKI 47%, chemo 32%, Table 1).

In total, data from 445 different treatments (regardless of the line of treatment, 'all lines' group) were available for analysis of PFS and OS (Figure 2).

Clinical outcomes of patients

Uncommon *EGFR* mutations (group 1). In patients with uncommon *EGFR* mutations (group 1), median PFS (mPFS) was significantly longer when patients were treated with EGFR-TKI in 1L compared to chemotherapy (HR 0.53; 95% CI 0.30-0.93, $P = 0.028$, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225). This effect was similar when all treatments were considered independent of treatment line (all lines, HR 0.54, 95% CI 0.35-0.81, $P = 0.003$, Figure 2A, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225). Afatinib was the most frequently applied EGFR-TKI in this group (36/79 treatments, 46%), followed by erlotinib (22/79 treatments, 28%), gefitinib (13/79 treatments, 16%) and osimertinib (8/79 treatments, 10%) (Figure 3A). Patients treated with afatinib had an mPFS of 12.0 months (95% CI 4.0-20.0 months) while mPFS with erlotinib was only 3.8 months (95% CI 2.7-5.0

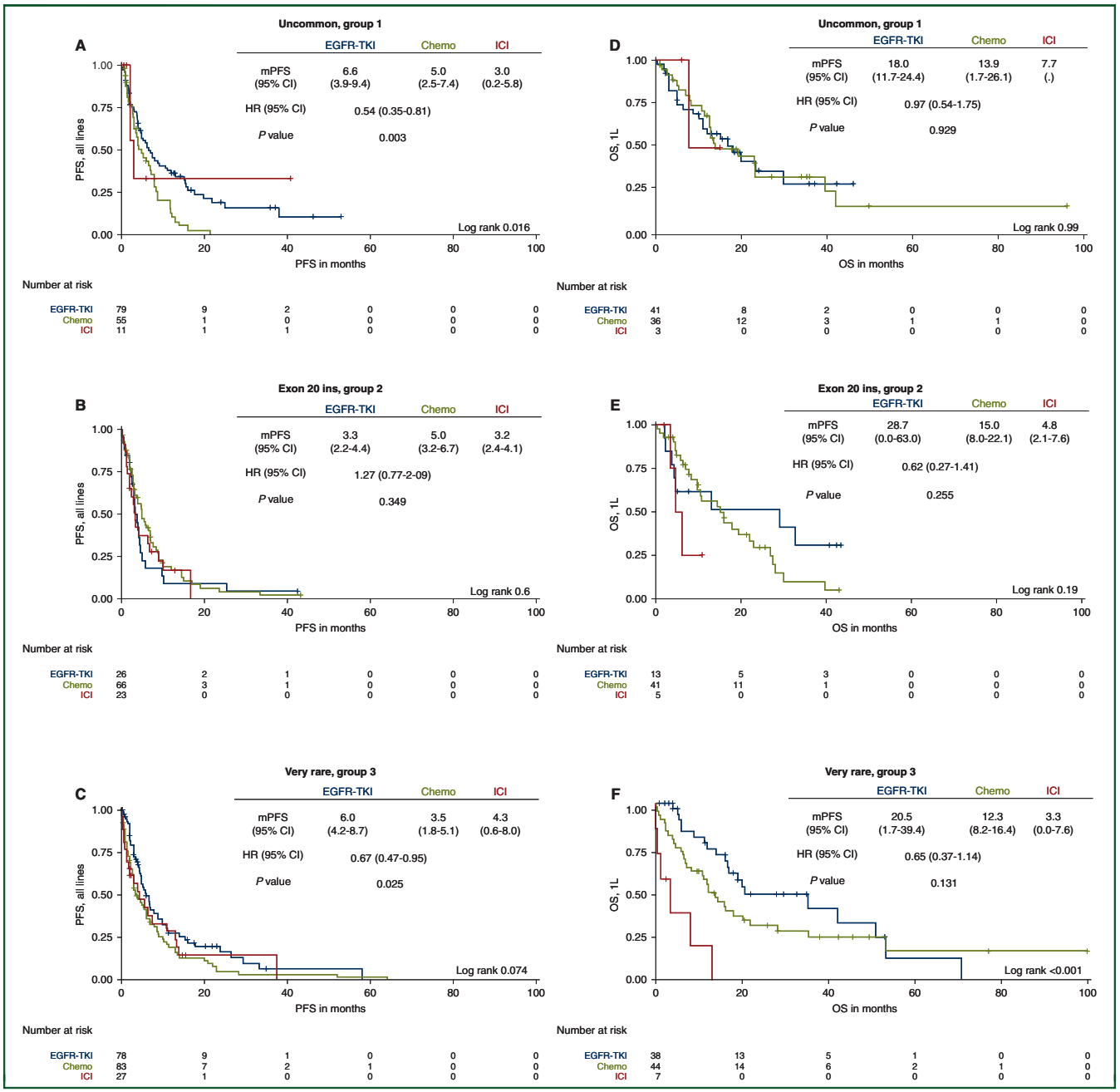


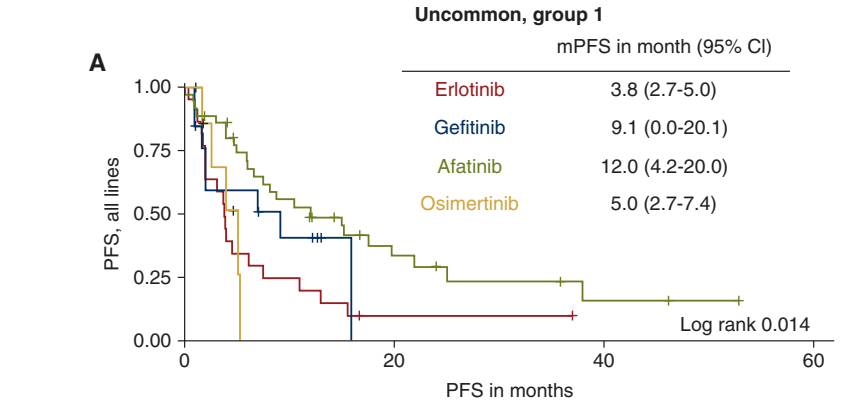
Figure 2. Progression-free (PFS) and overall survival (OS) with EGFR-TKI, chemotherapy and ICI according to the rare *EGFR* categories.
(A-C) Kaplan—Meier plot indicating PFS in patients with uncommon mutations, group1 (A), exon 20 insertions, group 2 (B) and very rare *EGFR* mutations, group 3 (C) calculated from any treatment regardless of treatment line ('all lines'). (D-F) Kaplan—Meier plot indicating OS for uncommon mutations, group 1 (D), exon 20 insertions, group 2 (E) and very rare *EGFR* mutations, group 3 (F) calculated for 1L treatments ('1L').
1L, first line; CI, confidence interval; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; HR, hazard ratio; ICI, immune checkpoint inhibitor; ins, insertions; mPFS, median PFS.

months). The number of patients treated with gefitinib and osimertinib was very low. However, the overall log-rank test indicated a significant difference in PFS among the different EGFR-TKIs (overall log rank 0.014, Figure 3A).

Data for treatment with ICI in 1L were available only for 3 patients and only for 11 patients considering all treatments regardless of treatment line ('all lines'). Upon treatment with ICI, mPFS in the 'all lines' group was 3.0 months (95% CI 0.2-5.8 months, Figure 2, Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225), which was lower than mPFS for EGFR-TKI and chemo (6.6 and 4.9 months, respectively, overall log rank 0.016).

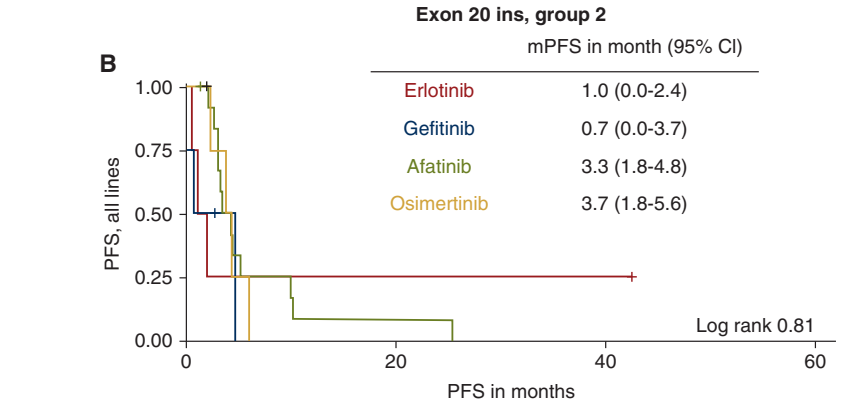
Median OS was numerically longer upon TKI treatment (18 months, 95% CI 11.7-24.4 months) compared to chemotherapy (13.9 months, 95% CI 1.7-26.1 months) and ICI (7.7 months) albeit this effect was not statistically significant (Figure 2D).

PFS outcomes upon treatment with different EGFR-TKIs per individual mutation for 1L and in the 'all lines' group are displayed in swimmer plots (Supplementary Figure S2,



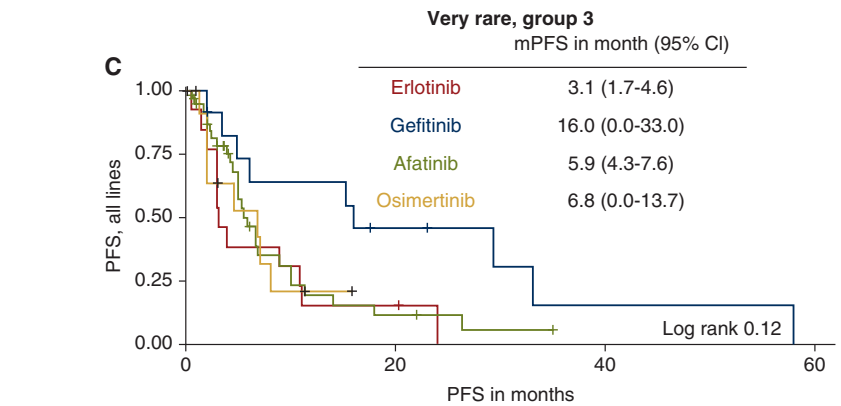
Number at risk

Erlotinib	22	1	0	0
Gefitinib	13	0	0	0
Afatinib	36	8	2	0
Osimertinib	8	0	0	0



Number at risk

Erlotinib	4	1	1	0
Gefitinib	4	0	0	0
Afatinib	13	1	0	0
Osimertinib	5	0	0	0



Number at risk

Erlotinib	13	2	0	0
Gefitinib	12	4	1	0
Afatinib	40	3	0	0
Osimertinib	13	0	0	0

Figure 3. Progression-free survival (PFS) according to type of EGFR-TKI.

available at https://doi.org/10.1016/j.annonc.2022.02.225). Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225, indicates mPFS for 1L and in the 'all lines' group in uncommon *EGFR* subgroups.

Exon 20 insertions (group 2). Patients with exon 20 insertions did not benefit from treatment with EGFR-TKI with a few exceptions (see below). In 1L, mPFS was numerically longer with chemotherapy compared to EGFR-TKI (6.9 versus 4.0 months, respectively, log rank 0.471, Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225). Considering all treatments, mPFS was 3.3 months upon treatment with EGFR-TKI and 5.0 months with chemotherapy. Possibly due to low numbers of patients treated with EGFR-TKI ($n = 26$) compared to chemotherapy ($n = 66$), this effect was not significant (HR 1.27, 95% CI 0.77-2.09, Figure 2B, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225). Afatinib was the most frequently administered EGFR-TKI in this group (13/27, 48.1% Figure 3B). Due to low numbers, no conclusion could be drawn regarding differences between EGFR-TKIs.

Median OS was longer in the EGFR-TKI group versus chemotherapy (28.7 versus 15.0 months, respectively). HR was 0.62, but the effect was not significant (95% CI 0.27-1.41, $P = 0.225$, Figure 2E, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225).

With regard to ICI treatment, only six patients were treated with ICI in the 1L. Considering all lines ($n = 23$), mPFS was similar in ICI-treated patients compared to EGFR-TKI-treated patients (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 2B).

Swimmer plots for individual exon 20 insertions confirmed the already documented sensitivity of 763_764AYinsFQEV to first-generation EGFR-TKI (Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225). In addition, one patient with a 773_774HVinsAH insertion had a remarkable response to afatinib as 1L treatment (PFS 25.4 months). The patient died 3.3 months after progression upon afatinib therapy without any further treatment. Also, a PFS of $\geq$10.0 months was observed in a patient with a 773_774HVinsGHPH insertion treated with afatinib. This patient had progressed from 1L chemotherapy after 7.7 months. These data suggest that afatinib can be effective in patients with these two specific exon 20 insertions in addition to the already documented 763_743AYinsFQEV mutation. For all other exon 20 insertions, mPFS was $<$9 months (mPFS 3.3 months, 95% CI 2.2-4.4 months, Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225).

Since NL insertions (AA767-772) may respond better to second-generation TKI than FL mutations, we also analyzed outcome in this subgroup of exon 20 insertion.[4,15] NL insertions occurred with a frequency of 79% ($n = 56$) and FL insertions with 20% ($n = 14$) of the exon 20 insertions. For patients with NL insertions, a mPFS of 8.4 months (95% CI 4.3-12.6 months) with chemotherapy, 3.3 months (95% CI 2.9-3.7 months) with EGFR-TKI and 3.4 months with ICI (95% CI 0.0-6.8 months) was observed in 1L (log rank 0.010, Supplementary Figure S1D, available at https://doi.org/10.1016/j.annonc.2022.02.225). In the 'all lines' cohort, we found an mPFS of 5.0 months (95% CI 3.1-6.8 months) with chemotherapy, 3.0 months (95% CI 2.3-3.7 months) with EGFR-TKI and 3.0 months with ICI (95% CI 1.5-4.3 months) (log rank 0.024, Supplementary Figure S1J, available at https://doi.org/10.1016/j.annonc.2022.02.225). The mPFS for afatinib in the 'all lines' cohort was 3.1 months (95% CI 2.7-3.5 months). However, the number of evaluable treatments for PFS was only 10 for afatinib, 4 for gefitinib and 3 for erlotinib and osimertinib (Supplementary Figure S1L, available at https://doi.org/10.1016/j.annonc.2022.02.225).

Due to the very low numbers of FL insertions in our cohort, no conclusions could be drawn in this subgroup concerning outcome of specific TKI (Supplementary Figure S1E and K, available at https://doi.org/10.1016/j.annonc.2022.02.225).

Very rare *EGFR* mutations (group 3). We observed a benefit for patients harboring mutations from this very heterogeneous group upon treatment with EGFR-TKI in comparison to chemotherapy. mPFS for patients treated with 1L EGFR-TKI was 6.7 versus 5.5 months for patients treated with chemotherapy (HR 0.71, 95% CI 0.42-1.18, $P = 0.187$, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225). When considering all lines, this effect was statistically significant (6.7 versus 3.5 months upon EGFR-TKI treatment compared to chemotherapy, HR 0.67, 95% CI 0.47-0.95, $P = 0.025$, Supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 2C). Interestingly, the PFS advantage translated into a numerical OS advantage with an mOS in 1L EGFR-TKI-treated patients of 20.5 months versus 12.3 months in patients treated with chemotherapy; however, this difference was not significant (HR 0.65, 95% CI 0.37-1.14, $P = 0.131$, Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2022.02.225, Figure 2F).

Again, afatinib was the most frequently given EGFR-TKI. Twenty-seven patients were treated with afatinib (60.0%), 8/45 with erlotinib (17.8%), 6/45 with gefitinib (13.3%) and 4/45 with osimertinib (8.9%, Figure 3C). Numerically, gefitinib-treated patients had the longest mPFS (16.0 months, Figure 3C), although the overall log-rank test comparing all four EGFR-TKIs was not significant ($P = 0.124$). Due to the heterogeneous nature and singular occurrence of many mutations in this group, further preclinical characterization is warranted in order to determine the functional impact of very rare *EGFR* mutations.

Kaplan–Meier plot indicating PFS for each EGFR-TKI in patients with uncommon mutations with known EGFR-TKI sensitivity, group 1 (A), exon 20 insertions, group 2 (B) and very rare *EGFR* mutations, group 3 (C) calculated from any treatment regardless of treatment line ('all lines').
CI, confidence interval; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; ins, insertions; mPFS, median PFS.

Table 2. nNGM classification of uncommon *EGFR* mutations

		mPFS upon EGFR-TKI	Recommendations
1. nNGM UC1 TKI-sensitive *EGFR* mutations			
A G719X, S768X, L861X mutations, alone or in complex with other uncommon mutations such as E709X or classical L858R or exon 19 deletions (group 1 in nNGM clinical FUP cohort)		6.6 months (Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225)	This group of *EGFR* mutations can be considered EGFR-TKI sensitive albeit these drugs are generally less effective compared to classical *EGFR* mutations.
B Complex mutations containing classical *EGFR* mutations L858R or exon19 deletions with very rare *EGFR* mutations		11.4 months (Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
C Exon 19 insertions		10.0 months (Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
D Specific exon 20 insertions		(Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
Y763_V764insFQEV	EGFR-TKI naive	Erlotinib, 42.5 months	
A767_V769dup	EGFR-TKI naive	Osimertinib, 13.4 months	
N771_H773dup	Second EGFR-TKI, switch from 1L gefitinib (instead of just gefinitinib) gefitinib due to toxicities	Afatinib, 10.1 months	
H773_V774insGHPH	EGFR-TKI naive	Afatinib, 10.0 months	
H773_V774insAH	EGFR-TKI naive	Afatinib, 25.4 months	
E Specific very rare single point mutations		(Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
E711Q	EGFR-TKI naive	Osimertinib, 11.4 months	
P733Q	EGFR-TKI naive	Afatinib, 14.3 months	
L747P	EGFR-TKI naive	Gefitinib, 33.1 months	
	EGFR-TKI naive	Osimertinib, 16.0 months	
P753L	5L (1L gefitinib PFS 2.0 months, 2L erlotinib PFS 3.0 months, 4L and 5L chemo)	Afatinib, 17.9 months	
E758G	EGFR-TKI naive	Afatinib, 34.9 months	
R776H	EGFR-TKI naive	Gefitinib, 58.1 months	
Q791H	EGFR-TKI naive	Erlotinib, 20.3 months	
F Specific complex mutations: uncommon with very rare		(Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
G719A, P753Q	EGFR-TKI naive	Afatinib, 10 months	
G Specific complex mutations: very rare with very rare		(Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225)	
L833V, H835L	EGFR-TKI naive	Gefitinib, 15.3 months	
2. nNGM UC2 T790M *de novo* mutations			
De novo T790M (not analyzed in this study)			T790M mutations are known to be resistant to first- and second-generation EGFR-TKIs. Osimertinib represents the current treatment of choice. However, information about T790M compound mutations with combination partner besides the classical *EGFR* mutations is rare. This is subject to further studies.
3. nNGM UC3 exon 20 insertions			
Exon 20 insertions		3.3 (2.2-4.4) months, Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225	Very heterogeneous group. In general, these mutations are insensitive toward classical EGFR-TKI, with the exception of specific exon 20 insertions mentioned in group 1 (nNGM UC 1D)
4. nNGM UC4 very rare *EGFR* mutations with insufficient functional and clinical data			
A Very rare single point mutations (except those mentioned in nNGM UC 1E)		5.0 (2.3-7.8) months, Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225	Discussion in molecular tumor boards and clinical decision making based on recommended guidelines based on consented evidence levels[32,33] taking into account case reports and also preclinical data. Preclinical *in vitro* and *in silico* testing should be considered in cases with no available information.
B Complex mutations: uncommon with very rare (except those mentioned in nNGM UC 1F)		3.0 (0.0-6.5) months, Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225	
C Complex mutations: very rare with very rare (except those mentioned in nNGM UC 1G)		7.0 (5.3-8.9) months, Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2022.02.225	

1L, first line; 2L, second line; 4L, fourth line; 5L, fifth line; CI, confidence interval; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; FUP, follow-up; ICI, immune checkpoint inhibitor; mPFS, median progression-free survival; nNGM, national Network Genomic Medicine.

As in groups 1 and 2, very few patients were treated with ICI in 1L ($n = 6$ patients, Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225). Considering all treatment lines, mPFS was 4.3 months for ICI-treated patients with very rare *EGFR* mutations (95% CI 0.6-8.4 months, Supplementary Table S4, available at https://doi.org/10.1016/j.annonc.2022.02.225).

Based on these data, we summarized the EGFR-TKI sensitivity in four groups of atypical *EGFR* mutations (nNGM UC1-4, Table 2). Of note, outcome data can be derived from one case only which especially applies to group 3 mutations.

Frequency of co-occurring mutations and their effect on outcome

The presence of co-occurring mutations such as *TP53* or *KRAS* mutations is known to influence outcome.[16,17] Co-mutations were reported for 310 patients in the nNGM cohort (36.2%) and occurred in 59% of these patients ($n = 183$) (Supplementary Figure S3A, available at https://doi.org/10.1016/j.annonc.2022.02.225). In 96% of the cases, one of three customized NGS panels was used (Supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2022.02.225). Here, not all genes were included in each panel. *BRAF, CTNNB1, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, PTEN* and *TP53* were sequenced with all three panels (Supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2022.02.225). Most frequently reported co-mutations were *TP53* mutations (132/310, 42.6%). In 34 of those patients (25.8%), *TP53* mutations were accompanied by other mutations such as *KRAS* (11/132 patients, 8.3%), *PIK3CA* (6/132 patients, 4.5%) *PTEN* (3/132 patients, 2.3%) and others (10/132 patients, 8.3%). *KRAS* was the second most frequently occurring co-mutation ($n = 29$, 9%, Supplementary Figure S3B, available at https://doi.org/10.1016/j.annonc.2022.02.225). Interestingly, significantly more co-mutations were reported in the group of very rare *EGFR* mutations, especially in the group of rare point mutations (Supplementary Figure S3C and D, available at https://doi.org/10.1016/j.annonc.2022.02.225), suggesting that these *EGFR* mutations may represent passenger mutations.

Since the co-occurrence of *KRAS* and other mutations was very rare, we chose to investigate only the effect of co-occurring *TP53* mutations on PFS in EGFR-TKI-treated patients in all treatment lines (Supplementary Figure S3F-I, available at https://doi.org/10.1016/j.annonc.2022.02.225). The KM plots indicated a detrimental effect of co-occurring *TP53* mutations on outcome in EGFR-TKI-treated patients with exon 20 insertions and a trend for a detrimental effect in very rare *EGFR* mutations but not in the EGFR-TKI-sensitive group 1 mutations (Supplementary Figure S3F-I, available at https://doi.org/10.1016/j.annonc.2022.02.225).

DISCUSSION

This nNGM multicenter, retrospective analysis represents the largest available real-world dataset of atypical *EGFR* mutations. Another main novelty lies in the analysis of clinical FUP data including outcome upon treatment with different EGFR-TKIs, chemotherapy and ICI while available studies mostly focus on single TKI.[5,6]

Our most important findings are (i) validation of EGFR-TKI efficacy in uncommon *EGFR* mutations generally considered TKI sensitive (group 1), (ii) TKI sensitivity of complex *EGFR* mutations containing exon 19 deletions or L858R mutations independent of the combination partner and (iii) report of the largest clinically annotated cohort of rare single and complex *EGFR* point mutations revealing a high degree of heterogeneity of TKI sensitivity.

Our findings are in line with previous data showing EGFR-TKI sensitivity for group 1 mutations (G719X, S768I, L861X and combination of these with other group 1 mutations or classical *EGFR* mutations).[3,5-7] Our analysis indicates increased efficacy of afatinib in these patients compared to gefitinib, erlotinib and osimertinib. This finding is in line with clinical trial data including NEJ002 and LUX-Lung 2, 3 and 6 and several smaller retrospective studies suggesting higher efficacy of afatinib compared to erlotinib or gefitinib.[3,7] In comparison, data from a recent phase II study (KCSG-LU15-09) indicated efficacy of osimertinib with an mPFS of 8.2, 15.2 and 12.3 months in patients with G719X, L861Q and S7681 mutations, respectively ($n = 19/9/8$).[8]

The reason for a lower mPFS observed with osimertinib in our cohort (mPFS = 5.0 months, Figure 3) might be the relatively low number of treatments with osimertinib ($n = 8$). Since most of the mutations in group 1 of our cohort were either PACC or classical-like mutations based on the structural approach by Robichaux et al., our data are in line with their predicted response model, as we observed a longer mPFS with afatinib compared to first-generation TKI and osimertinib. Although the number of osimertinib treatments was very low in our cohort preventing definitive conclusions, it is still remarkable that seven of eight osimertinib-treated patients had PACC mutations, predicted to respond less well to osimertinib compared to afatinib, which we also observed.[4] Structural modeling suggested that poziotinib, another second-generation TKI, may be an even better fit for PACC mutations compared to afatinib. However, this remains to be clinically validated.

Complex *EGFR* mutations occur relatively frequently (~30% in our cohort) and have not yet been extensively characterized. In addition to validating the already described sensitivity of complex uncommon compound mutations, our analysis revealed an overall EGFR-TKI sensitivity of complex mutations containing a classical *EGFR* mutation in combination with very rare point mutations (contained in group 3, mPFS 11.4 months, all lines, Supplementary Table S6 and Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225). We observed efficacy of afatinib, erlotinib and gefitinib in these patients. Again, due to low patient numbers, efficacy of osimertinib could not be assessed reliably in this group.

Our data also confirm low EGFR-TKI sensitivity in patients with exon 20 insertions in concordance with literature.

However, in this highly heterogeneous group, we observed a few exceptions sensitive to EGFR-TKI therapy, such as the already described 763_764YinsFQEV mutation (Friedlaender et al.,[15] PFS 42.5 months 1L erlotinib in our dataset). Additionally, we found four EGFR-TKI-sensitive mutations: 773_774HvinsAH (EGFR-TKI naive, PFS 25.4 months with afatinib), 767_769AVdup (EGFR-TKI naive, PFS 13.4 months with osimertinib), 771_773NHdup (EGFRI-TKI naive, PFS 10.1 months with afatinib) and 773_774HVinsGHPH (EGFR-TKI naive, PFS 10 months with afatinib, Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225). However, 771_773dup insertions and 767_769AVdup insertions did not respond to all TKIs in our study (Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225).

In the whole exon 20 insertion cohort (group 2), mPFS (all lines) of 3.3 months upon EGFR-TKI treatment was lower compared to the recently FDA-approved *EGFR* Exon20Ins mutation-targeting drugs mobocertinib (mPFS 7.3 months, EXCLAIM cohort) and amivantamab (mPFS 8.3 months).[11,12] Detailed information about response to specific exon 20 insertions from these studies was only available for one patient with a 763_764YinsFQEV mutation treated with amivantamab. Here, a PFS of ∼17 months was reported.[12]

We also investigated outcome especially in NL exon 20 insertions. In our cohort, most patients were treated with chemotherapy especially in 1L; therefore, any interpretation should be made with caution (Supplementary Figure S1B and H, available at https://doi.org/10.1016/j.annonc.2022.02.225). *In vitro* studies and clinical data suggest that some second-generation TKIs (mainly poziotinib) may have more efficacy in the NL than in the FL insertions.[4,13] Since treatments with first- and third-generation TKIs were very rare in our cohort and no patients have been treated with poziotinib, we cannot corroborate this. However, two exon 20 insertions from our cohort and two recent case reports showed that the second-generation TKI afatinib exerts efficacy in (some) FL insertions.[18,19] Altogether, mPFS upon EGFR-TKI treatment was lower in the subgroup of NL insertions upon EGFR-TKI treatment compared to chemotherapy and to reported outcome data with mobocertinib or amivantamab.[11,12]

Altogether, exon 20 insertions are very heterogeneous, and despite the recent approval of two new drugs specifically for this cohort, additional investigation is warranted in order to identify which type of insertion may respond to specific TKI or drugs. Of note, additional exon 20 insertion-specific drugs and antibody—TKI combinations such as CLN-081, BDTX-189 or anti-EGFR monoclonal antibody JMT101 in combination with EGFR-TKI are currently tested in early clinical trials.[20]

Very rare *EGFR* mutations represent almost one-third of patients in our study (group 3). Notably, this group was until now substantially underrepresented in existing cohorts and clinical trials with patients exhibiting rare *EGFR* mutations.[3,7,8,21] Additionally, in this group, the least knowledge about functional relevance of the mutations and clinical response to EGFR-TKI exists. As a note, this group, especially the group of very rare point mutations was also underrepresented in the Robichaux et al.'s dataset. In this thus far less well-investigated group, our data indicate clinical efficacy of EGFR-TKI compared to chemotherapy or ICI monotherapy. However, mPFS was numerically shorter (6.7 months, Figure 2C) compared to what is achievable with EGFR-TKI erlotinib or gefinitib (9.2-13.1 months), afatinib (11.0-11.1 months) or osimertinib (18.9 months) in patients with classical *EGFR* mutations.[22-28]

In contrast to patients with uncommon mutations (group 1), our data suggest a higher clinical efficacy of gefitinib (mPFS 16.0 months, $n = 12$) compared to osimertinib (6.8 months, $n = 13$), afatinib (5.9 months, $n = 40$) and erlotinib (3.1 months, $n = 13$). It is important, however, to acknowledge the high degree of heterogeneity and low numbers of patients with individual treatments in group 3. We therefore investigated response also in individual mutations and subgroups (Supplementary Table S6 and Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225). In the absence of other clinical information, information from one patient with a given very rare alteration who has responded to an EGFR-TKI is valuable to guide treatment decisions, although with a low level of evidence (in Germany, this corresponds to m1C case-report level of evidence leading to treatment recommendations[29]). European ESMO Scale for Clinical Actionability of molecular Target guidelines in this case refer to a IIa investigational evidence level in case of retrospective studies demonstrating clinical benefit of patients with a given alteration in the same cancer entity, however not specifying the number of patients.[30] We found a PFS >10 months in patients with the following mutations: R776H (gefitinib), L747P (gefitinib, osimertinib), E758G (afatinib), P753L (afatinib), P733Q (afatinib), Q791H (erlotinib), E711Q (osimertinib) (Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225, Table 2).

EGFR-TKIs also showed clinical activity in patients with exon 19 insertions (mPFS 10.0 months), but were less active in patients with exon 18 deletions (mPFS 4.0 months, Supplementary Table S4 and Figure S2, available at https://doi.org/10.1016/j.annonc.2022.02.225, Table 2), which is in line with other reports.[8,31]

It is known that co-mutations such as *TP53* can alter response to treatment and patients with classical *EGFR* mutations and *ALK* alterations with *TP53* mutations may have a detrimental effect on outcome with target-specific treatment.[32,33] Our data indicate a possible detrimental effect of co-occurring *TP53* mutations on outcome in EGFR-TKI-treated patients with exon 20 insertion and very rare *EGFR* mutations but not in the EGFR-TKI-sensitive group 1 mutations. However, this effect was not significant possibly due to low numbers and further analysis is warranted.

As a note, one limitation of our study is the retrospective nature and the investigator-assessed PFS data. Nevertheless, our data represent a large real-world dataset and will be helpful to guide treatment decisions.

Based on our findings, we suggest a novel nNGM classification of atypical *EGFR* mutations, nNGM uncommon (UC)

1, 3 and 4 (Table 2) according to their TKI sensitivity observed in our study. Based on the documented importance of *de novo* T790M mutations and their standard-of-care treatment option with osimertinib,[22] we included them as group UC2 even though we have not analyzed them in our study. It may well be that, in the future, we will have to split nNGM UC1 into subgroups based on how these mutations respond to different types of TKI, as Robichaux and colleagues are already suggesting. It will also be interesting to see how new third- and fourth-generation EGFR-TKIs may influence this. Some of these new drugs are already being tested in uncommon *EGFR* mutations [i.e. lazertinib in combination with amivantamb (CHRYSALIS)]. However, until now, clinical data on this are conflicting and the structure-based approach has to be validated especially for very rare mutations which have been mostly absent in their dataset.

Nevertheless, the combination of *in silico* and *in vitro* prediction validated with clinical data will be the way forward to predict response to targeted treatments for rare mutations even beyond *EGFR*.

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APPENDIX 1

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