FULL PAPER

BJC

British Journal of Cancer (2016) 115, 1504–1512 | doi: 10.1038/bjc.2016.372

Keywords: NSCLC; classic EGFR mutation; non-classic EGFR mutation; EGFR-TKI; TKI; EGFR

Non-classic *EGFR* mutations in a cohort of Dutch *EGFR*-mutated NSCLC patients and outcomes following EGFR-TKI treatment

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Background: Data on non-small-cell lung cancer (NSCLC) patients with non-classic *epidermal growth factor receptor* (*EGFR*) mutations are scarce, especially in non-Asian populations. The purpose of this study was to evaluate prevalence, clinical characteristics and outcome on EGFR-TKI treatment according to type of *EGFR* mutation in a Dutch cohort of NSCLC patients.

Methods: We retrospectively evaluated a cohort of 240 *EGFR*-mutated NSCLC patients. Data on demographics, clinical and tumour-related features, EGFR-TKI treatment and clinical outcome were collected and compared between patients with classic *EGFR* mutations, *EGFR* exon 20 insertions and other uncommon *EGFR* mutations.

Results: Classic *EGFR* mutations were detected in 186 patients (77.5%) and non-classic *EGFR* mutations in 54 patients (22.5%); 23 patients with an exon 20 insertion (9.6%) and 31 patients with an uncommon *EGFR* mutation (12.9%). Median progression-free survival (PFS) and overall survival (OS) on EGFR-TKI treatment were 2.9 and 9.7 months, respectively, for patients with an *EGFR* exon 20 insertion, and 6.4 and 20.2 months, respectively, for patients with an uncommon *EGFR* mutation. Patients with a double uncommon *EGFR* mutation that included G719X/L861Q/S768I had longer PFS and OS on EGFR-TKI treatment compared with patients with a single G719X/L861Q/S768I *EGFR* mutation (both $P = 0.02$).

Conclusions: In our Dutch cohort, prevalence and genotype distribution of non-classic *EGFR* mutations were in accordance with previously reported data. The PFS and OS on EGFR-TKI treatment in patients with an uncommon *EGFR* mutation were shorter compared with patients with classic *EGFR* mutations, but varied among different uncommon *EGFR* mutations.

Classic EGFR mutations. The discovery of mutations in the *epidermal growth factor receptor* (*EGFR*) gene as oncogenic driver in lung cancer patients has changed both the diagnostic process and treatment of such patients. The *EGFR* mutations are detected in ~10% of Caucasian patients with non-squamous non-small-cell lung cancer (NSCLC) and in up to 50% of Asian NSCLC patients (Dearden *et al*, 2013). In addition to the higher prevalence in people from Asian descent, there is a higher prevalence of *EGFR* mutations in women, nonsmokers and adenocarcinoma patients (Barlesi *et al*, 2016). The vast majority of *EGFR* mutations comprise microdeletions in exon 19 (45–50%) and the Leu858Arg (L858R) substitution, resulting from a point mutation in exon 21 (40–45%; Murray *et al*, 2008). These mutations are so-called sensitising *EGFR* mutations and hereafter referred to as 'classic *EGFR* mutations' (Supplementary Figure 1). The beneficial effect of treatment with EGFR tyrosine kinase inhibitors (TKIs) in NSCLC

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Received 10 June 2016; revised 24 September 2016; accepted 15 October 2016; published online 22 November 2016

patients who harbour a classic *EGFR* mutation in their tumour is well established (Lynch *et al*, 2004; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010; Fukuoka *et al*, 2011; Zhou *et al*, 2011; Han *et al*, 2012; Rosell *et al*, 2012; Sequist *et al*, 2013; Wu *et al*, 2014). However, resistance is inevitable and median progression-free survival (PFS) on EGFR-TKI treatment for NSCLC patients with a classic *EGFR* mutation is 8.0–13.1 months (Mok *et al*, 2009; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010; Fukuoka *et al*, 2011; Zhou *et al*, 2011; Han *et al*, 2012; Rosell *et al*, 2012; Sequist *et al*, 2013; Wu *et al*, 2014, 2015).

The T790M mutation. The T790M mutation is a distinct *EGFR* mutation that is located in exon 20. It interferes with binding of EGFR-TKI to EGFR, thereby prohibiting the inhibitory effect of these agents. Detection of the T790M mutation before EGFR-TKI treatment is rare (0.5%; Yu *et al*, 2014), although the detection rate of pretreatment T790M is higher with more sensitive detection methods (Rosell *et al*, 2011). However, the T790M mutation is detected in ~60% of *EGFR*-mutated NSCLC patients on or post treatment with an EGFR-TKI showing renewed tumour growth (Yu *et al*, 2013).

Non-classic *EGFR* mutations. *The EGFR* mutations other than the classic *EGFR* mutations and exon 20 T790M mutations are less prevalent (hereafter referred to as 'non-classic *EGFR*-mutations') (Supplementary Figure 1). The most prevalent non-classic *EGFR* mutations are insertions or duplications in *EGFR* exon 20 (further referred to as '*EGFR* exon 20 insertions') that are detected in ~2.2–5.0% of NSCLC patients (Wu *et al*, 2008a; Arcila *et al*, 2013; Oxnard *et al*, 2013; Beau-Faller *et al*, 2014). In the study of Arcila *et al* (2013), *EGFR* exon 20 insertions were furthermore mutually exclusive with mutations in other genes, such as *KRAS* and *BRAF*, except for *PIK3CA* and there were no associations with age, sex, race or stage. Patients with *EGFR* exon 20 insertions generally have a lower response rate to EGFR-TKI treatment and a poorer prognosis compared with NSCLC patients with classic *EGFR* mutations (Wu *et al*, 2008a; Oxnard *et al*, 2013). Other non-classic *EGFR* mutations include so-called uncommon mutations (Supplementary Figure 1), for example, in *EGFR* exon 18 (e.g., G719X; X = A, S or C), *EGFR* exon 20 (e.g., S768I) and *EGFR* exon 21 (e.g., L861Q). The proportion of uncommon *EGFR* mutations among *EGFR*-mutated NSCLC patients might be as high as 14%, but varies in different studies (Yokoyama *et al*, 2006; Zhang *et al*, 2007; Wu *et al*, 2008b, 2011; Hata *et al*, 2010; De Pas *et al*, 2011; Arcila *et al*, 2013; Kobayashi *et al*, 2013; Keam *et al*, 2014).

Multiple uncommon *EGFR* mutations or an uncommon *EGFR* mutation in combination with a classic *EGFR* mutation may co-exist in the same tumour. These so-called 'double' (or complex, or compound) mutations are reported to occur in 6.6% of *EGFR*-mutated NSCLC patients (Hata *et al*, 2010).

Data on results of EGFR-TKI treatment in Caucasian patients with non-classic *EGFR* mutations are scarce as they are commonly reported in small series, whereas the larger series typically include patients of Asian descent. We therefore evaluated a cohort of Dutch (i.e., predominant Caucasian) *EGFR*-mutated NSCLC patients retrospectively. The purpose of this study was to evaluate the prevalence and genotype distribution of non-classic *EGFR* mutations in this cohort, as well as clinical characteristics and outcome on EGFR-TKI treatment.

MATERIALS AND METHODS

Patients. All NSCLC patients in whom an *EGFR* mutation was detected in the VU University Medical Center (VUmc) between May 2006 and November 2014 ($N = 240$) were retrospectively evaluated. As the VUmc is a diagnostic referral centre, some patients were diagnosed at our centre, but follow-up and treatment were performed in other hospitals. Patients with missing data on follow-up were excluded from analysis of clinical characteristics and outcome on EGFR-TKI treatment. For all other patients, data on demographics, clinical and tumour-related features, treatments and clinical outcomes was extracted from the medical records.

Mutation analysis. All mutation analyses were part of the routine diagnostic procedures in VU University Medical Center, Amsterdam, The Netherlands. The molecular diagnostic modalities for *EGFR* mutation analysis included Sanger sequencing, HRM sequencing and cancer panel multiplexed targeted resequencing (Janmaat *et al*, 2006; Heideman *et al*, 2009; Sie *et al*, 2014). All assays are designed to identify deletions or insertions in *EGFR* exons 19 and 20, and hot spot mutations in *EGFR* exons 18 through 21.

For analytical purposes, deletions in *EGFR* exon 19 and the L858R point mutation in *EGFR* exon 21 are referred to as classic *EGFR* mutations. Among non-classic *EGFR* mutations, a distinction between exon 20 insertions and 'uncommon *EGFR*-mutations' was made (Supplementary Figure 1). The post-treatment T790M mutations are not included in our analyses, nor are common *EGFR* polymorphisms. All alterations that were detected were checked in Alamut Visual version 2.7 (Interactive Biosoftware, Rouen, France), the mycancergenome database (www.mycancergenome.org; accessed 1 April 2016) and the Cosmic database (cancer.sanger.ac.uk/cosmic; accessed 23 April 2016).

Treatment and outcomes. Patients who were alive at closing date (26 November 2015) or who were lost to follow-up were censored at the last date of follow-up. The EGFR-TKI treatment included treatment with erlotinib, gefitinib or afatinib in patients with advanced-stage disease. Survival was calculated from date of diagnosis of advanced-stage (stage IIIB or IV) disease until date of death. Objective response rate (ORR) on EGFR-TKI treatment was calculated as the proportion of patients with complete or partial response according to Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 (Eisenhauer *et al*, 2009). Disease control rate (DCR) on EGFR-TKI treatment was calculated as the proportion of patients with an objective response or stable disease (for at least 6 weeks) according to the RECIST 1.1 criteria (Eisenhauer *et al*, 2009). Progression-free survival on EGFR-TKI treatment was calculated as the time from first day of treatment until progression of disease or date of death (from any cause). Patients who had not progressed at data cutoff were censored at the last day of follow-up. Overall survival (OS) on EGFR-TKI treatment was either calculated as the time from the first day of EGFR-TKI treatment until date of death (from any cause), or patients were censored at last follow-up.

Statistical analyses. Comparison of categorical variables was performed with Pearson's χ^2 test. Comparison of three or more continuous variables was performed with one-way ANOVA. The Kaplan–Meier method was used for survival analyses and the log rank test was used to test for significance. Two-sided *P*-values of $\leqslant 0.05$ were considered significant and confidence intervals (CIs) were calculated at a 95% CI. The SPSS for Windows (version 20; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The medical ethical committee of VU University Medical Center (Amsterdam, The Netherlands) approved the protocol.

RESULTS

Classic *EGFR* mutations. In 186 out of 240 patients (77.5%), a classic *EGFR* mutation was detected (Figure 1): 134 patients (72.0%) with an exon 19 deletion and 52 patients (28.0%) with an exon 21 L858R point mutation.

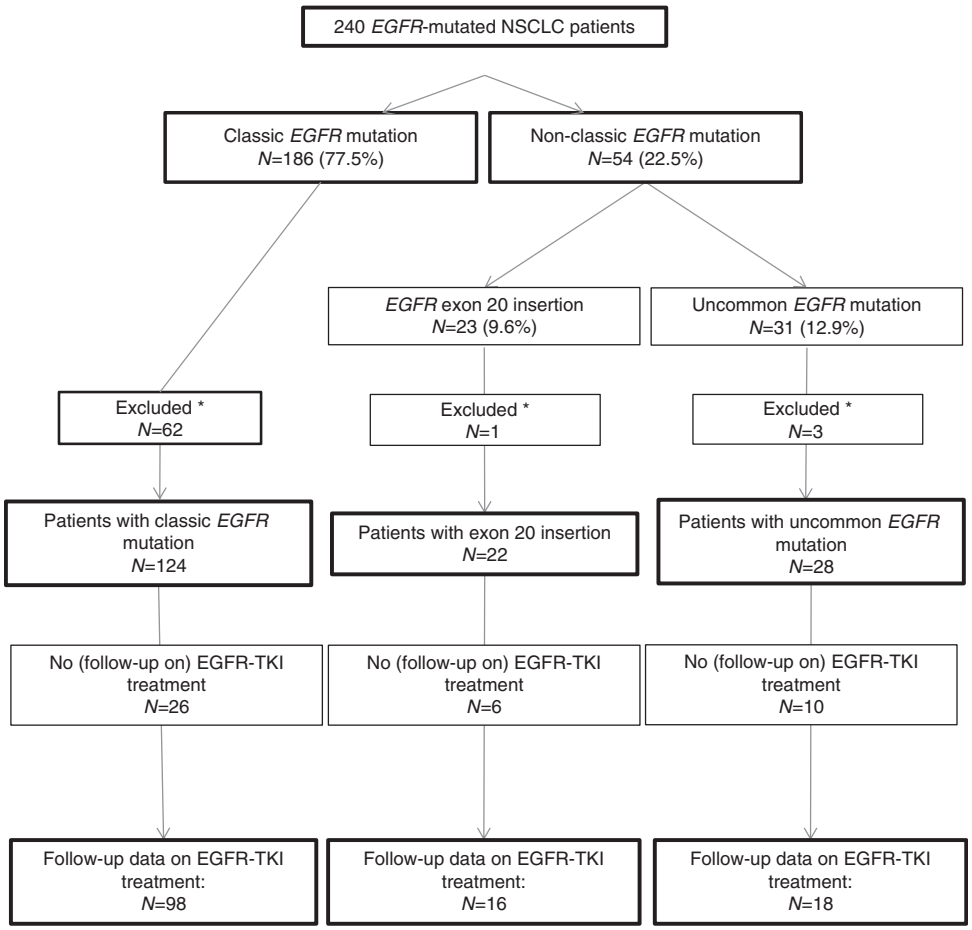

Figure 1. Flowchart. *No treatment and follow-up in VUmc.

Sixty-two patients with a classic *EGFR* mutation were not treated at our centre and were excluded from further analysis. Clinical characteristics of the remaining 124 *EGFR*-mutated NSCLC patients are described in Table 1. Median follow-up was 31.6 months (95% CI, 26.1–27.3). The EGFR-TKI treatment was started in 111 patients (89.5%) (Supplementary Table 1). Clinical outcome on EGFR-TKI treatment of this group of patients is described in Table 2. Supplementary Tables provide more detailed data on start and/or progression on EGFR-TKI treatment (Supplementary Table 2), survival after EGFR-TKI treatment (Supplementary Table 3) and response setting (Supplementary Table 4).

Non-classic *EGFR* mutations. A total of 54 patients (22.5%) harbouring a non-classic *EGFR* mutation were identified: 23 patients (9.6%) with an exon 20 insertion and 31 patients (12.9%) with an uncommon *EGFR* mutation in exons 18, 19, 20 and/or 21. In one patient, both an exon 20 insertion and an *EGFR* exon 20 V769L point mutation were detected. This patient was categorised in the *EGFR* exon 20 insertion group. All *EGFR* exon 20 insertions concerned insertions located on regions V769-N771 or H773-V774.

Of the group with uncommon *EGFR* mutations, 15 patients (6.3%) had a single uncommon *EGFR* mutation (Table 3) and 16 patients (6.7%) were identified with double uncommon *EGFR* mutations (Table 4). In three patients (1.3%) with a single uncommon *EGFR* mutation, a *KRAS* mutation was also detected (Table 3). In four patients (1.7%) with double *EGFR* mutations, one of these mutations concerned the classic *EGFR* mutation L858R on exon 21 (Table 4). There were two patients with a single G719X *EGFR* mutation and two patients with a single L861Q *EGFR* mutation (Table 3). Nine patients were identified with a double *EGFR* mutation that included G719X, L861Q and/or S768I (further referred to as 'double G719X/L861Q/S768I' *EGFR* mutations; Table 4).

Of the patients with non-classic *EGFR*-mutations, four were not treated in our centre and excluded from further analysis (i.e., one with an exon 20 insertion, one with L858R + V834L mutation, one with an exon 19 insertion and one with L861Q mutation).

Clinical characteristics of the remaining 22 patients with an *EGFR* exon 20 insertion and 28 patients with an uncommon *EGFR* mutation are described in Table 1. Median follow-up of these patients was 29.4 months (95% CI, 19.6–39.3). Baseline demographic characteristics were similar between the three groups, except for smoking ($P<0.01$).

EGFR-TKI treatment in patients with an EGFR exon 20 insertion. Sixteen patients with advanced-stage disease and an exon 20 insertion received EGFR-TKI treatment. Seven patients (43.8%) received EGFR-TKI as first-line treatment, but most patients received EGFR-TKI treatment as second-, third- or fourth-line treatment (Supplementary Table 5). Median PFS on EGFR-TKI treatment was 2.9 months (95% CI, 2.3–3.6). Median OS on EGFR-TKI treatment was 9.7 months (95% CI, 0.00–21.1). Both PFS and OS on EGFR-TKI treatment were significantly shorter in patients with an *EGFR* exon 20 insertion compared with patients with a classic *EGFR* mutation ($P<0.01$ and $P=0.01$, respectively; Figure 2A and B). The ORR was 0.0% and DCR was 56.3%.

EGFR-TKI treatment in patients with an uncommon EGFR exon 18, 19, 20 and 21 mutation. Twenty patients with an uncommon *EGFR* mutation received EGFR-TKI treatment. Sixteen patients (80%) received EGFR-TKI treatment as first-line

Table 1. Patient characteristics

Patient characteristics	Classic *EGFR* mutation (*N* = 124)		*EGFR* exon 20 insertion (*N* = 22)		Uncommon *EGFR* mutation (*N* = 28)		*P*-value
Median age[a] *(years)*			61.5 (range 29.5–83.0)		61.0 (range 41.4–81.5)		0.43
	Frequency	(percentage)	Frequency	(percentage)	Frequency	(percentage)	
Gender							
Male	29	23.4%	8	36.4%	9	32.1%	0.34
Female	95	76.6%	14	63.6%	19	67.9%	
Ethnicity							
Caucasian	110	89.4%	20	90.9%	24	85.7%	0.81
Other[b]	13	10.6%	2	9.1%	4	14.3%	
Unknown	1	—	0	—	0	—	—
Smoking[c]							
Current or previous smoker	50	43.5%	6	33.3%	20	74.1%	0.01
Never smoker	65	56.5%	12	66.7%	7	25.9%	
Unknown	9	—	4	—	1	—	—
Performance Status (PS)							
PS 0–1	95	92.2%	13	100.0%	18	92.6%	0.53
PS >1	8	7.8%	0	0.0%	2	7.4%	
Unknown	21	—	9	—	8	—	—
Histology							
Adenocarcinoma	114	91.9%	21	95.5%	26	92.9%	0.79
Squamous cell carcinoma	1	0.8%	0	0.0%	1	3.6%	
Adenosquamous carcinoma	2	1.6%	0	0.0%	0	0.0%	
Large-cell neuroendocrine carcinoma	4	3.2%	0	0.0%	0	0.0%	
Large-cell carcinoma	3	2.4%	1	4.5%	1	3.6%	
Stage[a]							
I–IIIA	25	20.7%	9	40.9%	3	10.7%	0.03
IIIB–IV	96	79.3%	13	59.1%	25	89.3%	
Unknown	3	—	0	—	0	—	—

Abbreviation: *EGFR* = *epidermal growth factor receptor.*
[a]At the time of first diagnosis of non-small-cell lung cancer (NSCLC).
[b]Afro-American or Oriental.
[c]Patients who smoked <100 cigarettes in a lifetime were considered as nonsmokers, patients who smoked within the last year before diagnosis were considered as current smokers and the remainder was considered as previous smoker.

and four patients (20%) as second-line treatment. For two patients, there was no registered date of progression. Median PFS on EGFR-TKI treatment for the remaining 18 patients (all advanced-stage disease) was 6.4 months (95% CI, 0.0–17.6). This was not significantly different compared with median PFS in patients with a classic *EGFR* mutation ($P = 0.39$). Median OS on EGFR-TKI treatment in patients with an uncommon *EGFR* mutation was 20.2 months (95% CI, 0.0–41.7). This was significantly shorter compared with the median OS on EGFR-TKI treatment in patients with a classic *EGFR* mutation ($P = 0.04$).

For 15 patients with uncommon *EGFR* mutations, data on response on EGFR-TKI treatment could be retrieved from the medical records: ORR was 53.3% and DCR was 86.7%.

Ten patients with single or double G719X/L861Q/S768I *EGFR* mutations were treated with an EGFR-TKI. Median PFS on EGFR-TKI treatment for patients with a double G719X/L861Q/S768I *EGFR* mutation ($N = 7$) was 6.4 months (95% CI, 0.0–17.6), and this was significantly longer ($P = 0.02$) than for patients with single-mutant status at these loci ($N = 3$; 1.6 months (95% CI, 1.5–1.7)). Median OS on EGFR-TKI treatment for patients with a double G719X/L861Q/S768I *EGFR* mutation was 28.6 months (95% CI, 11.3–45.8), and 3.9 months (95% CI, 0.5–7.4) for those with a single G719X/L861Q/S768I *EGFR* mutation ($P = 0.02$).

Table 2. Median PFS, OS, ORR and DCR on EGFR-TKI treatment in advanced-stage NSCLC patients with classic *EGFR* mutations

	N	Months	(95% CI)	*P*-value
Median PFS				
All patients	98[a]	12.2	(10.8–13.5)	
Exon 19	75	12.6	(11.2–14.1)	0.26
Exon 21	23	12.0	(7.5–16.6)	
Median OS				
All patients	107[b]	26.4	(22.8–30.1)	
Exon 19	79	28.2	(21.8–34.6)	0.04
Exon 21	28	21.0	(20.4–21.6)	
	N	%		*P*-value
ORR				
All patients	94[c]	84.0%		
Exon 19	70	84.3%		0.91
Exon 21	24	83.3%		
DCR				
All patients	94[c]	95.7%		
Exon 19	70	97.1%		0.25
Exon 21	24	91.7%		

Abbreviations: CI = confidence interval; DCR = disease control rate; EGFR = epidermal growth factor receptor; NSCLC = non-small-cell lung cancer; ORR = objective response rate; OS = overall survival; PFS = progression-free survival; TKI = tyrosine kinase inhibitor.
[a]For 13 patients, data on PFS on EGFR-TKI treatment were incomplete (Supplementary Table 2).
[b]For 4 patients, data on OS on EGFR-TKI treatment were incomplete (Supplementary Table 3).
[c]For 17, patients data on response on EGFR-TKI treatment were incomplete (Supplementary Table 4).

DISCUSSION

Targeted agents are being developed rapidly and their clinical use is increasing in NSCLC patients. Considering the toxicities and costs

Table 3. Patients with single uncommon *EGFR* mutations

EGFR mutation				Clinical characteristics					EGFR-TKI treatment	
Exon	Codon	Protein	*KRAS* mutation	Sex	Histology	Smoking	Stage[a]	PS[a]	PFS	Response
18	c.2124G>T	p.K708N	No	F	Adeno	Current	No stage IV	Unknown	No TKI	
18	c.2155G>A	p.G719S	No	M	Adeno	Previous	Stage IV	PS 1	1.6	PD
18	c.2156G>C	p.G719A	No	F	Adeno	Previous	Stage IV	Unknown	1.5	NA
18	c.2161G>T	p.G721C	Yes	F	SqCC	Previous	Stage IV	PS 1	No TKI	
19	c.2232 C>G	p.I744M	No	M	Adeno	Current	Stage IV	Unknown	No TKI	
20	c.2379 G>T	p.M793I	No	F	Adeno	Previous	Stage IV	PS 1	16.2	SD
20	c.2327G>A	p.R776H	No	M	Adeno	Previous	Stage IV	PS 1	9.8	PR
20	c.2327G>A	p.R776H	No	F	Adeno	Unknown	Stage IV	Unknown	No TKI	
20	c.2327G>T	p.R776L	No	F	Adeno	Current	Stage IV	PS 1	2.6	SD
20	c.2335_2336GG>TT	p.G779F	No	M	Adeno	Current	Stage IV	Unknown	No TKI	
21	c.2582T>A	p.L861Q	No	M	Adeno	Current	Stage IV	PS 2	1.8	PR
21	c.2513T>G	p.L838P	Yes	F	Adeno	Previous	Stage IV	PS 0	2.2	SD
21	c.2495G>A	p.R832H	Yes	M	Adeno	Previous	Stage IV	PS 1	No TKI	
19	c.2231 2232ins18	p.I744 K745insKIPVAI	No	F	Adeno	No clinical data				
21	c.2582T>A	p.L861Q	No	F	Adeno	No clinical data				

Abbreviations: Adeno = adenocarcinoma; *EGFR* = *epidermal growth factor receptor*; F = female; M = male; NA = not available; PD = progressive disease; PFS = progression-free survival; PR = partial response; PS = performance status; SD = stable disease; SqCC = squamous cell carcinoma; TKI = tyrosine kinase inhibitor.
[a]At the time of first diagnosis of non-small-cell lung cancer (NSCLC).

Table 4. Patients with double *EGFR* mutations (and at least one uncommon *EGFR* mutation)

EGFR mutation				Clinical characteristics					EGFR-TKI treatment	
Classic/uncommon	Exon	Codon	Protein	Gender	Histology	Smoking	Stage[a]	PS[a]	PFS	Response
Double uncommon	18+18	c.2155G>A+c.2125G>A	p.G719S+p.E709K	F	Adeno	Nonsmoker	Stage IV	Unknown	15.0	PR
Double uncommon	18+20	c.2155G>T+c.2303G>T	p.G719C+p.S768I	F	Adeno	Previous	Stage IV	Unknown	25.0	NA
Double uncommon	18+20	c.2155G>A+c.2303G>T	p.G719S+p.S768I	F	Adeno	Previous	Stage IV	PS 0	No TKI	
Double uncommon	18+20	c.2155G>A+c.2327G>A	p.G719S+p.R776H	M	Adeno	Previous	Stage IV	PS 0	59.1	PR
Double uncommon	18+20	c.2156G>C+c.2303G>T	p.G719A+p.S768I	F	Adeno	Previous	Stage IV	Unknown	1.9	PD
Double uncommon	19+21	c.2239-2253del15bp+c.2509G>T	p.del L747_T751+D837T	F	Adeno	Previous	Stage IV	PS 0	15.0	CR
Double uncommon	18+21	c.2156G>C+c.2582T>A	p.G719A+p.L861Q	F	Adeno	Current	Stage IV	PS 1	2.1	SD
Double uncommon	20+20	c.2303G>T+c.2305G>C	p.S768I+p.V769L	F	Adeno	Previous	Stage IV	PS 1	1.2	NA
Double uncommon	20+20	c.2303G>T+c.2320G>A	p.S768I+p.V774M	F	Adeno	Nonsmoker	Stage IV	PS 1	No TKI	
Double uncommon	21+21	c.2497T>G+c.2504A>T	p.L833V+p.H835L	F	Adeno	Nonsmoker	Stage IV	PS 2	11.7	PR
Double uncommon	21+21	c.2497T>G+c.2504A>T	p.L833V+p.H835L	F	Adeno	Nonsmoker	Stage IV	PS 1	NA	NA
Double uncommon	21+21	c.2512C>G+c.2582T>A	p.L838V+p.L861Q	F	Large-cell	Nonsmoker	Stage IV	PS 1	6.4	NA
Classic+uncommon	21+21	c.2573T>G+c.2618G>A	p.L858R+p.G873E	M	Adeno	Previous	Stage IV	PS 1	NA	PR
Classic+uncommon	21+21	c.2573T>G+c.2612C>A	p.L858R+p.A871E	F	Adeno	Nonsmoker	Stage IV	PS 0	18.0	PR
Classic+uncommon	21+20	c.2573T>G+c.2369C>T	p.L858R+p.T790M (pre-treatment T790M)	M	Adeno	Nonsmoker	Stage IV	PS 0	8.0	SD
Classic+uncommon	21+21	c.2573T>G+c.2500G>T	p.L858R+p.V834L	F	Large-cell	No clinical characteristics				

Abbreviations: Adeno = adenocarcinoma; CR = complete response; *EGFR* = *epidermal growth factor receptor*; F = female; Large-cell = large cell carcinoma; M = male; NA = not available; PD = progressive disease; PFS = progression-free survival; PR = partial response; PS = performance status; SD = stable disease; TKI = tyrosine kinase inhibitor.
[a]At the time of first diagnosis of non-small-cell lung cancer (NSCLC).

of these drugs, their usage should be restricted to patients who truly benefit from them. In lung cancer, the efficiency of EGFR-TKIs is well known for classic *EGFR* mutations, but less data are available for patients with non-classic *EGFR* mutations. Moreover, most studies were performed in Asian populations. This study, among Dutch *EGFR*-mutated NSCLC-patients, adds to the current knowledge on non-classic *EGFR* mutations and the outcome on EGFR-TKI treatment in this subgroup of lung cancer patients.

In our cohort of 240 *EGFR*-mutated NSCLC patients, 54 patients (22.5%) were identified with a non-classic *EGFR*-mutation: 23 patients (9.6%) with an *EGFR* exon 20 insertion and 31 patients (12.9%) with an uncommon *EGFR* mutation.

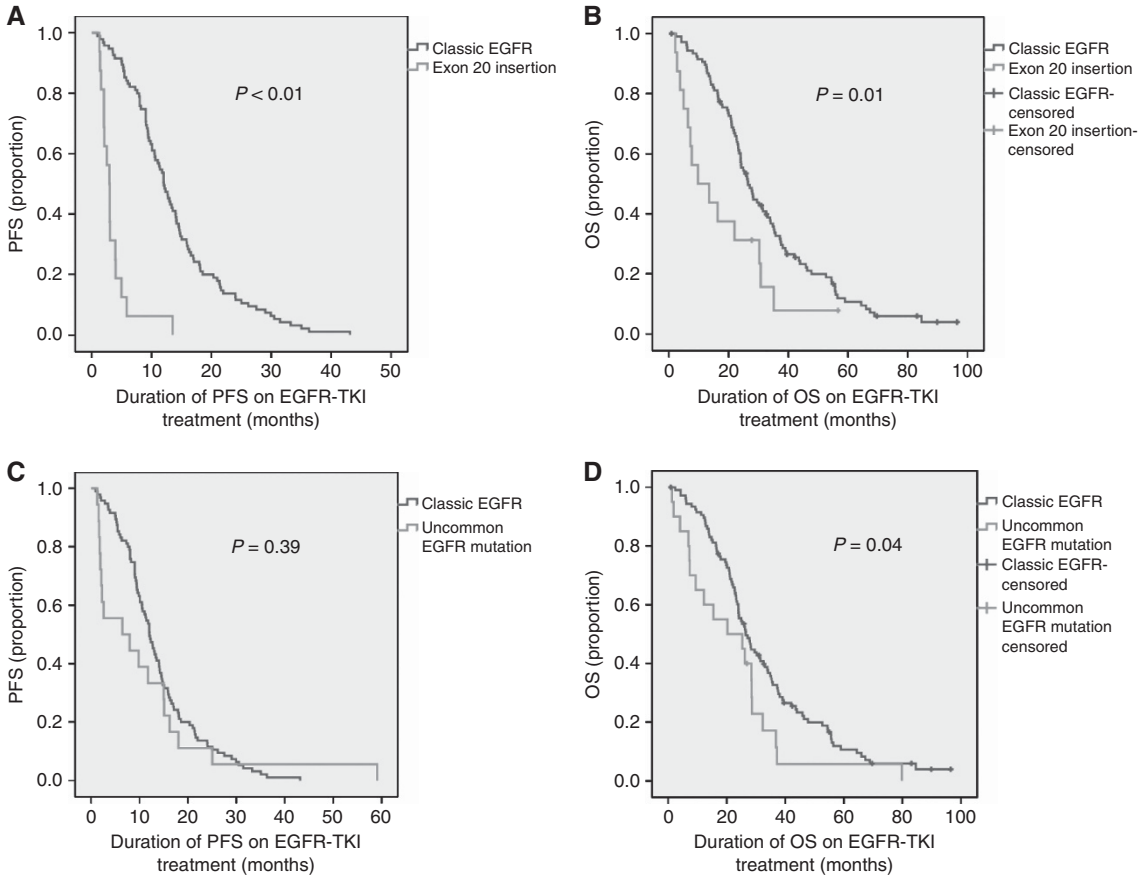

Figure 2. The PFS and OS on EGFR-TKI treatment in patients with a classic *EGFR* mutations *vs EGFR* exon 20 insertions or uncommon *EGFR* mutations. Difference between classic *EGFR* mutations *vs EGFR* exon 20 insertions in PFS (**A**) and OS (**B**) and between classic *EGFR* mutations and uncommon *EGFR* mutations in PFS (**C**) and OS (**D**). A full colour version of this figure is available at *British Journal Of Cancer* online.

Previous studies on *EGFR* exon 20 insertions in predominantly non-Asian *EGFR*-mutated NSCLC patients reported a rate of 9%, 4.0% and 9.2% (Arcila *et al*, 2013; Oxnard *et al*, 2013; Beau-Faller *et al*, 2014), hence incidence of *EGFR* exon 20 insertions in our cohort is approximately in line with these studies. The incidence of uncommon *EGFR* mutations among non-Asian *EGFR*-mutated NSCLC patients varies between 5.9% and 20.4% (Pallis *et al*, 2007; De Pas *et al*, 2011; Beau-Faller *et al*, 2014; Stone *et al*, 2014; Arrieta *et al*, 2015; Lohinai *et al*, 2015), and this is also in accordance with results from our study. However, comparison to other studies is difficult, as there is a large variance in ethnicity of patients included, detection method of *EGFR* mutations and categorisation of non-classic *EGFR* mutations.

Interestingly, we detected a numerical difference in PFS and OS between patients with a classic *EGFR* mutation (exon 19 *vs* exon 21 mutation) that was significantly different for OS in favour of patients with an *EGFR* exon 19 deletion. Although originally it was thought that there was no difference between these two subtypes of classical *EGFR* mutations (Igawa *et al*, 2014), a meta-analysis detected a difference in PFS in favour of patients with an *EGFR* exon 19 deletion (Zhang *et al*, 2014). We did not detect a significant difference in PFS, but we did detect a difference in OS between these groups in accordance with a recent study (Rossi *et al*, 2016). Further investigation is warranted.

Several studies reported a higher prevalence of *EGFR* exon 20 insertions among women, nonsmokers and Asians (Huang *et al*, 2004; Kosaka *et al*, 2004; Shigematsu *et al*, 2005; Sasaki *et al*, 2007; Wu *et al*, 2008a), although another study did not find a significant difference in age, sex, ethnic origin or stage at diagnosis when compared with both patients with a classic *EGFR* mutation as in patients lacking a mutation (Arcila *et al*, 2013). Survival of NSCLC patients with an *EGFR* exon 20 insertion has generally been reported to be poor (Oxnard *et al*, 2013). Most exon 20 insertions are insensitive for treatment with both reversible and irreversible EGFR-TKIs (except for the *EGFR* exon 20 insertion A763_Y764insFQEA; Yasuda *et al*, 2013). This insensitivity is probably the reason of the poorer survival of this category of patients compared with NSCLC patients with classic *EGFR* mutations (Wu *et al*, 2008a; Lund-Iversen *et al*, 2012; Woo *et al*, 2014). In our cohort, PFS of patients with an *EGFR* exon 20 insertion on EGFR-TKI treatment was 2.9 months, comparable to the PFS of 1.5–2.0 months on erlotinib or gefitinib (Wu *et al*, 2008a; Jackman *et al*, 2009) and 2.7 months on afatinib (Yang *et al*, 2015b) that were reported previously. This suggests that these patients should preferably be treated with cytotoxic chemotherapy instead of first- and second-generation EGFR-TKIs. Recently, favourable results of a clinical study with AUY922 in NSCLC patients with an *EGFR* exon 20 insertion were reported and may hopefully provide a better treatment option for *EGFR*-mutated NSCLC patients with exon 20 insertion (Piotrowska *et al*, 2015).

Likewise, it has been reported that patients with uncommon *EGFR* mutations have lower EGFR-TKI sensitivity (Arrieta *et al*, 2015). We did not detect a statistically significant difference between patients with uncommon and classic *EGFR* mutations with respect to PFS, but considering the large numerical difference (6.4 *vs* 12.0 months, respectively), this is probably because of the small sample size and wide variation in PFS among patients with an uncommon *EGFR* mutation (Tables 3 and 4).

In our study, G719X/L816Q/S768I *EGFR* mutations are the most frequently detected among uncommon *EGFR* mutations, in

line with previous reports (Mitsudomi and Yatabe, 2007; Shi *et al*, 2014). The G719X and the L861Q *EGFR* mutations were reported to have a shorter OS on gefitinib compared with classic *EGFR* mutations (Watanabe *et al*, 2014), although a recent study reported a PFS and OS of 13.8 and 26.9 months, respectively, for patients with a G719X *EGFR* mutation on first-line afatinib (Yang *et al*, 2015a). Chiu *et al* (2015) detected a statistically significant difference in median PFS on EGFR-TKI between patients ($N = 161$) with single and double G719X/L816Q/S768I *EGFR* mutations. In our cohort, patients with double G719X/L816Q/S768I *EGFR* mutations not only had a statistically significant longer PFS on EGFR-TKI treatment compared with patients with single-mutant status at these loci, but also a longer OS. However, groups were small in our cohort, and hence interpretation should be with caution. Further investigation on the difference between single and double G719X, L816Q and/or S768I *EGFR* mutations is warranted.

It has been suggested that platinum-doublet treatment might be the best first-line treatment option for patients with (both single and double) G719X/L816Q/S768I *EGFR* mutations (Watanabe *et al*, 2014). However, taking into account the durable responses on EGFR-TKI treatment of several patients with a double *EGFR* mutation that included G719X, L861Q and/or S768I, in our opinion EGFR-TKI treatment could be considered as first-line treatment for these patients. In addition, high response rates of patients with G719X/L816Q/S768I *EGFR* mutations to first-line afatinib were recently reported (Yang *et al*, 2015a). Prospective trials are needed to elucidate this question.

Several limitations should be taken into account for this study. Because of the retrospective design, bias cannot be excluded. In addition, in a considerable part of the patients (especially in patients with an *EGFR* exon 20 insertion) data on performance score and smoking could not be retrieved from the medical records and the line of EGFR-TKI treatment (i.e., first-, second-line and so on) varied. Perhaps therefore, we detected a significant difference between the groups in smoking. In addition, in the early days of *EGFR* testing, clinical characteristics were taken into account. Therefore, there might have been a screening bias for women and nonsmokers. However, from 2012, all stage IV adenocarcinoma patients were tested for *EGFR* mutations, irrespective of gender, smoking status and race. A large molecular heterogeneity existed among patients with non-classic *EGFR* mutations. The results of the subgroup analyses should therefore be interpreted with caution. Furthermore, in routine pathology, solely tumour tissue is evaluated, and for most cases of our study no normal DNA was available to confirm the somatic origin of the mutations identified. The R776H mutation (detected in three patients in our cohort), for example, has both been reported as somatic and germline (Nagalakshmi *et al*, 2013; van Noesel *et al*, 2013). For one of our patients, analysis of normal DNA confirmed somatic nature (data not shown), but for the others because of absence of normal DNA a germline nature cannot be excluded.

To summarise, in this cohort of Dutch *EGFR*-mutated NSCLC patients, the prevalence and genotype distribution of non-classic *EGFR* mutations was in accordance with previously published studies among non-Asian, *EGFR*-mutated NSCLC patients. Outcome on EGFR-TKI treatment was poor for patients with *EGFR* exon 20 insertions and varied widely in patients with uncommon *EGFR* mutations. Further (prospective) studies on patients with non-classic *EGFR* mutations are warranted to hopefully improve prognosis of these patients.

CONFLICT OF INTEREST

D Heideman has occasionally been member of the scientific advisory boards of Amgen and Pfizer.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)